Changes in Islet Capillary Angioarchitecture Coincide With Impaired B-Cell Function But Not With Insulin Resistance in Male Otsuka-Long-Evans-Tokushima Fatty Rats: Dimorphism of the Diabetic Phenotype at an Advanced Age

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The Otsuka-Long-Evans-Tokushima fatty (OLETF) rat is a genetic model of spontaneous development of non–insulin-dependent diabetes mellitus (NIDDM) established as an inbred strain after 20 generations of selective breeding. Although they are thought to be genetically homogeneous, they show a dimorphism regarding the diabetic phenotype at an advanced age, with one remaining obese and modestly diabetic while the other becomes lean and overtly diabetic. To clarify the causes for this divergence, we examined the physical, biochemical, and histopathological features in rats at 50 weeks of age, including an analysis of islet angioarchitecture. Sixty-one of 85 male OLETF rats lost weight, while the remainder remained obese. Mean nonfasting plasma glucose in the lean group was 21.8 ± 4.6 mmol/L, significantly higher versus the obese group (10.5 ± 1.4 mmol/L) and the age-matched control Long-Evans-Tokushima-Otsuka (LETO) group (7.1 ± 0.6 mmol/L). Morphological studies of the pancreas from the lean group showed enlarged multilobulated fibrotic islets with a paucity of B cells, whereas islets from the obese group appeared slightly enlarged and showed a relative abundance of B cells. The fine capillaries that form a network in the islets were extremely sparse in the lean group, resulting in a defective glomerular-like configuration, whereas those from the obese group were dense, forming a nearly typical glomerular-like configuration. Increased plasma insulin responses to oral and intravenous (IV) glucose and IV glucagon loads were nearly absent in the lean group, while they were evident in the obese group, although to a lesser extent compared with the LETO group. Mean insulin secretory output from the perfused pancreas in response to 11.1 mmol/L glucose in the lean group (3.5 ± 2.2 pmol/20 min) was significantly lower versus the obese group (8.8 ± 6.5 pmol/20 min) and LETO group (22.0 ± 10.8 pmol/20 min). Similarly, pancreatic insulin content was significantly lower in the lean group (9.3 ± 6.1 μg) versus the others (26.1 ± 17.3 μg for obese and 41.1 ± 24.8 μg for LETO). In vivo insulin-stimulated glucose uptake measured by a euglycemic clamp technique was significantly higher in the lean group compared with the obese group. These results demonstrate that the dimorphism regarding the diabetic phenotype in male OLETF rats at 50 weeks of age was due to differences in the number of islet B cells, which could be the result of a variation in the capacity for B-cell proliferation among male OLETF rats.

THE OLETF RAT (Otsuka-Long-Evans-Tokushima fatty) represents a genetic model for the spontaneous development of non–insulin-dependent diabetes mellitus (NIDDM). It was established as an inbred strain of rats after 20 generations of selective breeding.[1] Although they are thought to be homogeneous because of their genetic purity, not all become diabetic, and the cumulative incidence of diabetes in male OLETF rats greater than 23 weeks of age is 80% to 90%, even when bred under identical environments.[1] We have noted that some male OLETF rats remain obese to a relative old age, approximately 50 weeks, while most others become overtly diabetic and are characterized by weight loss, hyperglycemia, and polyuria. However, all show similar physical characteristics at 30 weeks of age. A thorough analysis of these phenotypic differences and their causes could provide insight into the pathophysiological factors responsible for the development of diabetes mellitus in this rat model, and could also aid in an assessment of the causes of heterogeneity with respect to human NIDDM.[2-5]

To clarify the causes and mechanisms for this observed dimorphism in the diabetic phenotype in older male OLETF rats, we examined physical, biochemical, and histological features of male OLETF rats at 50 weeks of age, including an analysis of islet angioarchitecture, which has not been performed to date.

MATERIALS AND METHODS

Animals

A spontaneously diabetic rat with polyuria, polyphagia, and slight obesity was discovered in an outbred colony of Long-Evans rats that were purchased from Charles River, St Constant, Canada, in 1983, and was subsequently maintained at the Tokushima Research Institute of Otsuka Pharmaceutical (Tokushima, Japan) After 20 generations of selective breeding, the diabetic strain, OLETF, was established in 1990.[1] As reported by Kawano et al,[1] the cumulative incidence of diabetes in male OLETF rats aged 24 weeks is nearly 90%, but the diabetes is mild with only a slight elevation of fasting plasma glucose at this age. A nondiabetic strain, Long-Evans-Tokushima-Otsuka (LETO), was used as a nondiabetic control. Eighty-five male OLETF and 24 male LETO rats were obtained from Tokushima Research Institute (Otsuka Pharmaceutical) and maintained in our animal facilities (Institute for Animal Experimentation, University of Tokushima) under specific pathogen-free conditions at controlled temperature (21° ± 2°C), humidity (55% ± 5%), and lighting (8:00 AM to 8:00 PM).

Experimental Design

After measuring body weight and fasting plasma glucose levels, an oral glucose tolerance test (OGTT) was performed after an overnight fast for 85 OLETF and 25 LETO rats at 30 weeks of age. All OLETF rats were judged to have diabetes or impaired glucose tolerance (IGT) according to our criteria. Rats were diagnosed as diabetic, when the peak plasma glucose was greater than 16.7 mmol/L and the 120-minute plasma glucose was greater than 11.1 mmol/L, and as having IGT when only one of these parameters exceeded the above-mentioned value.

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They were maintained under the same conditions until age 50 weeks to observe the natural course of the diabetes mellitus. At 50 weeks of age, they were divided into two groups depending on body weight, a lean group of 61 OLETF rats with body weight less than 630 g and an obese group of 24 OLETF rats with body weight greater than 670 g. Three groups of 24 rats representing the lean OLETF, obese OLETF, and control LETO groups, respectively, were subjected to the following experiments. Twenty-four rats were divided into five groups: the first group of six rats were subjected to an IV glucose + IV glucagon test (IVGGT) and pancreatic perfusion; the second group of six rats were designated for an OGTT, a hyperinsulinemic-euglycemic clamp test, and an analysis of fat deposition; the third group of six rats were used for measurements of pancreatic insulin content and histological examination; the fourth group of two rats were used in an analysis of islet angioarchitecture; and the fifth group of four rats were observed for the natural course of the disease until 80 weeks of age.

OGTT

After an overnight fast, D-glucose (2 g/kg body weight [BW]) was administered orally, and whole blood was taken from the tail vein without anesthesia at 0, 30, 60, and 120 minutes for measurement of plasma glucose levels in 85 OLETF and 24 LETO rats at 30 weeks of age. At age 52 weeks, an OGTT was performed in six rats from the second group under conscious conditions after an overnight fast. A few days before the OGTT, catheters were inserted into the femoral vein. Venous blood (0.25 mL) was withdrawn from the catheter using a heparinized syringe and collected into chilled tubes at 0, 30, 60, and 120 minutes for determination of plasma glucose and insulin levels.

IVGGT

At 51 weeks of age after an overnight fast, six rats from the first group were anesthetized by intraperitoneal injection of sodium pentobarbital and administered IV D-glucose (0.5 g/kg BW) at 0 minutes and glucagon (0.2 mg/whole body) at 75 minutes. Venous blood (0.25 mL) was withdrawn from the cervical vein using a heparinized syringe and collected into chilled tubes at 0, 3, and 6 minutes and at 75, 78, and 81 minutes for determination of plasma glucose and insulin levels.

Measurement of In Vivo Glucose Disposal by a Hyperinsulinemic-Euglycemic Clamp Test

At 54 weeks of age, insulin-mediated whole-body glucose uptake was measured in six anesthetized rats from the second group using a hyperinsulinemic-euglycemic clamp technique[6] 2 weeks after the OGTT. After an overnight fast, the rats were anesthetized by intraperitoneal injection of pentobarbital, and catheters were inserted into both femoral veins. The rats received an infusion of insulin (Novo Nordisk, Bagsvaerd, Denmark) at a rate of 60 pmol/kg/min for 1 hour. The infusion rate was adjusted to clamp plasma glucose at approximately 6.1 mmol/L. Blood samples for determination of glucose were obtained from the cervical veins by venous puncture at 2- to 5-minute intervals throughout the experiment. Data on total-body glucose uptake represent the mean value for the glucose infusion rate (GIR) during the last 20 minutes.

Measurement of Hepatic Glucose Output

Hepatic glucose output (HGO) was measured in the second group of rats during a hyperinsulinemic-euglycemic clamp test. At time 0, 2.5 μCi (10 μCi/mL) D-[U-^{14}C]glucose (Amersham International, Buckinghamshire, England) was infused as a bolus followed by continuous infusion at a rate of 10 μCi/h. Blood samples for determination of D-[U-^{14}C]glucose specific activity were obtained at 55 and 60 minutes. Blood was deproteinized in $Ba(OH)_2/ZnSO_4$ as described by Somogyi[7] and centrifuged (2 minutes at 16,000 × *g*). D-[U-^{14}C]glucose in the supernatant was determined by liquid scintillation counting (LSC-700; Aloka, Tokyo, Japan). The glucose disappearance rate (Gd) and HGO were determined by Steel's method.[8] Gd and HGO were calculated as follows:

$$Gd = \frac{\text{blood glucose (mg/dL)} \times \text{flow rate (μL/min)} \times (\text{total count/plasma count})}{\text{body weight (kg)}} \times 0.2 \times 10^{-4}.$$

The mean HGO at 55 and 60 minutes was calculated.

Determination of Insulin-Stimulated Glucose Utilization Index

The glucose utilization index for skeletal muscle tissue was measured in the same group of rats during a hyperinsulinemic-euglycemic clamp test using the 2-deoxy-D-[1-^{3}H]glucose technique as described by Ferre et al[9] and James et al.[10] In brief, 2-deoxy-D-[1-^{3}H]glucose (25 μCi, 1 mCi/mL; Amersham) was injected in 250 μL 0.9% NaCl as a bolus through the femoral vein. A blood sample (50 μL) for determination of the plasma tracer concentration was obtained 60 minutes after the bolus injection. At the end of the hyperinsulinemic-euglycemic clamp test, skeletal muscle tissues were rapidly removed and frozen in liquid N_2. Blood was deproteinized in $Ba(OH)_2/ZnSO_4$ as already described, and the supernatant was used for determination of 2-deoxy-D-[1-^{3}H]glucose by liquid scintillation counting. Tissue samples were weighed and placed into 1 mol/L NaOH (2.5 mL/g tissue) and heated at 60°C for 45 minutes to totally digest the tissues, after which 1 mol/L HCl (2.5 mL/g tissue) was added. One milliliter of 6% $HClO_4$ was added to 200 μL of the neutralized solution, and 1 mL $Ba(OH)_2/ZnSO_4$ was added to another 200 μL of the neutralized solution. After centrifugation, the supernatants (800 μL) of these solutions were used for determination of radioactivity, after the addition of 10 mL ATOMLIGHT scintillation solution (Biotechnology Systems, Boston, MA), by liquid scintillation counting. Because 2-deoxyglucose and 2-deoxyglucose 6-phosphate are both soluble in 6% $HClO_4$ and only 2-deoxyglucose is soluble in the Somogyi reagent [$Ba(OH)_2/ZnSO_4$], the content of 2-deoxy-D-[1-^{3}H]glucose 6-phosphate in an individual skeletal muscle sample was obtained by subtracting the radioactivity (dpm) in the $Ba(OH)_2/ZnSO_4$ supernatant from that in the $HClO_4$ supernatant. Tissue glucose uptake (defined as the glucose metabolic index, Rg′) was calculated using the following equation described by Kragen et al[11]:

$$Rg'\ (\mu mol/100\ g/min) = \frac{Cp \times Cm^*(60)}{\int Cp^*(t)\,dt}.$$

Cp is the plasma glucose concentration at steady state over a 60-minute period of observation (mmol/L); Cm* is tissue accumulation of 2-deoxy-D-[1-^{3}H]glucose 6-phosphate per unit mass at 60 minutes (dpm/mg wet weight); Cp* is the plasma [^{3}H]2-deoxy glucose concentration (dpm/mL); and *t* equals 0 when the tracer is administered as a bolus.

Pancreatic Perfusion

Pancreata prepared from six rats from the first group at 53 weeks of age were perfused through the celiac artery via a catheter inserted into the aorta, according to the method of Grodsky et al.[12] The basal medium, which was used for the perfusion, was Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with 0.2% bovine serum albumin (Sigma Chemical, St Louis, MO) and 4.5% dextran (average MW, 71 k; Sigma). The pancreas was perfused at 37°C at a flow rate of 2.5 mL/min and equilibrated continuously with 95% O_2/5% CO_2. Perfusion with the basal medium supplemented with 3.3 mmol/L D-glucose was performed during the first 25 minutes, followed by perfusion with medium supplemented with 11.1 mmol/L D-glucose for 20 minutes, then perfusion with the basal medium supplemented with 3.3 mmol/L

D-glucose for 10 minutes, and after this procedure, perfusion with medium supplemented with 11.1 mmol/L D-glucose and glucagon-like peptide-1(7-36) amide (tGLP-1) at a rate of 1 nmol/L for 20 minutes. The effluent was collected at 2-minute intervals in chilled tubes containing aprotinin (500 KU) and EDTA disodium salt (5 mg).

Histology

At 54 weeks of age, six rats from the third group were killed after anesthesia, and the pancreas was excised and cleared of extraneous lymph nodes and fat. The pancreas of each rat was divided into two equal portions after weighing. One portion was then placed in 10% formalin for histological study, and the other was frozen in liquid N_2 for measurement of insulin content. The fixed pancreas was embedded in paraffin, and sections (3 to 5 μm) were then cut. The sections were first deparaffinized and stained with hematoxylin eosin (HE) or immunostained using antiinsulin antibody as the first antibody (Dokopatls, Grosyreep, Denmark) with an ABC kit (Amersham).

Insulin Content

All pancreatic samples were stored at −80°C and individually homogenized using a polytron homogenizer (Kinematica, Luzern, Switzerland) with 6 mL cold acid-ethanol (750 mL absolute ethanol, 250 mL water, and 150 mL concentrated hydrochloric acid) per gram of tissue, kept at 4°C overnight, and centrifuged at 600 × *g* for 30 minutes. The supernatant was then stored at −80°C until assayed for immunoreactive insulin (IRI).

Analysis of Islet Angioarchitecture Using Scanning Electron Microscopy

At 54 weeks of age, two rats from the fourth group were anesthetized using pentobarbital and perfused with 0.9% NaCl solution containing heparin (10 U/mL) via a catheter inserted into the left cardiac ventricle for 20 minutes, accompanied by infusion of 10 mL Mercox (Dainippon Ink, Osaka, Japan) under a flow pressure of less than 100 cm H_2O. After resin infusion and laparotomy, the pancreas was kept for 4 hours at room temperature and then removed and digested in a 20% KOH solution for 24 hours at room temperature until it was free of tissues. Twenty-four hours later, the samples were washed twice with distilled water and dried completely. The pancreatic casts were kept in a desiccator until used for SEM analysis. The islet casts were mounted on aluminum specimen stubs with double-sided cellophane tape and coated with a thin layer of platinum using a Poralon 5150 sputter-coater (Poralon, Hertfordshire, England). The coated specimens were observed and photographed in a Hitachi S-800 field emission scanning electron microscope (Hitachi, Tokyo, Japan) at 400× magnification for at least 10 islets per specimen.

Assays

Plasma glucose levels were determined by the glucose oxidase method using Antsense II (Bayer-Sankyo, Tokyo, Japan). Plasma immunoreactive insulin (IRI) levels were measured with a commercial radioimmunoassay kit (Eiken Chemical, Tokyo, Japan) with rat insulin as a standard (Novo Nordisk).

Calculations and Statistical Analysis

The results are expressed as the mean ± SD unless otherwise indicated, together with the number of individual observations (n). The statistical significance of differences was evaluated by ANOVA, followed by the multiple *t* test for individual comparison of mean values.

RESULTS

Body Weight and Plasma Glucose Response to OGTT in OLETF Rats at 30 Weeks of Age

For OLETF rats at age 30 weeks, the body weight was 680 to 730 g, and all were either diabetic (77 of 85) or had IGT (eight of 85) based on our criteria for diabetes using the OGTT.

Body Weight and Nonfasting Plasma Glucose Levels in OLETF Rats at 50 Weeks of Age

At 50 weeks of age, male OLETF rats were divided into two groups according to body weight: one group with body weight less than 630 g (n = 61, lean group) and the other greater than 670 g (n = 24, obese group). Nonfasting plasma glucose levels for all rats in the former group were higher than 14.0 mmol/L, but those in the latter group were lower than 11.7 mmol/L. Having discarded 37 lean OLETF rats, we used 24 each of the obese, lean OLETF, and LETO rats for the following experiments.

Physical and Biochemical Characteristics of Obese, Lean OLETF, and LETO Rats at 54 Weeks of Age

Fat deposition. The weight of abdominal fat and subcutaneous fat was significantly higher in the obese group versus the lean group, which was nearly equal to the LETO group (Table 1).

Plasma lipid, glucose, and insulin concentrations. Hyperlipidemia was evident in the OLETF rats. Nonfasting plasma glucose levels were twice as high in the lean group versus the obese groups. Fasting plasma IRI levels were significantly lower in the lean group compared with the obese group and the LETO group (Table 1).

Pancreatic B-Cell Function

Plasma insulin response to OGTT. Plasma insulin responses during the OGTT were dramatically decreased in the lean group, while the obese group showed elevated basal plasma IRI levels and a sluggish increase in response to the oral glucose load (Fig 1A).

Table 1. Physical and Biochemical Characteristics of Obese and Lean OLETF and LETO Rats at 54 Weeks of Age

Characteristic	OLETF Obese (n = 12)	OLETF Lean (n = 12)	LETO (n = 12)
Body weight (g)	710.1 ± 27.2†§	584.9 ± 39.1	580.2 ± 23.3
Abdominal fat (g)	102.9 ± 12.1†§	39.2 ± 19.4	47.8 ± 4.8
Subcutaneous fat (g)	112.0 ± 21.3†§	53.5 ± 30.2	58.3 ± 12.6
Triglyceride (mmol/L)	3.76 ± 0.73†§	2.09 ± 0.46‡	0.35 ± 0.27
Total cholesterol (mmol/L)	5.77 ± 0.58*§	6.49 ± 0.63§	2 59 ± 0.18
FPG (mmol/L)	6.6 ± 0.7§	6.3 ± 0.5§	4.6 ± 0.6
NFPG (mmol/L)	10.5 ± 1.4†§	21.8 ± 4.6§	7.1 ± 0 6
F-IRI (pmol/L)	350.8 ± 131.5†	102.9 ± 59.0§	264.8 ± 77.6

Abbreviations: FPG, fasting plasma glucose; NFPG, nonfasting plasma glucose; F-IRI, fasting plasma IRI.

*$P < .01$, †$P < .001$ *v* lean OLETF.

‡$P < .01$, §$P < .001$ *v* LETO.

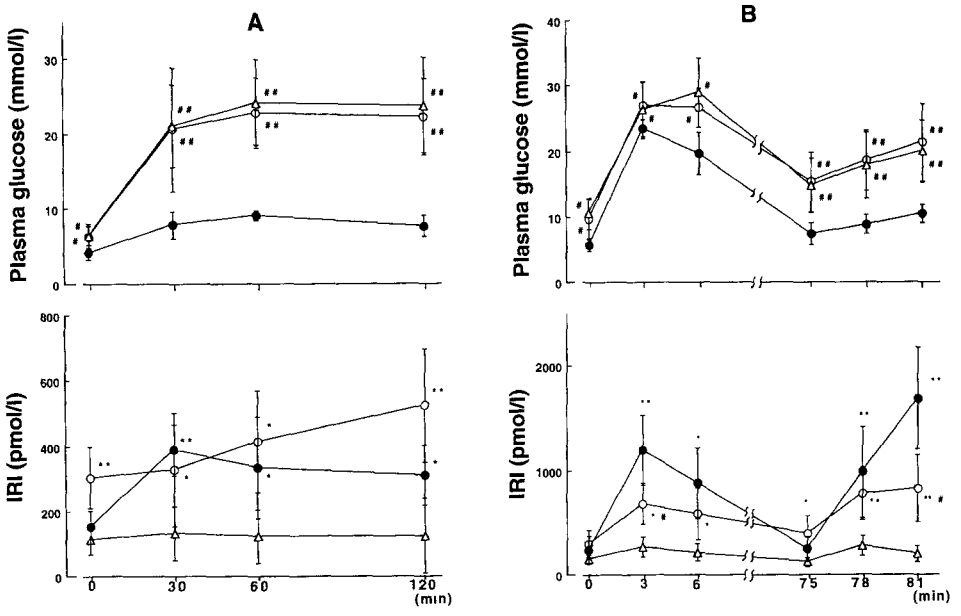

Fig 1. Plasma glucose and IRI responses to an OGTT (A) and an IVGGT (B) in 6 each of the obese (○), lean (△), and LETO (●) rats at >50 weeks of age. Points and bars represent the mean ± SD. *$P < .05$, **$P < .01$ *v* lean OLETF; #$P < .05$, ##$P < .01$ *v* LETO.

Plasma insulin response to IVGGT. Plasma insulin responses to both glucose and glucagon were dramatically decreased in the lean group, but plasma insulin levels after administration of both stimuli were much higher in the obese group versus the lean group, although not as high as in the LETO group (Fig 1B).

Insulin release from perfused pancreas. Mean total insulin secretory output from the pancreas in response to glucose and to tGLP-1 stimulation of the obese group was twice and six times the output of the lean group, respectively, although the insulin response to glucose stimulation of the obese group reached a level as low as 40% of that of the LETO group (Table 2).

Measurement of In Vivo Glucose Disposal

The GIR, which represents total-body glucose uptake, was reduced 74% in the obese group and 32% in the lean group compared with the LETO group. In the obese group, HGO during a hyperinsulinemic-euglycemic clamp test was not decreased but instead, increased compared with the LETO group. HGO for the lean group was higher than for the LETO group. However, this difference was not statistically significant. 2-Deoxyglucose uptake in muscle during a hyperinsulinemic-euglycemic clamp test in the obese group was approximately 25% of that in the LETO group, and the difference was statistically significant. 2-Deoxyglucose uptake in muscle in the lean group was lower than in the LETO group, but the difference was not statistically significant (Table 3).

Table 2. Mean Insulin Secretory Output (pmol/20 min) From the Perfused Pancreas in Response to 11.1 mmol/L Glucose and to 1 nmol/L tGLP-1

	OLETF		
Stimulation	Obese (n = 6)	Lean (n = 6)	LETO (n = 6)
Glucose (11.1 mmol/L)	8.8 ± 6.5*†	3.5 ± 2.2‡	22.0 ± 10.8
tGLP-1 (1 nmol/L)	92.7 ± 73.3*	16.2 ± 8.4†	36.8 ± 23.4

*$P < .05$ *v* lean OLETF.
†$P < .05$, ‡$P < .01$ *v* LETO.

Insulin Content in Whole Pancreas

The insulin content in the whole pancreas of the lean group was significantly lower versus the obese group ($P < .05$) and the LETO group ($P < .01$). The pancreatic insulin content of the obese group was lower than that of the LETO group, but the difference was not statistically significant (Table 3).

Morphometrics

HE and immunostaining. The light-microscopic appearance for random sections of the pancreas of obese and lean OLETF and LETO rats is shown in Fig 2. Islets from the obese group are slightly enlarged and fibrotic, but the fundamental islet structures are preserved, whereas the islets from the lean group show notable alterations in architecture due to the extensive proliferation of connective tissue in which clusters of endocrine cells are widely separated from one another, resulting in a multinodular appearance. The middle panel of Fig 2 shows sections of islets from obese and lean OLETF and LETO rats that have been immunochemically stained for insulin. The B

Table 3. Parameters for Insulin Sensitivity and Pancreatic Insulin Content in Obese and Lean OLETF and LETO Rats at 54 Weeks of Age

	OLETF		
Parameter	Obese (n = 6)	Lean (n = 6)	LETO (n = 6)
GIR (mmol/min)	16.3 ± 14.1*‖	42.4 ± 16.3‡	62.2 ± 10.6
HGO (mmol/min)	35.9 ± 19.1‡	22.3 ± 14.9	16.4 ± 8.4
Glucose uptake in muscle (μmol/100 g/min)	5.1 ± 3.9§	10.8 ± 8.4	19.4 ± 7.1
Insulin content per whole pancreas (μg)	26.1 ± 17.3†	9 3 ± 6.1§	41.1 ± 24.8

*$P < .05$, †$P < .01$ *v* lean OLETF.
‡$P < .05$, §$P < .01$, ‖$P < .001$ *v* LETO.

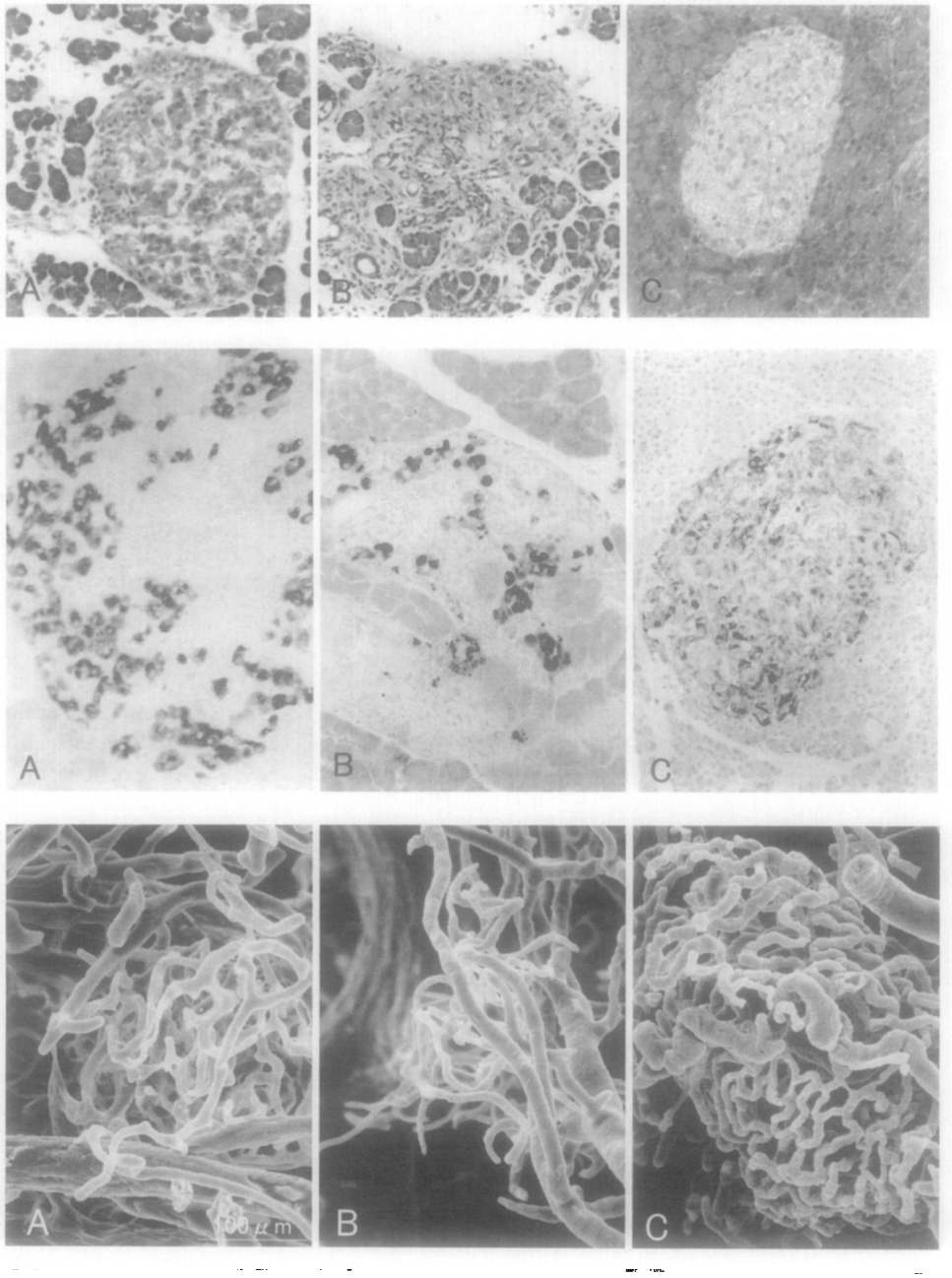

Fig 2. Histological analysis (original magnification ×100) of the islets (top), immunohistochemical analysis (original magnification ×200) of the islets for insulin (middle), and scanning electron micrograph (original magnification ×400) of islet angioarchitecture (bottom) in obese (A), lean OLETF (B), and LETO (C) rats at 54 weeks of age. Bar in lower panel indicates 100 μm length.

cells are dispersed but relatively abundant in islets from the obese group but sparse in islets from the lean group.

Islet angioarchitecture. Analysis for islet corrosion casts of the obese group revealed an angioarchitecture similar to that of the LETO group, consisting of dense capillaries compacted into spherical- to elliptical-shaped conglomerates and forming a typical glomerular-like configuration. On the other hand, the fine capillaries that form a network are sparse, resulting in a defective glomerular-like configuration in islet angioarchitecture of the lean group (Fig 2).

Clinical Features of OLETF Rats at 80 Weeks of Age

The body weight of the obese group gradually decreased after 60 weeks of age and became indistinguishable from that of the lean group at 80 weeks of age. The obese group showed hyperglycemia and hypoinsulinemia to an extent similar to the lean group at 80 weeks of age (Table 4).

DISCUSSION

It has been reported that male OLETF rats eventually become hypoinsulinemic and develop IDDM-like diabetes,[1] and require insulin therapy for survival after the chronic course of diabetes. However, the present study clearly shows that the natural course of the disease after adulthood, at approximately 30 weeks of age, was heterogeneous among individuals, with one group following a typical course and the other remaining obese with relative hyperinsulinemia and insulin resistance until an advanced age.

Plasma insulin responses to both oral and IV glucose loads were nearly absent in lean OLETF rats at 50 weeks of age, while

Table 4. Physical and Biochemical Characteristics of the Rats at 80 Weeks of Age

Characteristic	OLETF Obese (n = 4)	OLETF Lean (n = 4)	LETO (n = 4)
Body weight (g)	561.2 ± 49.3	525.1 ± 22.3*	602.1 ± 41.1
FPG (mmol/L)	6.79 ± 1.12*	7.14 ± 0.94*	4.9 ± 0.8
NFPG (mmol/L)	21.1 ± 5.6†	19.9 ± 5.0†	7.8 ± 1.62
F-IRI (pmol/L)	97.8 ± 72.5*	82.6 ± 69.1*	281.6 ± 94.4

NOTE. Abbreviations are as in Table 1.
*$P < .05$, †$P < .01$ *v* LETO.

they were moderately reduced but preserved in the age-matched obese OLETF rats. The same was true for the data on glucose-stimulated insulin release from the perfused pancreas. However, tGLP-1–stimulated insulin release from the perfused pancreas of obese OLETF rats at age 50 weeks was exaggerated compared with the age-matched control LETO rats. These characteristics of insulin secretion observed in obese OLETF rats resemble those of patients with mild NIDDM, in whom attenuated or absent early-phase and delayed and exaggerated late-phase insulin responses to an oral glucose load were observed,[13,14] together with an undiminished response to nonglucose secretagogues such as tGLP-1.[15,16]

The differences in the characteristics of the insulin response to various stimuli between obese and lean OLETF groups at 50 weeks of age can be reasonably explained by differences in the insulin content of the pancreas and the histopathological appearance of the islets of both groups. These changes in pancreatic islets may be a result of persistent hyperglycemia, so-called glucotoxicity,[17,18] and/or insulin resistance,[19] which forces pancreatic B cells to secrete more insulin to overcome the loss of normal insulin sensitivity, leading to their damage and death[20,21] and infiltration of connective tissue. However, our previous experiment[22] demonstrated that OLETF rats have a poor capacity for proliferation of pancreatic B cells, and this change may be the critical pathogenetic event prior to the onset of overt diabetes.[23] Based on the finding that the insufficient compensatory capacity for B-cell growth was unaffected by the presence or absence of hyperglycemic stimulation when phlorizin was administered,[22] a poor capacity for proliferation of B cells may be genetically determined in this animal model. The insufficient proliferation of B cells leading to a decrease in B-cell mass and a reduction in insulin content in the pancreas may be a cause of diabetes mellitus in this animal model. In other animal models, the restriction of the B-cell mass is also thought to be a primary and crucial event in the sequence leading to overt diabetes.[24,25] Based on the data herein, it is possible that the capacity for proliferation of pancreatic B cells varies among male OLETF rats, although this has not yet been absolutely proven, which leads one group to hypoinsulinemic diabetes characterized by loss of body weight and the other to relatively hyperinsulinemic hyperglycemia with obesity.

Insulin resistance is also an important risk factor for NIDDM.[26,27] Insulin sensitivity was not evaluated for rats at 30 weeks, at which age all rats were similarly obese and the body weight did not differ significantly, suggesting that no significant difference in insulin sensitivity exists among them, since such sensitivity has been reported to be inversely correlated with body weight.[28,29] At 50 weeks of age, the obese group was more resistant to the action of insulin than the lean group (Table 3). If insulin resistance is more profoundly related to the late development of diabetes than other factors, diabetes mellitus would be more severe in obese OLETF rats versus the lean ones. However, this was not the case, and suggests that the difference in insulin sensitivity between these groups did not exclusively affect the difference in the subsequent course of the disease.

To our knowledge, no morphological microvascular abnormalities of the islet in the case of diabetes have been reported, although a few studies on hemodynamic alterations in pancreatic islet capillaries of animal models with diabetes mellitus have been reported.[30-32] Our results show that the glomerular-like networks of the islet capillaries from lean OLETF rats differ dramatically from their normal and obese diabetic counterparts. The alterations in the angioarchitecture of islets observed in lean OLETF rats may be secondary to persistent hyperglycemia, just as a common denominator for the numerous late microvascular complications of diabetes such as retinopathy and nephropathy is a chronically increased blood glucose level. However, the possibility cannot be excluded that these angioarchitectural changes in islets of lean OLETF rats are partially related to their genetic vulnerability. In any case, an insufficient blood supply to the islets as a result of a paucity of capillary vessels deprives them of an adequate supply of oxygen and nutrients, resulting in an acceleration in the damage to and death of B cells, which are vulnerable to the stress of overwork. Furthermore, an alteration of the islet's physiologic core to mantle capillary perfusion[33,34] causes a dysfunction of pancreatic endocrine cells, such as an impairment of A-cell responsiveness to glucopenia, in these model rats.[35]

In summary, the majority of OLETF rats, a genetic model of the spontaneous development of NIDDM, eventually became hypoinsulinemic and develop IDDM-like diabetes, while some remain hyperglycemic with obesity with relative hyperinsulinemia and insulin resistance to approximately 50 weeks of age. The insulin content of the pancreas and histopathologic appearance and microangioarchitecture of the islets differed significantly between the groups: a lower insulin content in the pancreas and a sparse B-cell population and islet capillaries, all of which resulted in more severe derangement of the insulin response to various stimuli in the former versus the latter. The dimorphism for the diabetic phenotype observed in male OLETF rats might be attributed to subtle variations in the capacity for proliferation of pancreatic B cells.

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