Microvascular Versus Macrovascular Dysfunction in Type 2 Diabetes: Differences in Contractile Responses to Endothelin-1

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Vascular dysfunction characterized by a hyperreactivity to vasoconstrictors and/or impaired vascular relaxation contributes to increased incidence of cardiovascular disease in diabetes. Endothelin (ET)-1, a potent vasoconstrictor, is chronically elevated in diabetes. However, the role of ET-1 in resistance versus larger vessel function in mild diabetes remains unknown. Accordingly, this study investigated vascular function of third-order mesenteric arteries and basilar arteries in control Wistar and Goto-Kakizaki (GK) rats, a model of mild Type 2 diabetes. Six weeks after the onset of diabetes, contractile responses to 0.1–100 n*M* ET-1 and relaxation responses to 1 n*M*–10 μ*M* acetylcholine (ACh) in vessels preconstricted (baseline + 60%) with serotonin (5-HT) were assessed by myograph studies in the presence or absence of a nitric oxide synthase (NOS) inhibitor, *N*-nitro-L-arginine (L-NNA). Maximum contractile response to ET-1 was augmented in mesenteric vessels (155 ± 18% in GK vs. 81 ± 6% in control; *n* = 5–7) but not in the basilar artery (134 ± 29% in GK vs. 107 ± 17% in control; *n* = 4 per group). However, vascular relaxation was impaired in the basilar arteries (22 ± 4% in GK vs. 53 ± 7% in control; *n* = 4 per group) but not in mesenteric arteries of GK rats. Inhibition of NOS decreased the relaxation response of basilar arteries to 15 ± 8% and 42 ± 5% in GK and control rats, respectively; whereas, in resistance vessels, corresponding values were 56 ± 7% and 89 ± 3% (vs. 109 ± 2% and 112 ± 3% without NOS blockade), indicating the involvement of different vasorelaxation-promoting pathways in these vascular beds. These findings provide evidence that the ET system is activated even under mild hyperglycemia and that it contributes to the hyperreactivity of resistance vessels, therefore, the ET system may play an important role in elevated blood pressure in Type 2 diabetes.
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Key words: endothelin-1; type 2 diabetes; vascular dysfunction; Goto-Kakizaki

Introduction

Diabetes mellitus is a major health issue and, with nearly 75,000 deaths in the United States in 2002, is also a leading cause of mortality. Of those deaths, it is estimated that 65%–75% can be attributed to cardiovascular disease (CVD), including hypertension and stroke (1). Although it is known that diabetes is a major risk factor for CVD (2, 3), the mechanisms that potentiate this risk are not fully understood.

Vascular dysfunction, characterized by impaired relaxation to vasodilators or exacerbated response to vasoconstrictors, coexists in many disease states, such as hypertension and diabetes. In general, these diseases alter vascular responses to vasoactive substances, leading to increased basal tone and an inability to respond normally to stimuli. Studies in streptozotocin (STZ)-induced Type 1 diabetes have demonstrated decreased endothelium-mediated relaxation in both mesenteric and cerebral (basilar) arteries (4–6). Additionally, similar results have been obtained in superior mesenteric arteries from the Type 2 diabetic Goto-Kakizaki (GK) rat (7–9). However, the effect of Type 2 diabetes on mesenteric microvessels and cerebral vessels remains unknown.

The potent vasoconstrictor, endothelin (ET)-1, is chronically upregulated in diabetes (10–12) and may contribute to CVD and diabetic complications (13, 14). Studies have demonstrated enhanced contractile responses in the mesenteric and basilar arteries in STZ-treated rats (15, 16), as well as in basilar arteries from alloxan-induced diabetic rabbits (17). Furthermore, Dumont *et al.* (18) showed that chronic ET receptor antagonism abolishes increased myogenic tone in diabetic cerebral arteries.

The endogenous vasodilator nitric oxide (NO) plays an

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Table 1. Metabolic Parameters of Control Wistar and GK Rats

Metabolic parameters	Control	GK
Body weight (g)	502 ± 11	359 ± 7*
Blood glucose (mg/dl)	116 ± 5	239 ± 23*
Mean arterial pressure (mm Hg)	101 ± 2	110 ± 7

* $P < 0.05$ vs. control.

important role in the regulation of vascular tonus in response to both molecular and physical signals to increase levels of cGMP and dilation. Decreased NO bioavailability, either through decreased production, primarily by endothelial NO synthase (NOS), or increased scavenging by reactive oxygen species, impairs vascular relaxation (19–22). Thus, within the scope of diabetes, it is important to understand the relative dependence of the vasculature on NO.

The objective of the current study was to compare and contrast the effects of hyperglycemia on different vascular beds in a model of Type 2 diabetes. Specifically, maximal contractile responses and endothelium-dependent relaxation responses were investigated in third-order mesenteric and basilar arteries from Type 2 diabetic GK rats. Additionally, we sought to determine the relative contribution of NO-mediated relaxation in these vessels.

Materials and Methods

Animals. All experiments were performed on male Wistar (Harlan, Indianapolis, IN) and GK (in-house bred, derived from the Tampa colony) rats (23, 24). The animals were housed at the Medical College of Georgia animal care facility, which is approved by the American Association for Accreditation of Laboratory Animal Care. All protocols were approved by the Institutional Animal Care and Use Committee. Animals were fed standard rat chow and tap water *ad libitum* until sacrifice at 18 weeks of age.

Blood Pressure Monitoring. Blood pressure was measured by telemetry. Animals were implanted with telemetry transmitters at week 12, and allowed to recover for 2 weeks. Mean arterial pressure was recorded from Week 14 through Week 18.

Surgical Procedures. Animals were anesthetized and decapitated. The brain was quickly excised for isolation of basilar arteries. The mesenteric bed was harvested and third-order mesenteric arteries were isolated for vascular function studies.

Determination of Vascular Function. Isometric tension exerted by the vessels was recorded *via* a force transducer using the wire-myograph technique (Danish Myo Technologies, Denmark). The myograph chambers were filled with Kreb's buffer (118.3 m*M* NaCl, 25 m*M* $NaHCO_3$, 4.7 m*M* KCl, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4, 1.5 m*M* $CaCl_2$, and 11.1 m*M* dextrose), gassed with 95% O_2 and 5% CO_2, and maintained at 37°C. Vessel segments were mounted in the chamber using 40-μm-thin wires and adjusted to a baseline tension (~0.5 g for basilar and 1 g for mesentery). After stabilization, the vessels were challenged with 70 m*M* KCl and only those vessels that had a 70% response above the baseline were considered viable. Cumulative dose-response curves to 0.1–100 n*M* ET-1 were generated and the force generated was expressed as percent change from baseline. Endothelium-dependent relaxation to 1 n*M*–1 μ*M* acetylcholine (ACh) was assessed after vessels were constricted to 60% of the baseline tension with serotonin (5-HT), either alone or with a 30-min pre-incubation with the NOS inhibitor, *N*-nitro-L-arginine (L-NNA; 100 n*M*). Sensitivity (median effective concentration [EC_{50}]) and maximum response (R_{max}) values were calculated from the respective dose-response equations.

Statistical Analyses. EC_{50} and R_{max} within-group differences were determined by *t* tests. For multiple group comparisons within and between vascular beds, a one-way or two-way analysis of variance (ANOVA) was performed accordingly, with a *post hoc* Bonferroni test. A repeated measures ANOVA was used to determine group differences (diabetic vs. control groups) across the ET-1 concentrations. *Post hoc* group comparisons at each concentration used a Tukey's adjustment for the multiple comparisons. Statistical significance was determined at $P < 0.05$. SAS 9.1.3 (SAS Inc, Cary, NC) was used for all analyses.

Results

Animal Data. Metabolic parameters for control and diabetic (GK) animals are summarized in Table 1. Diabetic animals were significantly smaller than control animals, and displayed mildly elevated blood glucose and a small, but not significant, increase in blood pressure. Plasma and local mesenteric and basilar artery ET-1 levels were increased as compared with controls (data not shown).

ET-1 Dose Response. Mesenteric arteries of GK rats were hyperreactive to ET-1 (R_{max}, 155 ± 18%) compared with the control rats (81 ± 6%), although their sensitivity was not significantly altered (Fig. 1A; Table 2). There was a significant interaction between groups across the ET-1 concentration range ($P < 0.0001$), indicating a differential effect on responses to ET-1 between diabetic and control rats. The response was significantly different (all $P < 0.0001$) for concentrations from 1 to 100 n*M*. Contractile responses to 5-HT were not different between groups (data not shown). However, in the basilar arteries, sensitivity to ET-1 was greatly augmented (4.2 ± 2.7 n*M* vs. 13.1 ± 2.0 n*M*), whereas the maximum constriction was not significantly different (134 ± 29% vs. 107 ± 17%; Fig. 2A; Table 2).

Endothelium-Dependent Relaxation. As shown in Figure 1B, there was no impaired relaxation to ACh in the mesenteric arteries of GK rats, similar to in the controls. In the presence of L-NNA, there was a greater decrease in relaxation in the GK rats (56 ± 7%) compared with the control animals (89 ± 3%), suggesting a greater involve-

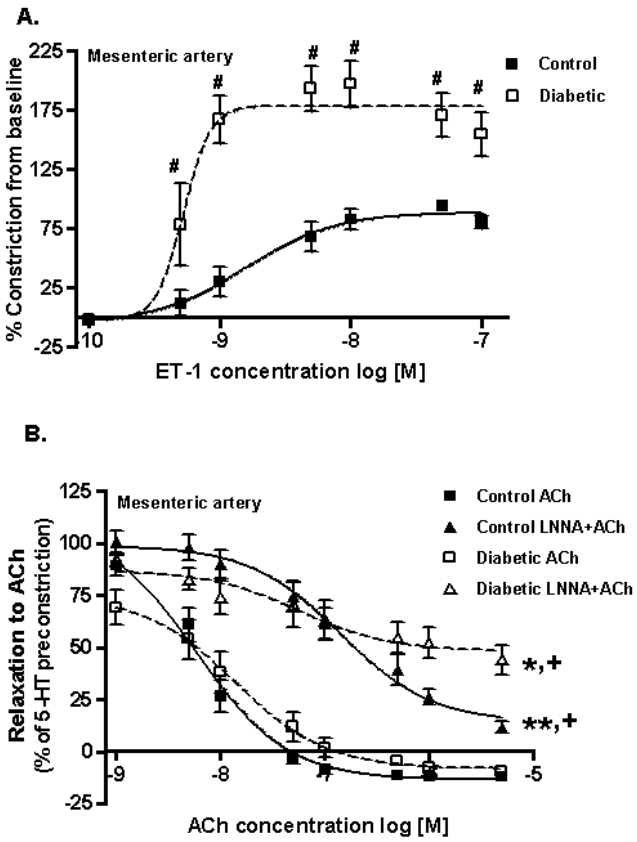

Figure 1. (A) Constriction responses to ET-1 in mesenteric arteries of control (Wistar) and diabetic (GK) rats. The magnitude of constriction (R_{max}) was increased in GK rats, although sensitivity (EC_{50}) to ET-1 was not significantly altered. (B) Relaxation responses to ACh in the absence or presence of the endothelial (e)-NOS inhibitor, L-NNA (100 n*M*) in mesenteric arteries of control and GK rats after preconstriction with 5-HT. #, Percent constriction vs. control; *, R_{max} $P < 0.05$ vs. control L-NNA + ACh or diabetic rats; **, R_{max} $P < 0.05$ vs. control; +, percent relaxation $P < 0.05$ vs. control or diabetic rats across the 5 n*M*–5 μ*M* ACh concentration range.

ment of NO-mediated relaxation in the diabetic group. Although the EC_{50} values increased in both groups, indicating decreased sensitivity in the presence of L-NNA, there was no significant difference between controls and GKs. In the cerebral circulation, endothelium-dependent relaxation was impaired to a greater degree (Fig. 2B; Table 2). The basilar arteries of GKs relax only 22 ± 4%, whereas the control rat arteries relax 53 ± 7% after preconstriction with 5-HT. Sensitivity to ACh is also greatly reduced in the GK rats in comparison with the control rats (20.4 ± 2 n*M* and 63 ± 2 n*M*, respectively; Fig. 2B; Table 2). In the presence of L-NNA, impaired relaxation of the basilar arteries is augmented even further in the GK rats compared with controls.

Vascular Responses in Mesenteric Arteries Versus Basilar Arteries. Vascular constriction and relaxation responses were greatly altered in the two vascular beds in the GK rats. In the mesenteric arteries, the sensitivity to ET-1 was augmented nearly 4-fold in comparison with the basilar arteries (Fig. 3A; Table 2); the magnitude of constriction, however, increasing comparably. In relaxation responses to ACh, there was no impairment of endothelium-dependent relaxation in the mesenteric bed, in contrast to a decreased relaxation response in the basilar arteries of GK rats (Fig. 3B; Table 2).

Discussion

Although it is well established that endothelial dysfunction underlies vascular complications of diabetes (19, 25), past studies focused mostly on STZ-induced Type 1 diabetes or in Type 2 models associated with obesity, such as the Zucker rat (26, 27). This study investigated the hypotheses that vascular responses to the potent constrictor ET-1 and the endothelium-dependent dilator, ACh, differ in small resistance vessels versus larger vessels, in a nonobese model of Type 2 diabetes, which displays mild hyperglycemia. When compared with control rats, ET-1–induced contractions in the mesenteric resistance vessels are greater, whereas basilar arteries are more sensitive to the same stimulus in the GK rat. Moreover, when vascular beds are compared within groups, mesenteric vessels seem to be more sensitive to ET-1. We also provided evidence that after 6 weeks of mild diabetes, endothelium-dependent relaxation is not impaired in the mesenteric bed but is decreased by approximately 80% in the cerebral vessels.

The endothelium plays an important role in the control of vascular tone, in part through the release of vasoactive factors, and, in diabetes, the imbalance of vasodilators and vasoconstrictors contributes to the increased risk of CVD, including hypertension and stroke in diabetes (28). Because resistance arteries play an important role in the regulation of blood pressure, and because relatively larger arteries, such as the basilar artery, participate in the regulation of cerebral vascular resistance that is critical in the pathophysiology of stroke, this study was designed to compare and contrast the vascular reactivity of these two beds in diabetic GK and control rats. Cheng *et al.* (7) reported that the GK rats display endothelial dysfunction and impaired vasorelaxation of superior mesenteric arteries that is associated with salt-sensitive hypertension. Witte *et al.* (9) demonstrated increased blood pressure and decreased endothelium-dependent relaxation in the mesenteric bed. In the current study, we did not observe any impairment of the relaxation response to ACh, and the data indicate a greater involvement of the NO pathway in the relaxation response in the GK rats compared with controls, as evidenced by 50% versus 10% blockade, respectively, in the presence of L-NNA. Another group also reported normal endothelium-dependent relaxation in this model (29). These differences may be caused by the age of the animals used or the size of the mesenteric arteries. In the current study, we used segments between 200 and 250 μm in diameter, which allowed the insertion of two pieces of 40-μm wire for the myograph. Witte *et al.* (9) reported using 120-μm wire in

Table 2. Sensitivity (EC_{50}) and Magnitude (R_{max}) of Vascular Responses to ET-1 and ACh in the Absence or Presence of the e-NOS Inhibitor L-NNA (100 n*M*) in Mesenteric and Basilar Arteries of Control Wistar and GK Rats

	Mesentery artery		Basilar artery	
	Control	GK	Control	GK
EC_{50} (n*M*)				
ET-1	3.2 ± 1.5***	0.5 ± 0.1***	13.1 ± 2	4.2 ± 2.7*
ACh	6.0 ± 1.2	16.1 ± 14.0	62.6 ± 1.8	20.4 ± 2.2*
L-NNA + ACh	92.2 ± 13.5	56.6 ± 22.4**	9.7 ± 1.8	>1000
R_{max} (%)				
ET-1	81 ± 6	155 ± 18*	107 ± 17	134 ± 29
ACh	112 ± 2	109 ± 2**	53 ± 7	22 ± 4*
L-NNA + ACh	89 ± 3	56 ± 7*,**	42 ± 5	15 ± 8*

*$P < 0.05$ vs. control ($n = 4$–9 /group); **$P < 0.05$ vs. GK basilar artery ($n = 4$–9 /group); ***$P < 0.05$ vs. respective control or GK basilar ($n = 4$–9/group).

their system, indicating the use of a superior mesenteric artery segment.

Several studies reported impaired relaxation of the middle cerebral artery in insulin-resistant rats before the development of overt diabetes, however, vascular reactivity changes in the cerebral circulation in Type 2 diabetes are largely unknown. Recently, Matsumoto *et al*. (16) reported that contraction induced by blockade of NOS is significantly less in the basilar artery of STZ-diabetic rats, suggesting that

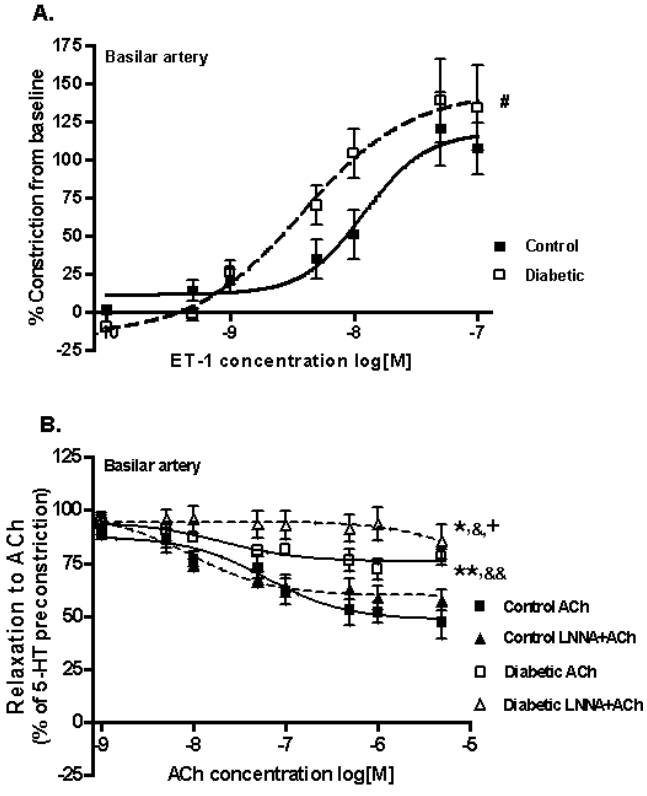

Figure 2. (A) Constriction responses to ET-1 in basilar arteries of control and GK rats. (B) Relaxation to ACh in the absence or presence of the e-NOS inhibitor, L-NNA (100 n*M*), in basilar arteries after preconstriction with 5-HT. #, EC_{50} $P < 0.05$ vs. control; *, R_{max} $P < 0.05$ vs. control L-NNA + ACh; **, R_{max} $P < 0.05$ vs. control; &, EC_{50} $P < 0.05$ vs. control L-NNA + ACh; &&, EC_{50} $P < 0.05$ vs. control; +, percent relaxation $P < 0.05$ vs. control ACh, GK ACh, or control LNNA + ACh across the 50 n*M*–1 μ*M* ACh concentration range.

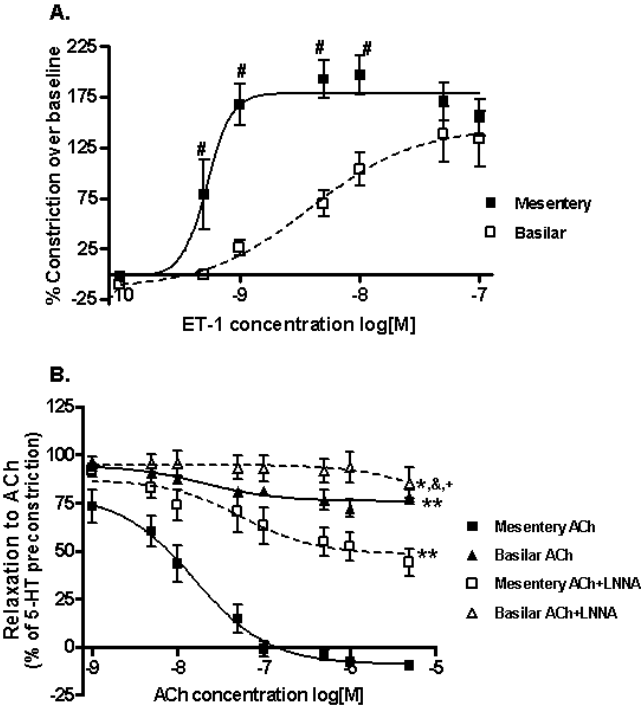

Figure 3. (A) Comparison of constriction responses to ET-1 in mesenteric and basilar arteries of GK rats. GK rats were more sensitive to ET-1, however, the magnitude of constriction was unaffected. Relaxation responses to ACh in the absence or presence of the e-NOS inhibitor, L-NNA, demonstrated that relaxation in response to ACh was impaired in the basilar arteries but not in the mesenteric arteries. With NO blockade, the approximately 50% relaxation was abolished in the mesentery artery, but relaxation in the basilar artery was almost null. #, EC_{50} $P < 0.05$ vs. basilar artery; *, R_{max} $P < 0.05$ vs. mesentery artery LNNA + ACh; **, R_{max} $P < 0.05$ vs. mesentery artery; &, EC_{50} $P < 0.05$ vs. mesentery artery LNNA + ACh; +, percent relaxation $P < 0.05$ vs. mesentery artery ACh, mesentery artery LNNA + ACh, or basilar artery LNNA + ACh across the 50 n*M*–1 μ*M* ACh concentration range.

the NO pathway is already dysfunctional in the cerebral circulation. The current study also demonstrated that endothelium-dependent relaxation is severely impaired in basilar arteries. In the presence of L-NNA, basilar arteries of GK rats fail to relax, whereas there is an approximately 50% relaxation in the mesenteric circulation, providing evidence that the NOS pathway is dysfunctional in the cerebral circulation of Type 2 diabetic animals as well, and further blockade with L-NNA does not have an impact on maximal relaxation.

It is well established that circulating and local ET-1 levels are elevated in both clinical and experimental Type 2 diabetes (11, 30–32). Antagonism of ET receptors prevents diabetic complications (33–35). In the current study, we found that the ET-1 response is enhanced in the mesenteric bed. Katakam *et al.* (36) reported an augmented ET-1 response and ET receptor expression in insulin-resistant rats, providing support for an activated ET system in diabetes. The current data demonstrate that mesenteric arteries are more sensitive to ET-1. Matsumoto *et al.* (16) reported an enhanced contractile response in Type 1 diabetes, providing further support for the findings of the current study.

In conclusion, this study demonstrates differences in macro versus microvascular dysfunction in a mild model of Type 2 diabetes. The lack of impaired function of the mesenteric microvessels in contrast to a decreased relaxation response in the basilar arteries of GK rats could possibly suggest that, in diabetes, there is a greater degree of impaired relaxation in the cerebral circulation than in the gut, and that this impairment occurs at an earlier time. Hyperreactivity to ET-1, however, is augmented in the mesenteric and not in the basilar arteries, suggesting that different pathways could be involved in different vascular beds, each contributing independently to vascular dysfunction.

1. American Heart Association. Heart Disease and Stroke Statistics—2005 Update. Dallas: American Heart Association, 2005.
2. Pyorala K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiologic view. Diabetes Metab Rev 3:463–524, 1987.
3. Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. Diabetes 48:937–942, 1999.
4. Taylor PD, Oon BB, Thomas CR, Poston L. Prevention by insulin treatment of endothelial dysfunction but not enhanced noradrenaline-induced contractility in mesenteric resistance arteries from streptozotocin-induced diabetic rats. Br J Pharmacol 111:35–41, 1994.
5. Diederich D, Skopec J, Diederich A, Dai FX. Endothelial dysfunction in mesenteric resistance arteries of diabetic rats: role of free radicals. Am J Physiol 266:H1153–1161, 1994.
6. Mayhan WG, Trauernicht AK, Irvine SD. Insulin reverses impaired acetylcholine-induced dilatation of the rat basilar artery during diabetes mellitus. Brain Res 893:195–201, 2001.
7. Cheng ZJ, Vaskonen T, Tikkanen I, Nurminen K, Ruskoaho H, Vapaatalo H, Muller D, Park J-K, Luft FC, Mervaala EMA. Endothelial dysfunction and salt-sensitive hypertension in spontaneously diabetic Goto-Kakizaki rats. Hypertension 37:433–439, 2001.
8. Witte K, Jacke K, Stahrenberg R, Arlt G, Reitenbach I, Schilling L, Lemmer B. Dysfunction of soluble guanylyl cyclase in aorta and kidney of Goto-Kakizaki rats: influence of age and diabetic state. Nitric Oxide 6:85–95, 2002.
9. Witte K, Reitenbach I, Stolpe K, Schilling L, Kirchengast M, Lemmer B. Effects of the endothelin a receptor antagonist darusentan on blood pressure and vascular contractility in type 2 diabetic Goto-Kakizaki rats. J Cardiovasc Pharmacol 41:890–896, 2003.
10. Haak T, Jungmann E, Felber A, Hillmann U, Usadel KH. Increased plasma levels of endothelin in diabetic patients with hypertension. Am J Hypertens 5:161–166, 1992.
11. Takahashi K, Ghatei MA, Lam HC, O'Halloran DJ, Bloom SR. Elevated plasma endothelin in patients with diabetes mellitus. Diabetologia 33:306–310, 1990.
12. Collier A, Leach JP, McLellan A, Jardine A, Morton JJ, Small M. Plasma endothelin-like immunoreactivity levels in IDDM patients with microalbuminuria. Diabetes Care 15:1038–1040, 1992.
13. Goto K, Hama H, Kasuya Y. Molecular pharmacology and pathophysiological significance of endothelin. Jpn J Pharmacol 72:261–290, 1996.
14. Khan ZA, Chakrabarti S. Endothelins in chronic diabetic complications. Can J Physiol Pharmacol 81:622–634, 2003.
15. Kiff RJ, Gardiner SM, Compton AM, Bennett T. The effects of endothelin-1 and NG-nitro-L-arginine methyl ester on regional haemodynamics in conscious rats with streptozotocin-induced diabetes mellitus. Br J Pharmacol 103:1321–1326, 1991.
16. Matsumoto T, Yoshiyama S, Kobayashi T, Kamata K. Mechanisms underlying enhanced contractile response to endothelin-1 in diabetic rat basilar artery. Peptides 25:1985–1994, 2004.
17. Alabadi JA, Miranda FJ, Llorens S, Centeno JM, Marrachelli VG, Alborch E. Mechanisms underlying diabetes enhancement of endothelin-1-induced contraction in rabbit basilar artery. Eur J Pharmacol 486: 289–296, 2004.
18. Dumont AS, Dumont RJ, McNeill JH, Kassell NF, Sutherland GR, Verma S. Chronic endothelin antagonism restores cerebrovascular function in diabetes. Neurosurgery 52:653–660; discussion 659–660, 2003.
19. Maritim AC, Sanders RA, Watkins JB III. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 17:24–38, 2003.
20. Turko IV, Marcondes S, Murad F. Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase. Am J Physiol 281:H2289–2294, 2001.
21. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. Circ Res 86:E85–90, 2000.
22. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, Channon KM. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 105:1656–1662, 2002.
23. Standaert ML, Sajan MP, Miura A, Kanoh Y, Chen HC, Farese RV Jr, Farese RV. Insulin-induced activation of atypical protein kinase C, but not protein kinase B, is maintained in diabetic (ob/ob and Goto-Kakazaki) liver. Contrasting insulin signaling patterns in liver versus muscle define phenotypes of type 2 diabetic and high fat-induced insulin-resistant states. J Biol Chem 279:24929–24934, 2004.
24. Farese RV, Standaert ML, Yamada K, Huang LC, Zhang C, Cooper DR, Wang Z, Yang Y, Suzuki S, Toyota T. Insulin-induced activation of glycerol-3-phosphate acyltransferase by a chiro-inositol–containing insulin mediator is defective in adipocytes of insulin-resistant, type II diabetic, Goto-Kakizaki rats. Proc Natl Acad Sci U S A 91:11040–11044, 1994.
25. Ergul A, Schultz Johansen J, Stromhaug C, Harris AK, Hutchinson J, Tawfik A, Rahimi A, Rhim E, Wells B, Caldwell RW, Anstadt MP. Vascular dysfunction of venous bypass conduits is mediated by reactive oxygen species in diabetes: role of endothelin-1. J Pharmacol Exp Ther 313:70–77, 2005.

26. Makino A, Ohuchi K, Kamata K. Mechanisms underlying the attenuation of endothelium-dependent vasodilatation in the mesenteric arterial bed of the streptozotocin-induced diabetic rat. Br J Pharmacol 130:549–556, 2000.
27. Erdos B, Snipes JA, Miller AW, Busija DW. Cerebrovascular dysfunction in Zucker obese rats is mediated by oxidative stress and protein kinase C. Diabetes 53:1352–1359, 2004.
28. Klein RL, Colwell JA. Altered endothelial function in diabetes mellitus. In: Sowers JR, Ed. Contemporary Endocrinology: Endocrinology of the Vasculature. Totowa, NJ: Humana Press Inc, 1996.
29. Brondum E, Nilsson H, Aalkjaer C. Functional abnormalities in isolated arteries from Goto-Kakizaki and streptozotocin-treated diabetic rat models. Horm Metab Res 37(Suppl 1):56–60, 2005.
30. Laurenti O, Vingolo EM, Desideri G, Ferri C, Bellini C, Cassone-Faldetta M, Santucci A, Mattia GD. Increased levels of plasma endothelin-1 in non-insulin dependent diabetic patients with retinopathy but without other diabetes-related organ damage. Exp Clin Endocrinol Diabetes 105(Suppl 2):40–42.1997.
31. Perfetto F, Tarquini R, Tapparini L, Traquini B. Influence of non-insulin dependent diabetes mellitus on plasma endothelin-1 levels in patients with advanced atherosclerosis. J Diabetes Comp 12:187–192, 1998.
32. Ak G, Buyukberber S, Sevine S, Turk HM, Ates M, Sari R, Savli H, Cigli A. The relation between plasma endothelin-1 levels and metabolic control, risk factors, treatment modalities, and diabetic microangiopathy in patients with Type 2 diabetes mellitus. J Diabetes Comp 15: 150–157, 2001.
33. Chakrabarti S, Cukiernik M, Mukherjee S, Chen S. Therapeutic potential of endothelin receptor antagonists in diabetes. Expert Opin Invest Drugs 9:2873–2888, 2000.
34. Balsiger B, Rickenbacher A, Boden PJ, Biecker E, Tsui J, Dashwood M, Reichen J, Shaw SG. Endothelin A-receptor blockade in experimental diabetes improves glucose balance and gastrointestinal function. Clin Sci (Lond) 103(Suppl 48):430S–433S, 2002.
35. Verma S, Arikawa E, McNeil J. Long-term endothelin receptor blockade improves cardiovascular function in diabetes. Am J Hypertens 14:679–687, 2001.
36. Katakam PV, Pollock JS, Pollock DM, Ujhelyi MR, Miller AW. Enhanced endothelin-1 response and receptor expression in small mesenteric arteries of insulin-resistant rats. Am J Physiol 280:H522–527, 2001.