The Influence of Dietary Restriction on the Development of Diabetes and Pancreatitis in Female WBN/Kob-Fatty Rats

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Abstract: Original WBN/Kob male rats commonly develop chronic pancreatitis by the age of 3 months, while diabetes mellitus occurs at 9 months. In contrast, female rats of this strain do not show pancreatitis or diabetes. The WBN/Kob-fatty rat is a homozygous (fa/fa) congenic strain for the fa allele of the leptin receptor gene (Lepr). In WBN/Kob-fatty rats, both females and males provide a model of non-insulin-dependent diabetes with obesity. The leptin receptor fatty gene (Lepr\(^fa\)) induces obesity and hyperphagia. In the present study, we examined the effect of dietary restriction on pancreatitis and diabetes in female WBN/Kob-fatty rats. Five female fatty rats comprised a restricted feeding group with paired-feeding from 3 to 13 weeks of age, and five female lean rats comprised a control group with paired-feeding. At 13 weeks of age, two of the five female fatty rats of the control group developed diabetes mellitus, while no female fatty rats of the restricted feeding group developed diabetes mellitus. At this stage, pathological changes of the pancreas were observed in female fatty rats. All female fatty rats showed severe interlobular, intra-lobular and intra-islet fibrosis. In female fatty rats of the restricted feeding group, pathological changes of the pancreas were milder those of the free-feeding fatty group. Although dietary restriction could not completely prevent pancreatitis in female fatty rats, the development of diabetes was inhibited by its reduction of the severity of pancreatitis.

Key words: diabetes, leptin receptor, obesity, pancreatitis, WBN/Kob rat

Introduction

The WBN/Kob-fatty rat is a homozygous (fa/fa) congenic strain for the fa allele of the leptin receptor gene (Lepr). The leptin receptor fatty gene (Lepr\(^fa\)) is a recessive mutation that leads to leptin receptor deficiency, and homozygous animals (fa/fa) show obesity, hyperphagia, insulin resistance, hyperinsulinemia, and glucose intolerance [5, 15, 23, 27, 28, 31]. Zucker diabetic fatty rats are homozygous for the fa allele of the leptin receptor gene and develop type 2 diabetes with obesity. Type 2 diabetes is a metabolic syndrome caused by an imbalance between insulin secretion by \(\beta\)-cells and the insulin sensitivity of peripheral tissues. There are many animal
models in which diabetes mellitus develops spontaneously, and its onset is usually secondary to hypoinsulinemia. In various strains of rat, obesity and insulin resistance secondary to leptin receptor deficiency induce diabetes [7, 11, 17, 19]. Original WBN/Kob male rats spontaneously develop diabetes, although its onset is secondary to chronic pan-pancreatitis [2, 13, 20, 22, 24, 29]. In contrast, female rats of this strain do not show pancreatitis or diabetes. Male rats of this strain commonly develop chronic pancreatitis by the age of 3 months [2, 13, 20, 22], while diabetes mellitus (characterized by hyperglycemia, hypoinsulinemia, and glycosuria) occurs at 9 months of age [20, 22, 29]. In WBN/Kob-fatty rats, both females and males provide a model of non-insulin-dependent diabetes with obesity [1]. WBN/Kob-fatty rats commonly develop pancreatitis and show reduced glucose tolerance at the age of 7–9 weeks. WBN/Kob-fatty rats develop diabetes mellitus by the age of 3–4 months [1].

In the present study, we examined the inhibitory effect of dietary restriction on diabetes in female WBN/Kob-fatty rats.

**Materials and Methods**

**Animals**

WBN/Kob-fatty (fa/fa) and WBN/Kob-lean (+/fa, +/+)

rats were produced by mating heterozygous (+/fa) males with heterozygous (+/fa) females of the WBN/Kob-Lepr(α) strain. WBN/Kob-Lepr(α) strain was established by introducing the leptin receptor fatty gene (Lepr(α)) of the Zucker fatty rat into the original WBN/Kob rat genome [1]. Ten female fatty rats and five female lean rats aged 3 weeks were used. The ten female fatty rats were subdivided into two groups [fatty and fatty-restricted (FR)]. FR rats were subjected to restricted feeding with paired-feeding. Five female lean rats were used as the control group with paired-feeding. The daily food intake of female lean rats was measured. On the next day, the same volume of food consumed by the female lean rats was fed to the restricted feeding group. The animals were given a commercial diet (MF, Oriental Yeast Co., Tokyo, Japan) and tap water, and were housed in an air-conditioned room (24 ± 2°C, 50–60% relative humidity, and lights on for 14 h per day from 6:00 to 20:00).

**Examinations**

Female fatty rats and female lean rats were checked for rectal temperature and glycosuria using a Tes-Tape (Shionogi & Co., Tokyo, Japan), and blood glucose levels were measured using a portable glucose meter (Ascensia Breeze, Bayer-Sankyo, Osaka, Japan). Detection of glycosuria was used as a diagnosis of diabetes. At 13 weeks of age, animals from the lean, fatty and FR groups were anesthetized with ether; they were weighed, had their body lengths measured (from anus to nose), and were then exsanguinated. Organs, including the gastrocnemius muscle, retroperitoneal fat pad, liver, kidney, adrenal glands, spleen, pancreas, ovary, and uterus were removed and weighed.

**Histological examination**

The pancreas was removed, fixed overnight in Bouin’s solution, and embedded in paraffin for histopathological studies. Serial 4-μm sections were cut and then stained with hematoxylin-eosin for histological examination.

**Statistics**

Data are expressed as the mean ± SD. ANOVA and the Tukey’s HSD (Honestly Significant Difference) test were used to analyze differences among groups, and P<0.05 was considered to be significant.

This study was approved by the Animal Experiments Ethical Review Committee of Nippon Medical School.

**Results**

The progress of daily food intake and body weight during dietary restriction are shown in Fig. 1. From 3 to 7 weeks of age, the daily food intake of FR rats increased, while after 7 weeks the daily food intake remained constant. FR rats had a significantly lower body weight than to lean female rats from 27 to 59 days (P<0.05). At 13 weeks of age, the body weights of FR rats and lean rats were significantly lower than that of fatty rats (P<0.05) (Fig. 2A). The body length of FR rats was significantly smaller than that of lean rats, while the body length of lean rats was significantly smaller than that of fatty rats (P<0.05) (Fig. 2B). The body mass index of FR rats was significantly higher than that of
lean rats, and significantly lower than that of fatty rats ($P<0.05$) (Fig. 2C). The rectal temperature of FR rats was not significantly lower than that of either lean rats or fatty rats (Fig. 2D). The blood glucose levels of FR rats and lean rats were not significantly lower than that of fatty rats (Fig. 2E). Glycosuria was detected in two of the five fatty rats (Fig. 2F). The weight of the gastrocnemius muscle in FR rats was significantly lower than those of lean rats and fatty rats ($P<0.05$) (Fig. 3A). The total weight of the retroperitoneal fat pad in FR rats was significantly higher than that of lean rats, and significantly lower than that of fatty rats ($P<0.05$) (Fig. 3B). The weight of the liver in FR rats and lean rats was significantly lower than that of fatty rats ($P<0.05$) (Fig. 3C). The total weight of the kidney in FR rats was significantly lower than that of lean rats, and in lean rats was significantly lower than that of fatty rats ($P<0.05$) (Fig. 3D). The total weight of the adrenal organ in FR rats was not significantly lower than that of either lean rats or fatty rats (Fig. 3E). The weight of the spleen in FR rats was significantly lower than those of lean rats and fatty rats ($P<0.05$) (Fig. 3F). The weight of the pancreas in FR rats was significantly lower than that of lean rats ($P<0.05$) (Fig. 3G). The total weight of the ovary in FR rats was significantly lower than that of either lean rats or fatty rats ($P<0.05$) (Fig. 3H). Additionally, the weight of the uterus in FR rats was significantly lower than that of lean rats ($P<0.05$) (Fig. 3I).

Histological findings of the pancreas are shown in Fig. 4. At 13 weeks of age, three of the five FR rats showed pathological changes related to mild chronic pancreatitis, while all fatty rats showed pathological changes related to severe chronic pancreatitis. FR rats showed only focal interstitial edema and fibrosis with mild inflammatory cell infiltration in interlobular, intra-lobular and intra-islet areas (Fig. 4C and 4D). At this stage, fatty rats showed severe inflammatory cell infiltration fibrosis, extra hemorrhage and hemosiderin deposition in interlobular, intra-lobular and intra-islet areas (Fig. 4E and 4F). The pancreata of lean rats, including islets
and pancreatic acini, were normal (Fig. 4A and 4B). The degree of pancreatitis for each group was described as the percentage of lesion area (Fig. 5). The percentage of area with histopathological abnormalities in FR rats was significantly lower than that in fatty rats ($P<0.05$).

**Discussion**

FR rats had a significantly lower body weight than lean rats from 27 to 59 days. At 13 weeks of age, the body weights and liver weights of FR rats were similar to those of lean rats. The BMI and weight of the retroperitoneal fat pads of FR rats were significantly higher than those of lean rats and significantly lower than those of fatty rats. Other organ weights (gastrocnemius muscle, adrenal, spleen, pancreas, ovary, and uterus) and the body length of FR rats were lower than those of lean rats and fatty rats. With respect to relative organ weight per body weight, the gastrocnemius muscle, adrenal glands and pancreas, ovary and uterus of FR rats were greater than those of fatty rats (data not shown). Fatty rats had markedly higher body weights than FR rats. The relative weight per body weight for other organs showed a tendency to be the same as the organ weight (data not shown).

At 13 weeks of age, the body weight of fatty rats was greater than that of lean rats allowed the same food consumption (data not shown). The body weights of mature Zucker fatty rats are greater than those of lean rats when given the same caloric intake [10]. Therefore, the period of paired-feeding employed in this experiment was maintained up to 13 weeks of age to eliminate any differences in body weight between FR rats and lean rats.

Female WBN/Kob-fatty rats were obese and developed pancreatitis and diabetes [1]. At 13 weeks of age, two of the five female fatty rats had developed diabetes mellitus, while no female fatty rats developed diabetes mellitus in the restricted feeding group. At this stage,
pathological changes of the pancreas were observed in all the female fatty rats. All female fatty rats showed severe interlobular, intra-lobular and intra-islet fibrosis. In female fatty rats of the restricted feeding group, pathological changes of the pancreas were milder compared with those of the free-feeding fatty group. Obesity was recently proposed as a risk factor for acute pancreatitis [16]. Furthermore, several studies have shown that obesity is strongly associated with the onset of complications and increased mortality in patients with acute pancreatitis [8, 18], and a decreased survival rate has been reported in genetically obese Zucker rats following induction of experimental acute pancreatitis [25]. These results suggest that obesity could accelerate pancreatitis in WBN/Kob-fatty rats. In the present study, dietary restriction controlled obesity in female WBN/Kob-fatty rats. Thus, pancreatitis was alleviated by dietary restrictions applied to female WBN/Kob-fatty rats.
In the present study, female WBN/Kob-fatty rats developed diabetes at three months of age, as observed in lean male WBN/Kob rats at 9 months of age [1]. Obesity and hyperphagia accelerate and/or promote the development of pancreatitis and diabetes in this strain. The leptin receptor fatty gene (*Lepr*<sup>fa</sup>) induces insulin resistance and hyperinsulinemia [15]. Hyperinsulinemia in Zucker fatty rats is not affected by dietary restriction [6], but dietary restriction retards the decrease of serum insulin levels in male SDT-fatty rats, which are derived from a strain congenic for the leptin receptor fatty gene [21]. At 12 weeks of age, a dietary restriction group of male SDT-fatty rats showed hyperinsulinemia. This indicates that dietary restriction does not affect insulin resistance caused by the leptin receptor fatty gene (*Lepr*<sup>fa</sup>). Furthermore, dietary restriction retards increase of blood glucose levels in male SDT-fatty rats [21]. In female WBN/Kob-fatty rats, lesions of the pancreas were re-
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Reduced by dietary restriction. These findings suggest that blood glucose levels and insulin secretion were maintained by reduction of pancreatitis in FR rats. Two of the five fatty rats developed diabetes, while none of the five FR rats developed diabetes. Although there was no significant difference between the onset of diabetes in the two groups, we consider the development of diabetes in FR rats was inhibited by the reduction in severity of pancreatitis.

The growth curve, body weight, body length and organ weights of FR rats were similar to those of restricted Zucker fatty rats [10]. Although the adipose deposit weight of restricted Zucker fatty rats was higher than that of lean rats, the weights of other organs in restricted Zucker fatty rats were lower than those of lean rats [10]. The leptin receptor fatty gene responds to caloric restriction with preservation of fat cell volume. Thus, the inhibition of obesity and fat deposition were incomplete in the FR rats, although the body weight of female WBN/Kob-fatty rats was controlled by dietary restriction.

Acute pancreatitis leads to increased production of TNFα [4] by macrophages and pancreatic acinar cells [9], as well as the premature activation of enzymes within acinar cells and the destruction of pancreatic tissue [26]. In caerulein-induced pancreatitis (CIP), plasma leptin levels are increased and the onset of pancreatitis is accompanied by increased pancreatic expression of the leptin gene [14]. In CIP rats, leptin decreases the plasma level of TNFα and increases the plasma level of IL-4, an anti-inflammatory cytokine [12]. Furthermore, Jaworek et al. suggested that leptin protects the pancreas by the inhibiting of pancreatic enzyme secretion [12]. In the present study, dietary restrictions did not completely prevent pancreatitis in female WBN/Kob-fatty rats. These findings suggest that lack of leptin protection may accelerate the development of pancreatitis in WBN/Kob-fatty rats.

In summary, dietary restriction inhibited body weight, hyperphagia and diabetes. Based on the relationship between obesity and histological changes of the pancreas in female WBN/Kob-fatty rats, it appears that diabetes was secondary to severe degeneration of the islets of Langerhans due to diffuse pancreatitis. Thus, diabetes in FR rats was inhibited by the reduction in severity of pancreatitis. However, inhibition of pancreatitis, obesity and fat deposition was incomplete in FR rats.

![Fig. 5. Percentage area of histopathological abnormalities in the pancreas. Histopathological abnormalities included inflammatory cell infiltration, fibrosis, interstitial edema, hemorrhage, or hemosiderin deposition. The total area of histopathological abnormalities was determined by observation of ten points per area of 1 mm² in one section. The percentage area was calculated per area of 10 mm². Data are means ± SD. (n=5). *: The calculated area of the fatty rats in the dietary restriction group was significantly lower than that of the fatty rats with unrestricted feed (P<0.05). In the lean rats group, histopathological abnormalities were not observed.]

References


