Plasma Profiles of Glucose, Insulin and Lipids in the Male WBN/Kob-\(Lepr^{fa}\) Rat, a New Model of Type 2 Diabetes with Obesity

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**Abstract.** Plasma profiles of glucose, insulin and lipids were examined in the male WBN/Kob-\(Lepr^{fa}\) (fa/fa) rat, a new model of type 2 diabetes (T2D), in comparison with age-matched original male WBN/Kob (lean) rats. The fa/fa rats developed hypertriglyceridaemia, obesity and hyperglycaemia from 5, 7, and 9 weeks of age, respectively. Plasma insulin levels in fa/fa rats were significantly higher than those in lean rats at 5 weeks of age, but after 11 weeks of age gradually declined to the levels in lean rats. HOMA-IR, a measure of insulin resistance status, showed that fa/fa rats had insulin resistance. The fa/fa rat has the potential to become an important animal model of T2D with obesity.

**Key Words:** glucose, insulin, lipid, type 2 diabetes, WBN/Kob-\(Lepr^{fa}\) rat.

Type 2 diabetes (T2D) is a major global health problem, and rapidly increasing in prevalence worldwide [3, 9, 10, 14]. T2D is a multifactorial disease caused by a hormonal imbalance between insulin secretion and peripheral insulin sensitivity [5]. Animal models of T2D are useful to elucidate the pathogenesis of the disease and to evaluate the therapeutic activity of new medications. The currently established rodent T2D models are divided into 2 broad categories: experimentally induced nonsprontaneous models, and genetically induced spontaneous models.

The WBN/Kob-\(Lepr^{fa}\) (fa/fa) rat is a new congenic strain that has been developed by introducing the \(fa\) gene of the Zucker fatty (ZF) rat into the original WBN/Kob (lean) rat genome [1, 2]. The male, but not female, lean rat is a hereditary model of diabetes mellitus and chronic pancreatitis [13, 15]. The leptin receptor fatty gene (\(Lepr^{fa}\)) is a recessive mutation which was caused as a result of a deficiency of leptin receptor signaling followed by obesity, hyperinsulaemia and dyslipidaemia [6, 11, 16]. The fa/fa rat has been reported as a model of T2D obesity [1, 2], but little is known about profiles of plasma insulin and lipids in this strain. In order to further characterize the fa/fa rat as a T2D model, we evaluated the plasma profiles of glucose, insulin and lipids in fa/fa rats in comparison with those in lean rats.

Male WBN/Kob-\(Lepr^{fa}\) (fa/fa) rats and age-matched male WBN/Kob (lean) rats (Japan SLC Inc., Shizuoka, Japan) were used in the present study. The fa/fa rats were confirmed by genotyping of the \(fa\) locus, performed according to a PCR-restriction fragment length polymorphism method [8]. The rats were housed in a climate-controlled room with a temperature of 23 ± 3°C, humidity of 55 ± 5%, and 12-hr lighting cycle. A commercial rat chow (CRF-1®, Oriental Yeast, Tokyo, Japan) and water were provided ad libitum. All study protocols were approved by the Animal Research Committee of Azabu University.

Body weight and plasma levels of glucose, insulin, triglyceride (TG) and non-esterified fatty acids (NEFA) were analyzed in rats from the age of 5 weeks to 19 weeks at 2-week intervals. Blood was collected from the tail vein of non-fasted conscious rats into a heparinized glass capillary, and centrifuged to remove plasma. Plasma samples were kept frozen at −40°C until assays.

Intravenous glucose tolerance tests (IVGTTs) were performed on anesthetized rats at 5, 9 and 21 weeks of age. After animals were fasted overnight (16 hr), they were anesthetized with pentobarbital sodium (50 mg/kg, ip). Once a fasting sample of blood (0.2 ml) was collected from the jugular vein into heparinized tubes for measurements of baseline glucose and insulin, a dose of 0.5 g/kg body weight of glucose (20 wt/vol% Otsuka Pharmaceutical, Tokyo, Japan) was injected into the femoral vein. Subsequently, 0.2-ml blood samples were taken 2, 5, 10, and 20 min after the glucose injection. Blood samples were centrifuged at 12,000 rpm for 15 min at 4°C, and the plasma was removed and flash-frozen for later analyses.

The areas under the curves (AUCs) for plasma glucose and plasma insulin, which represented the total glucose level and total insulin secretion during IVGTT (from 0 to 20 min), were determined according to the trapezoidal rule. The fasting plasma glucose (FPG) and insulin (FPI) levels were used to calculate the homeostasis model assessment of insulin resistance (HOMA-IR), a measure of
insulin resistance status [12]. The equation was as follows: HOMA-IR=(FPG × FPI)/405, where FPI was in microunits per milliliter and FPG in milligram per deciliter.

At the end of experiments, animals were euthanized with an overdose of pentobarbital sodium, and epididymal fat was dissected out and weighed.

Plasma glucose, TG and NEFA levels were measured by enzymatic colorimetric tests (Wako Pure Chemicals, Osaka, Japan). The plasma immunoreactive insulin was measured using a rat insulin enzyme-linked immunosorbent assay kit (Morinaga Institute of Biological Science, Kanagawa, Japan).

The results were expressed as the mean ± standard error of the mean (SEM). The statistical analysis of differences between mean values was performed using the unpaired Student’s t-test. A P value less than 0.05 was considered statistically significant.

Body weight: There was no significant difference in body weight between fa/fa rats and lean rats at 5 weeks of age (130 ± 7 g in fa/fa rats vs. 114 ± 5 g in lean rats). From 6 to 19 weeks of age, the body weights of fa/fa rats were significantly greater than those of lean rats (Fig. 1A).

Plasma glucose: Plasma glucose levels of fa/fa rats were comparable to those of lean rats at 5 and 7 weeks of age, and significantly (P<0.05) but moderately higher than those of lean rats between 9 and 11 weeks of age. Plasma glucose levels in fa/fa rats were elevated beyond 300 mg/dl from 13 weeks of age, and 500 mg/dl from 15 weeks of age. In contrast, plasma glucose levels in lean rats remained constant (Fig. 1B).

Table 1. Comparison of HOMA-IR between male fa/fa rats and lean rats at 5, 9 and 21 weeks of age

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>fa/fa</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>28.1 ± 2.8</td>
<td>12.7 ± 2.3</td>
</tr>
<tr>
<td>9</td>
<td>25.2 ± 2.0</td>
<td>4.4 ± 1.2</td>
</tr>
<tr>
<td>21</td>
<td>10.9 ± 1.3</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SEM (n=10). HOMA-IR: homeostasis model assessment of insulin resistance.

Plasma insulin: The fa/fa rats in the non-fasting state showed hyperinsulinemia from 5 to 11 weeks of age, but after 11 weeks the insulin levels decreased to those in lean rats (Fig. 1C). There were no statistically significant differences in plasma insulin levels between fa/fa rats and lean rats between 13 and 19 weeks of age (Fig. 1C).

Plasma TG and NEFA: Plasma concentrations of TG were significantly (P<0.01) higher in fa/fa rats than lean rats throughout experiments (Fig. 1D). Plasma NEFA levels were significantly higher in fa/fa rats than lean rats between 13 and 19 weeks of age (Fig. 1E).

Glucose tolerance test: The IVGTT was performed on rats at 5, 9 and 21 weeks of age. The levels of FPG and FPI were significantly higher in fa/fa rats than lean rats (Fig. 2A-2F), and accordingly, the fa/fa rats had significantly (P<0.01) higher values of HOMA-IR calculated from basal plasma levels of glucose and insulin than did lean rats (Table 1).

Glucose loading elevated plasma levels of glucose and
insulin in both groups (Fig. 2A-2F). Plasma levels of glucose were significantly higher in fa/fa rats at 9 and 21, but not 5, weeks of age (Fig. 2A-2C). In contrast, plasma levels of insulin after glucose loading were comparable to those in lean rats at 9 and 21, but not 5, weeks of age (Fig. 2D-2F).

The AUC of plasma glucose levels was greater in fa/fa rats at 5, 9 and 21 weeks of age (Fig. 3A-3C). In contrast, the AUC of plasma insulin levels of fa/fa rats and lean rats were comparable at 21 weeks of age (P>0.05), whereas fa/fa rats had significantly (P<0.05) higher plasma insulin levels at 5 and 9 weeks of age (Fig. 3D-3F).

**Visceral fat weight:** Epididymal fat was isolated at 21 weeks of age and weighed. The epididymal fat was significantly (P<0.01) heavier in fa/fa rats than lean rats when compared both in absolute terms (13.7 ± 0.3 g in fa/fa rats vs. 5.7 ± 0.2 g in lean rats) and relative to body weight (3.70 ± 0.05% in fa/fa rats vs. 1.70 ± 0.04% in lean rats).

The fa/fa(WBN/Kob-Leprd) rat, a new congenic strain derived by crossing ZF rats with lean rats, has been claimed to be a new model of T2D with obesity [1, 2]. We have elucidated the plasma profiles of glucose, insulin and lipids to further characterize the pathophysiologic features of the fa/fa rat as a T2D model in comparison with lean (the original WBN/Kob) rats. Since previous studies reported that female lean rats do not develop T2D [13, 15], we used male rats of both strains in this study. In addition, we selected an experimental period that covered up to 20 weeks of age, since a previous study [1] showed that obesity-related changes including the body weight and plasma glucose levels in male fa/fa rats reached a plateau between 16 and 24 weeks of age.

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Fig. 2. The intravenous glucose tolerance test (IVGTT) in male fa/fa rats and lean rats at 5, 9 and 21 weeks of age. A-C: plasma glucose, D-F: plasma insulin. Data are expressed as the mean ± SEM (n=10). #: P<0.01.
Obesity is a critical risk factor for the development of T2D. The present study showed that fa/fa rats developed obesity, and visceral fat weight was heavier in fa/fa rats than the lean rats (Fig. 3) indicating that fa/fa rats develop abdominal obesity. The profile of obesity in fa/fa rats is similar to that in ZDF rats [16], indicating the important role of the fa mutation of the leptin receptor for the development of obesity in both strains.

The lean rats used in our study as the control did not develop hyperglycemia up to 19 weeks of age, whereas the lean rat is known as a diabetic model. However, this is in line with earlier reports that male lean rats develop mild diabetes at 9 months of age [13, 15]. In contrast, all fa/fa rats used in our study developed severe hyperglycemia from 9 to 11 weeks of age. This is consistent with the previous study [1]. T2D has multiple forms, each of which is characterized to variable degrees by insulin resistance and β-cell dysfunction, which together lead to hyperglycemia.

The present study demonstrated the profile of plasma insulin levels in fa/fa rats. T2D is a disease characterized by reduced insulin sensitivity in peripheral tissues and secretion from pancreatic β-cells [5]. The fa/fa rats showed remarkable hyperinsulinemia at 5–11 weeks of age, but after 11 weeks of age, no significant difference in plasma insulin levels compared to the original lean rats. The increase in plasma insulin levels at 5–11 weeks of age and subsequent decrease may be due to insulin resistance and dysfunction of pancreatic β-cells, respectively.

Insulin resistance is the other important factor characterizing T2D, and can be assessed from fasting plasma levels of glucose and insulin by the HOMA-IR equation [12]. Our study showed that fa/fa rats had a higher value of HOMA-IR than lean rats at 5, 9 and 21 weeks of age. In addition, IVGTT demonstrated that fa/fa rats at 9 and 21 weeks of age had impaired glucose tolerance, although insulin secretion in response to glucose loading was comparable between fa/
fa rats and the lean rats. These results suggest that fa/fa rats have insulin resistance over their life-time, and insulin resistance may play a critical role in fa/fa rats. Taken together, the results that the decrease in plasma insulin and increase in plasma glucose were seen at 7–9 weeks of age suggest that pancreatic islet injury leads to a decrease in insulin secretion which fails to compensate for the increased insulin demand, and then leads to hyperglycemia. Previous observations suggest that damage to pancreatic islets precedes the occurrence of hyperglycemia [1, 2].

The Zucker diabetic fatty (ZDF) rat and the Goto-Kakizaki (GK) rat are commonly used models of T2D. It is well known that GK rats develop mild to moderate hyperglycemia with marked insulin resistance, whereas hyperinsulinemia before the occurrence of hyperglycemia is not evident [4]. It is reported that ZDF rats develop hyperinsulinemia soon after birth like fa/fa rats, but the subsequent decrease in plasma insulin levels is not as rapid as in fa/fa rats [7]. Taken together, the plasma profiles of glucose and insulin in fa/fa rats are similar to those in ZDF rats rather than GK rats.

This study also showed plasma lipid profiles in fa/fa rats. The result that fa/fa rats developed dyslipidemia is consistent with the general notion that obese T2D is related with dyslipidemia. It should be noted that time courses of the increase in plasma levels of TG and NEFA were different (Fig. 1D, 1E). TG and NEFA are mainly produced in the liver and the adipose tissue, respectively. Although the exact mechanism remains to be elucidated, the different time courses of increases in plasma TG and NEFA may be due to different time courses of metabolic dysfunction of the liver and adipose tissue.

Our study has several important limitations, the major one being a lack of histological analysis. In addition, the experimental period was relatively short for a metabolic study. Thus, we are currently performing longitudinal histological studies.

In summary, this study shows that the fa/fa rat develops hyperglycemia with impaired glucose tolerance and insulin secretion, insulin resistance, and dyslipidemia, similar to human T2D. Taken together, the fa/fa rat is considered to be a useful model for the analysis of diabetes and its complications.

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