Effect of Exercise Training and Food Restriction on Endothelium-Dependent Relaxation in the Otsuka Long-Evans Tokushima Fatty Rat, a Model of Spontaneous NIDDM

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We investigated whether endothelial function may be impaired in the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, a model of spontaneous NIDDM. The effect of exercise training and food restriction on endothelial function was also studied. OLETF rats were divided into three groups at age 16 weeks: sedentary, exercise trained, and food restricted (70% of the food intake of sedentary rats). Otsuka Long-Evans Tokushima rats were used as the age-matched nondiabetic controls. Endothelium-dependent relaxation of the thoracic aorta induced by histamine was significantly attenuated in the sedentary or food-restricted rats, and exercise training improved endothelial function. Relaxation induced by sodium nitroprusside, a donor of nitric oxide, did not differ significantly among groups. Both exercise training and food restriction significantly suppressed plasma levels of glucose and insulin and serum levels of triacylglycerol and cholesterol and reduced the accumulation of abdominal fat. Insulin sensitivity, as measured by the hyperinsulinemic-euglycemic clamp technique, was significantly decreased in sedentary rats but was enhanced in exercise-trained and food-restricted rats. The urinary excretion of nitrite was significantly decreased in sedentary and food-restricted rats compared with nondiabetic rats and was significantly increased in exercise-trained rats. These results indicate that exercise training, but not food restriction, prevents endothelial dysfunction in NIDDM rats, presumably due to the exercise-induced increase in the production of nitric oxide. ***Diabetes*** **47:82–86, 1998**

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GIR, glucose infusion rate; KRBB, Krebs-Ringer bicarbonate buffer; OGTT, oral glucose tolerance testing; OLETF rat, Otsuka Long-Evans Tokushima Fatty rat; STZ, streptozotocin.

Atherosclerosis is the principal cause of death and disability in diabetic patients. The proposed factors that contribute to atherosclerosis in the presence of diabetes include hyperglycemia, high insulin levels, and insulin resistance. Exercise training and food restriction are first-line treatments for NIDDM patients. However, few studies have examined the direct effects of exercise and food intake on the vasculature endothelial function of aorta.

Endothelium-derived relaxing factor plays an important role in the maintenance of vascular tone (1,2), and impairment of endothelial function is closely related to the development of atherosclerosis (3). Endothelial function is impaired in various pathological conditions, including hypertension (4,5), atherosclerosis (6,7), and diabetes (8,9) in both humans and animal models. Acetylcholine-induced endothelium-dependent relaxation is decreased in rats with streptozotocin (STZ)-induced diabetes, an impairment reversed by insulin treatment (10). However, most studies that have examined endothelium-dependent relaxation have used animal models of IDDM (11–15). The Otsuka Long-Evans Tokushima Fatty (OLETF) rat (16), a model of NIDDM, develops hyperglycemia, mild obesity, and insulin resistance similar to humans with NIDDM. The present study investigated the effects of exercise training and food restriction on impaired endothelial function in this rat model of NIDDM.

RESEARCH DESIGN AND METHODS

Animals and experimental design. Male OLETF rats were fed standard rat diet (Oriental Yeast, Tokyo, Japan) and tap water ad libitum until age 16 weeks, when they were randomly assigned to one of the following three groups of seven rats each: sedentary, exercise trained, or food restricted. Body weight and fasting blood glucose levels did not differ significantly among groups. A nondiabetic rat strain (Otsuka Long-Evans Tokushima) was used as the age-matched control group. Separate groups of seven rats, each with the same interventions, were used to measure the response to oral glucose tolerance testing (OGTT), and were also used in a euglycemic insulin clamp study.

Each exercise-trained rat was placed in an individual cage with an exercise wheel (Nishin, Tokushima, Japan) and allowed to run at its own pace. The number of revolutions of the wheel per day was recorded with a cyclometer attached to the axis of the wheel to measure the running activity. All but the food-restricted rats were allowed free access to rat diet. Food intake in the food-restricted group was limited to 70% of the intake in the sedentary group. Food intake in a 24-h period and body weight were measured once weekly. These interventions were performed until age 25 weeks. Then rats were anesthetized with pentobarbital sodium (50

mg/kg) and killed by exsanguination after an overnight fast. Blood samples were collected from the abdominal aorta for determination of serum levels of lipids and immunoreactive insulin. The mesenteric, epididymal, and retroperitoneal fat pads were surgically removed and weighed separately.

Assessment of vascular function. At ages 16 and 25 weeks, the thoracic aorta was dissected following exsanguination, and the adherent perivascular tissue was carefully removed. The ring segment of the aorta was mounted isometrically at a resting tension of 1.0 g in a 5-ml organ bath (Micro Easy Magnus; Medical Kishimoto, Kyoto, Japan) containing Krebs-Ringer bicarbonate buffer (KRBB) (maintained at 36°C and bubbled with 95% O_2–5% CO_2 throughout the experiment). The solution consisted of (in mmol/l): NaCl, 118.4; KCl, 4.9; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; and glucose, 11.1.

Vascular responses were measured after an 80-min period of equilibration at a resting tension of 1.0 g with an isometric transducer. The KRBB in the organ bath was changed every 20 min. The vessels were submaximally precontracted with phenylephrine (1 μmol/l) and were relaxed with cumulative doses of acetylcholine, histamine, and sodium nitroprusside. Relaxation responses to acetylcholine, histamine, and sodium nitroprusside are expressed as the percentage of the precontracted tension induced by 1 μmol/l of phenylephrine. The sensitivity to agonists is expressed as the dosage required to produce 50% of the maximal response (EC_{50}). Thoracic aortas were examined histopathologically.

OGTT and euglycemic insulin clamp studies. At ages 16 and 20 weeks, rats underwent an OGTT after an overnight fast. Glucose, 2 g/kg body wt, was administered orally. Blood was drawn from a tail vein at 0, 30, 60, and 120 min for measurement of the plasma level of glucose. Insulin-mediated whole-body glucose uptake was determined in 25-week-old anesthetized rats using a euglycemic insulin clamp (17). After an overnight fast, rats were anesthetized by the intraperitoneal injection of pentobarbital sodium, and catheters were inserted in the jugular and femoral veins. Rats received a 1-h infusion of insulin (60 pmol · kg^{-1} · min^{-1}). A glucose solution (100 g/l) was initiated at t = 0. The rate of infusion was adjusted to maintain the plasma concentration of glucose at approximately 6.1 mmol/l. The whole-body glucose uptake represents the mean glucose infusion rate (GIR) during the last 20 min.

Urinary NO_2 analysis. To determine the NO_2 excretion rate, 24-h urine samples were collected in bottles containing an antibiotic solution (1 mg/ml of penicillin G, 1 mg/ml of streptomycin, and 0.25 mg/ml of amphotericin B) from 23-week-old rats. NO_3 in urine was converted into NO_2 with NO_3 reductase and measured with the Griess reagent (Cyman Chemical, Ann Arbor, MI). The NO_2 measured in this manner reflected the combination of NO_2 and NO_3 in the original sample. NO_2 values were normalized by body weight.

Statistical analysis. Data in the text are expressed as means ± SD, and data in figures are expressed as means ± SE. Data were analyzed by analysis of variance plus Bonferroni multiple comparison tests. A level of $P < 0.05$ was accepted as statistically significant.

RESULTS

Body weight and food intake. Body weight was significantly greater in the sedentary rats than in the nondiabetic rats (Table 1). The mean running distance of the exercise-trained group was 4,199 ± 519 m/day. With no decrease in food consumption, physical exercise reduced the body weight of this group compared with that of sedentary rats. The food-restricted rats lost body weight, even though their food intake did not differ significantly from that of nondiabetic rats.

Endothelium-dependent relaxation. Histamine and nitroprusside caused a similar relaxation of aortic rings in 16-week-old OLETF and nondiabetic rats (Fig. 1*A* and *B*). At age 25 weeks, histamine-induced vascular relaxation was impaired in the aortic rings obtained from sedentary rats compared with those from nondiabetic rats (EC_{50}: 5.9 ± 1.7 vs. 2.6 ± 1.0 μmol/l; $P < 0.05$) (Fig. 1*C*). Histamine-induced relaxation in exercise-trained rats was significantly improved, and did not differ from that in nondiabetic rats. Food restriction did not improve histamine-induced relaxation (EC_{50}: 9.4 ± 5.7 vs. 5.9 ± 1.7 μmol/l). Similar results were obtained with acetylcholine, although the differences were less marked (data not shown).

To determine whether the impairment of endothelial function was caused by a decreased response to NO, we examined the effect of nitroprusside, a donor of NO. There were no significant differences in sodium nitroprusside–induced relaxation among groups (Fig. 1*D*).

Abdominal fat and serum concentrations of lipids. The amount of mesenteric, epididymal, and retroperitoneal fat was significantly increased in sedentary rats compared with nondiabetic rats (Table 1). Fat deposition was decreased in exercise-trained and food-restricted rats.

Serum levels of cholesterol, triacylglycerol, and phospholipids were significantly higher in sedentary than in nondiabetic rats (Table 2), but were significantly decreased in exercise-trained and food-restricted rats. There was no significant difference in serum concentrations of lipids between exercise-trained and food-restricted rats.

Glucose tolerance and in vivo glucose disposal. At age 16 weeks, the OLETF rats had significantly higher plasma levels of glucose than nondiabetic rats (Fig. 2). At age 20 weeks, the OGTT score was significantly improved in the exercise-trained and food-restricted rats. There was no significant difference in the plasma concentration of glucose between exercise-trained and food-restricted rats. The fasting level of insulin tended to be higher in sedentary rats than in nondiabetic rats (200 ± 24 vs. 163 ± 41 pmol/l). The insulin level was significantly lower in exercise-trained and food-restricted rats than in the sedentary group, but was not significantly different between exercise-trained and food-restricted rats (80 ± 16 vs. 105 ± 23 pmol/l).

The GIR was decreased in sedentary rats compared with nondiabetic rats (Fig. 3), and was significantly increased in the exercise-trained and food-restricted rats, although there was no significant difference between exercise-trained and food-restricted rats.

The urinary NO_2 excretion rate. The normalized urinary NO_2 excretion rate was significantly decreased in sedentary

TABLE 1
Effects of exercise training and food restriction on abdominal fat deposition

	Nondiabetic group	Sedentary group	Exercise-trained group	Food-restricted group
Body weight (g)	516 ± 27	643 ± 41*	519 ± 47†	518 ± 53†
Food intake (g/day)	22.7 ± 1.0	31.0 ± 1.4*	34.3 ± 4.1*	22
Mesenteric fat (g/kg body wt)	17.8 ± 0.8	23.7 ± 2.4*	12.8 ± 3.9*†	13.6 ± 3.5†
Epididymal fat (g/kg body wt)	22.8 ± 2.9	30.1 ± 4.1*	16.9 ± 3.8†	22.3 ± 0.4†
Retroperitoneal fat (g/kg body wt)	21.1 ± 2.4	61.2 ± 8.2*	28.9 ± 9.6†	40.7 ± 11.1†
Total abdominal fat (g/kg body wt)	61.8 ± 4.6	115.0 ± 10.0*	58.6 ± 17.0†	76.7 ± 17.9†

Data are means ± SD. *$P < 0.05$ vs. nondiabetic rats; †$P < 0.05$ vs. sedentary rats.

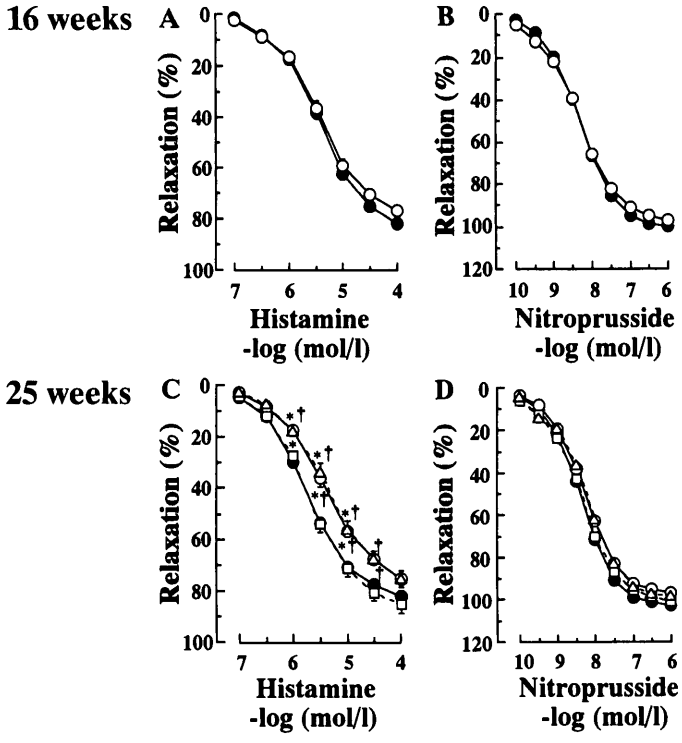

FIG. 1. Histamine (*A*) and nitroprusside (*B*) induced relaxation in thoracic aortic rings from sedentary (n = 7, ○), and nondiabetic (n = 7, ●) rats at age 16 weeks (top). Histamine (*C*) and nitroprusside (*D*) induced relaxation in thoracic aortic rings from sedentary (n = 7, ○), exercise-trained (n = 7, □), food-restricted (n = 7, △), and nondiabetic (n = 7, ●) rats at age 25 weeks (bottom). Data are means ± SE. *P < 0.05 vs. nondiabetic rats; †P < 0.05 vs. exercise-trained rats.

and food-restricted rats compared with nondiabetic rats. The urinary NO_2 excretion rate was significantly greater in exercise-trained rats than in sedentary or food-restricted rats (Fig. 4).

DISCUSSION

The endothelium-dependent vasodilatation induced by histamine was decreased in OLETF rats. This endothelial dysfunction was improved by exercise training but not by food restriction, although food restriction, like exercise, improved hyperglycemia, lessened abdominal fat content, and increased sensitivity to insulin, suggesting that mild hyperglycemia and insulin resistance are not related to the beneficial effect of exercise on endothelial dysfunction in OLETF rats. Urinary NO_2 excretion did not differ between food-restricted and sedentary rats, but was increased in exercise-trained rats. These results indicate that exercise training prevents endothelial dysfunction by increasing the production of NO.

Bohlen and Lash (18) reported that endothelium-dependent relaxation induced by acetylcholine was not impaired in Zucker fatty rats, an NIDDM rat model associated with obesity. However, this result must be interpreted cautiously, because the stage of diabetes may have influenced the results. In the present study, endothelium-dependent relaxation was not impaired in 16-week-old OLETF rats but was impaired in 25-week-old rats.

IDDM models (e.g., STZ-induced diabetes) exhibit more severe hyperglycemia-related metabolic abnormalities, hyperfiltration, and weight loss due to the lack of insulin. The 25-week-old diabetic OLETF rats used in the present study had hyperinsulinemic hyperglycemia due to obesity associated with hyperphagia, but did not show polyuria or loss of body weight. Therefore, the present results suggest that endothelial dysfunction may occur even in the early stages of NIDDM.

Exercise and food restriction are the first steps in the treatment of NIDDM. Sedentary rats in the present study had hyperglycemia, hypertriglyceridemia, hypercholesterolemia, insulin resistance, and obesity. Exercise training and food restriction improved those conditions, but only exercise training increased the endothelium-dependent vasodilatation induced by histamine.

The differences in endothelial relaxation induced by histamine between the exercise-trained and food-restricted rats could not be explained by the serum levels of glucose, insulin, or lipids. Previous studies have shown that the forearm vasodilator response to acetylcholine is not correlated with levels of LDL cholesterol, fasting glucose, or GHb, but is inversely correlated with the serum concentration of insulin (8,9). In the present study, the GIR was slightly higher in the exercise-trained than in the food-restricted rats, which could, in part, explain the difference in endothelial function between these groups. However, the difference in the GIR was not significant. Similarly, the abdominal fat content, which is closely related to insulin resistance (19), was slightly lower in exercise-trained than in food-restricted rats, but the difference was not significant. Thus these two factors were not responsible for the difference in endothelial function between groups.

Although the endothelium-dependent relaxation induced by histamine was impaired and the urinary excretion of NO_2 was decreased in sedentary rats, these values were significantly increased in exercise-trained rats. Thus the production of NO may explain the difference between these groups. NO_2 and NO_3 are the stable degradation products of NO in biological solutions and are accurate indicators of NO produc-

TABLE 2
Effects of exercise training and food restriction on serum levels of lipids

	Nondiabetic group	Sedentary group	Exercise-trained group	Food-restricted group
Total cholesterol (mmol/l)	1.40 ± 0.08	2.00 ± 0.11*	1.23 ± 0.15†	1.40 ± 0.10†
HDL cholesterol (mmol/l)	0.91 ± 0.05	1.18 ± 0.02*	0.88 ± 0.08†	0.93 ± 0.06†
Triglycerides (mmol/l)	0.35 ± 0.07	1.45 ± 0.32*	0.44 ± 0.22†	0.36 ± 0.11†
Phospholipids (mmol/l)	1.68 ± 0.08	2.45 ± 0.21*	1.65 ± 0.21†	1.87 ± 0.11†
Free fatty acids (mmol/l)	0.73 ± 0.13	0.80 ± 0.15	0.73 ± 0.03	0.50 ± 0.10†

Data are means ± SD. *P < 0.05 vs. nondiabetic rats; †P < 0.05 vs. sedentary rats.

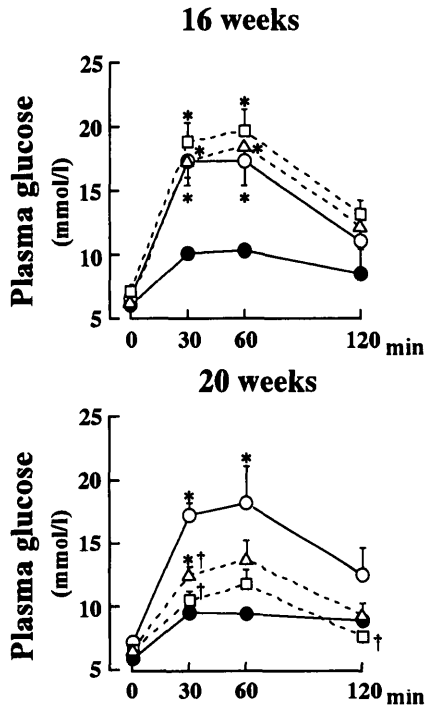

FIG. 2. Changes in the plasma levels of glucose in response to an oral glucose load in nondiabetic (n = 7, ●), sedentary (n = 7, ○), exercise-trained (n = 6, □), and food-restricted (n = 6, △) rats. After an overnight fast, glucose at 2 g/kg body wt was administered orally. Blood samples were obtained from a tail vein without anesthesia. Data are means ± SE. *P < 0.05 vs. nondiabetic rats; †P < 0.05 vs. sedentary rats.

tion in vivo (20). Taylor et al. (21) reported that abnormalities in endothelium-dependent relaxation in diabetic rats were due, at least in part, to the decreased production and release of NO. Moreover, NO-dependent cyclic guanosine monophosphate is impaired in the glomeruli and arteries from rats with STZ-induced diabetes (13,22). However, Tolins et al. (23) and Wu (24) found that STZ-induced diabetic rats and spontaneously diabetic BB rats showed an increase in the urinary excretion of NO_2, and suggested that a decreased responsiveness of the smooth muscle cells to NO was

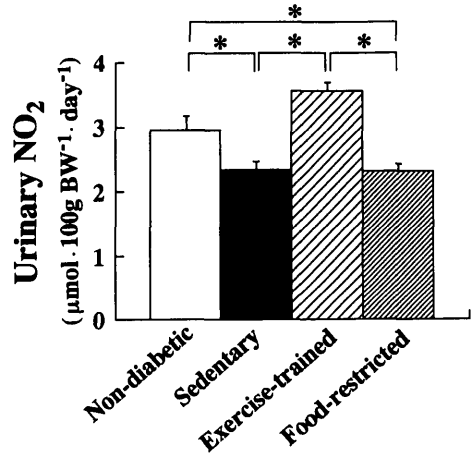

FIG. 4. Effects of exercise training and food restriction on urinary excretion of NO_2 in nondiabetic, sedentary, exercise-trained, and food-restricted rats. Data are means ± SE (n = 7 in each group). *P < 0.05.

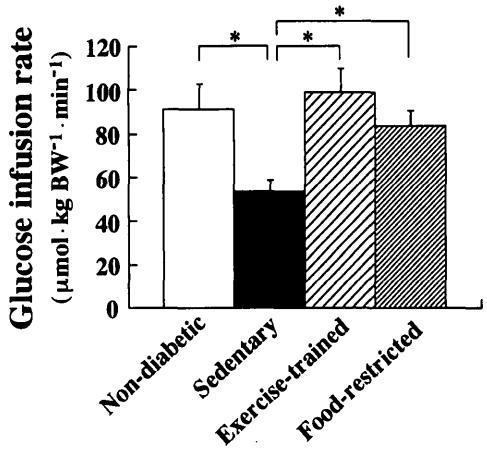

FIG. 3. Effects of exercise training and food restriction on insulin-stimulated glucose disposal in vivo. GIR is shown in nondiabetic, sedentary, exercise-trained, and food-restricted rats. Data are means ± SE (n = 7 in each group). *P < 0.05.

responsible for the decrease in acetylcholine-induced relaxation. Bucala et al. (25) demonstrated that, in rats with STZ-induced diabetes, advanced glycosylation products quench NO via a rapid chemical reaction. In a recent study, however, Lawrence and Brain (26) reported that the local production of NO was decreased in the cutaneous microvasculature of rats with STZ-induced diabetes.

A recent study showed that repeated increases in blood flow or a chronic elevation of blood flow significantly affects the function of the vascular endothelium (27, 28). In the present study, the urinary excretion of NO_2 was increased in exercised-trained rats, suggesting that exercise improved the function of the endothelium in aortic rings by increasing the production of NO.

REFERENCES

1. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373–376, 1980
2. Van Voorde J, Leusen I: Role of the endothelium in the vasodilator response of rat thoracic aorta to histamine. *Eur J Pharmacol* 87:113–120, 1983
3. Cohen RA, Zintnay KM, Haudenschild CL, Cuningham LD: Loss of selective endothelial cell vasoactive functions caused by hypercholesterolemia in pig coronary arteries. *Circ Res* 63:903–910, 1988
4. Linder L, Kiowski W, Buhler FR, Luscher TF: Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo: blunted response in essential hypertension. *Circulation* 81:1762–1767, 1990
5. Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 323:22–27, 1990
6. Zeiher AM, Drexler H, Wollschlagr H, Just H: Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 83:391–401, 1991
7. Shimokawa H, Nakaike R, Takeshita A: Significance of defective endothelial signal transduction in impaired endothelium-dependent relaxation in atherosclerosis. *Gerontology* 41 (Suppl. 1):28–33, 1995
8. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA: Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 88:2510–2516, 1993
9. McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW, Hayes JR: Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35:771–776, 1992
10. Taylor PD, Oon BB, Thomas CR, Poston L: Prevention by insulin treatment of endothelial function but not enhanced noradrenaline-induced contractility in mesenteric resistance arteries from streptozotocin-induced diabetic rats. *Br J Pharmacol* 111:35–41, 1994

11. Oyama Y, Kawasaki H, Hattori Y, Kanno M: Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. *Eur J Pharmacol* 131:75–78, 1986
12. Cameron NE, Cotter MA: Impaired contraction and relaxation in aorta from streptozotocin-diabetic rats: role of polyol pathway. *Diabetologia* 35:1011–1019, 1992
13. Kamata K, Miyata N, Kasuya Y: Impairment of endothelium-dependent relaxation and changes in levels of cyclic GMP in aorta from streptozotocin-induced diabetic rats. *Br J Pharmacol* 97:614–618, 1989
14. Agrawal DK, McNeill JH: Vascular responses to agonists in rat mesenteric artery from diabetic rats. *Can J Physiol Pharmacol* 65:1484–1490, 1987
15. Durante W, Sen AK, Sunahara FA: Impairment of endothelium-dependent relaxation in aortae from spontaneously diabetic rats. *Br J Pharmacol* 94:463–468, 1988
16. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T: Spontaneous long-term hyperglycemic rat with diabetic complications, Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes* 41:1422–1428, 1992
17. Rossetti L, Smith D, Shulman GI, Papachriston D, Defronzo RA: Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. *J Clin Invest* 79:1510–1515, 1987
18. Bohlen HG, Lash JM: Endothelial-dependent vasodilation is preserved in non-insulin-dependent Zucker fatty diabetic rats. *Am J Physiol* 268:H2366–H2374, 1995
19. Shima K, Shi K, Sano T, Iwami T, Mizuno A, Noma Y: Is exercise training effective in preventing diabetes mellitus in the Otsuka-Long Evans-Tokushima Fatty rat, a model of spontaneous non-insulin-dependent diabetes mellitus? *Metabolism* 42:971–977, 1993
20. Shultz PJ, Raij L: Endogenously synthesized nitric oxide prevents endotoxin-induced glomerular thrombosis. *J Cin Invest* 90:1718–1725, 1992
21. Taylor PD, McCarthy AL, Thomas CR, Poston L: Endothelium-dependent relaxation and noradrenaline sensitivity in mesenteric resistance arteries of streptozotocin-induced diabetic rats. *Br J Pharmacol* 107:393–399, 1992
22. Craven PA, Studer RK, DeRubertis FR: Impaired nitric oxide-dependent cyclic guanosine monophosphate generation in glomeruli from diabetic rats. *J Clin Invest* 93:311–320, 1994
23. Tolins JP, Shultz PJ, Raij L, Brown DM, Mauer SM: Abnormal renal hemodynamic response to reduced renal perfusion pressure in diabetic rats: role of NO. *Am J Physiol* 265:F886–F895, 1993
24. Wu G: Nitric oxide synthesis and the effect of aminoguanidine and N^G-monomethyl-L-arginine on the onset of diabetes in the spontaneously diabetic BB rat. *Diabetes* 44:360–364, 1995
25. Bucala R, Tracey KJ, Cerami A: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest* 87:432–438, 1991
26. Lawrence E, Brain SD: Altered microvascular reactivity to endothelin-1, endothelin-3 and N^G-nitro-L-arginine methylester in streptozotocin-induced diabetes mellitus. *Br J Pharmacol* 106:1035–1040, 1992
27. Koller A, Huang A, Sun D, Kaley G: Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles: role of endothelial nitric oxide and prostaglandins. *Circ Res* 76:544–550, 1995
28. Miller VM, Vanhoutte PM: Enhanced release of endothelium-derived relaxing factor(s) by chronic increases in blood flow. *Am J Physiol* 255:H446–H451, 1988