The High-Fat Diet–Fed Mouse

A Model for Studying Mechanisms and Treatment of Impaired Glucose Tolerance and Type 2 Diabetes

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This study characterizes the high-fat diet–fed mouse as a model for impaired glucose tolerance (IGT) and type 2 diabetes. Female C57BL/6J mice were fed a high-fat diet (58% energy by fat) or a normal diet (11% fat). Body weight was higher in mice fed the high-fat diet already after the first week, due to higher dietary intake in combination with lower metabolic efficiency. Circulating glucose increased after 1 week on high-fat diet and remained elevated at a level of ~1 mmol/l throughout the 12-month study period. In contrast, circulating insulin increased progressively by time. Intravenous glucose challenge revealed a severely compromised insulin response in association with marked glucose intolerance already after 1 week. To illustrate the usefulness of this model for the development of new treatment, mice were fed an orally active inhibitor of dipeptidyl peptidase-IV (LAF237) in the drinking water (0.3 mg/ml) for 4 weeks. This normalized glucose tolerance, as judged by an oral glucose tolerance test, in association with augmented insulin secretion. We conclude that the high-fat diet–fed C57BL/6J mouse model is a robust model for IGT and early type 2 diabetes, which may be used for studies on pathophysiology and development of new treatment. *Diabetes* 53 (Suppl. 3):S215–S219, 2004

There is a need for new treatment modalities of type 2 diabetes in view of the progressive deterioration of metabolic control that occurs in spite of intense treatment with existing modalities (1). New treatment should aim at normalizing the basic defects in the disease, which are islet dysfunction in combination with insulin resistance (2). There is, however, also a need for more knowledge of the molecular mechanisms underlying these basic defects. These two needs require reliable and clinically relevant experimental models. Most animal models do not, however, fulfill such requirements, since they are based on monogenic disorders of little relevance for human diabetes (3–5) or on chemical destruction of β-cells, which is also of less clinical relevance (6,7). An important and relevant model, however, is the high-fat diet–fed C57BL/6J mouse model. This model was originally introduced by Surwit et al. in 1988 (8). The model has shown to be accompanied by insulin resistance, as determined by intravenous glucose tolerance tests, and of insufficient islet compensation to the insulin resistance (9). The model has, accordingly, been used in studies on pathophysiology of impaired glucose tolerance (IGT) and type 2 diabetes (10–12) and for development of new treatments (13–16). Here we report the characteristics of this model and illustrate its relevance in studies on developing new treatment modes by showing beneficial influences of a novel and efficient orally active inhibitor of dipeptidyl peptidase-IV (DPP-IV), which is a new mode for treating type 2 diabetes by preventing the degradation of glucagon-like peptide-1 (GLP-1) (17–19).

RESEARCH DESIGN AND METHODS

Female C57BL/6J mice were purchased from Taconic (Skensved, Denmark). The animals were maintained in a temperature-controlled room (22°C) on a 12-h light-dark cycle. The study was approved by the Animal Ethics Committee at Lund University, Sweden. One week after arrival, mice were divided into two groups and were fed either a high-fat diet (Research Diets, New Brunswick, NJ) or received continuous feeding of a normal diet (Lactamin, Stockholm, Sweden) for up to 12 months. On caloric basis, the high-fat diet consisted of 58% fat from lard, 25.6% carbohydrate, and 16.4% protein (total 23.4 kJ/g), whereas the normal diet contained 11.4% fat, 62.8% carbohydrate, and 25.8% protein (total 12.6 kJ/g). Food intake and body weight were measured once a week, and blood samples were taken at indicated time points from the intraorbital retrobulbar plexus from nonfasted anesthetized mice.

Glucose tolerance tests and insulin release. For intravenous glucose tolerance tests (IVGTTs), 4-h fasted mice were anesthetized with 7.2 mg/kg fluanison/fentanyl (Hypnorm; Janssen, Beerse, Belgium) and 15.3 mg/kg midazolam (Dormicum; Hoffman-LaRoche, Basel, Switzerland). Thereafter a blood sample was taken from the retrobulbar, intraorbital, capillary plexus, after which D-glucose (1 g/kg) was injected intravenously in a tail vein (volume load 10 μl/g). Additional blood samples were taken at 1, 5, 10, 20, 50, and 75 min after injection. Following immediate centrifugation at 4°C, plasma was separated and stored at −20°C until analysis. For oral glucose tolerance tests (OGTTs), 16-h fasted anesthetized mice were given 150 mg glucose by gavage through a gastric tube (outer diameter 1.2 mm), which was inserted in the stomach. Blood samples were taken at 0, 15, 30, 60, 90, and 120 min after glucose administration and handled as above.

Administration of DPP-IV inhibitor. Five-week-old mice were fed a high-fat or a normal diet for 8 weeks. After 4 weeks, the mice were additionally given the DPP-IV inhibitor LAF237 in their drinking water (0.3 mg/ml, ~3 μmol LAF237 · day^{-1} · $mouse^{-1}$). LAF237 (1-[[(3-hydroxy-1-adamantyl)amino-]acetyl]-2-cyano-(*S*)-pyrrolidine) is an orally active, highly efficient inhibitor of

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AIR, acute insulin response; DPP-IV, dipeptidyl peptidase-IV; GLP-1, glucagon-like peptide-1; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

DPP-IV (19) (a kind gift from Novartis Pharmaceuticals, Boston, MA). Control groups were given tap water without LAF237. After another 4 weeks, the mice were subjected to an OGTT as described above.

Insulin and glucose measurements. Insulin was determined radioimmunochemically using a guinea pig anti-rat insulin antibody, ^{125}I-labeled human insulin as tracer, and rat insulin as standard (Linco Research, St. Charles, MO). Plasma glucose was determined by the glucose oxidase method.

Statistical analyses. All results are expressed as means ± SE. Metabolic efficiency was calculated as the energy intake divided by the body weight gain over a certain period of time. In the IVGTT, the acute insulin response (AIR) to intravenous glucose was calculated as the mean of suprabasal 1- and 5-min values, and the glucose elimination was quantified as the K_G (i.e., the glucose elimination constant), the reduction in circulating glucose between minute 1 and 20 after intravenous administration after logarithmic transformation of the individual plasma glucose values, and expressed as percent elimination of glucose per minute. In the OGTT, the early insulin response was estimated as the increase in plasma insulin at 15 min above basal, and the K_G as the glucose elimination rate between minute 15 and 60. Linear relationships were estimated using Pearson's moment correlation coefficient. Statistical comparisons were performed with Student's unpaired and paired t tests; when multiple comparisons were performed, ANOVA was used.

RESULTS

Body weight and food intake. In this large experimental series of animals comprising of 10 separate experiments with ~50 mice in each experiment, high-fat diet was introduced at 4 weeks of age in half of the animals, while the other half was maintained on the normal, low-fat diet. At this age, body weight was 16.3 ± 0.1 g in both mice switched to high-fat diet (n = 259) and in mice maintained on normal diet (n = 240). Already during the first week after introduction of high-fat diet, body weight increased significantly more in the high-fat diet–fed mice (+1.6 ± 0.1 g) than in the normal diet–fed mice (+0.2 ± 0.1 g; $P <$ 0.001). The weight gain continued thereafter to be progressively higher in high-fat–fed mice (Fig. 1*A*). The growth curves showed, however, similar patterns in the two groups, with a larger body weight gain over the first 12 weeks, followed by a slower weight gain during the subsequent weeks. Both these patterns were linear in both groups, as illustrated in Fig. 1*A*. The growth rate in normal diet–fed mice during the first 12 weeks was 0.40 ± 0.03 g/week (r = 0.98; $P <$ 0.001) and this was increased to 0.68 ± 0.04 g/week (r = 0.99; $P <$ 0.001) in high-fat diet–fed mice, i.e., the weight gain was augmented by ~70% by the high-fat diet ($P <$ 0.001). The growth rate during the second phase, i.e., from week 13 and onwards, was 0.10 ± 0.01 g/week in normal diet–fed mice (r = 0.93; $P <$ 0.001) versus 0.18 ± 0.03 g/week in high-fat diet–fed mice (r = 0.87; $P <$ 0.001); hence, the augmented growth rate was ~80% during this phase ($P <$ 0.001). The break point between the two linear curves was identical in mice fed high-fat and normal diet (occurring at 12 weeks after introduction of high-fat diet, i.e., at 16 weeks of age).

The statistical power was high in this study due to the inclusion of the large number of animals (n = ~500). When analyzed in each of the 10 separate experiments (n = ~50 in each), however, it was clear that body weight had not increased significantly after 1 week in all individual experiments. A robust increase in body weight (i.e., having a probability level of random difference of $P <$ 0.001 when n = 25 in each group) is not seen until week 3, when in this study it was observed in 9 of the 10 individual experimental series. In rare occasions, however, it took an even longer period of time to reach a significant difference between the two groups. Yet, at 8 weeks of age and 4 weeks on the diet, all separate experiments showed a highly significant difference in body weight between the groups.

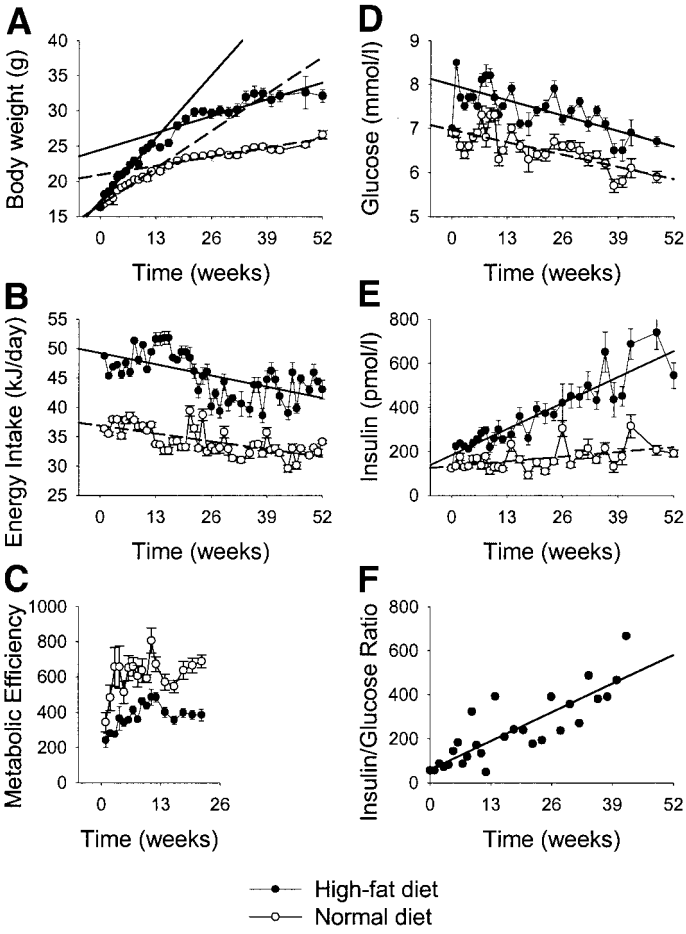

FIG. 1. Body weight, energy intake, metabolic efficiency, baseline levels of glucose and insulin, and the baseline insulin/glucose ratio in female C57BL/6J mice given a high-fat diet or a normal diet for 12 months. Ten different experimental series with a total of ~500 mice were included in the figures. The mice were 4 weeks old at the start of the experiment. Data are means ± SEM. Linear regressions are also estimated.

The energy intake was increased in high-fat diet–fed mice compared with normal diet–fed mice throughout the study period (Fig. 1*B*). By time, energy intake declined linearly, with, however, the difference between the two groups being stable. Metabolic efficiency (i.e., energy intake divided by body weight gain) was calculated for the initial 24-week study period, when weight gain was high (Fig. 1*C*). This was significantly reduced in high-fat diet–fed mice ($P <$ 0.001).

Baseline glucose and insulin. Weekly samples were collected from nonfasting, anesthetized mice for measurements of plasma levels of glucose and insulin. At the start of the study, i.e., at 4 weeks of age, basal glucose was 7.0 ± 0.1 mmol/l (n = 499) and basal insulin was 127 ± 4 pmol/l with no difference between the two groups. After already 1 week on high-fat diet, both glucose (by 1.8 ± 0.2 mmol/l) and insulin increased (by 78 ± 15 pmol/l; both $P <$ 0.001), whereas no difference was observed after 1 week on maintained normal diet. Circulating glucose declined throughout the 1-year study period in both groups of animals (Fig. 1*D*). The reduction was linear by time with a similar rate (slope of the curve) in both groups (−0.022 ± 0.004

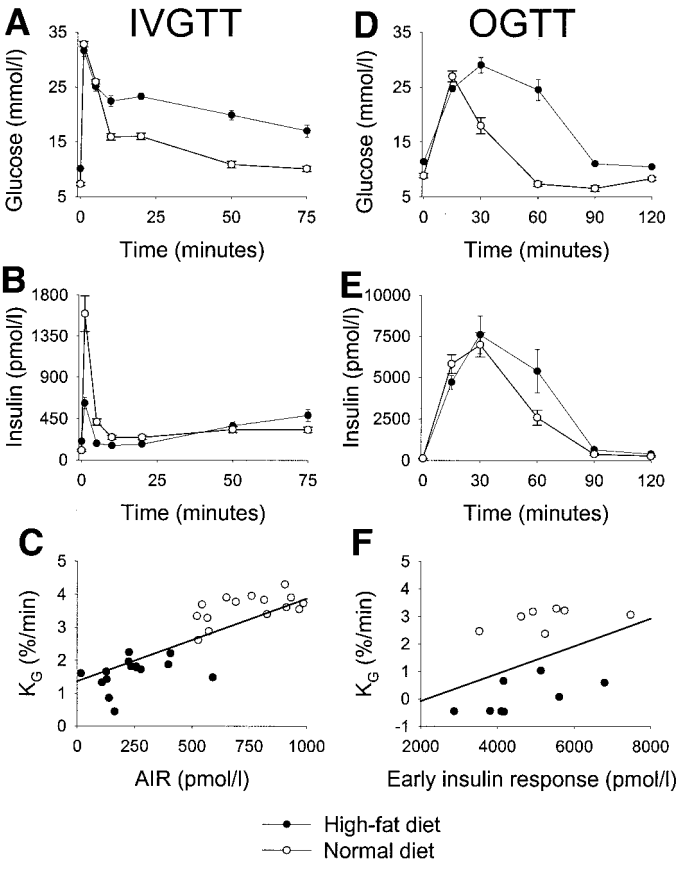

FIG. 2. Plasma levels of glucose and insulin and the glucose elimination constant as a function of AIR or early insulin response in female C57BL/6J mice given high-fat diet or a normal diet during an IVGTT performed 1 week after starting the high-fat feeding or in an OGTT performed 3 weeks after starting the high-fat feeding. Data are means ± SEM.

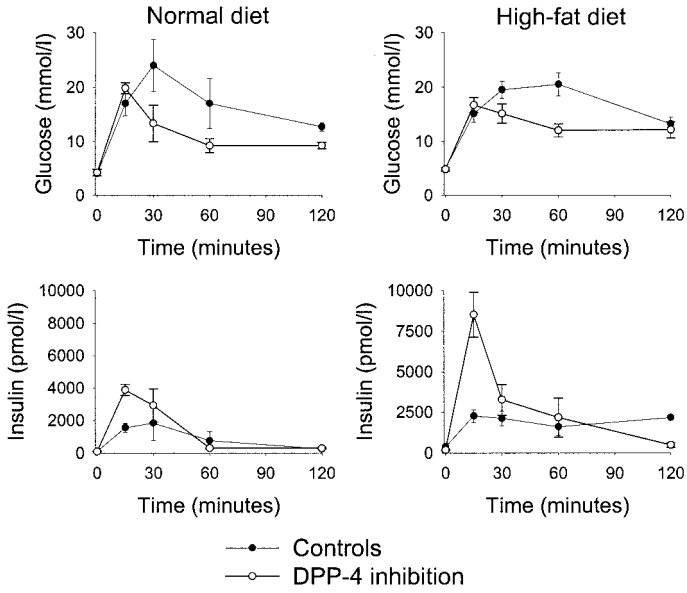

FIG. 3. Plasma levels of glucose and insulin during an OGTT in C57BL/6J mice given high-fat diet or normal diet for 8 weeks. In the last 4 weeks, mice given the DPP-IV inhibitor LAF237 in the drinking water (controls given plain water). Data are means ± SEM.

mmol/l/week in normal diet–fed mice versus −0.027 ± 0.005 mmol/l/week in high-fat diet–fed mice [NS]). The difference in glucose between the two groups was thus unchanged throughout, being 0.91 ± 0.05 mmol/l for the 10 batches of experiments as a mean ($P < 0.001$). Insulin levels progressively increased throughout the 1-year study period in both groups of mice (Fig. 1*E*). The rate of increase was, however, markedly different, being 8.9 ± 0.8 pmol/l/week in high-fat diet–fed mice versus only 1.6 ± 0.6 pmol/l/week in normal diet–fed mice ($P < 0.001$). The pattern of progressive separation of insulin levels in high fat–fed mice in association with a similar time-dependent reduction in glucose in the two groups suggests a progressive worsening of insulin resistance during high-fat feeding. This is illustrated in Fig. 1*F*, where the increase in mean insulin levels in high-fat diet–fed mice over mean insulin in normal diet–fed mice at each time point is plotted versus the corresponding increase in mean glucose levels in high-fat diet–fed mice. It is seen that this ratio, which indirectly estimates augmented insulin resistance in high-fat diet–fed mice, is linearly increased by time [$r = 0.85$, $P < 0.001$, slope 9.6 ± 1.1 (pmol/l insulin)/(mmol/l glucose)/week].

IVGTT. IVGTT was performed at 1 week after introduction of high-fat diet (Figs. 2*A* and *B*). It is seen that already at this early time point, high-fat diet–fed mice were glucose intolerant and had impaired glucose-stimulated insulin secretion. In particular, the AIR was impaired. Glucose elimination, as estimated by the K_G, was 3.9 ± 0.2%/min in normal diet–fed mice ($n = 20$) versus only 1.6 ± 0.1%/min in high-fat diet–fed mice ($n = 18$; $P < 0.001$). Similarly, AIR was markedly lower in high-fat diet–fed mice (196 ± 37 pmol/l) than in normal diet–fed mice (899 ± 92 pmol/l; $P < 0.001$). Figure 2*C* shows that there was a highly significant linear relation between AIR and K_G across all animals, such that when AIR was increased, so was K_G ($r = 0.87$; $P < 0.001$).

OGTT. Figs. 2*D* and *E* show the results of the OGTTs in the two groups of mice, performed after 3 weeks of high-fat feeding. In normal diet–fed mice, plasma glucose levels reach the maximum at 15 min after glucose challenge; thereafter, a first-order kinetic of glucose elimination occurs until minute 60. In contrast, there was hardly any glucose elimination between minute 15 and 60 in high-fat diet–fed mice, which suggests severe glucose intolerance. The K_G between minute 15 and 60 was 2.9 ± 0.1%/min in normal diet–fed mice ($n = 8$) versus 0.1 ± 0.2%/min in high-fat diet–fed mice ($n = 8$; $P < 0.001$). The 15-min insulin response to the oral glucose challenge was blunted after high-fat feeding (2.8 ± 0.4 nmol/l) compared with after normal diet feeding (4.1 ± 0.4 nmol/l, $n = 14$). Plotting the early insulin response versus the 15- to 60-min K_G revealed a significant relation ($r = 0.51$; $P = 0.048$), although this was partially explained by the complete separation of the K_G values between the groups (Fig. 2*F*).

Inhibition of DPP-IV. After 4 weeks on high-fat diet or normal diet, mice were given the DPP-IV inhibitor LAF237 in the drinking water; controls were given water alone. Four weeks later, an OGTT was undertaken. It was found that the administration of LAF237 improved glucose tolerance in association with markedly augmented insulin secretion. This was seen in both the normal diet–fed mice (Fig. 3*A* and *B*) and the high-fat diet–fed mice (Fig. 3*C* and *D*).

DISCUSSION

This study characterizes the high-fat diet–fed mouse as a robust model for IGT and early type 2 diabetes. This model was initially described by Surwit et al. in 1988 (8), and the model has been shown to be most efficient in C57BL/6J mice compared with other strains (20–22). We show here by accumulated data on a large number of animals belonging to this strain that a high-fat diet results in increased body weight gain and over time a stable hyperglycemia but a progressively increased hyperinsulinemia, indicating progressive worsening of insulin resistance. Furthermore, already after 1 week on the diet, baseline plasma glucose and insulin were significantly elevated and IVGTTs showed reduced glucose elimination and impaired insulin secretion (particularly the AIR). The model thus shows two important mechanistic characteristics for IGT and type 2 diabetes: insulin resistance and islet dysfunction.

The growth curves for this 1-year study could be divided into two phases—one initial phase with more rapid growth, which lasted until 16 weeks of age, and a second phase with slower growth. Energy intake was higher in the high-fat diet–fed mice. We estimated a parameter, metabolic efficiency, by calculating the ability of ingested energy to be metabolized. During the rapid growth phase, energy intake was stable while metabolic efficiency increased over the time period for both groups (i.e., by the time ingested energy less likely resulted in body weight gain). Metabolic efficiency index was lower in high-fat diet–fed mice compared with normal diet–fed mice. This is the inverse parameter of the feed efficiency (i.e., weight gain per ingested energy unit), which has been shown to be elevated in high-fat diet–fed mice (23). This indicates that the weight gain observed in high-fat–fed mice is not fully explained by increased energy intake but is also caused by a reduced metabolic rate. After the rapid growth period, both body weight gain and energy intake decreased in both feeding groups, which was reflected in a slight reduction in the metabolic efficiency.

Baseline glucose was significantly higher in high-fat diet–fed mice already after 1 week with the diet, the difference being 1.9 mmol/l. This was followed by a reduction of the difference to ~1 mmol/l after 2 weeks, which was a difference being stable throughout the 1-year study period. In contrast, insulin levels increased progressively in high-fat diet–fed mice. This suggests that insulin resistance progressively increased but that this was compensated under baseline conditions to keep the hyperglycemia stable at ~1 mmol/l. It may be speculated that the compensation requires slight hyperglycemia and that mechanisms responsible for the hyperinsulinemia do not work properly if circulating glucose is not increased by even a low degree. The initiation of the compensation under basal conditions may, however, require a slightly higher hyperglycemia, as is evident from the higher glucose levels at 1 week.

In contrast to the near-normal compensation under baseline conditions in glucose levels, IVGTT revealed a marked deterioration of glucose elimination, and this was seen in association with marked suppression of insulin secretion. The tight correlation between AIR and K_G shows that the AIR is of major importance for glucose elimination and that the mechanism of the IGT is the defective AIR. The present study shows that this is seen already after 1 week on high-fat diet. The OGTT showed similarly that high-fat diet–fed mice had IGT and that this was associated with defective insulin secretion. Hence, the model is suitable for studies on IGT and early type 2 diabetes.

In this study, we also show that the model is suitable for examining novel therapeutic interventions. Thus, we demonstrate that DPP-IV inhibition is a robust mode for treating glucose intolerance, which is seen in association with improved insulin secretion. The rationale for developing DPP-IV inhibition for treatment of type 2 diabetes is that GLP-1 has been proposed as a new therapeutic agent in the treatment of type 2 diabetes (13,17–19). The problem in developing GLP-1 as a new treatment, however, is that the hormone is rapidly degraded in the circulation by DPP-IV, which limits the duration of the GLP-1 effect. Thus, by inhibiting DPP-IV, the inactivation of GLP-1 is prevented; therefore, the effect of GLP-1 is prolonged (13). Previous studies have shown this mode of treatment to be efficient in both rodent models of diabetes (13,15,18) and human subjects with type 2 diabetes (24,25). In this study we show that the efficient DPP-IV inhibitor LAF237 is efficient in improving glucose tolerance and insulin secretion in the high-fat diet–fed mouse model.

In conclusion, we show here that the high-fat diet–fed C57BL/6J mice is a robust and efficient model for IGT and early type 2 diabetes and may therefore be used for both mechanistic studies and as a tool for developing novel therapeutic interventions. We also show specifically that IGT is apparent already after 1 week on the high-fat diet, that adequate islet compensation under baseline conditions requires a minimal hyperglycemia, and that treatment with DPP-IV inhibition by LAF237 does improve the condition.

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