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Suppression of Body Weight Gain Preserves Acute Insulin Response to Glucose in the Portal Vein of Spontaneously Type 2 Diabetic Rats with Visceral Obesity

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The age-related changes in acute insulin response after glucose loading and the influence of suppression of body weight gain were investigated by using blood samples from portal and peripheral veins. We placed indwelling catheters in the portal vein of 12- and 24-wk-old Otsuka Long–Evans Tokushima fatty (OLETF) rats (*n* = 8, 12), and age-matched control Long–Evans Tokushima Otsuka (LETO) rats (*n* = 8, 6). To suppress the body weight gain, 6 out of 12 OLETF rats were fed chow containing 50 ppm voglibose (VOG) from 8 until 24 wk of age. After fasting for 17 h, rats underwent 1 g/kg oral glucose tolerance test (OGTT). Peripheral glucose levels after glucose loading were significantly higher in 12- and 24-wk-old OLETF rats than in the age-matched LETO rats. Values for delta insulin 15 min/delta glucose 15 min (delta $I_{15\ min}$/delta $G_{15\ min}$) in portal blood were 0.029 ± 0.011 and 0.009 ± 0.009 (12 wk of age) and 0.03 ± 0.03 and –0.01 ± 0.01 (24 wk of age) in the LETO rats and OLETF rats. At the age of 24 wk, the body weights in VOG-treated OLETF rats were significantly lower than those in the OLETF rats. And there was significantly greater acute insulin response to glucose in VOG-treated OLETF rats than in the OLETF rats. Acute insulin response to glucose decreased with advancing age and the suppression of body weight gain preserved the response in spontaneously type 2 diabetic rats with visceral fat obesity.

Key Words: Acute insulin response; portal vein; peripheral vein; voglibose; OLETF rats.

Introduction

The Otsuka Long–Evans Tokushima Fatty (OLETF) rats originated from the outbred strain of Long–Evans rats that were purchased from Charles River Canada in 1982 and maintained at Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). In 1983, a spontaneously diabetic rat with polyuria, polydipsia, and slight obesity was first discovered in this strain, and, after the 20th generation of selective breeding, the diabetic strain (OLETF) has been established in 1990 *(1)*. Obesity, hyperlipidemia and glucose intolerance in this rat strain were shown to be regulated by multiple genes *(2–5)*. As an animal model of spontaneous type 2 diabetes accompanied by obesity *(6)*, the OLETF rat is characterized by insulin resistance *(7)* and accumulated intra-abdominal fat *(8)*, and progression from impaired glucose tolerance (IGT) to type 2 diabetes *(8)*. Hyperglycemia, hyperinsulinemia, and hyperlipidemia, as they characterize this rat, are more noticeable under nonfasting than fasting conditions. The OLETF rat can be used as a model for various forms of human postprandial disease including postprandial hyperglycemia, hyperinsulinemia, and hyperlipidemia, providing an optimal animal model that helps to elucidate the pathological conditions involved.

On the other hand, the significance of decrease in early phase insulin secretion associated with progression to type 2 diabetes was clinically reported in Japanese–American patients *(9)*, in Finnish patients *(10)*, and Japanese patients *(11)*.

To shed further light on the significance of early phase insulin secretion after glucose loading, we performed the present study to observe age-related changes in acute insulin response to glucose in OLETF rats and to examine the influences of suppression of body weight gain on the response to glucose. As a major portion of the insulin that was secreted into portal vein is taken by liver before it reaches the peripheral circulation and insulin secretion dynamics after glucose loading is more remarkable in portal blood than in peripheral blood, we investigated the acute insulin response to glucose using samples from portal vein in this study.

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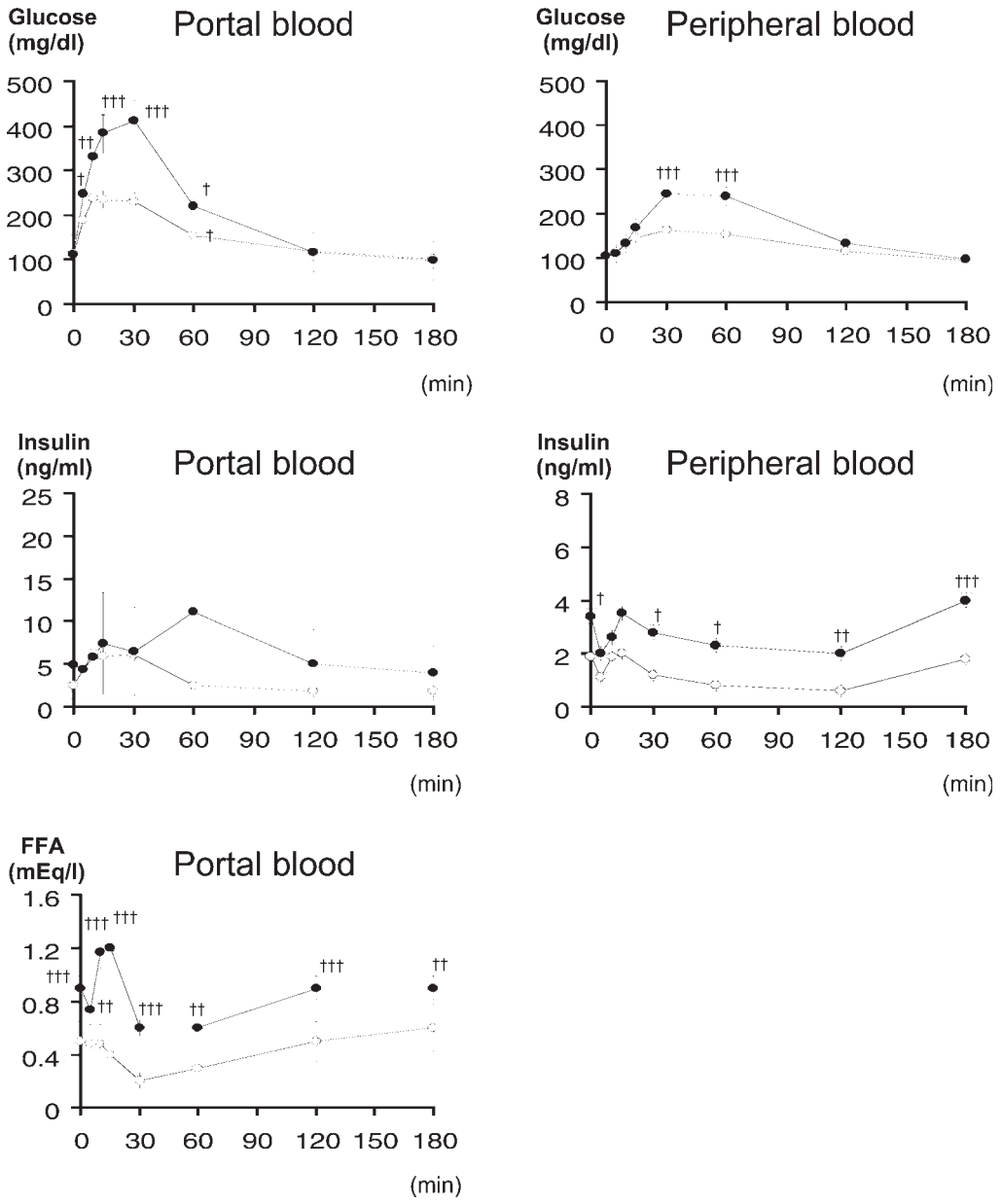

Fig. 1. Portal and peripheral levels of blood glucose and plasma insulin, and portal plasma levels of FFA after glucose loading. Closed circle, 12-wk-old OLETF rats ($n = 8$); open circle, age-matched LETO rats ($n = 8$). $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$, vs LETO group.

Results

Acute Insulin Response to Glucose in Prediabetic Rats (Experiment 1)

Animal weights during the experiment were 325.0 ± 19.6 g in the LETO group and 405.0 ± 39.1 g in the OLETF group at the age of 12 wk. Body weight was significantly higher in the OLETF group than in the LETO group ($p < 0.001$).

Portal blood glucose levels were significantly higher in the OLETF group than in the LETO group 5, 10, 15, 30, and 60 min after glucose loading, but significantly higher values in peripheral blood were noted only at 30 and 60 min after glucose loading (Fig. 1). The area under the glucose curve (AUC-glucose, mg·min/dL) for both portal and peripheral blood glucose was significantly higher in the OLETF group than in the LETO group (Table 1).

Insulin secretion in portal blood peaked 60 min after glucose loading in the OLETF group, suggesting a delay in insulin secretion compared to that in the LETO group, while these values for peripheral blood were significantly higher in the OLETF group at baseline and at 30, 60, 120, and 180 min (Fig. 1). We also found no significant difference between the OLETF and LETO groups in area under the insulin curve (AUC-insulin, ng·min/mL) for portal blood, although these values were significantly higher in the OLETF group than in the LETO group for peripheral blood (Table 1).

Table 1
AUC for Glucose, Insulin, and FFA During OGTT in 12-wk-Old Rats

	12-wk-old LETO (n = 8)	12-wk-old OLETF (n = 8)
AUC for glucose (mg·min/dL)		
Portal blood	26,966 ± 4485	36,070 ± 2315*
Peripheral blood	23,247 ± 2979	30,223 ± 3317*
AUC for insulin (ng·min/mL)		
Portal blood	544 ± 210	1206 ± 775
Peripheral blood	196 ± 57	466 ± 210*
AUC for FFA (mEq·min/L)		
Portal blood	74.8 ± 16.1	139.4 ± 11.0**

*$p < 0.01$, **$p < 0.001$, vs value of LETO.

In portal blood, the ratio of delta insulin 15 min/delta glucose 15 min (delta $I_{15\ min}$/delta $G_{15\ min}$) was lower for the OLETF group than for the LETO group although it was not statistically significant. Both portal and peripheral values for delta $I_{30\ min}$/delta $G_{30\ min}$ showed tendencies similar to those seen for portal delta $I_{15\ min}$/delta $G_{15\ min}$, with delta $I_{30\ min}$/delta $G_{30\ min}$ values in the OLETF group lower than those in the LETO group (Fig. 2).

Values for free fatty acids (FFA) and for area under the FFA curve (AUC-FFA, mEq·min/L) were significantly higher in the OLETF group than in the LETO group (Fig. 1, Table 1).

In prediabetic OLETF rats aged 12 wk, acute insulin response to glucose was already lower and portal FFA levels was higher than those in the control LETO rats.

Acute Inslin Response to Glucose in Overt Diabetic Rats and the Influence of Suppression of Body Weight Gain (Experiment 2)

Animal weights during the experiment were significantly higher ($p < 0.001$) in the OLETF group than in the LETO group and significantly lower ($p < 0.001$) in VOG-treated OLETF group than in the OLETF group (487.2 ± 24.6 g in the LETO group, 589.3 ± 65.5 g in the OLETF group, and 490.9 ± 35.2 g in the VOG-treated OLETF group).

Portal blood glucose levels were significantly higher in the OLETF group than in the LETO group at baseline and at 15, 30, and 60 min after glucose loading, and peripheral blood glucose levels were significantly higher at baseline and at 15, 30, 60, and 120 min after glucose loading (Fig. 3). AUC-glucose was significantly higher in the OLETF group than in the LETO group for both portal and peripheral blood (Table 2). Portal blood glucose levels were significantly lower in the VOG-treated OLETF group than in the OLETF group at baseline and at 15, 30, and 60 min after glucose loading, and peripheral blood glucose levels were significantly lower at baseline and at 15, 30, 60, and 120 min after glucose loading. AUC-glucose was significantly lower in the VOG-treated OLETF group than in the OLETF group for both portal and peripheral blood (Table 2).

Portal insulin levels at baseline were significantly higher in the OLETF group than in the LETO group, and peripheral insulin levels were significantly higher in the OLETF group than in the LETO group at baseline and at 120 and 180 min after glucose loading (Fig. 3). We found no significant difference in portal AUC-insulin between the OLETF and LETO groups, but, for peripheral blood, these values were significantly higher in the OLETF group than in the LETO group (Table 2). Although there was no significant difference in portal insulin levels between the OLETF group and the VOG-treated OLETF group, peripheral insulin levels were significantly lower in the VOG-treated OLETF group than in the OLETF group at baseline and at 120 and 180 min after glucose loading (Fig. 3). We found no significant difference in portal AUC-insulin between the VOG-treated OLETF and OLETF groups, but for peripheral blood these values were significantly lower in the VOG-treated OLETF group than in the OLETF group (Table 2).

In portal blood, the ratios of delta $I_{15\ min}$/delta $G_{15\ min}$ and delta $I_{30\ min}$/delta $G_{30\ min}$ were lower for the OLETF group than for the LETO group. Similar findings were noted in peripheral blood (Fig. 2). In portal blood, the ratio of delta $I_{15\ min}$/delta $G_{15\ min}$ was higher for the VOG-treated OLETF group than for the OLETF group. As for the ratio of delta $I_{30\ min}$/delta $G_{30\ min}$, the similar findings were noted as the ratio of delta $I_{15\ min}$/delta $G_{15\ min}$ in portal blood and it was significantly higher for the VOG-treated OLETF group than for the OLETF group in peripheral blood (Fig. 2).

Values for FFA and for AUC-FFA in portal blood were significantly higher in the OLETF group than in the LETO group at baseline and at 15, 30, and 60 min after glucose loading (Fig. 3; Table 2). Values for FFA and for AUC-FFA in portal blood were significantly lower in the VOG-treated OLETF group than in the OLETF group at baseline

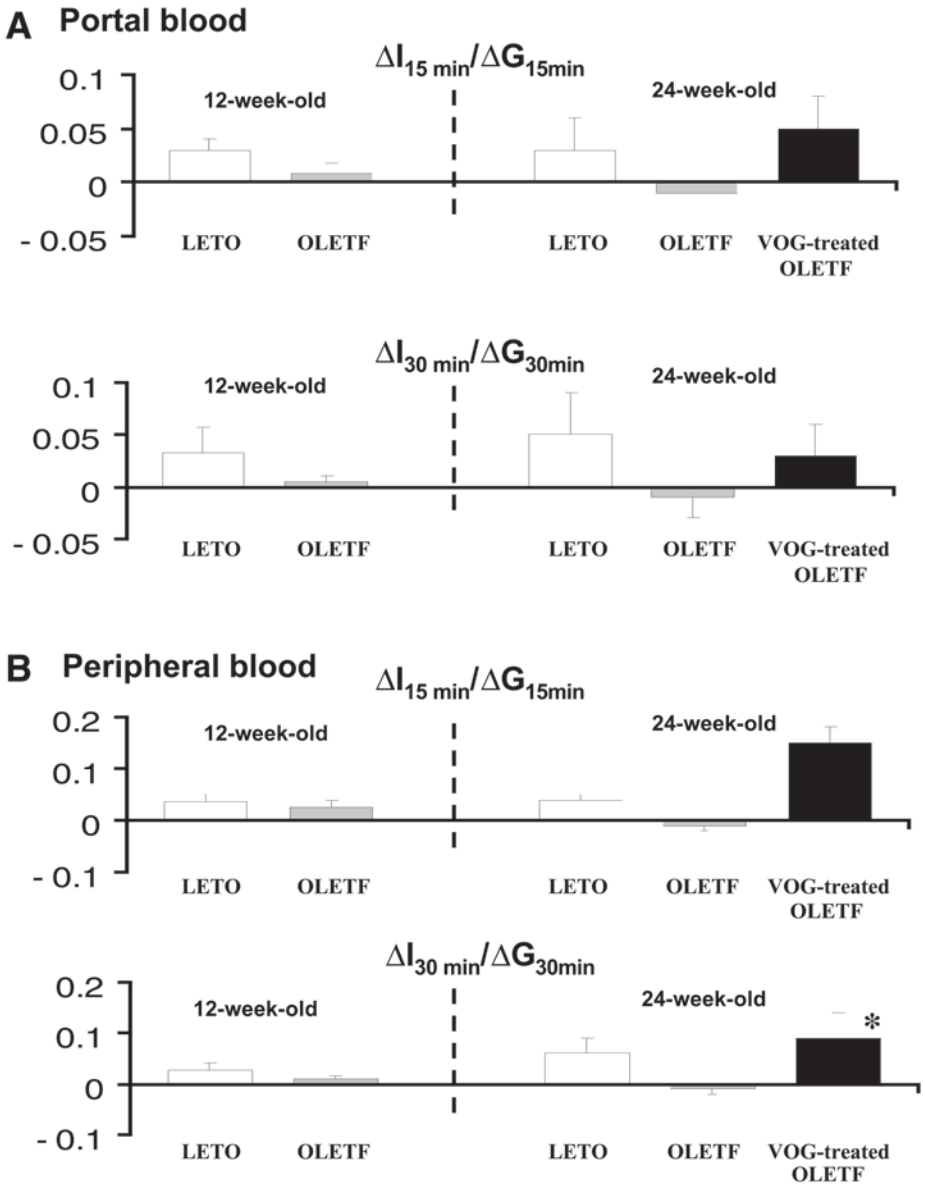

Fig. 2. The ratios of delta insulin/delta glucose for 15 min and 30 min after glucose loading in the portal blood (**A**) and the peripheral blood (**B**). *$p < 0.001$, vs OLETF group.

and at 15, 30, and 60 min after glucose loading (Fig. 3; Table 2).

Both in hepatic and pancreatic tissues, the OLETF group showed a significantly higher triglyceride content than the LETO group ($p < 0.001$ and $p < 0.05$, respectively) and the VOG-treated OLETF group showed a significantly lower triglyceride content than the OLETF group ($p < 0.001$, $p < 0.05$ respectively) (Fig. 4).

Histological examination of the pancreas from OLETF rats aged 24 wk showed the remarkably hypertrophic islets compared with those from the age-matched control LETO rats and the fibrous changes in the islets were observed. In contrast, the pancreatic islets from OLETF rats that were treated with VOG from 8 to 24 wk of age showed that the hypertrophy of islets were suppressed and the fibrous changes were mild (Fig. 5).

In spontaneously type 2 diabetic OLETF rats with visceral obesity, acute insulin response to glucose was reduced with advancing age and then eliminated at the age of 24 wk. The body weights in VOG-treated OLETF rats were significantly lower than those in the OLETF rats, and there was significantly greater acute insulin response to glucose in VOG-treated OLETF rats than in the OLETF rats.

Discussion

In type 2 diabetes, there is a reduction in the early insulin response to oral glucose loading, as represented by a low

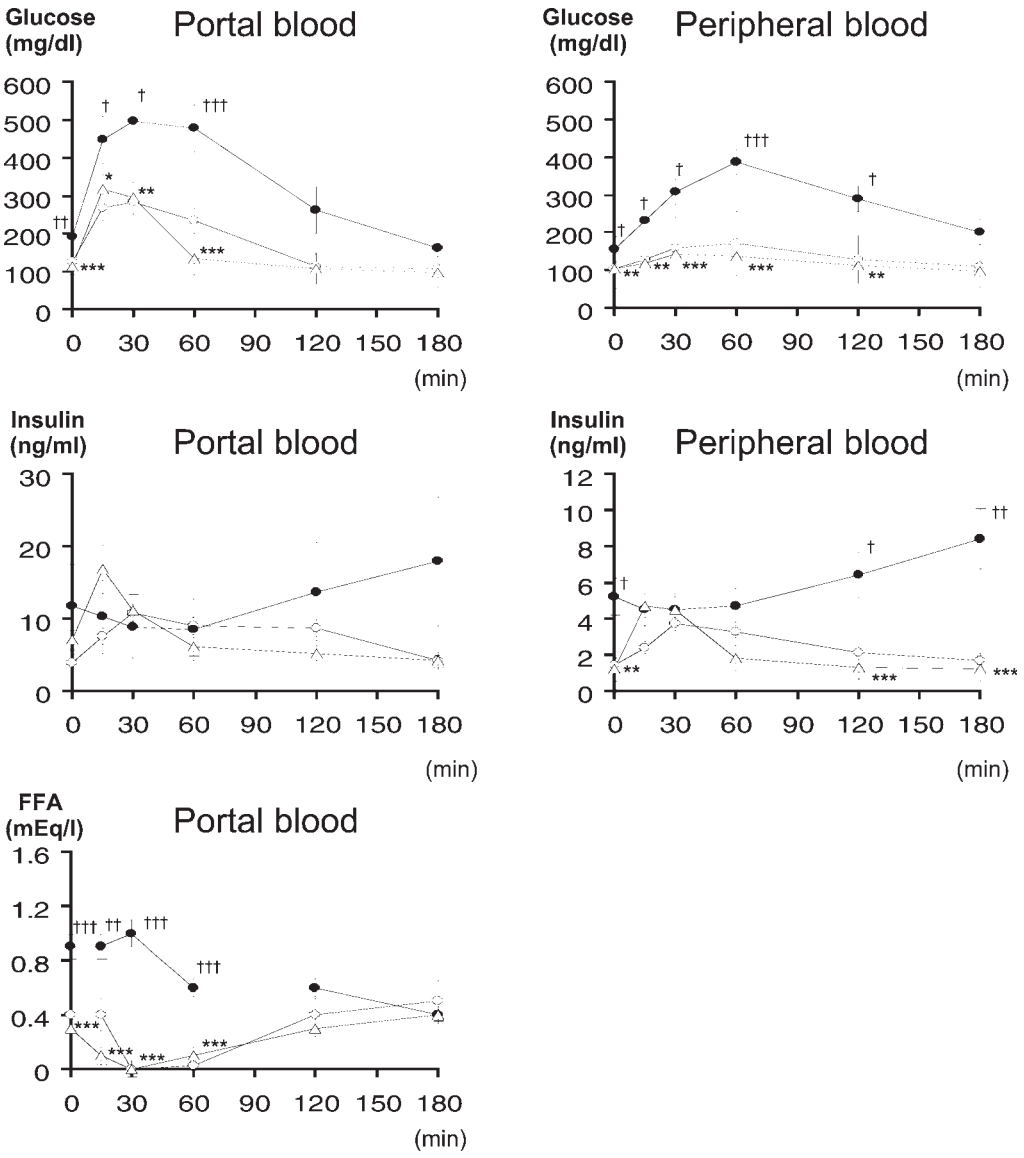

Fig. 3. Portal and peripheral levels of blood glucose and plasma insulin, and portal levels of FFA after glucose loading. Closed circle, 24-wk-old OLETF rats ($n = 6$); open triangle, age-matched VOG-treated OLETF rats ($n = 6$); open circle, age-matched LETO rats ($n = 6$). $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$, vs LETO group. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, vs OLETF group.

Table 2
AUC for Glucose, Insulin, and FFA During OGTT in 24-wk-Old Rats

	24-wk-old LETO ($n = 6$)	24-wk-old OLETF ($n = 6$)	24-wk-old VOG-treated OLETF ($n = 6$)
AUC for glucose (mg·min/dL)			
Portal blood	31,773 ± 2818	61,317 ± 16,873††	27,618 ± 4737**
Peripheral blood	24,951 ± 3220	52,316 ± 11,906†††	21,662 ± 3221***
AUC for insulin (ng·min/mL)			
Portal blood	1434 ± 620	2186 ± 495	1260 ± 528
Peripheral blood	458 ± 60	1053 ± 428††	378 ± 90**
AUC for FFA (mEq·min/L)			
Portal blood	50 ± 7.8	118 ± 26†††	38 ± 12***
Peripheral blood	146 ± 27	130 ± 28	78 ± 26

$^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, vs value of LETO. $**p < 0.01$, $***p < 0.001$, vs value of OLETF.

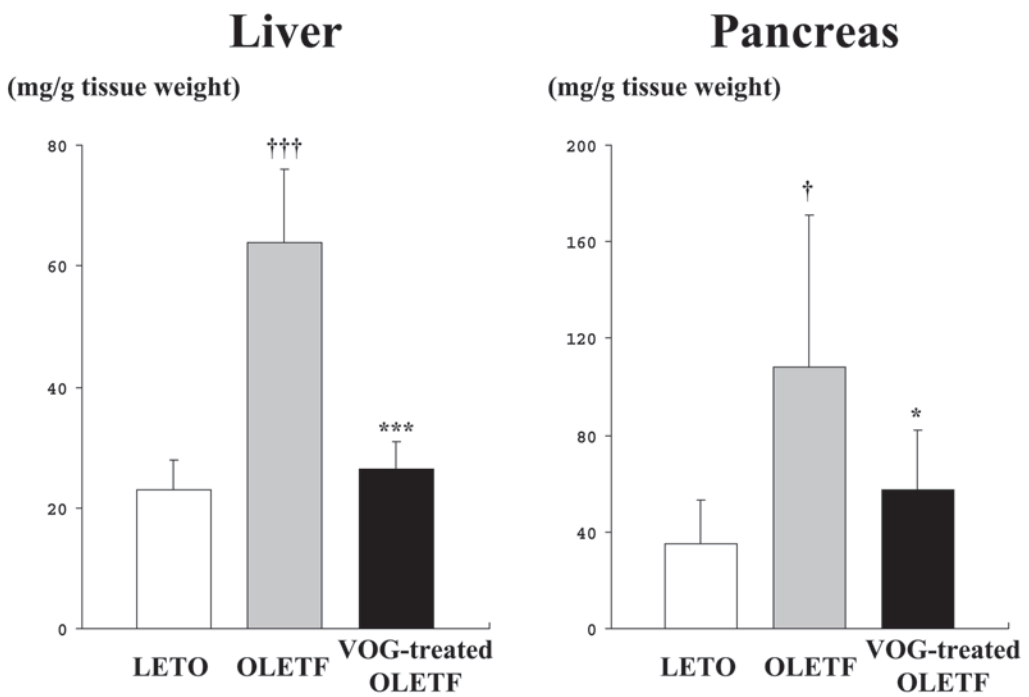

Fig. 4. Triglyceride contents in hepatic and pancreatic tissue in 24-wk-old OLETF rats (n = 6), 24-wk-old VOG-treated OLETF rats (n = 6) and the age-matched control LETO rats (n = 6).

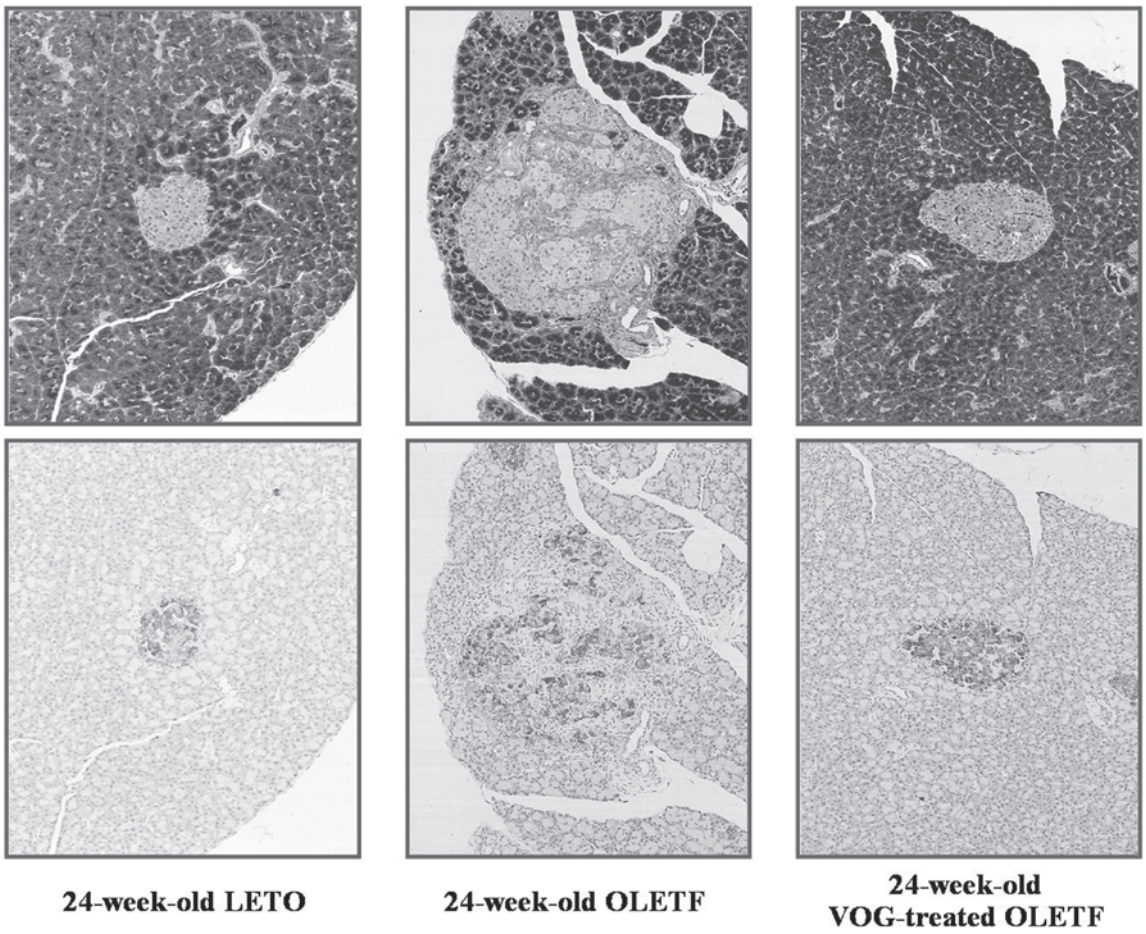

Fig. 5. Histologic features of the pancreatic islets from 24-wk-old OLETF rats, 24-wk-old VOG-treated OLETF rats, and the age-matched control LETO rats (×300).

insulinogenic index *(11)*. In our present study of spontaneously diabetic OLETF rats, an animal model of type 2 diabetes with visceral obesity, we found the values for portal delta $I_{15\ min}$/delta $G_{15\ min}$ and delta $I_{30\ min}$/delta $G_{30\ min}$ after glucose loading showed a tendency to decrease in 12-wk-old OLETF rats than in age-matched control LETO rats, even though the OLETF rats at this age have not yet manifested diabetes and show only a slight elevation of peripheral blood glucose after glucose loading. At 24 wk of age, when diabetes was almost fully developed, there was negligible portal insulin elevation 15 min and 30 min after glucose loading. These findings confirm the loss of early phase insulin secretion to glucose that accompanies the progression of diabetes associated with aging. These experimental results strongly support the findings of Kahn and colleagues in Japanese–American patients *(9)*, as well as findings from the Botnia study in Finnish patients *(10)*, regarding the significance of decreases in early phase insulin secretion associated with progression to type 2 diabetes. Moreover, high triglyceride accumulation in the pancreas of 24-wk-old OLETF rats might be also related to the loss of glucose-induced early phase insulin secretion through lipotoxicity *(12,13)*.

Voglibose, a alpha-glucosidase inhibitor, inhibits the breakdown of disaccharides into monosaccharides by acting competitively on the activities of disaccharidase (a alpha-glucosidase) in the final stage of carbohydrate digestion. This delays the digestion and absorption of carbohydrates, controlling postprandial hyperglycemia *(14)*. Moreover, it is reported that high-dose voglibose inhibits the digestion and absorption of carbohydrate, showing antiobesity effects *(15)*. In the present study, long-term (from 8 to 24 wk of age) treatment of high-dose voglibose suppressed the body weight gain and prevented the development of obesity in OLETF rats. In portal blood, the ratio of delta $I_{15\ min}$/delta $G_{15\ min}$ and the ratio of delta $I_{30\ min}$/delta $G_{30\ min}$ was higher for the VOG-treated OLETF group than for the OLETF group. These results might be related to the histological findings of pancreatic islets from the VOG-treated OLETF rats and the decreased triglyceride content in the pancreas of VOG-treated OLETF rats. Long-term treatment of high-dose voglibose prevented the development of obesity and ameliorated insulin resistance. Subsequently, the pancreatic beta cell load might be reduced and the acute insulin response to glucose might be preserved.

In this study we found significantly higher portal blood glucose levels after glucose loading in 12-wk-old and 24-wk-old OLETF rats than in age-matched LETO controls. In particular, although we detected no significant difference in peripheral blood glucose levels between OLETF rats and LETO rats at 5, 10, and 15 min after glucose loading, these rats in the OLETF group already showed significantly higher portal blood glucose levels than age-matched LETO animals at 5, 10, and 15 min. These findings suggest the possibility that blood glucose elevation is evidenced in portal blood than in peripheral blood. Some researchers have reported that the final digestion and absorption of carbohydrates in the small intestine is accelerated under diabetic conditions *(16,17)*, and this abnormal glucose absorption in the small intestine could contribute to the portal blood glucose elevation after glucose loading seen in these rats. And another possibility is that high glucose levels in portal vein after glucose loading was caused by glucose intolerance, secondary to insulin resistance in these rats.

In both of 12-wk-old and 24-wk-old rats, portal AUC-insulin values showed a tendency to increase than the age-matched LETO group, although they were not statistically significant. Moreover, we found significantly higher peripheral AUC-insulin values in the OLETF group than in the LETO group. These findings are related to the significant elevation of FFA in portal blood in the OLETF group in comparison with the LETO group, and indicated that in addition to the hypersecretion of insulin from pancreatic beta cells, there was a reduced hepatic insulin clearance in the OLETF rats compared to the LETO rats. These findings agree with those for dietary obesity in rats *(18)* and for obese patients *(19)*. In contrast, although there was no significant difference in the portal AUC-insulin values between OLETF group and VOG-treated OLETF group, the peripheral AUC-insulin values were significantly lower in VOG-treated OLETF group than in OLETF group. This result suggest that hepatic insulin clearance might be higher in VOG-treated OLETF group than OLETF group, which was related to the lower FFA levels in portal vein and the lower hepatic triglyceride contents in VOG-treated OLETF group.

In spontaneously type 2 diabetic OLETF rats with visceral obesity, as early phase insulin secretion after glucose loading is first reduced and then eliminated with advancing age, the animal progresses from IGT to type 2 diabetes. The amelioration of insulin resistance by suppression of body weight gain might reduce pancreatic beta cell load and preserve the acute insulin response to glucose. And the preservation of acute insulin response might be also related to the removal of lipotoxicity.

These results suggest that in the patients with IGT, body weight management might be the best therapeutic approach to preserve the early phase insulin secretion and to prevent the progression to type 2 diabetes.

Materials and Methods

Animals

OLETF rats and control Long–Evans Tokushima Otsuka (LETO) rats *(1,6–8)* were obtained at 4 wk of age from the Tokushima Research Institute (Otsuka Pharmaceutical Co., Tokushima). The rats were housed in plastic cages (320 × 270 × 175 mm) in an animal room with a controlled temperature (23 ± 2°C) and relative humidity (55 ± 15%) and a 12-h light/12-h dark cycle (lights on at 0700). They were given free access to standard rat chow (CE-2; CLEA Japan,

Inc., Tokyo) and tap water until 12 wk of age (n = 8,8) and 24 wk of age (n = 12, 6). Six out of 12 OLETF rats were fed with the chow containing 50 ppm voglibose *(15)* (Takeda Pharmaceutical, Co. Ltd, Osaka, Japan) from 8 until 24 wk of age. The care and use of the animals in this study were in accordance with the guidelines of the Laboratory Animal Facilities of the Jikei University School of Medicine.

Experimental Design

Experiment 1

An indwelling catheter was surgically implanted in the portal vein in 12-wk-old OLETF rats (n = 8) and age-matched, control LETO rats (n = 8), with the exposed end of the catheter positioned behind the head. Rats were fasted for 17 h, and were then given 1 g/kg of glucose orally (oral glucose tolerance test, OGTT). The rats were not anesthetized, and their movement was not restricted. Blood samples were drawn from the indwelling catheter (portal blood) and the caudal vein (peripheral blood) at baseline and at 5, 10, 15, 30, 60 120, and 180 min after glucose loading. Plasma glucose and insulin levels were measured in both portal and peripheral blood samples, and FFA in portal blood were assessed.

Experiment 2

Experiment 2 compared findings in 24-wk-old OLETF rats (n = 6), in voglibose (VOG)-treated OLETF rats (n = 6) and in age-matched LETO rats (n = 6). After fasting for 17 h, the unanesthetized and unrestrained rats underwent 1 g/kg OGTT just as in Experiment 1. Portal and peripheral blood samples were taken at baseline and at 15, 30, 60, 120, and 180 min after glucose loading, and plasma glucose and insulin levels were measured. Plasma FFA levels were measured in the portal blood. After this experiment was completed, the liver and pancreas were removed from the experimental animals and homogenized: the Folch method *(20)* was used to extract lipids and measure triglycerides, and tissue triglyceride content (milligram per gram of tissue weight) was calculated. Several rat pancreata were collected for histologic studies and fixed in 10% formalin solution. Paraffin-embedded and consecutive sections were stained with hematoxylin and eosin (H&E) and immunostained using anti-insulin antibody by avidine–biotin peroxidase complex (ABC) method.

Blood glucose levels were determined by the glucose oxidase methods and triglyceride levels were determined by glycerol phosphodehydroxygenase method, both of which were performed using a Fuji Dri-Chem 5500 auto analyzer (Fuji Medical Sysytems, Tokyo, Japan). Plasma FFA levels were measured by an enzyme technique, where the samples were incubated with enzyme reagent (NEFA-SS, Eiken, Japan) and the optical density determined. Plasma insulin levels were determined with a commercial enzyme immunoassay kit (Ultra Sensitive Rat Insulin Kit, Seikagaku Co., Tokyo, Japan) using rat insulin as the standard.

All numerical values were expressed as mean ± SEM. We applied one-way analysis of variance (ANOVA), and followed up with Scheffe's method as a post-hoc test for any significant differences among the groups ($p < 0.05$). The level of $p < 0.05$ was considered to indicate statistical significance.

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