Metabolic Abnormalities in the Genetically Obese and Diabetic Otsuka Long-Evans Tokushima Fatty Rat Can Be Prevented and Reversed by α-Glucosidase Inhibitor

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The recently developed Otsuka Long-Evans Tokushima Fatty (OLETF) rat is known to develop insulinopenic diabetes after a prolonged period in a condition resembling non–insulin-dependent diabetes mellitus (NIDDM). We examined the effect of pharmacological intervention with a potent intestinal α-glucosidase inhibitor, acarbose, on the metabolic and histopathologic changes in this rat model. The first two groups of rats received an acarbose-rich diet (150 mg/100 g normal chow) from 12 weeks of age (ie, before the onset of diabetes) or from 28 weeks of age (ie, after the onset of diabetes), while a third group received the acarbose-rich diet for the initial 16 weeks only (from 12 to 28 weeks of age). A control group received standard rat chow. Acarbose-fed rats gained less weight or lost weight despite increased food intake when switched to the acarbose-rich diet. Acarbose also reduced visceral adipose depots and fasting triglyceride (TG), glucose, and insulin levels. At the end of the study at 72 weeks, the pancreatic wet weight and insulin content were significantly higher in the treated groups versus control rats. The morphological changes observed in control rats, such as atrophy of the pancreas and reduced number and size of islets, were not present in acarbose-treated rats. Rats fed acarbose from 12 to 28 weeks of age gradually gained weight after switching to standard chow, and hyperinsulinemia, hyperglycemia, and hyperlipidemia appeared (in that order). The pancreatic insulin content in these rats was significantly higher and the visceral adipose depot was significantly smaller than in control rats. Our study demonstrates that acarbose prevented and reversed the metabolic derangement and histopathological changes in genetically diabetic rats. Moreover, treatment with acarbose even for a short period produced a marked delay in the development of insulin insensitivity and frank diabetes.

THE RECENTLY ESTABLISHED spontaneously diabetic strain, the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, develops obesity and shows glucose intolerance associated with hyperinsulinemia after 18 weeks of age. This is followed by a gradual increase in plasma glucose and insulin, reaching levels that are three to four times higher than normal. During the chronic course of non–insulin-dependent diabetes mellitus (NIDDM)-like diabetes, OLETF rats eventually become hypoinsulinemic and develop insulin-dependent diabetes mellitus (IDDM)-like diabetes. Extreme atrophy of the pancreas and a significant reduction in the number and size of islets occur in rats older than 70 weeks of age.[1] Thus, OLETF rats exhibit some metabolic and histopathologic characteristics similar to those of NIDDM in humans.

Acarbose is a potent competitive inhibitor of α-glucosidases in the intestinal brush border, and thus delays carbohydrate and disaccharide digestion after meals at the level of the small intestine.[2-4] Several studies have demonstrated the ability of acarbose to decrease postprandial blood glucose, insulin, and C-peptide levels in experimental animals,[3,5-9] healthy subjects,[3,5,10-12] and patients with IDDM or NIDDM.[10-12] Treatment with acarbose is also known to prevent hyperglycemia and to decrease insulin levels in genetically obese animals (*fa/fa* and SHR/N-*cp* rats or *db/db* mice).[5,7-9,13] In addition, it also reduces the prevalence of diabetic nephropathy in acarbose-treated *db/db* mice.[8] Thus, acarbose is useful for the treatment of certain metabolic disorders such as diabetes, hyperlipidemia, and obesity.[3,10-13]

Considering the potential effects of acarbose to reduce hyperinsulinemia and hyperglycemia,[3,5,9-12] the present study was designed to examine whether pharmacological intervention with acarbose, either at a time when plasma glucose concentrations are still normal or after the onset of diabetes mellitus, can delay, prevent, or reverse the metabolic and histopathologic changes in genetically obese and diabetic OLETF rats.[1]

MATERIALS AND METHODS

Animals and Diet

A spontaneously diabetic strain, the OLETF rat, was established at the Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan) in 1990.[1] OLETF male rats aged 5 weeks were kindly supplied by the Tokushima Research Institute and maintained in a temperature (23° ± 2°C)- and humidity (55% ± 5%)-controlled room with a 12-hour light/dark cycle (lights on at 7:00 AM). The animals were provided ad libitum standard rat chow consisting of (as a percentage of calories) 61% carbohydrate, 26% protein, and 13% fat with 5% cellulose (wt/wt) (3.596 kcal/g diet; Oriental Yeast, Tokyo, Japan) and tap water. The rats were maintained according to the ethical guidelines of our institution, and the experimental protocol was approved by the animal welfare committee.

Administration of Acarbose

Standard rat chow was pulverized to a fine powder, and acarbose (a generous gift from Bayer Pharmaceutical, Osaka, Japan) was added and thoroughly mixed to a final concentration of 150 mg/100 g food. This drug concentration was chosen based on our previous studies.[14,15] The drug-chow powder mixture was reconstituted into pellets with a normal appearance. Chow for control rats was prepared in a similar fashion but without the addition of acarbose.

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Experimental Protocol

Animals were fed standard rat chow until the start of the experiment. Male OLETF rats were randomly divided into four groups at 12 weeks of age (the start of the experiments) (Fig 1). Group A12 (n = 10) was maintained on the acarbose-rich chow diet from 12 weeks of age, ie, before the onset of diabetes, until the end of the study at 72 weeks. Group A28 (n = 10) received standard chow without drug until 28 weeks of age, and thereafter, ie, after the onset of diabetes, they received acarbose-rich chow until the end of the study. Group A12-28 (n = 10) was maintained on a diet containing acarbose from 12 to 28 weeks of age and then changed to standard rat chow without drug until 70 weeks of age. The control (C) group (n = 10) received standard rat chow free of acarbose.

All groups were allowed free access to food and water throughout the study. Animals were weighed on a weekly basis, and food intake was determined every 2 weeks over a 48-hour period by weighing the full food cups and then weighing the food cups again 48 hours later, correcting for spillage. The average food intake was estimated as the amount of food consumed per cage. Because the animals were fed as groups and housed two per cage, the value obtained for 48 hours was then divided by four to obtain the approximate estimate of daily food consumption per rat.

Intravenous Glucose Tolerance Test

At 12, 20, 28, 36, 44, 52, 60, and 70 weeks of age, an intravenous glucose tolerance test (IVGTT) was performed after an overnight fast. Animals were weighed before the experiments, and anesthesia was induced using sodium pentobarbital (50 mg/kg body weight intraperitoneally [IP]). A bolus dose of glucose 200 mg/kg body weight was injected into the right jugular vein immediately after blood sampling for measurement of serum concentrations of insulin, glucose, TG, and cholesterol. Blood samples were collected again from the left jugular vein at 5, 10, 30, and 60 minutes for measurement of serum concentrations of glucose and insulin.

Analysis of Pancreatic Content and Measurement of the Weight of Adipose Depots

Two weeks after the last IVGTT (72 weeks of age), the rats were treated with sodium pentobarbital and the abdomen was quickly opened to remove the pancreas. Retroperitoneal, mesenteric, and epididymal white adipose depots were dissected and weighed. The pancreas was excised, cleared of lymph nodes and fat, and weighed. Portions of each tissue with similar anatomic orientation were used for histologic examination and biochemical determinations.

A portion of the pancreatic tissue was homogenized in saline using a motor-driven Teflon-coated glass homogenizer at 3,000 rpm (eight passes). The homogenates were filtered through three layers of gauze and then sonicated for 1 minute. The aqueous phase obtained after 15 minutes was used for protein and DNA assay. Insulin was extracted by a modified method of Davoren.[16]

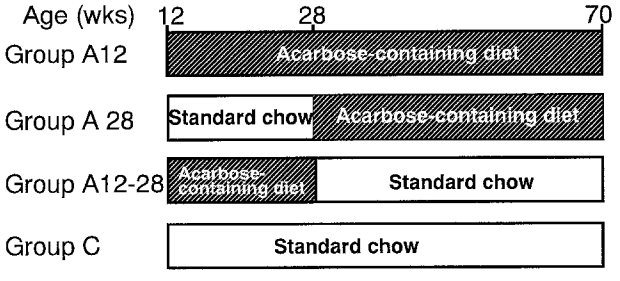

Fig 1. Experimental design. Male OLETF rats at 12 weeks of age were randomly divided into 4 groups. Group A12 was maintained on a diet containing acarbose (150 mg/100 g diet) from 12 weeks of age, ie, before the onset of diabetes, until the end of the study. Group A28 received standard chow without drug until age 28 weeks, and thereafter, ie, after the onset of diabetes, they received acarbose-rich chow until the end of the study. Group A12-28 was maintained on a diet containing acarbose from 12 to 28 weeks of age, and then changed to standard rat chow without drug until the end of the study. Group C received standard rat chow without drug throughout the experimental period.

Histologic Examination

A portion of the pancreatic tissue was fixed overnight in 10% formaldehyde solution for hematoxylin and eosin staining and light-microscopic examination. All histologic samples were examined in a single-blind fashion by the pathologist without awareness of the treatment.

Assays

Protein and DNA concentrations in pancreatic homogenates were determined by the method of Lowry et al[17] using bovine plasma albumin as a standard and the method of Labarca and Paigen[18] using the fluorescent dye H-33258 (Hoechst, Frankfurt, Germany) and calf thymus DNA (type I; Sigma Chemical, St Louis, MO) as a standard, respectively. Serum glucose concentrations were determined by the glucose-oxidase method using a glucose kit (Glucose-E reagent; International Reagents, Kobe, Japan).[19] Insulin concentrations in the serum and pancreatic homogenates were measured by radioimmunoassay using the double-antibody method[20] with a commercially available radioimmunoassay kit (ShionoRIA; Shionogi Pharmaceutical, Osaka, Japan) using crystalline rat insulin as a reference standard. Serum TG and cholesterol concentrations were analyzed enzymatically using commercially available kits (Wako TG and cholesterol kits; Wako Pure Chemical, Tokyo, Japan).

Statistical Analysis

Results are presented as the mean ± SEM. Differences between groups were tested for statistical significance using ANOVA followed by Tukey's test. A P value less than .05 denoted a statistically significant difference.

RESULTS

During the experiment, six rats died, four rats in group C at 52 (one rat), 60 (two rats), and 70 (one rat) weeks of age and two rats in group A12-28 at 40 weeks of age.

Food Consumption and Body Weight

Group C OLETF rats at 12 weeks of age consumed 26.2 ± 0.2 g food/rat/d, and the mean food intake remained nearly the same until 56 weeks of age, when it gradually increased to 33.6 ± 0.3 g food/rat/d at 70 weeks of age ($P < .05$ v C; Fig 2). The addition of acarbose to the diet resulted in a significant increase in food intake (group A12, 33.7 ± 0.9 g food/rat/d at 16 weeks of age), and the intake remained almost the same until the end of the study. However, in group A12-28, the high food consumption gradually returned to control levels after switching to standard rat chow (27.6 ± 0.8 g food/rat/d at 36 weeks of age).

The appearance of the feces was different in acarbose-treated rats versus C rats. The volume was greater, the size was larger,

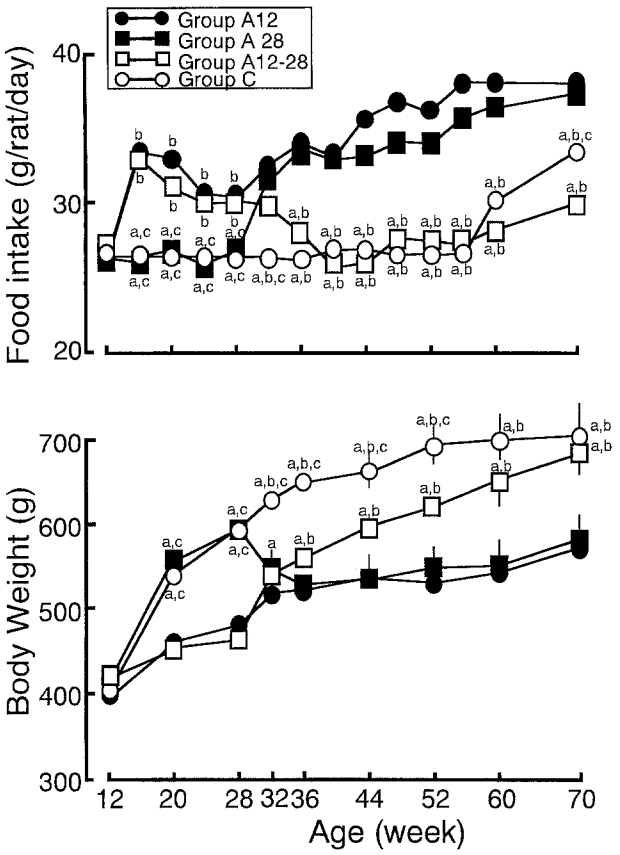

Fig 2. Serial changes in daily food intake and body weight of untreated C and acarbose-treated OLETF rats. Results are the mean ± SEM of 6-10 rats. [a]Significantly different *v* group A12, [b]significantly different *v* group A28, [c]significantly different *v* group A12-28.

and the solidity was softer than for the C rats, although the shape was similar. At the end of this study (72 weeks of age), the cecum and intestine appeared to be greatly enlarged compared with group C.

The body weight of C rats increased progressively from 405 ± 6.1 g at 12 weeks of age to 702 ± 41 g at 70 weeks of age (Fig 2). The addition of acarbose to the diet diminished the gain in body weight (group A12) or reduced the body weight despite the high food consumption (group A28, 593 ± 15.9 g at 28 weeks of age *v* 542 ± 15.5 g at 32 weeks of age, $P < .05$). At 70 weeks of age, the body weight of these rats (570 ± 11.5 g) and of group A28 (581 ± 27.4 g) was significantly less than in group C. On the other hand, the body weight of rats treated with acarbose for the initial 16 weeks only (group A12-28) gradually increased after switching to the standard rat chow and became similar to C rats at 70 weeks of age (679 ± 12.6 g).

Fasting Serum Cholesterol and TG Levels

Fasting serum TG levels in group C increased progressively with age from 1.73 ± 0.12 mmol/L at 12 weeks to 3.96 ± 0.07 mmol/L at 70 weeks (Fig 3). Supplementing the diet with acarbose led to a decrease in serum TG to less than the initial values obtained on standard rat chow (group A12, 1.76 ± 0.11 mmol/L at 12 weeks *v* 0.64 ± 0.07 mmol/L at 28 weeks, $P < .001$). However, in group A12-28, it gradually increased to the initial levels and higher after switching to standard rat chow (0.64 ± 0.05 mmol/L at 28 weeks *v* 3.85 ± 0.11 mmol/L at 70 weeks, $P < .001$).

Fasting serum cholesterol in the C group remained constant until 36 weeks of age, when it gradually increased to 6.98 ± 0.05 mmol/L at 70 weeks (Fig 3). Supplementing the diet with acarbose (groups A12 and A28) completely prevented the increase in serum cholesterol. Serum cholesterol in group A12-28 remained at the initial level for 24 weeks (until 52 weeks of age) even after switching to the C diet; thereafter, it gradually increased but was still significantly low compared with group C at 70 weeks of age (4.29 ± 0.21 mmol/L).

Serum Insulin and Glucose Response to IVGTT

Changes in the baseline concentration of serum glucose and immunoreactive insulin and changes in response to IV glucose administration are shown in Figs 4 and 5. Baseline values were similar in all four groups (C, A12, A12-28, and A28) at 12 weeks of age. At this age, the basal serum glucose concentration in group C was 5.66 ± 0.33 mmol/L and increased gradually

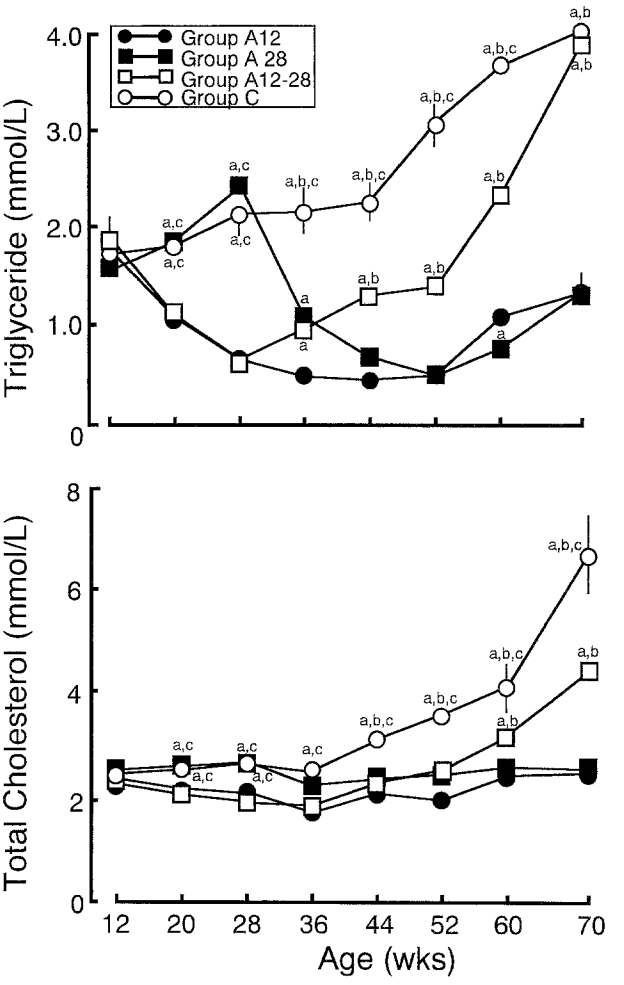

Fig 3. Serial changes in fasting serum TG and cholesterol in untreated C and acarbose-treated OLETF rats. Results are the mean ± SEM of 6-10 rats. [a]Significantly different *v* group A12, [b]significantly different *v* group A28, [c]significantly different *v* group A12-28.

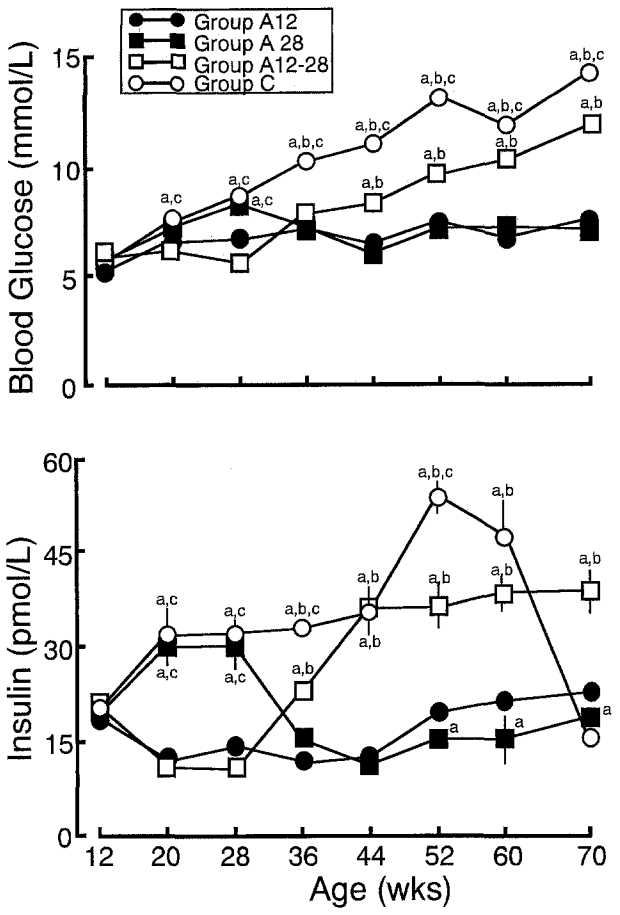

Fig 4. Serial changes in fasting serum glucose and insulin in untreated C and acarbose-treated OLETF rats. After an overnight fast, blood samples for measurement of serum insulin and glucose were collected from the left jugular vein under sodium pentobarbital anesthesia (50 mg/kg body weight IP). Results are the mean ± SEM of 6-10 rats. [a]Significantly different *v* group A12, [b]significantly different *v* group A28, [c]significantly different *v* group A12-28.

with age, reaching 8.66 ± 0.33 mmol/L at 28 weeks (Fig 4). After an IV glucose load, serum glucose increased, reaching a peak at 5 minutes, and then decreased to the baseline level at 60 minutes in rats of all ages in all four groups, except group C at age 70 weeks (Fig 5). In group C, the glycemic response to an IV glucose load, as well as fasting serum glucose, gradually increased with age, reaching peak values at 70 weeks (basal, 15.32 ± 0.24 mmol/L; peak, 21.20 ± 1.05 mmol/L). Acarbose almost completely prevented (group A12) or normalized (group A28) the fasting serum glucose level (Fig 4) and maintained the normal glycemic response to the IVGTT at levels equivalent to those observed at 12 weeks of age until age 70 weeks (Fig 5). However, in group A12-28, baseline serum glucose and the levels during IVGTT increased gradually after switching to standard rat chow, although each level was significantly lower than the corresponding value in group C at similar time points, even at 70 weeks of age.

Basal serum insulin in group C significantly increased from 20.59 ± 1.44 pmol/L at 12 weeks to 33.79 ± 2.87 pmol/L at 20 weeks ($P < .001$) and further increased to a peak value of 56.25 ± 2.73 pmol/L at 52 weeks, but it markedly decreased to 15.21 ± 0.93 pmol/L at 70 weeks (Fig 4). At 20 weeks of age, basal serum insulin in group C was significantly higher and the insulin secretory response to the IV glucose load showed significantly greater increases versus groups A12 and A12-28 (Figs 4 and 5). At 44 weeks of age, serum glucose in group C increased from 10.16 ± 0.42 mmol/L to 16.04 ± 0.41 mmol/L at 5 minutes after an IV glucose load, whereas the serum insulin level showed no significant increase from 32.86 ± 4.16 pmol/L at baseline to a peak of 41.62 ± 2.15 pmol/L at 5 minutes. At 70 weeks of age, the basal insulin concentration decreased and showed no significant increase even after the IVGTT (basal, 15.21 ± 0.93 pmol/L; peak at 30 minutes, 22.82 ± 4.16 pmol/L). Acarbose treatment prevented the increase in fasting serum insulin in group A12 and markedly decreased the fasting serum insulin in group A28. However, after 44 weeks of age, the fasting serum insulin concentration gradually increased and the serum insulin response to IVGTT was augmented, although there were no significant differences in serum glucose concentrations at all time points. In A12-28 rats, fasting serum insulin significantly increased after switching to standard rat chow and remained persistently high even at 70 weeks of age (39.75 ± 4.31 pmol/L; Fig 4). In these rats, an exaggerated insulin response was observed at 36 weeks of age, but thereafter, secretory responsiveness over the basal value was reduced (basal, 40.25 ± 4.81 pmol/L; peak, 55.89 ± 6.96 pmol/L at 70 weeks of age).

Pancreatic Weight and Protein, DNA, and Insulin Content

At 72 weeks of age, the pancreatic wet weight and pancreatic protein and insulin content in acarbose-treated groups A12, A28, and A12-28 were significantly higher than the values in untreated C rats (Table 1). Acarbose treatment doubled the insulin content, whether expressed as the total amount of insulin or the amount relative to the DNA content (Table 1).

Abdominal Fat

The addition of acarbose to the diet significantly decreased the white adipose tissue mass compared with the mass in untreated OLETF rats (Table 2). The reduction was greater in retroperitoneal and epididymal versus mesenteric white adipose tissue. Treatment with the acarbose diet for the initial 16 weeks only (group A12-28) was associated with a significant reduction in retroperitoneal and epididymal adipose depots, but not mesenteric adipose tissue, at 72 weeks of age.

Histopathologic Changes

Representative photomicrographs of randomly selected sections of the pancreas taken at 72 weeks of age for the four different treatment groups are shown in Fig 6 using the same magnification. The pancreas of the group C rat was extremely atrophic and the tissue was replaced by fatty and connective tissue. Both the number and size of islets were decreased significantly (Fig 6A). The islets of group A12 (Fig 6B) and group A28 (Fig 6C) were almost normal in size, and no

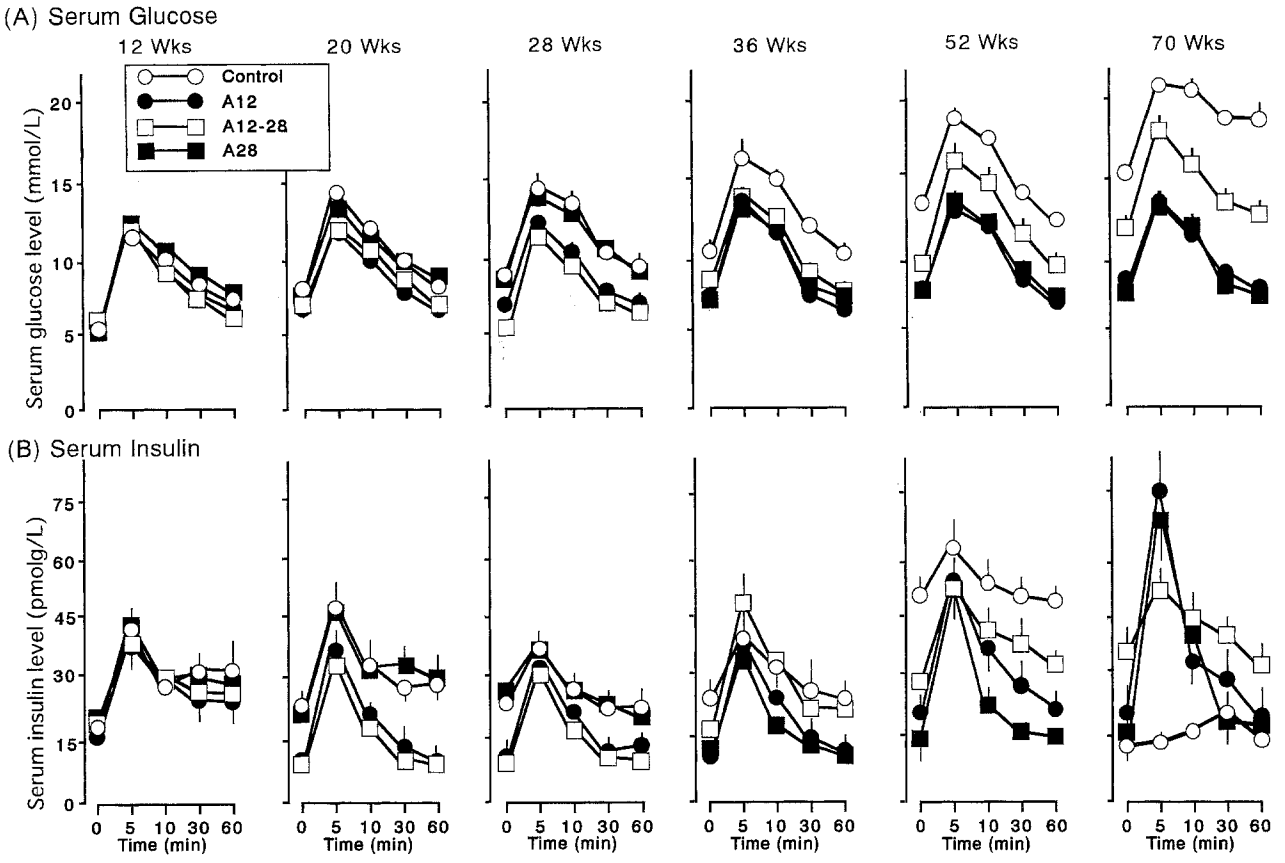

Fig 5. Serial changes in serum glucose (A) and insulin (B) responses to an IV glucose load. After an overnight fast, glucose in a bolus dose of 0.2 g/kg body weight was injected into the right jugular vein, and blood samples were collected from the left jugular vein at 0, 5, 10, 30, and 60 minutes for measurement of serum glucose and insulin. Results are the mean ± SEM of 6-10 rats. [a]Significantly different *v* group A12, [b]significantly different *v* group A28, [c]significantly different *v* group A12-28.

connective tissue proliferation was noted. Histological changes in the islets remained minimal. On the other hand, the islets of A12-28 rats (Fig 6D-1 and -2) were clearly enlarged. More than half of the islets in individual rats were 250 to 500 μm in diameter, and islets greater than 500 μm were observed in the pancreas of each rat in group A12-28. A variable degree of connective tissue proliferation was detected in these enlarged islets in which clusters of endocrine cells were widely separated from each other by transverse bands of connective tissue, resulting in a multinodular appearance (Fig 6D, 1).

Table 1. Effect of Acarbose Treatment on Body and Pancreatic Wet Weight and Pancreatic Protein, DNA, and Insulin Content in Fed OLETF Rats at 72 Weeks of Age

Parameter/Treatment	A12	A28	A12-28	C
Body weight (g)	593 ± 15	593 ± 25	685 ± 33*†	684 ± 41*†
Pancreatic wet weight				
mg per rat	1,396 ± 38.7	1,384 ± 36.2‡	1,158 ± 42.6*†	965 ± 54.7*†‡
mg/g body weight	2.35 ± 0.07	2.33 ± 0.06	1.69 ± 0.06*†	1.39 ± 0.08*†‡
Pancreatic contents				
Protein (mg/pancreas)	220.3 ± 29.6	227.5 ± 19.1	166.4 ± 11.2*†	142.9 ± 7.1*†
DNA (mg/pancreas)	6.12 ± 0.79	6.78 ± 0.26	5.45 ± 0.29*†	4.78 ± 0.21*†‡
Protein/DNA (mg/mg)	33.3 ± 1.9	33.1 ± 2.3	30.7 ± 1.8	29.9 ± 0.2
Insulin				
μg/pancreas	58.5 ± 3.7	53.2 ± 3.4	48.3 ± 3.7	21.3 ± 0.8*†‡
μg/mg DNA	9.17 ± 0.71	7.90 ± 0.53	9.32 ± 0.82	4.46 ± 0.36*†‡

NOTE. Values are the mean ± SEM of 6-10 rats. Treatment groups are the same as indicated in Fig 1.
*Significant difference *v* corresponding A12 group.
†Significant difference *v* corresponding A28 group.
‡Significant difference *v* corresponding A12-28 group.

Table 2. Effect of Acarbose Treatment on Adipose Depot in Fed OLETF Rats at 72 Weeks of Age

Depot (g)	A12	A28	A12-28	C
Mesenteric	13.6 ± 1.0	10.6 ± 1.1	20.0 ± 0.6*†	17.9 ± 1.4*†
Retroperitoneal and epididymal	32.1 ± 3.1	27.4 ± 3.8	51.2 ± 3.5*†	84.7 ± 7.5*†‡
Total dissected white adipose tissue	45.7 ± 3.9	41.5 ± 3.8	71.2 ± 3.2*†	102.5 ± 7.9*†‡

NOTE. Values are the mean ± SEM of 6-9 rats. Treatment groups are the same as indicated in Fig 1.

*Significant difference *v* corresponding A12 group.

†Significant difference *v* corresponding A28 group.

‡Significant difference *v* corresponding A12-28 group.

DISCUSSION

The results of the present study using a new rat model of human NIDDM are consistent with previous findings, and provide further support for the therapeutic potential of the potent intestinal α-glucosidase inhibitor, acarbose, in treating NIDDM and hyperlipidemia in humans.[11,12] Pharmacologic intervention with acarbose not only prevented but also improved the metabolic derangements such as hyperglycemia, hyperinsulinemia, hyperlipidemia, and obesity and the characteristic histopathological changes of the genetically diabetic OLETF rat up to 70 weeks of age. Our results also show that diet plays a major etiological role in the development of diabetes in OLETF rats, as previously reported with caloric restriction for a short period.[21]

In contrast to our previous studies in normal Wistar rats,[14,15] addition of acarbose to the diet resulted in a significant increase in food consumption and a concomitant decrease in body weight in the OLETF rat. The effects of acarbose on body weight in rats reported by other investigators have been inconsistent and differ according to the type of diet, the dose of acarbose, and the rat model selected.[3,5-8,14,15] Although specific measurements of carbohydrate absorption were not conducted, based on the present findings of the increased size and decreased solidity of the feces and the enlarged cecum and intestine in acarbose-treated rats compared with control rats, it is likely that significant energy loss due to carbohydrate maldigestion and malabsorption was responsible for the reduction in body weight. There is therefore a possibility that the reduced postprandial glycemic response increased the consumption of the acarbose-rich diet. In support of this view, Maggio and Vasselli[22] have demonstrated that acarbose decreases the satiety effect of cornstarch in lean and obese Zucker rats, leading to increased food intake. Indeed, acarbose has no effect on the food intake or rate of weight gain in rats fed a high-glucose diet.[8] In contrast to our observations, an anorexic effect of acarbose has been described in genetically obese Zucker[5] and diabetic SHR/N-*cp*[9] rats and lean streptozotocin-induced diabetic rats.[6] However, other investigators reported that the drug does not change food intake in diabetic *db/db* mice[8] and streptozotocin-induced diabetic rats.[23] Differences between our results and those of previous studies could be attributed to differences in the dose of acarbose. OLETF rats weighing approximately 500 g consumed about 34 g acarbose-containing diet, which is equivalent to 4.76 kg dry food per day in 70-kg human terms (10,681 kcal by carbohydrate; total, 17,117 kcal/d). Although the dose of acarbose given to OLETF rats in the present study is much higher than the dose used for humans, based on the volume and calories of daily intake, the dose of acarbose appears acceptable.

Since obesity is a known potent diabetogenic factor,[24] the diminished body weight gain with less fat deposition in the abdominal cavity may be related to an improvement of insulin action in these rats. A reduction of body weight, especially fat deposition in the abdominal cavity, may itself lead to enhanced insulin sensitivity, and this phenomenon would assist in main-

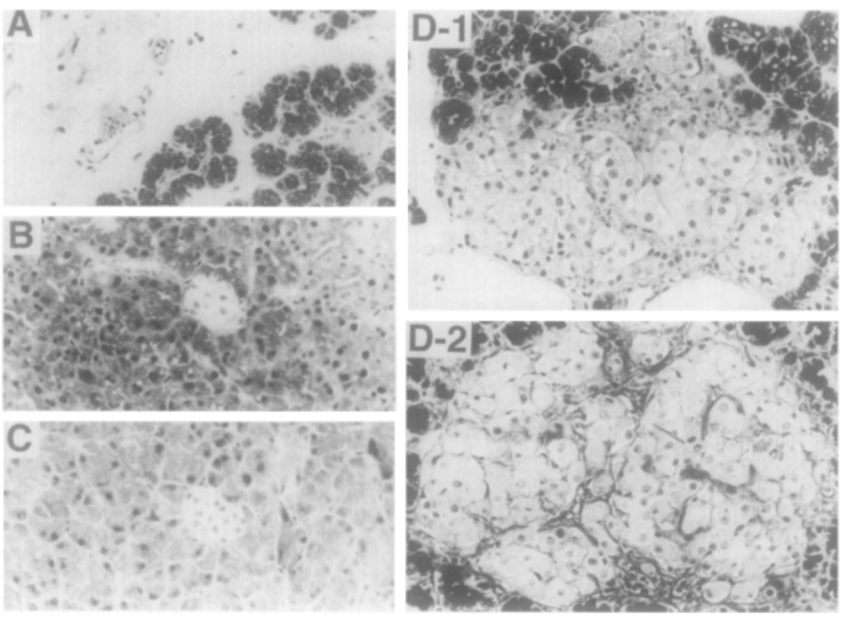

Fig 6. Representative photomicrographs of islets from groups C (A), A12 (B), A28 (C), and A12-28 (D) at 72 weeks of age. Note differences in islet size and degree of connective tissue proliferation. (A) Pancreas of a representative rat from group C shows extreme atrophy and replacement of normal tissue by fatty and connective tissue. Number and size of islets are decreased significantly. Islets of groups A12 (B) and A28 (C) are of normal size. Note the lack of connective tissue proliferation. (D) Islets of a representative rat from group A12-28. Note marked enlargement and presence of multinodularity due to intrainsular proliferation of fibrous connective tissue (D-2, Azan stain). H&E stain. Original magnification ×100.

taining normal glucose tolerance. Since serum insulin levels are known to be directly associated with the rate of production of very–low-density lipoprotein (VLDL)[25] and since lipoprotein lipase (LPL) activity in adipose tissue is also regulated by insulin,[26] a reduction in insulin and/or improvement of insulin action may be associated with a decline in the rate of VLDL production and an increase in LPL activity. Thus, the low levels of serum TG are elicited by a series of changes in insulin resistance that include a reduction of serum insulin levels and enhancement of insulin responsiveness in adipose tissue. It is therefore conceivable that the favorable effects of acarbose on glucose and lipid metabolism may be attributed, at least in part, to improvements in peripheral insulin sensitivity.[27] Nestel[28] found that the hepatic VLDL production rate was more than 25% lower during acarbose treatment compared with placebo. The correction of hyperinsulinemia probably contributed to the reduction of hepatic TG synthesis. Since there is increasing evidence that hypertriglyceridemia is an independent coronary risk factor in NIDDM,[29] the reduction of postprandial hypertriglyceridemia induced by acarbose may be an added benefit of acarbose therapy. Although glycemic control and body weight change are closely linked, weight loss is not normally observed in the human therapeutic situation with acarbose. The relative lack of weight gain in OLETF rats fed acarbose may be due to malabsorption. Although it is difficult to extrapolate results from an animal model to humans, there is a possibility that acarbose at a much higher dosage than the current therapeutic dosage reduces body weight.

Our results also show that acarbose substantially decreased basal glucose levels and insulin secretion. In A12 and A28 rats, basal insulin and serum glucose, both before and after the IV glucose load, remained at the initial levels observed at 12 weeks of age, even when animals reached age 70 weeks, whereas augmentation of the insulin response to IVGTT was noted after 44 weeks. These results indicate that the initial step in the development of NIDDM is a defect in insulin action. The resulting resistance to insulin-mediated glucose disposal is compensated for by overproduction of insulin. If hyperinsulinemia in the presence of normoglycemia is considered an indicator of insulin resistance, it begins at 44 weeks of age in both A12 and A28 rats despite continuous acarbose treatment. Since prolonged administration of acarbose increases sucrase activity in the small intestine in a dose-dependent manner and induces intestinal adaptation, resulting in a reduction of the effect of the drug,[30,31] it is possible that the onset of insulin resistance in OLETF rats may be further delayed or completely prevented by increasing the dosage of acarbose. In support of this view, fasting serum insulin and adipose depots were lower in group A28 versus group A12. Our present observation indicates that the onset of diabetes in the OLETF rat is clearly delayed by acarbose, but it cannot be excluded that diabetes may occur soon after 70 weeks of age.

In rats treated with acarbose during the initial 16 weeks only (from 12 to 28 weeks of age, group A12-28), augmentation of the insulin secretory response to the IVGTT in the presence of a normal glycemic response was first observed at 36 weeks of age, 8 weeks after switching to the standard rat chow, and increased with age. Thus, insulin resistance in group A12-28 appeared to begin at age 36 weeks. Abnormalities in serum glucose values in basal measurements and in response to the IVGTT became apparent at 44 weeks of age, whereas serum concentrations of TG and cholesterol increased over the initial values at 60 weeks of age. Moreover, the visceral adipose depot at age 72 weeks was significantly lower in this group versus the untreated control rats. Although hyperlipidemia is thought to induce insulin resistance in peripheral tissues[32] and contributes to the development of β-cell failure in NIDDM,[13] the results of group A12-28 clearly suggest that insensitivity to insulin action appears before the onset of abnormal lipid metabolism and precedes hyperglycemia. It is therefore conceivable that hyperinsulinemia causes hyperlipidemia and visceral adipocyte deposition. These results suggest that treatment with acarbose before the early stage of NIDDM, even for a short period, produces a marked delay in the development of insulin insensitivity and frank diabetes. Moreover, there is a possibility that intermittent administration of acarbose to humans with a strong genetic predisposition for NIDDM, before the onset of diabetes, delays or prevents the development of insulin resistance and diabetes.

Pancreatic wet weight and insulin content were significantly higher in acarbose-treated rats versus untreated OLETF rats. Moreover, none of the morphologic changes observed in untreated rats were found in A12 and A28 rats at 72 weeks of age. The islets were almost normal in size and histologic changes were minimal. The low rate of weight gain and reduced visceral adipose depots, as well as the reduced increase in postprandial blood glucose, in these rats would undoubtedly help to maintain β-cell function, preventing enlargement or atrophy of these islets by protecting the β cells from overactivity and exhaustion. The combination of reduced hyperglycemia, protective β-cell effect, and reduced hyperinsulinemia may also exert further beneficial effects on peripheral insulin resistance and possibly improve long-term insulin secretion. On the other hand, the enlarged and multilobulated islets in A12-28 rats could be the result of an overactivity of the β cells to compensate for the insensitivity to insulin action.

Although it is difficult to transfer the present observations made in a particular animal model to the human situation, our long-term study in the genetically obese and diabetic OLETF rat—a model of spontaneous NIDDM—shows that pharmacological intervention with acarbose prevents the development of diabetes when given early in life before the onset of diabetes, and also reverses hyperglycemia, hyperinsulinemia, dyslipidemia, and obesity even when given after the onset of diabetes. The results of the present study further suggest that intermittent treatment with acarbose before the onset of diabetes could prevent or delay the onset in humans with a genetic predisposition for diabetes. Acarbose has demonstrated a similar degree of glycemic control when compared with sulfonylureas and biguanides in comparative studies.[5,10-12] In contrast to the sulfonylureas, acarbose does not induce hypoglycemia but does decrease TG and body weight. Although β-cell dysfunction and insensitivity to insulin are typically present in NIDDM and genetic predisposition is clearly important, our results suggest that environmental factors, especially diet, play a major etiological role in the development of NIDDM in OLETF rats, as in human NIDDM.

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