

Islet Hyperperfusion During Prediabetic Phase in OLETF Rats, a Model of Type 2 Diabetes

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Although it has been hypothesized that initial hyperperfusion followed by late hypoperfusion in islet circulation occurs in rodent models of type 2 diabetes, islet blood flow has not been measured during prediabetic phase. We studied islet blood flow in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of slowly progressive obese type 2 diabetes. Islet blood flow was measured by the two-color microsphere method under anesthesia at different ages. Islet blood flow was significantly higher in young OLETF rats compared with control Long-Evans Tokushima Otsuka (LETO) rats when the former were not obese or diabetic and had normal insulin secretion at 5 weeks of age (LETO 4.6 ± 1.1 , OLETF 8.8 ± 1.2 ml \cdot min⁻¹ \cdot mg⁻¹, $P < 0.01$). At 6 months of age, islet hyperperfusion was observed in OLETF rats, and >40% of whole pancreatic blood flow was diverted into islets in OLETF rats. Prevention of obesity by food restriction increased basal islet blood flow. On the other hand, long-term hyperglycemia induced by sucrose feeding decreased fractional islet blood flow as well as glucose-stimulated islet blood flow. Our results indicate that hyperperfusion is present during the preobese and prediabetic phase in our type 2 diabetes rats. *Diabetes* 51:2530–2535, 2002

An extensive network of capillaries provides abundant blood flow to pancreatic islets (1). Islet blood flow is important for supplying nutrients and oxygen to islet cells, preventing accumulation of metabolic waste in islet interstitium, and transporting islet hormones to systemic circulation (2–4). The regulatory mechanisms that control islet blood flow are complex and include neural, hormonal, and local mechanisms that are independent of the surrounding exocrine pancreas (5). It has been reported that islet blood flow is altered in rodent models with type 1 (6) or type 2 diabetes (7,8), obesity (9–11), and hypertension (12). Although it has been suggested that changes in islet blood flow may be closely relevant to changes in islet function and metabolism, the relationship between flow and function is not fully understood.

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CCK, cholecystokinin; ELISA, enzyme-linked immunosorbent assay; IRI, immunoreactive insulin; L-NMMA, N^G-monomethyl-L-arginine; NOS, nitric oxide synthase.

The Otsuka-Long-Evans-Tokushima fatty (OLETF) rats are derived from spontaneously diabetic Long-Evans rats and characterized by mild obesity and late-onset hyperglycemia (after 18 weeks of age) with complications related to chronic diabetes (13). Although multiple loci have been implicated in genetic analysis, the cause of diabetes in these rats seems to be a combination of insulin resistance and impaired insulin secretion (14), bearing resemblance to human type 2 diabetes. In these rats, insulin sensitivity decreases with aging, i.e., it is normal at 6 weeks and reduced by 40% at 12 weeks and 80% after 18 weeks compared with age-matched control Long-Evans Tokushima Otsuka (LETO) rats (15). Insulin secretion is impaired at 40 weeks (14) and lipotoxicity to islet β -cells may be involved in the pathogenesis of islet dysfunction in hypertriglyceridemic OLETF rats (16). In addition, OLETF rats exhibit reduced regenerative capacity of pancreatic β -cells compared with LETO rats (17). Because of the chronic progressive form of diabetes, OLETF rats are suitable for studying the pathophysiological changes during the prediabetic phase.

The present study was designed to investigate the age-related changes in islet blood flow in OLETF rats during the prediabetic phase. Although a number of studies have previously measured islet blood flow in various hyperglycemic animal models, islet blood flow has not been investigated during the prediabetic phase in type 2 diabetic models.

RESEARCH DESIGN AND METHODS

Animals. Four-week-old male LETO rats ($n = 41$) and OLETF rats ($n = 49$) were supplied by Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). All rats were kept in specific pathogen-free conditions at Kyushu University Animal Center in rooms controlled for both temperature and light (on from 8:00 A.M. to 8:00 P.M.). The experiments were approved by the local animal ethics committee. Some rats (LETO $n = 10$, OLETF $n = 9$) were provided with sucrose (20% sucrose solution as drinking water) for 4–20 weeks of age. In OLETF rats ($n = 10$), food intake was restricted to 70% of average daily consumption for 8–16 weeks of age. Otherwise, the rats had free access to tap water and a diet of standard chow (Clea Japan, Tokyo).

Measurement of basal and glucose-stimulated islet blood flow rate. Islet blood flow in basal and glucose-stimulated states was measured using the two-color microsphere method (18). Age-related changes in islet blood flow were investigated at 5 weeks (LETO $n = 10$, OLETF $n = 9$), 12 weeks (LETO $n = 7$, OLETF $n = 8$), and 6 months of age (LETO $n = 7$, OLETF $n = 7$, sucrose-fed LETO $n = 10$, sucrose-fed OLETF $n = 9$, food-restricted OLETF $n = 10$). Pentobarbital sodium (Abbott Laboratories, Chicago) was injected intraperitoneally at a dose of 50 mg/kg. Body temperature was maintained at 37.5°C using a body temperature controller (Fine Science Tools, Foster City, CA). Polyethylene tubing (PE-50) was implanted in the ascending aorta via the right carotid artery, and the femoral artery and vein were cannulated. The former catheter was connected to a pressure transducer (Nihon Koden, Tokyo) for monitoring blood pressure. Heparin (100 IU; Shimizu, Shizuoka, Japan) was administered via the right jugular vein. After stabilization of arterial blood pressure and body temperature, islet blood flow was measured

TABLE 1
Body weight, pancreatic weight, and islet volume in OLETF and control LETO

Age	Groups	<i>n</i>	Body weight (g)	Pancreatic weight (g)	Islet volume (%)
5 weeks	LETO	10	131 ± 4	0.54 ± 0.02	1.30 ± 0.06
	OLETF	9	119 ± 5	0.41 ± 0.01*	1.46 ± 0.11
12 weeks	LETO	7	326 ± 7	0.88 ± 0.02	2.06 ± 0.11
	OLETF	8	416 ± 11*	0.72 ± 0.03*	2.91 ± 0.09*
6 months	LETO	7	439 ± 8	0.93 ± 0.02	2.24 ± 0.19
	Sucrose-fed LETO	10	524 ± 8*†	1.08 ± 0.01*†	3.74 ± 0.11*
	OLETF	7	579 ± 12*	0.84 ± 0.03	3.63 ± 0.29*
	Sucrose-fed OLETF	9	575 ± 7*	0.71 ± 0.03*‡	4.08 ± 0.25*‡
	Food-restricted OLETF	10	444 ± 3†	0.76 ± 0.05*	2.54 ± 0.12†

Data are means ± SE. **P* < 0.001 vs. LETO of the same age; ‡*P* < 0.05, †*P* < 0.001 vs. OLETF of the same age.

using green and black 10 μm-microspheres (E-Z TRAC microsphere; Interactive Medical Technology, San Diego, CA). Glucose solution (50%, 5 g/kg) was injected via the femoral vein catheter 8 min after the first microsphere injection. The second microsphere was injected 3 min after injection of glucose solution. Microspheres were suspended in saline and sonicated before injection. A total of 400,000 microspheres were injected and flushed with 350 μl saline into the ascending aorta over 25 s. Starting 10 s before the microsphere injection, the reference blood sample was withdrawn from the femoral artery catheter into a syringe at a rate of 0.5 ml/min using a constant withdrawal pump (Model 120; KD Scientific, Boston, MA). Preliminary experiments showed that the 10-μm microspheres (*N* = 800,000) did not affect blood pressure or heart rate in young rats (before 85 ± 7, after 85 ± 10 mmHg; before 425 ± 6, after 438 ± 13 beats/min, respectively, mean ± SE). Blood was obtained for the determination of blood glucose and serum immunoreactive insulin (IRI). Blood glucose was measured by the electrode method (Glucose Ace; Kyotodaiichikagaku, Kyoto, Japan), and serum IRI was measured by an enzyme-linked immunosorbent assay (ELISA) commercial kit using rat insulin as a standard (Morinaga, Yokohama, Japan). The rats were then sacrificed and the whole pancreas and adrenal glands were removed, blotted, and weighed. The whole pancreas was carefully dissected and freed of fat and lymph nodes under a stereomicroscope (Leica MZ8; Leica, Heerbrugg, Switzerland). Each pancreas was cut into a small piece and placed between object slides and then treated with the freeze-thawing technique, which allows visualization of microspheres and pancreatic islets. The percentage of islet volume was determined using the point-counting method (10). Intersections of overlapping islets were counted at a magnification of 400×. Whole pancreas was digested with 2 mol/l NaOH at 70°C overnight. The microsphere contents of the organ and reference blood sample were determined by transferring parts of the samples after vigorous stirring to glass microfiber filters (GF/A; Whatman, Kent, U.K.) and counting the microspheres under a stereomicroscope. By determining the number of microspheres present in the organ and the arterial reference sample, the blood flow values were calculated using the formula $Q_{org} = N_{org} \times 0.5 / N_{ref}$, where Q_{org} is the organ blood flow (ml/min), 0.5 is the withdrawal rate of the reference sample (ml/min), N_{org} is the number of microspheres in the organ, and N_{ref} is the number of microspheres in the reference sample. A difference in microsphere content <10% in the adrenal glands was taken to indicate sufficient mixing of the microspheres with the arterial bloodstream. When the islet blood flow was expressed per islet weight, islet weight was estimated by multiplying the pancreatic weight with the islet volume fraction of the whole pancreas. Fractional islet blood flow was expressed as a percentage of whole pancreatic blood flow.

Effect of nitric oxide synthase (NOS) inhibitor on islet blood flow. Five-week-old male LETO (*n* = 7) and OLETF rats (*n* = 7) were used in these experiments. After the first microsphere injection, 10 mg/kg body wt of *N*^G-monomethyl-L-arginine (L-NMMA) was administered via the femoral vein catheter. The second microsphere was injected at 7 min after L-NMMA injection.

Statistical analysis. Values are expressed as mean ± SE. Student's paired *t* test was used for the comparison of data from the same animal. Student's unpaired *t* test was used for comparison of two groups, and ANOVA and Scheffé's *F* test as a post hoc test were used for comparison of multiple groups. Correlation coefficients were determined by univariate Spearman correlation. Differences were considered significant when the *P* value was <0.05.

RESULTS

Changes in body weight and pancreas weight. Body weight was not significantly different between LETO and OLETF rats at 5 weeks of age, but was significantly greater in OLETF than in LETO rats at 12 weeks of age (Table 1). At 6 months of age, OLETF rats developed moderate obesity compared with LETO rats. Sucrose load significantly increased body weight in LETO but not in OLETF rats. Food restriction prevented the development of obesity in OLETF rats; their body weight was close to that of LETO rats. Pancreatic weight was consistently lower in OLETF than in LETO rats at each age (Table 1). Pancreatic weight significantly increased after sucrose load in LETO rats, but significantly decreased in OLETF. Pancreas was smaller in food-restricted OLETF than in LETO rats despite similar body weight.

Islet volume was not different between LETO and OLETF rats at 5 weeks of age, but was significantly greater in OLETF compared with LETO rats at 12 weeks and 6 months (Table 1). Sucrose feeding increased islet volume in both LETO and OLETF rats, although the percent increase was significantly greater in LETO (LETO 67.1 ± 5.0%, OLETF 12.5 ± 7.0%, *P* < 0.0001). Food restriction reduced islet volume in OLETF rats, close to that in LETO rats.

Changes in blood glucose concentrations. Blood glucose concentrations were not significantly different between LETO and OLETF rats at basal states as well as after glucose load at 5 and 12 weeks of age (Table 2). At 6 months of age, blood glucose was mildly increased in OLETF compared with LETO rats. Sucrose feeding markedly increased blood glucose in OLETF rats, whereas in LETO rats, it only marginally increased blood glucose after glucose load. Blood glucose in food-restricted OLETF rats was significantly lower than in OLETF rats fed ad libitum and similar to that in LETO rats.

Changes in serum IRI concentrations. Serum IRI concentrations at basal and glucose-stimulated states were not different between LETO and OLETF rats at 5 and 12 weeks of age (Table 2). At 6 months of age, basal serum IRI was markedly increased in OLETF rats but the response to glucose load was attenuated compared with that in LETO rats. Although sucrose feeding did not significantly change serum IRI in LETO rats, it significantly reduced serum IRI and abolished glucose-stimulated increase in OLETF rats. In food-restricted OLETF rats,

TABLE 2
Blood glucose, serum IRI, and mean blood pressure in OLETF and control LETO

Age	Groups	Blood glucose (mmol/l)		Serum IRI (ng/ml)		Mean blood pressure (mmHg)	
		Basal	Glucose i.v.	Basal	Glucose i.v.	Basal	Glucose i.v.
5 weeks	LETO	5.4 ± 0.1	14.3 ± 0.3	1.0 ± 0.1	8.1 ± 0.8	95 ± 3	90 ± 3
	OLETF	6.3 ± 0.2	15.4 ± 0.4	1.4 ± 0.2	9.2 ± 1.1	84 ± 2	82 ± 2
12 weeks	LETO	5.4 ± 0.2	15.8 ± 0.3	3.5 ± 0.6	10.7 ± 0.5	131 ± 2	125 ± 5
	OLETF	6.3 ± 0.3	17.3 ± 0.3	4.0 ± 0.8	8.5 ± 0.7	105 ± 9	117 ± 5
6 months	LETO	5.7 ± 0.3	18.5 ± 0.7	3.7 ± 0.3	24.6 ± 3.3	111 ± 8	119 ± 4
	Sucrose-fed LETO	7.1 ± 0.3	20.8 ± 0.4*	6.1 ± 0.8†	18.5 ± 1.5†	111 ± 6¶	120 ± 3
	OLETF	7.9 ± 0.6*	21.5 ± 0.9§	22.7 ± 4.6§	36.2 ± 5.8*	78 ± 3	105 ± 5
	Sucrose-fed OLETF	21.5 ± 1.3§†	32.3 ± 0.6§†	15.5 ± 2.9§‡	18.9 ± 3.0†	90 ± 4	101 ± 3
	Food-restricted OLETF	6.2 ± 0.2¶	19.3 ± 0.4‡	2.8 ± 0.4†	12.3 ± 1.8§†	87 ± 3	99 ± 3

Data are means ± SE. The number of animals is shown in Table 1. † $P < 0.05$, * $P < 0.01$, § $P < 0.001$ vs. LETO of the same age; ¶ $P < 0.05$, ‡ $P < 0.01$, † $P < 0.001$ vs. OLETF of the same age.

serum IRI at basal and glucose-stimulated states was significantly reduced compared with OLETF rats fed ad libitum, and serum IRI after glucose load was significantly lower than that in LETO rats.

Changes in blood pressure and pancreatic blood flow. Mean blood pressure at measurement of islet blood flow in anesthetized rats was not different between LETO and OLETF rats at 5 or 12 weeks of age (Table 2). At 6 months of age, however, blood pressure was significantly lower in OLETF than in LETO rats.

Whole pancreatic blood flow was not different between LETO and OLETF rats at 5 weeks of age (Table 3). At 12 weeks of age, whole pancreatic blood flow was reduced by half in OLETF compared with LETO rats. Whole pancreatic blood flow decreased with advancing age in OLETF and LETO rats (5 weeks vs. 6 months in LETO, $P < 0.001$; 5 vs. 12 weeks in OLETF, $P < 0.001$). There were no significant differences between the groups at 6 months of age. Intravenous glucose load did not alter whole pancreatic blood flow in any group at any age.

When islet blood flow was not corrected by estimated islet mass weight, there were no significant differences in whole islet blood flow between LETO and OLETF rats at each age (Table 3). However, islet blood flow corrected by estimated islet weight was significantly higher in OLETF

rats at basal and glucose-stimulated states compared with LETO rats at 5 weeks of age (Table 3). At 12 weeks of age, basal and glucose-stimulated islet blood flow was not different in LETO and OLETF rats. At 6 months of age, glucose-stimulated islet blood flow was markedly increased in OLETF compared with LETO rats. Although sucrose feeding did not significantly alter basal or glucose-stimulated islet blood flow in LETO rats, it did not significantly change basal islet blood flow in OLETF rats but reduced glucose-stimulated islet blood flow compared with OLETF fed ad libitum. Basal islet blood flow in food-restricted OLETF rats was significantly increased compared with that in LETO and OLETF rats fed ad libitum, but glucose-stimulated islet blood was not affected by food-restriction. Intravenous glucose significantly increased islet blood flow in both LETO and OLETF rats of all age-groups.

Fractional islet blood flow increased with age in both LETO and OLETF rats (Table 3). Although fractional islet blood flow was not different between LETO and OLETF rats at 5 weeks of age, it was significantly higher in OLETF than in LETO rats at 12 weeks of age. At 6 months of age, fractional islet blood flow was markedly increased in OLETF rats fed ad libitum. Sucrose feeding and food restriction reduced fractional islet blood flow, but re-

TABLE 3
Whole pancreatic blood flow, islet blood flow, and fractional islet blood flow in OLETF and control LETO

Age	Groups	Whole pancreatic blood flow (ml · min ⁻¹ · g ⁻¹ pancreas)		Islet blood flow (μl/min)		Islet blood flow (μl · min ⁻¹ · mg ⁻¹ islet tissue)		Fractional islet blood flow (%)	
		Basal	Glucose i.v.	Basal	Glucose i.v.	Basal	Glucose i.v.	Basal	Glucose i.v.
5 weeks	LETO	2.0 ± 0.3	1.8 ± 0.2	35 ± 9	50 ± 8	4.6 ± 1.1	7.0 ± 1.0	2.9 ± 0.3	4.9 ± 0.4
	OLETF	2.4 ± 0.4	2.3 ± 0.5	53 ± 9	78 ± 17	8.8 ± 1.2*	12.8 ± 2.5†	5.7 ± 0.6	8.8 ± 1.0
12 weeks	LETO	2.2 ± 0.4	2.4 ± 0.4	98 ± 20	206 ± 22	5.5 ± 1.1	11.6 ± 1.4	5.3 ± 0.7	11.0 ± 1.5
	OLETF	1.1 ± 0.1*	1.1 ± 0.3*	98 ± 7	151 ± 43	4.8 ± 0.5	7.8 ± 2.8	13.6 ± 1.1†	19.0 ± 2.2†
6 months	LETO	1.0 ± 0.3	1.4 ± 0.4	146 ± 27	294 ± 62	6.9 ± 1.2	14.1 ± 2.7	19.1 ± 4.5	23.9 ± 3.1
	Sucrose-fed LETO	1.4 ± 0.2	1.6 ± 0.3	288 ± 36	487 ± 72	7.2 ± 0.9	12.0 ± 1.6‡	20.4 ± 1.7§	28.2 ± 1.4§
	OLETF	0.8 ± 0.1	1.7 ± 0.3	274 ± 26	687 ± 169	9.1 ± 0.6	21.9 ± 4.0†	42.0 ± 2.4	48.2 ± 2.5
	Sucrose-fed OLETF	1.4 ± 0.1	1.8 ± 0.2	278 ± 33	423 ± 60	9.9 ± 1.2	14.5 ± 1.8¶	28.0 ± 1.4*§	32.9 ± 2.0*§
	Food-restricted OLETF	1.3 ± 0.1	1.5 ± 0.2	266 ± 27	373 ± 45	14.2 ± 1.5 ‡	20.6 ± 3.0	28.2 ± 3.3*§	33.4 ± 2.9*§

Data are means ± SE. The number of animals is shown in Table 1. † $P < 0.05$, * $P < 0.01$, ‡ $P < 0.001$ vs. LETO of the same age. ¶ $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$ vs. OLETF of the same age.

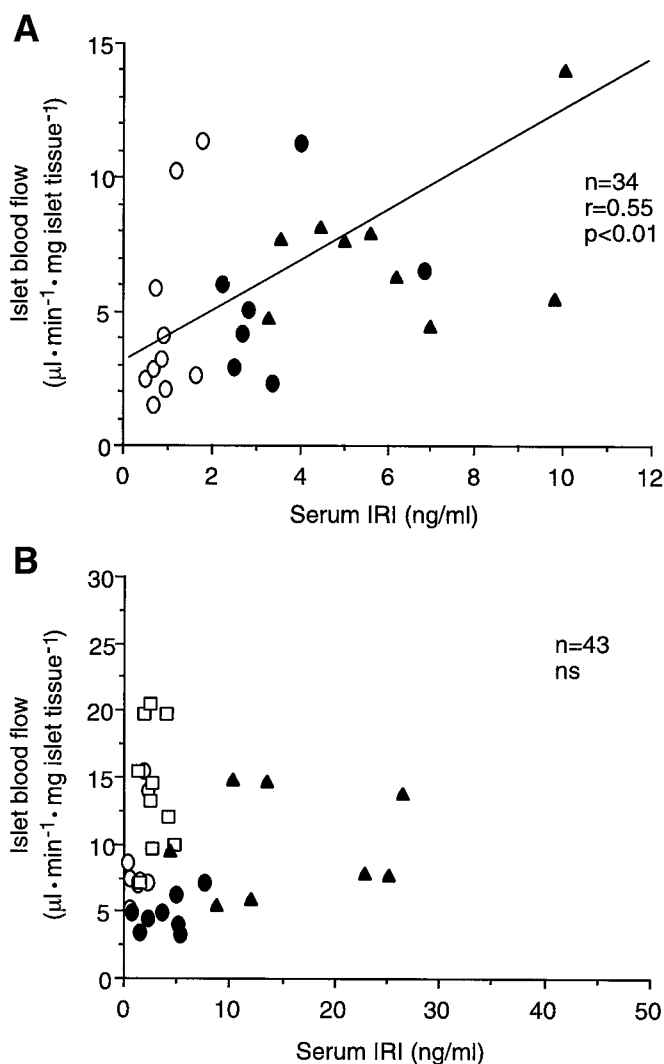


FIG. 1. Correlation between serum IRI concentration and islet blood flow during the basal state in LETO rats (A) and OLETF rats (B). \circ , 5 weeks of age; \bullet , 12 weeks of age; \blacktriangle , sucrose feeding at 6 months; \square , food restriction at 6 months.

mained at higher levels than that in LETO rats. Sucrose feeding in LETO rats did not affect its fractional islet blood flow. Administration of glucose increased fractional islet blood flow in all age-groups.

Further analysis showed a significant correlation between basal islet blood flow and serum IRI in LETO rats (Fig. 1A, $n = 34$, $r = 0.55$, $P < 0.01$), but not in OLETF rats (Fig. 1B, $n = 43$, NS). There was no significant correlation within the individual groups.

Effects of L-NMMA on pancreatic blood flow. L-NMMA significantly increased mean blood pressure in both LETO and OLETF rats (LETO: before 95 ± 3 , 2 min after 117 ± 4 , 4 min after 112 ± 3 , 6 min after 116 ± 3 mmHg; OLETF: before 83 ± 1 , 2 min after 96 ± 4 , 4 min after 96 ± 5 , 6 min after 101 ± 3 mmHg). L-NMMA did not affect blood glucose in LETO and OLETF rats (data not shown). As shown in Fig. 2, L-NMMA significantly decreased whole pancreatic and islet blood flow in both LETO and OLETF rats ($P < 0.01$, $P < 0.01$, respectively). Islet blood flow was not significantly different between LETO and OLETF rats after administration of L-NMMA.

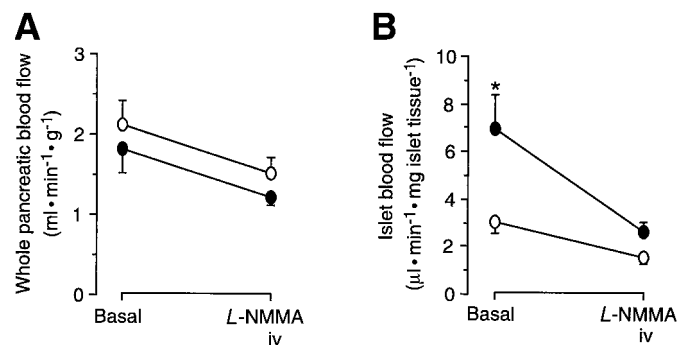


FIG. 2. Effect of NOS inhibitor, L-NMMA, on whole pancreatic blood flow (A) and islet blood flow (B). Data are means \pm SE. \circ , LETO rats ($n = 7$); \bullet , OLETF rats ($n = 7$). $P < 0.01$ vs. LETO.

DISCUSSION

The major findings of the present study were as follows: First, islet blood flow was significantly higher in OLETF rats than control LETO rats when OLETF rats were not obese or diabetic and had normal insulin secretion at 5 weeks of age. Second, NOS inhibitor reduced basal islet blood flow in OLETF as well as LETO, suggesting that NO system is important in maintaining basal islet blood flow in both strains. Third, the age-related decrease in whole pancreatic blood flow occurred earlier in OLETF than in LETO rats at 12 weeks of age. When total pancreatic blood flow was similar at 6 months of age, a higher islet blood flow was observed in OLETF rats. Fourth, sucrose feeding induced obesity in LETO rats but did not affect islet blood flow, whereas sucrose feeding caused marked hyperglycemia in OLETF rats and reduced glucose-stimulated islet blood flow. Lastly, in contrast, food restriction in OLETF rats prevented the development of obesity and increased basal islet blood flow.

Islet hyperperfusion has been reported in animal models of diabetes and obesity, e.g., obese hyperglycemic (*ob/ob*) mice (10), lean type 2 diabetic GK rats (7,8), genetically obese Zucker rats, and obese rats induced by lesioning ventromedial hypothalamus (9). Age-related changes in islet blood flow have been studied in *ob/ob* mice (19) and GK rats (20). In these studies, islet blood flow was higher in 1-month-old *ob/ob* mice and in 5- and 12-week-old GK rats than in age-matched controls, when these animals had already developed obesity and/or overt diabetes. With aging, however, islet blood flow decreased in *ob/ob* mice at 6 months of age and in GK rats at 1 year of age. Thus, it was hypothesized that the initial hyperperfusion followed by late hypoperfusion of the islets might be a common phenomenon observed in type 2 diabetes models (19). The late hypoperfusion may contribute to further deterioration of pancreatic islet function. Islet hypoperfusion in *ob/ob* mice was reversed by normalization of hyperglycemia with phlorizin treatment (19). The present finding of islet hyperperfusion during preobese and prediabetic period in 5-week-old OLETF may expand the initial hyperperfusion back into the prediabetic period in that hypothesis. Hypoperfusion was not observed in OLETF up to 6 months of age. However, electron microscopic studies using the corrosion cast technique reported by Mizuno et al. (21) showed sparse capillary network in the islets of severely hyperglycemic 54-week-old OLETF rats, while age-matched LETO rats showed normal typical glomerular-like

configuration. The mechanism(s) of islet hyperperfusion is controversial. In *ob/ob* mice lacking appetite suppressing hormone leptin, islet hyperperfusion was corrected by systemic administration of leptin (19). Islet hyperperfusion was reversed by vagotomy in diabetic GK rats, genetically Zucker rats, and hypothalamic obese rats (8,9). On the other hand, Svenson et al. (22) reported that the NOS inhibitor, *N*^G-nitro-L-arginine, decreased islet blood flow in GK rats. Because NOS was constitutively expressed in cultured rat islet capillary endothelial cells (23) and NOS inhibitors reduced islet blood flow in denervated transplanted pancreas (24), it is conceivable that islet vasculature is dependent on NO formation to maintain its normal high blood perfusion and NO may regulate islet blood flow through a local mechanism. Our results confirmed the sensitivity of the islet vasculature to NO, but provided no evidence for any causative role of this substance in islet hyperperfusion seen in OLETF rats. However, increased sensitivity of the vasculature to NO cannot be ruled out.

Atef et al. (8) reported that islet blood flow correlated positively with plasma insulin concentrations in nondiabetic and diabetic GK rats. This finding suggests that islet blood flow may be regulated by the state of endogenous insulin secretion irrespective of presence or absence of hyperglycemia. In the present study, however, such a correlation was found only in control LETO rats. The lack of this correlation in OLETF rats suggests that islet blood flow was mismatched with the state of endogenous insulin secretion, i.e., mismatched hyperperfusion. The dissociation between islet blood flow and insulin secretion was previously reported under certain conditions (25). A combination of increased islet blood flow and impaired insulin secretion was observed in starved rats and after the administration of mannoheptulose, an inhibitor of islet glucose metabolism (25). What is the significance of islet hyperperfusion? Islet hyperperfusion may be associated with islet capillary hypertension, as reported in diabetic GK rats (26). Capillary hypertension may subsequently lead to capillary endothelial damage in the islets. The extent to which the changes in islet microcirculation affects islet function remains unclarified at present. Insulin secretion was normal in 5-week-old OLETF rats *in vivo*, whereas isolated islets were hyper-responsive to high glucose *in vitro* (27). Therefore, islet hyperperfusion may reflect the underlying hyperfunctioning β -cells. Islet hyperperfusion during the stage of normal insulin secretion and sensitivity *in vivo* suggests that islet hyperperfusion may be an early *in vivo* marker for the future development of obesity or diabetes.

Although sucrose feeding induced obesity in LETO rats, islet blood flow remained unchanged. This finding is in disagreement with the previous studies, which showed increased islet blood flow in genetically Zucker obese rats and hypothalamic obese rats (9). In these studies, however, it should be noted that islet blood flow was expressed as per pancreatic weight. Because obesity induced compensatory islet hypertrophy, islet blood flow should be corrected by islet mass weight. The increase in islet volume induced by sucrose feeding was greater in LETO rats than in OLETF rats. This confirmed the poorer capacity for β -cell proliferation in OLETF rats subjected to 70%

pancreatectomy (17). In OLETF rats, sucrose feeding markedly exacerbated hyperglycemia and reduced serum IRI levels. Glucose-stimulated islet blood flow and fractional islet blood flow decreased in sucrose-fed OLETF rats, but basal islet blood flow remained unchanged. This suggests that islet perfusion was relatively constant in the basal condition, even under persistent hyperglycemia. In contrast, it has been reported that acute glucose challenge does not increase islet blood flow in diabetic GK rats and neonatal streptozotocin diabetic rats (8). It seems that long-term hyperglycemia affects islet circulation of animal models in a different manner.

The distribution of blood flow within the pancreas, i.e., fractional islet blood flow, was markedly different in LETO and OLETF rats at 6 months of age. More than 40% of whole pancreatic blood flow was diverted into islets in OLETF rats. The reported value for fractional islet blood flow using a microsphere technique was 2–4% in young rats, 10% in 3- to 4-month-old rats, 15% in rats older than 1 year (28), and 20% in obese 12-week-old Zucker rats (9). Although the mechanism of the markedly increased fractional islet blood flow seen in OLETF rats was not investigated in the present study, it was partially reversed by food restriction. Thus, the marked increase in fractional islet blood flow seems to be an adaptive response to the increased demand for insulin secretion, which should compensate for insulin resistance.

Pancreatic weight was consistently smaller in OLETF rats than in LETO rats, and whole pancreatic blood flow was reduced earlier during growth in OLETF rats compared with LETO rats at 12 weeks of age. The reduction in total pancreatic blood flow may affect islet blood flow because islets are widely distributed in the pancreas. Although fractional islet blood flow was increased in OLETF rats compared with LETO rats at 12 weeks of age, islet blood flow did not significantly differ between the two. Previous studies indicated that OLETF rats genetically lack cholecystokinin (CCK) receptor A, a major form expressed in the rat pancreas (29,30). Because CCK stimulates pancreatic growth and increases pancreatic blood flow (31), the absence of CCK action may contribute to the small pancreas and hemodynamic changes seen in OLETF rats. Tachibana et al. (30) reported that pancreatic protein and trypsin contents were reduced in OLETF rats compared with LETO rats, while DNA content was similar and amylase content was rather increased in OLETF rats. Although exocrine pancreas failed to respond to CCK stimulation in OLETF rats, basal exocrine secretion was not impaired. The greater body weight of OLETF rats may exclude any major changes in the exocrine function. Regarding the role of endogenous CCK in maintaining basal islet blood flow, we examined the effect of a specific CCK-A receptor antagonist in normal rats. Our results showed no significant changes in pancreatic or islet blood flow (unpublished data). This suggests that endogenous CCK does not seem to have a major role in maintaining basal pancreatic and islet blood flow.

The finding that blood pressure was lower in OLETF than in LETO rats (significant only at 6 months) was somewhat unexpected. Yagi et al. (32) reported that blood pressure measured using the tail cuff method was elevated in unanesthetized OLETF rats. This difference may be due

to the use of pentobarbital anesthesia in the present study. Alternatively, the stressful condition in measuring blood pressure by the tail cuff method may erroneously raise blood pressure in OLETF rats, since OLETF rats are more sensitive to stress than LETO rats (K. Kawano, personal communication). Direct measurement of blood pressure in conscious rats is necessary to identify the true change in blood pressure in OLETF rats.

In conclusion, we demonstrate in the present study that islet blood flow was significantly increased in OLETF rats compared with control LETO rats during the preobese and prediabetic period. Surprisingly, >40% of whole pancreatic blood flow was diverted into islets in 6-month-old OLETF rats. Prevention of obesity by food restriction increased basal islet blood flow. On the other hand, long-term hyperglycemia induced by sucrose feeding decreased fractional islet blood flow as well as glucose-stimulated islet blood flow. Our findings indicated that hyperperfusion commenced from the preobese and prediabetic phase and that it was followed by late hypoperfusion in islet circulation of a type 2 diabetes model.

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