

# Effect of partial pancreatectomy on $\beta$ -cell mass in the remnant pancreas of Wistar fatty rats

Tomoe Ogino, Min Zhu, Takashi Murakami, Masamichi Kuwajima,  
and Kenji Shima

*Department of Laboratory Medicine, The University of Tokushima School of Medicine, Tokushima,  
Japan*

**Abstract :** Wistar fatty rat, which has been established by transferring the *fa* gene of Zucker fatty rat to the Wistar Kyoto rat, has many features in common with human NIDDM. It exhibits hyperglycemic obesity with hyperinsulinemia and insulin resistance. It is unclear, however, whether a defect in the  $\beta$ -cell proliferation is related to the onset of diabetes mellitus together with insulin resistance in this model rat. To determine this, we compared non-fasting plasma glucose levels, insulin content and  $\beta$ -cell mass in the remnant pancreas of Wistar fatty rats with those in their diabetic-resistant lean counterparts after a 70% partial pancreatectomy. We also examined whether such a defect, if present, could be improved by either phlorizin or nicotinamide. We further investigated if there were any differences in these parameters between the phenotypically identical but genotypically different Wistar lean rats with a gene type of homogeneous *Fa/Fa* and that of heterogeneous *Fa/fa*. Male rats, 6 weeks of age, were allocated at random into two groups : 70% pancreatectomy (Px) and sham-pancreatectomy (sham). A sustained hyperglycemia was evident in the Px Wistar fatty rats after surgery, which was accompanied by a reduction of insulin content and  $\beta$ -cell mass in the remnant pancreas. The changes in insulin content and  $\beta$ -cell mass were unaffected by restoration of normoglycemia, induced by phlorizin injection. The administration of nicotinamide partially ameliorated the sustained hyperglycemia by a slight but not significant increase in  $\beta$ -cell mass. No discernible difference in the above parameters was observed between the Wistar lean rats with *Fa/Fa* and those with *Fa/fa*. These findings suggest that Wistar fatty rats have a poor capacity for proliferation of pancreatic  $\beta$ -cells, which causes the onset of overt diabetes along with insulin resistance due to extreme obesity. *J. Med. Invest.* 45 : 103-110, 1998

**Key words :** *pancreatectomy,  $\beta$ -cell mass, Wistar fatty rat*

## INTRODUCTION

Established non-insulin dependent diabetes mellitus (NIDDM) is associated with profound insulin secretory defects, which occur along with insulin resistance. These two fundamental defects, which are major factors in the pathogenesis of NIDDM, are caused by a combination of genetic and environmental factors

(1). Little is known of the molecular basis for these abnormalities and it is difficult to study them in human subjects. Animal models, however, do provide an opportunity to study this problem. The development of diabetes in the Wistar fatty male rat, which has been established by transferring the *fa* gene of the Zucker fatty rat to the Wistar Kyoto rat (2), has many features in common with human NIDDM, in that the animals exhibit hyperglycemic obesity with hyperinsulinemia and insulin resistance. Ikeda *et al.* (2) have reported insulin resistance as a cause for diabetes mellitus in this model rat. Recently, we reported a failure of pancreatic  $\beta$ -cell proliferation

Received for publication July 3, 1998 ; accepted July 15, 1998.

<sup>1</sup> Address correspondence and reprint requests to Kenji Shima, M.D., Ph.D., Department of Laboratory Medicine, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-886-31-9495.

as a cause of sustained hyperglycemia, induced by a 70% pancreatectomy in a model of spontaneous NIDDM, the Otsuka Long-Evans Tokushima Fatty (OLETF) rat (3, 4). This may represent a fundamental etiological factor in the onset of overt diabetes. Impaired  $\beta$ -cell proliferation has been also reported to be etiologically related to the development of NIDDM in the other NIDDM model animals (5, 6). To determine whether  $\beta$ -cell proliferation is impaired, whether the subsequent reduction of  $\beta$ -cell mass is also related to NIDDM in the Wistar Fatty model, and whether any differences in the capacity for proliferation of  $\beta$ -cell between Wistar lean rats with a homogeneous genotype (*Fa/Fa*) and a hetero-geneous genotype (*Fa/fa*) exist, we used partially pancreatectomized rats as a model as was previously done for the case of OLETF rats. For this purpose, the compensatory capacity of the pancreatic remnant was determined by comparing alterations in blood glucose levels and compensatory proliferation of the remnant pancreas in Wistar fatty and non-fatty litter mates, Wistar lean with *Fa/Fa* and *Fa/fa*. In addition, we examined whether diabetes was caused by partial pancreatectomy in these model rats, and if so, whether it could be prevented by the administration of nicotinamide.

## RESERCH DESIGN AND METHODS

### *Animals*

The Wistar fatty rat, which was established by transferring the *fa* gene of Zucker fatty rat to the Wistar Kyoto rat, genetically develops both obesity and diabetes. According to Ikeda *et al.* (2), plasma glucose reaches a hyperglycemic level in excess of 16.7 mmol/l at as early as 8 weeks of age in male Wistar fatty rats, but this does not occur in their lean litter mates. Male Wistar fatty and lean rats, 4 weeks of age, were obtained from the Takeda Pharmaceutical Co., and were maintained in our animal facilities under specific pathogen-free conditions (Institute of Animal Experimentation, Tokushima University). The temperature ( $21 \pm 2^\circ\text{C}$ ), humidity ( $55 \pm 5\%$ ), lighting (07:00-19:00), and air conditioning were all controlled. The animals were supplied with a standard rat diet (Oriental Yeast, Tokyo, Japan) and tap water ad libitum.

### *Experimental design*

Experiments were performed on rats of 6 weeks of age at the beginning of the study. The rats were allocated at random into two groups: partial pancreatectomy (Px) and sham-pancreatectomy (sham).

The animals from the Px group were treated with either phlorizin, nicotinamide or normal saline. A total of 5-7 rats were used for each condition for each strain.

### *Genotyping*

In order to determine the genotype of Wistar rats, we followed the method developed by Phillips *et al.* (7). Genomic DNA was extracted from the tail by proteinase K digestion followed by phenol/chloroform extraction and ethanol precipitation (8). Primers 5'-GTT TGC GTA TGG AAG TCA CAG-3' and 5'-ACC AGC AGA GAT GTA TCC GAG-3' were used to amplify the OB-R gene from 5ng of genomic DNA. The PCR conditions were  $94^\circ\text{C}$  for 30s,  $60^\circ\text{C}$  for 30s and  $72^\circ\text{C}$  for 1min. The PCR products were digested with Hap II and analyzed on an agarose gel (9).

### *70% pancreatectomy (Px)*

After an overnight fast, animals were anesthetized with ether and given additional ether, if required during surgery. All pancreatic tissue was removed by gentle abrasion with a cotton applicator, except for an anatomically well-defined remnant, which is bordered by a branch of the hepatic portal vein and the first portion of the duodenal loop. The sham operation was performed by disengaging the pancreas from the mesentery and gently rubbing it between the fingers. After surgery, the rats were given access to food and water ad libitum.

In the first week after surgery, body weight and non-fasting plasma glucose concentrations were measured daily at 4:00-5:00 P.M. and thereafter once a week at the same time of day. Blood samples were obtained by tail snipping. Phlorizin (400mg/kg body wt. per day), prepared as a 20% solution in propylene glycol, was administered subcutaneously and divided into three equal doses at 8-h intervals to ensure continuous day-long inhibition of renal tubular glucose reabsorption in Wistar fatty rats alone. Nicotinamide (350mg/kg body wt.) and normal saline were injected intraperitoneally once a day. The injections were initiated 2 days after surgery and were continued until 28 days after surgery. The last injection was given 15 h before sacrifice.

### *Body composition*

The lean body mass was measured with an EM-SCAM model ISA-2 (EM-SACAN, IL). The total fat volume was calculated from the body weight and the value for the lean body mass.

### *Tissue processing*

At 4 weeks after treatment (10 weeks of age), animals were fasted overnight and then sacrificed under deep anesthesia. The abdomen was quickly opened, and the pancreas or remnant pancreas was excised, cleared of extraneous lymph nodes and fat, weighed, and divided into two lengthwise portions to avoid bias due to regional variation in islet distribution and cell composition. One of the portions was cassetted in the same anatomic orientation; placed in Bouin's fixation; and processed for paraffin embedding using a standard protocol. The other portion was frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . On a single day, all samples of pancreas or Px remnant stored at  $-80^{\circ}\text{C}$  were individually homogenized using a polytron homogenizer (Kinematica, Switzerland) with 20ml cold acidified-ethanol per gram of tissue, kept at  $4^{\circ}\text{C}$  overnight, and centrifuged at 600 g for 30min, and the supernatant was then stored at  $-80^{\circ}\text{C}$  until assay for insulin immunoreactivity (IRI).

### *Assays*

Non-fasting plasma glucose values were determined by the glucose oxidase method (Toecho Super, Kyoto Daiichi Kagaku, Kyoto, Japan). The measurement of plasma IRI and IRI in tissue extracts was determined using a commercially available IRI kit (Eiken Kagaku Co., Tokyo, Japan) with rat insulin (Novo, Bagsvared, Denmark) as a standard.

### *Quantitative morphometrics*

Two sets of three serial sections (3-5 $\mu\text{m}$  thick) were obtained at intervals of about 300 $\mu\text{m}$ . The sections were first deparaffined, and then immunostained using an ABC kit (Amersham, UK) for insulin immunostaining. The primary antibody used was polyclonal guinea pig anti-porcine insulin antibody (1 : 400, Dako Carpinteria, USA). Using Weibel's point counting morphometrics (10), the relative volume (%) of  $\beta$ -cell mass was quantitated at a 200 x magnification using a 96-point grid with a minimum of 9600 points in 100 fields. Starting at a random point in one corner of the section,  $\beta$ -cell mass was scored in every other field. The relative volume (%) of  $\beta$ -cell mass was taken as the number of intercepts over that specific tissue as a proportion of the total counts over pancreatic tissues. To obtain an absolute  $\beta$ -cell mass (mg), the relative volume (%) of  $\beta$ -cell mass for each pancreas was multiplied by its tissue weight. All observations were made by one person (T.O.).

### *Statistics*

All data are expressed as means  $\pm$  S.E. The insulin content was calculated for each rat as the product of insulin concentration and tissue weight. The statistical significance of difference was evaluated using one-way analysis of variance (ANOVA). Differences were considered statistically significant at  $P < 0.05$ .

## RESULT

### *Abdominal fat and fasting IRI*

Abdominal fat deposits, which consist of mesenteric, retroperitoneal and epididymal fat in the sham Wistar fatty rats, were about three times those in sham Wistar lean rats (Table 1). In addition, fasting plasma IRI levels in the sham Wistar fatty rats were about 10 times those in the sham Wistar lean rats. However in the case of partial pancreatectomy, the fasting plasma IRI levels in the Px Wistar fatty rats were only double those in the Px Wistar lean rats, suggesting that insulin secretion in the Px Wistar fatty rats failed to meet the demand.

### *Non-fasting blood glucose concentrations and Body weights*

Figure 1 shows the effects of 70% pancreatectomy on non-fasting plasma glucose concentrations and body weight in the various groups during the experiment. In the Px Wistar fatty rats, a marked hyperglycemia ( $>20\text{mmol/l}$ ) was detected the first day after surgery, which was maintained for the remainder of the experiment. The rate of body weight increase in the Px Wistar fatty rats was significantly slower than that in the sham Wistar fatty rats. In addition, in the Px Wistar lean rats, neither hyperglycemia nor smaller increment in body weight compared with the sham Wistar lean rats was noted after surgery. In the Px Wistar fatty rats, non-fasting plasma glucose values sharply declined after phlorizin injection and subsequently remained at levels comparable to the sham rats. However, non-fasting plasma glucose values in the nicotinamide treated Px Wistar fatty rats decreased gradually and remained at a level that was significantly higher than that of the sham rats but lower than that of the saline-treated Px rats (Fig. 2A). The body weight of the Px group was significantly lower than that of sham group (Fig. 2B).

### *Insulin content and pancreatic $\beta$ -cell mass*

Table 2. shows the insulin content and pancreatic  $\beta$ -cell mass in the Px remnant pancreas, remnant

equivalent, and whole pancreas of the sham-operated rats. In addition, insulin content and  $\beta$ -cell mass in the remnant pancreas of the Px rats and the remnant equivalent pancreas of the sham-operated rats as a percentage of those in whole pancreas are shown in Fig. 3. Both insulin content and  $\beta$ -cell mass of the Px Wistar fatty rats treated with saline were significantly less than 30% of the whole pancreas. The same was true for the Px Wistar fatty rats, which had been treated with phlorizin or nicotinamide, although the  $\beta$ -cell mass tended to be higher in the latter groups. In the Px Wistar lean rats, the  $\beta$ -cell mass volume was maintained at the same level as that of the sham-operated rats, but the insulin content in the remnant pancreas was considerably higher than the expected value of 30%. As shown in Table 2, the insulin content per unit  $\beta$ -cell mass in the remnant pancreas of the Px Wistar lean rats treated with either saline or nicotinamide was significantly greater than that in the remnant equivalent pancreas of the sham rats, while that of Px Wistar fatty rats treated with saline or nicotinamide was decreased, compared to their sham-operated counterparts. Although the number of animals was too small to properly evaluate the data statistically, no discernible differences in the various parameters shown in Table 3 between the Wistar lean rats with *Fa/Fa* and those with *Fa/fa*, were observed.

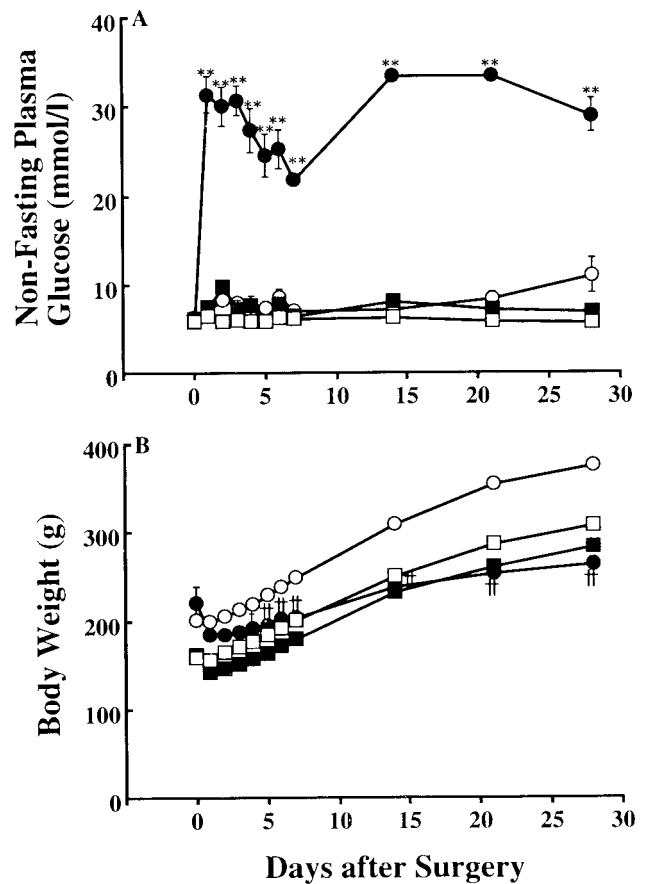


Fig. 1. Effects of 70% pancreatectomy on non-fasting plasma glucose levels (A) and body weights (B) of Px Wistar fatty (●●), sham Wistar fatty (○○), Px Wistar lean (■●), and sham Wistar lean (□□) rats during the period of observation. Points and bars are expressed as means  $\pm$  S.E. \*\* $p < 0.01$  vs. all other groups in A. †  $p < 0.05$  and ††  $p < 0.01$  vs. sham Wistar fatty in B.

Table 1. Effect of phlorizin and nicotinamide on fasting plasma glucose and IRI levels, and anthropometric parameters in Px Wistar fatty and lean rats.

Rat	Fasting plasma			LBM (g)	Abdominal fat (g)	Fat (%)
	Glucose (mmol/l)	IRI (pmol/l)				
Px						
Fatty	Saline (5)	13.2 $\pm$ 1.04 <sup>##</sup>	451.53 $\pm$ 55.57	208.16 $\pm$ 7.05	15.03 $\pm$ 0.71	13.49 $\pm$ 1.44
	Nicotinamide (6)	11.05 $\pm$ 1.30	624.63 $\pm$ 82.92	217.10 $\pm$ 7.04	15.83 $\pm$ 1.06	13.71 $\pm$ 1.39
	Phlorizin (7)	7.06 $\pm$ 0.59	356.62 $\pm$ 37.93	212.63 $\pm$ 7.05	15.43 $\pm$ 1.05	13.60 $\pm$ 1.42
	Sham (6)	10.38 $\pm$ 2.33	3480.11 $\pm$ 955.39 <sup>**</sup>	252.01 $\pm$ 4.44 <sup>**</sup>	24.09 $\pm$ 1.03 <sup>**</sup>	27.92 $\pm$ 1.07 <sup>**</sup>
Px						
Lean	Saline (6)	6.69 $\pm$ 0.32	200.24 $\pm$ 30.23	249.62 $\pm$ 3.70	6.74 $\pm$ 0.38	7.01 $\pm$ 1.20
	Nicotinamide (6)	5.93 $\pm$ 0.32	323.55 $\pm$ 17.71	239.38 $\pm$ 9.84	6.24 $\pm$ 0.47	6.83 $\pm$ 1.08
	Sham (6)	5.75 $\pm$ 0.36	338.08 $\pm$ 57.37	265.19 $\pm$ 3.56	8.49 $\pm$ 0.64	7.21 $\pm$ 0.95

Data are means  $\pm$  S.E. The samples were taken at the end of the experiment, 28 days after surgery.

LBM : Lean body mass.

Nicotinamide : Nicotinamide (350mg/kg body weight) was injected intraperitoneally daily from 2 days after surgery until 28 days after surgery. Phlorizin : Phlorizin (400 mg/kg body weight per day) was injected subcutaneously three times per day in the same manner as that of nicotinamide.

\*\* $p < 0.01$  vs. all other Wistar fatty groups, and ## $P < 0.01$  vs. phlorizin-treated Px Wistar fatty group.

DISCUSSION

The 70% pancreatectomy caused a sustained hyperglycemia in the Wistar fatty rats, but it did not result in the elevation of non-fasting plasma glucose levels in the Wistar lean rats. These findings, which are similar to those of spontaneous NIDDM rats, OLETF as reported previously (4), suggest that the remnant pancreas cannot meet insulin demand in the Wistar fatty rats but does so in the Wistar lean rats, despite the similar remaining pancreatic mass just after surgery. According to Ikeda *et al.* (2), in a glucose tolerance test at 8 weeks of age, Wistar fatty rats showed a comparable value to Wistar lean rats for the incremental glucose area but a higher value for the incremental insulin area. This suggests that the former rats were less sensitive to insulin, and, as a result, required more insulin to meet the demand.

It is quite possible to presume that the  $\beta$ -cell mass in the remnant pancreas in the Wistar fatty rats is not sufficiently large to meet this increased demand for insulin, resulting in a relative insulin insufficiency and, hence, a sustained hyperglycemia. Consistent with this hypothesis, our results show that both  $\beta$ -cell mass and insulin content are significantly decreased in the remnant pancreas of the Px Wistar fatty rats compared with those in the remnant equivalent of the sham rats after surgery, resulting in an insufficient increase in plasma insulin level in the Px Wistar fatty rats in the face of an increased demand for insulin due to insulin insensitivity.

There was, however, no significant decrease in  $\beta$ -cell mass and insulin content in the Px Wistar lean rats after surgery. The remaining  $\beta$ -cell mass and insulin content in the remnant pancreas might be sufficiently large to meet the demand for less insulin and maintain the normal plasma glucose level because of less resistance to insulin in these rats, compared to the

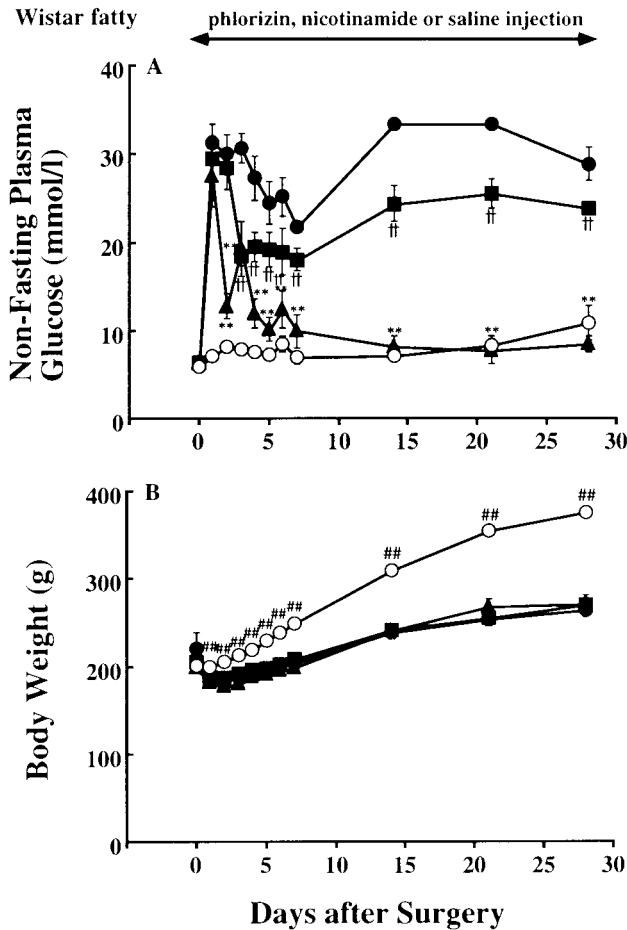


Fig.2. Effect of phlorizin (400mg/kg wt per day) and nicotinamide (350mg/kg wt per day) on non-fasting plasma glucose levels (A) and body weight (B) in phlorizin-treated (▲-▲), nicotinamide-treated (■-■), and saline-treated (●-●) Px and sham (○-○) rats after 70% pancreatectomy. Injections were begun 2 days after surgery and continued at daily intervals until 28 days after surgery. The findings for control Wistar lean rats after surgery and injections were the same as those sham in Fig.1. (data not shown). \*\*p<0.01 and p<0.01 vs. saline treated rats in A, and ##p<0.01 vs. all other groups in B.

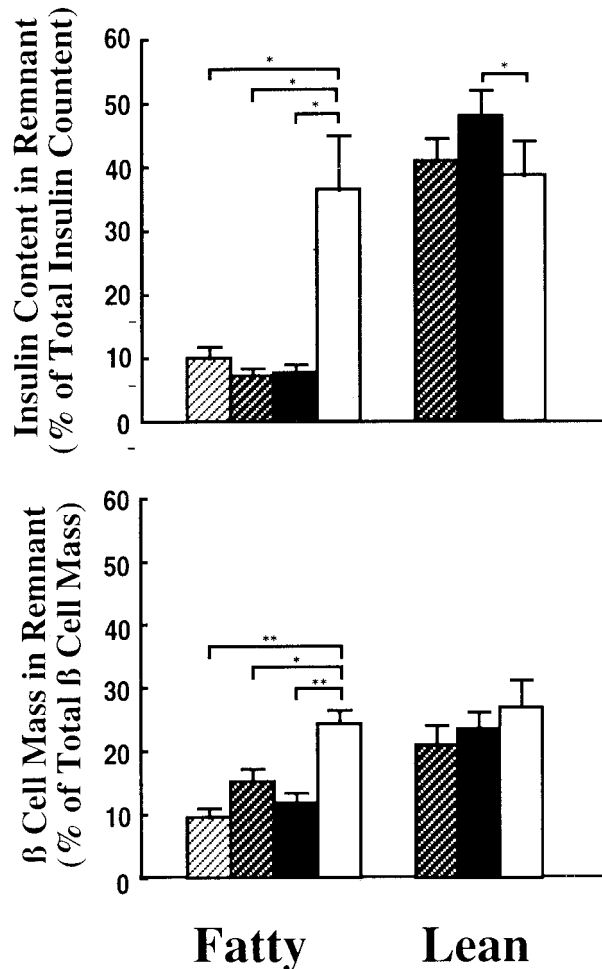


Fig.3. Insulin content and  $\beta$ -cell mass in remnant pancreas of phlorizin ( ), nicotinamide ( ), saline-treated (■), Px Wistar rats and remnant equivalent of the counterpart sham rats (□) 28days after surgery. Values are mean  $\pm$  S.E. \*p<0.05, \*\*p<0.01 Vs. sham Wistar fatty.

fatty counterparts. However, contrary to the control Px LETO rats, which demonstrated a compensatory increase in  $\beta$ -cell mass after surgery, the Px Wistar lean rats maintained  $\beta$ -cell mass at the same level as that of the sham rats. An important issue, then, is why the  $\beta$ -cell mass in the Px of Wistar lean rats was not increased after surgery as it was in the Px LETO rats. It is possible that the pancreatic  $\beta$ -cell per se may have some genetic defects in proliferative capacity in the Wistar rat strain. This possibility is supported by Ikeda's finding (2) that Zucker fatty rats show euglycemia with a compensatory hyperinsulinemia while the Wistar fatty rats demonstrate hyperglycemia, which is accompanied by a lower plasma insulin level relative to that in the Zucker fatty rats. The  $\beta$ -cells of the Wistar

strain might be incapable of increasing their mass in order to meet an increased demand for insulin in the presence of insulin resistance. Because of insulin insensitivity due to extreme obesity, the islets are forced to secrete more insulin to overcome the loss of normal insulin sensitivity, which may put a stress on pancreatic  $\beta$ -cells, leading to their damage and death resulting in a reduction in  $\beta$ -cell mass and insulin content. This might also be the case in Px Wistar fatty rats.

We also found that this insufficient compensatory capacity for  $\beta$ -cell growth was unaffected by the presence or absence of hyperglycemic stimulation in the Px Wistar rats at 28 days after surgery, as evidenced by the administration of phlorizin. It has

Table 2. Effect of phlorizin (400mg/kg wt per day) and nicotinamide (350mg/kg wt per day) on insulin contents and  $\beta$ -cell mass in remnant pancreas of 70% Px rats and in remnant equivalent and whole pancreas of sham-pancreatectomized rats.

Rat	Insulin		$\beta$ -cells		Insulin content per unit $\beta$ -cell mass ( $\mu\text{g}/\text{mg}$ )
	Content ( $\mu\text{g}$ )	Concentration ( $\mu\text{g}/\text{g}$ )	$\beta$ -cell mass (mg)	Relative $\beta$ -cell mass (%)	
Fatty Px					
Saline (5)	8.67 $\pm$ 1.53**	24.01 $\pm$ 4.17**	4.56 $\pm$ 0.61**	1.24 $\pm$ 0.13**	2.21 $\pm$ 0.68
Nicotinamide (6)	8.27 $\pm$ 1.39**	17.50 $\pm$ 3.13**	5.84 $\pm$ 0.80**	1.24 $\pm$ 0.18**	1.73 $\pm$ 0.59
Phlorizin (7)	11.47 $\pm$ 2.02**	34.38 $\pm$ 7.43**	3.69 $\pm$ 0.54**##	1.08 $\pm$ 0.14	3.57 $\pm$ 0.73
Sham					
Remnant (6)	34.76 $\pm$ 4.53	120.18 $\pm$ 20.17	8.85 $\pm$ 0.23	3.00 $\pm$ 0.17	3.91 $\pm$ 0.47
Whole (6)	113.90 $\pm$ 13.55	235.74 $\pm$ 26.21	38.32 $\pm$ 3.86	7.21 $\pm$ 0.17	3.08 $\pm$ 0.54
Lean Px					
Saline (6)	30.33 $\pm$ 2.58	86.93 $\pm$ 8.09	3.36 $\pm$ 0.37	0.96 $\pm$ 0.10	9.38 $\pm$ 0.96
Nicotinamide (6)	25.66 $\pm$ 2.34	71.44 $\pm$ 6.99	2.98 $\pm$ 0.44	0.83 $\pm$ 0.12	9.59 $\pm$ 1.57
Sham					
Remnant (6)	23.40 $\pm$ 2.64	66.72 $\pm$ 6.32	3.72 $\pm$ 0.53	1.08 $\pm$ 0.14	7.11 $\pm$ 1.45
Whole (6)	31.54 $\pm$ 4.55	106.41 $\pm$ 6.39	14.46 $\pm$ 1.34	2.35 $\pm$ 0.17	4.59 $\pm$ 0.63

Data are means $\pm$ S.E. Nicotinamide and phlorizin doses were the same as for Table 1. The insulin content was calculated for each rat as the product of insulin concentration and tissue weight. The  $\beta$ -cell mass was calculated for each rat as the product of relative volume of  $\beta$ -cell mass(%), which was obtained by point-counting morphometrics and tissue weight. \*\*p<0.01 vs. remnant equivalent of Fatty sham, ##p<0.01 vs. nicotinamide-treated Px Fatty.

Table 3. Differences in plasma glucose, IRI levels and anthropometric parameters between Wistar lean rats with homogeneous (*Fa/Fa*) and heterogeneous (*Fa/fa*) genotype after partial pancreatectomy.

Rat	Fasting plasma					
	Bs (mmol/l)	IRI (pmol/l)	LBM (g)	Abdominal fat (g)	Fat (%)	$\beta$ -cell mass (mg)
Px Lean Nicotinamide						
<i>Fa/Fa</i> (3)	5.52 $\pm$ 0.20	324.45 $\pm$ 28.41	240.40 $\pm$ 21.07	5.87 $\pm$ 0.91	3.53 $\pm$ 1.30	2.87 $\pm$ 0.52
<i>Fa/fa</i> (3)	6.33 $\pm$ 0.53	322.65 $\pm$ 27.56	238.35 $\pm$ 6.23	6.62 $\pm$ 0.33	4.13 $\pm$ 2.01	3.09 $\pm$ 0.83
Saline						
<i>Fa/Fa</i> (3)	6.20 $\pm$ 0.37	168.97 $\pm$ 61.12	248.32 $\pm$ 6.15	6.65 $\pm$ 0.59	7.86 $\pm$ 1.71	3.22 $\pm$ 0.65
<i>Fa/fa</i> (3)	7.17 $\pm$ 0.39	221.09 $\pm$ 35.47	250.91 $\pm$ 5.37	6.15 $\pm$ 1.87	6.84 $\pm$ 0.60	3.50 $\pm$ 0.49

Data are means $\pm$ S.E. LBM and Nicotinamide doses were the same as for Table 1.

been suggested that hyperglycemia accelerates  $\beta$ -cell mass growth by increasing the number of  $\beta$ -cells undergoing mitosis (11, 12). In addition, a prolonged hyperglycemia impairs, not only  $\beta$ -cell function, but also proliferation, which is the result of the effect of glucose, glucose toxicity (13). Phlorizin was used to eliminate both of these effects of hyperglycemia on  $\beta$ -cells. The results of the present study suggest that the abnormality of the proliferative capacity in Wistar fatty rats is unrelated to both the stimulative and toxic effects of hyperglycemia. It can, therefore, be concluded that the poor capacity for proliferation of  $\beta$ -cells in this strain may be genetically determined.

The administration of nicotinamide, which is known to induce the outgrowth of  $\beta$ -cells from undifferentiated epithelial cells in human (14) and to increase  $\beta$ -cell mass largely due to  $\beta$ -cell replication (15), induced an increase in the size, but not the significant growth of  $\beta$ -cell mass in the Px Wistar fatty rats. This is reflected by the slight reduction of hyperglycemia after surgery in the Px Wistar fatty rats treated with nicotinamide, which was quite different from the results obtained in the nicotinamide-treated Px OLETF rats. The degree of obesity is far greater in the Wistar fatty rats than the OLETF rats, which might result in greater resistance to, and demand for insulin in the former than the latter. This might in turn lead to more extensive  $\beta$ -cell death and reduction of  $\beta$ -cell mass exceeding the proliferation of  $\beta$ -cells induced by nicotinamide in the Wistar fatty rats. Recently, we (16, 17) and others (7, 18) discovered the substitution at codon 269 of the leptin receptor cDNA in Zucker fatty and Wistar fatty rats, which is thought to be a cause of their hyperphagia and obesity (19). These phenotypes are seen in rats with homogenous genotype, *fafa* but not in the heterogeneous, *Fa/?* rats. It is now possible to subgroup phenotypically identical lean litter mates into those with a homogeneous *Fa/Fa* and those with a heterogeneous, *Fafa* genotype by genotype analysis. Thus facilitating a comparison of any difference in physical characteristics and parameters related to the carbohydrate metabolism. The results showed no significant differences in these parameters between both groups, suggesting that the defect of leptin receptor per se is not related to the poor capacity for proliferation of  $\beta$ -cell growth and, hence, leads to a reduction in  $\beta$ -cell mass.

In summary, 70% pancreatectomy caused an immediate and sustained hyperglycemia in Wistar fatty rats, which was associated with a reduction in  $\beta$ -cell mass and a low plasma insulin concentration. This defect was unaffected by restoration of normoglycemia when

phlorizin was used. These findings suggest that Wistar fatty rats have a poor capacity for proliferation of pancreatic  $\beta$ -cells, which causes the onset of overt diabetes along with insulin resistance due to extreme obesity.

## ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (NO.09671001) from the Ministry of Education, Science and Culture.

## REFERENCE

1. Kahn CR : Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes* 43 : 1066-1084, 1994
2. Ikeda H, Shino A, Mastuo T, Iwatsuka H, and Suzuoki Z : A new genetically obese-hyperglycemic rat (Wistar Fatty). *Diabetes* 30 : 1045-1050, 1981
3. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T : Spontaneous long-term hyperglycemic rat with diabetic complications, Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes* 41 : 1422-1428, 1992
4. Zhu M, Noma Y, Mizuno A, Samo T, and Shima K: Poor capacity for proliferation of pancreatic  $\beta$ -cell mass in, Otsuka-Long Evans Tokushima-Fatty (OLETF) rat, model of spontaneous non-insulin-dependent diabetes mellitus (NIDDM). *Diabetes* 45 : 941-946, 1996
5. Pick A, Bonner-Weir S, Clark J, Polonsky K: Failure of  $\beta$ -cell mass to compensate for insulin resistance and  $\beta$ -cell defects in Zucker Diabetic Fatty (ZDF) rats. *Diabetes* 45 (suppl. 2) : 158A, 1996
6. Movassat J, Saulnier C, Portha B :  $\beta$ -cell mass depletion precedes the onset of hyperglycemia in the GK rat, a genetic model of non-insulin-dependent diabetes mellitus. *Diabetes & Metab* 21 : 365-370, 1995
7. Phillips M S, Liu Q, Hammond H A, Dugan V, Hey PJ, Caskey CT, Hess JF : Leptin receptor missense mutation in the fatty Zucker rat. *Nature Genet* 13 : 18-19, 1996
8. Hogan B, Costantini F, Lacy E : Manipulating the mouse embryo : A laboratory manual. Cold spring harbor, New York, Cold Spring Harbor Laboratory Press, 1986
9. Sambrook J, Fritsch E F, Maniatis T : Molecular

- cloning : A laboratory manual. 2nd ed. Cold spring harbor, New York, Cold Spring Harbor Laboratory Press, 1989
10. Weibel ER : Principles and methods for the morphometric studies of the lung and other organs. *Lab Invest* 12 : 131-155, 1963
  11. Swenne I : The role of glucose in the in vitro regulation of cell cycle kinetics and proliferation of fetal pancreatic  $\beta$ -cells. *Diabetes* 31 : 754-760, 1982
  12. McDowell EM, DeSanti Am, Strum JM : Correlation between blood glucose levels and bromodeoxyuridine labeling indices of pancreatic islet cells following streptozotocin administration to pregnant Syrian golden hamsters. *Virchows Arch B cell Pathol* 57 : 223-230, 1989
  13. Robertson RP, Olson LK, Zhang HJ : Differentiating glucose toxicity from glucose desensitization ; a new message from the insulin gene. *Diabetes* 43 : 1085-1089, 1994
  14. Otonkoski T, Beattie GM, Mally MI, Ricordi C, Hayek A : Nicotinamide is a potent inducer of endocrine differentiation in cultured human fetal pancreatic cells. *J Clin Invest* 92:1459-1466, 1993
  15. Brockenbrough JS, Weir GC, Bonner-Weir S : Discordance of exocrine and endocrine growth following 90%partial pancreatectomy in the rat. *Diabetes* 37 : 232-236, 1988
  16. Iida M, Murakami T, Ishida K, Mizuno A, Kuwajima M, Shima K: Phenotypelinked amino acid alteration in leptin receptor cDNA from Zucker fatty (fa/fa) rat. *Biochem Biophys Res Commun* 222 : 19-26, 1996
  17. Iida M, Murakami T, Ishida K, Mizuno A, Kuwajima M, Shima K : Substitution at codon 269 (Glutamine $\rightarrow$ Proline) of the leptin receptor (OB-R) cDNA is the only mutation found in the Zucker fatty (fa/fa) rat. *Biochem Biophys Res Commun* 224 : 597-604, 1996
  18. Chua SC, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, Leibel RL : Phenotypes of mouse diabetes and rat fatty due to mutation in the Ob (leptin) receptor. *Science* 271 : 994-996, 1996
  19. Yamashita T, Murakami T, Iida M, Kuwajima M, Shima K : Leptin receptor of Zucker fatty rat performs reduced signal transduction. *Diabetes* 46 : 1077-1080, 1997