Synergistic Effects of C-Peptide and Insulin on Coronary Flow in Early Diabetic Rats

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The aims of the present study are (1) to examine whether coronary flow is increased and (2) to examine the role of C-peptide in relation to nitric oxide (NO) production and coronary flow in a rat heart (Wistar) during the early stages of type 1 diabetes. Coronary flow increased by 36.4% ±10.6% ($P < .05$) during the early stages of streptozotocin-induced diabetes of isolated perfused rat hearts, but NO production increased without significance. C-peptide alone did not change coronary flow, but increased NO production in diabetes. In the presence of insulin, C-peptide reversed both flow and NO production to the control level of normal rats ($P < .05$). In conclusion, during the early stages of type 1 diabetes, coronary flow was increased, and C-peptide in the presence of insulin synergistically normalized the excessive flow and NO production induced by C-peptide to the control level of normal rats.

DIABETES MELLITUS is an independent risk factor for the development of and extent of coronary artery disease, and diabetic patients are at increased risk of coronary disease morbidity and mortality.[1] C-peptide is a connecting segment of proinsulin and is secreted from pancreatic β cells into the circulation along with insulin after the cleavage of proinsulin. Although C-peptide was formerly considered biologically inactive, more recent studies have suggested that it is a biologically active peptide and might be involved in the development of diabetic microangiopathy and neuropathy.[2,3]

Impairment of endothelium-dependent relaxation and consequent reduction of blood flow occur in diabetes, especially at the later stage.[4-8] Most studies on experimental diabetes have shown a progressive worsening of endothelial dysfunction. The importance of duration of diabetes has been pointed out, and it is now hypothesized that a phasic response of increased, unaltered, and impaired endothelium-dependent relaxation within the same model, but dependent on the duration of disease.[6] Based on this, we define the early stage as the period of diabetes with increased endothelium-dependent relaxation observed in the present study. At the early stage, it is still under debate whether coronary flow increases or not. Some in vivo studies have reported that renal cortical expression of all nitric oxide synthase (NOS) isoforms, including endothelial NOS (eNOS), are markedly increased in streptozotocin (STZ)-diabetic rats.[9] It is likely that eNOS expression at the early stage of diabetes is elevated. We hypothesized that coronary flow is increased in relation to NO during the early stages of diabetes.

Administration of C-peptide to insulin-dependent diabetic patients or diabetic animals has been shown to decrease glomerular hyperfiltration,[10-12] diminish the leakage of albumin or fluorescein across the blood-retina barrier,[10] increase glucose uptake in skeletal muscle,[13,14] and to improve autonomic nerve and microvascular disease.[10,12,14-16] All of these findings appear to be beneficial. As to the effects of C-peptide on cardiac vasculature, it remains unclear. We hypothesized that C-peptide may have another beneficial normalizing function to reduce increased flow during the early stage of diabetes. This study examines whether coronary flow is increased and whether C-peptide in the presence or absence of insulin reduces coronary flow during the early stages of diabetes.

MATERIALS AND METHODS

Only male Wistar rats were used in this study. At the age of 10 weeks, the diabetic group was injected with 50 mg/kg STZ, and the control group was given sham saline shots by tail-vein injection. Four weeks later, at the age of 14 weeks, the rats (n = 8 and 10, body weight = 340 ± 13 g and 283 ± 4 g, control and diabetic groups respectively, $P < .01$, Table 1) were anesthetized by diethyl-ether inhalation, and isolated hearts (wet heart weight, control *v* diabetes: 1.06 ± 0.05 g and 1.08 ± 0.03 g) were perfused in a Langendorff mode at a perfusion pressure of 100 cm H_2O. We adopted this preparation because the perfusion pressure can be strictly controlled and the oxygen saturation among other contents in the perfusate can be kept constant. A modified Krebs-Henseleit buffer solution (pH, 7.4) that was saturated by a mixed gas of 95% O_2 and 5% CO_2 was used. All the STZ-injected rats showed a high concentration of blood glucose, 400 ± 25 mg/dL, while that of the control rats was 102 ± 6 mg/dL ($P < .01$, Table 1).

After a 20-minute stabilization period perfusing a Krebs-Henseleit buffer, C-peptide (10 nmol/L) was then perfused. Another 15 minutes were allowed for stabilization.[15] Finally, insulin (1 μU/mL) of subphysiologic concentration was coperfused with C-peptide of 10 nmol/L concentration.[17] Coronary effluent flow rate from the pulmonary artery

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Table 1. Characteristics of the Study Rat Groups

	Control (n = 8)	Diabetes (n = 10)
Body weight (g)	340 ± 13	283 ± 4*
Wet heart weight (g)	1.06 ± 0.05	1.08 ± 0.03
BS before experiment (mg/dL)	102 ± 6	400 ± 25†
Basal coronary flow (mL/g heart weight/min)	6.6 ± 0.4	9.0 ± 0.7*
Basal NO production (nmol/g heart weight/min)	1.29 ± 0.43	1.71 ± 0.18
Effect of insulin	(n = 6)	(n = 7)
Coronary flow (%)	97.8 ± 6.9	96.4 ± 3.9
NO production (%)	102.6 ± 16.5	98.4 ± 15.5

NOTE. Values are expressed as means ± SD.
Abbreviation: BS, blood sugar level.
*$P < .05$ *v* basal control, †$P < .01$ *v* basal control.

was measured as coronary flow by an electromagnetic blood flowmeter (MFV-3200; Nihon Kohden, Tokyo, Japan). Effluent of 200 to 300 μL was collected for concentration measurement of oxidative products of NO derivatives, ie, nitrite and nitrate anions (NOx). NOx concentrations were measured by a NOx analyzer (ENO-20; Eicom, Kyoto, Japan).[18] This analyzer is, in principle, a combination of the Griess method[19] and high-performance liquid chromatography, allowing respective measurement of nitrite and nitrate concentration at a high sensitivity. NOx concentration was calculated by subtracting buffer NOx concentration from perfusate NOx concentration and used to calculate the NO production rate, which was evaluated as a product of the NOx concentration and coronary flow rate.

To confirm the absence of a vasoactive effect by 1 μU/mL insulin, a sole application of low-dose insulin at 1 μU/mL after perfusing a Krebs-Henseleit buffer was performed in normal and diabetic rats (n = 6 and 7, respectively).

Housing and anesthesia concurred with the guidelines set out by the committee of animal research at the Kawasaki Medical School, in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Modified Krebs-Henseleit bicarbonate buffer (11 D-glucose, 1.2 $MgSO_4$, 12 KH_2PO_4, 4.7 KCl, 120 NaCl, 25 $NaHCO_3$, 2.5 $CaCl_2 \cdot 2H_2O$, all in millimolars) was purchased from Sigma Chemical, St Louis, MO. STZ was purchased from Wako Pure Chemical Industries, Osaka, Japan. C-peptide was donated by Eli Lilly, Indianapolis, IN.

Data were expressed as means ± SE. The analysis of data on group characteristics and sole insulin perfusion experiments was performed either by the paired or unpaired *t* test for comparison. Data of coronary flow and NO production were analyzed by 1-way analysis of variance (ANOVA) followed by Fisher post hoc test. A *P* value less than .05 was considered statistically significant.

RESULTS

Coronary flow was significantly increased in diabetic rats compared with that of normal rats by 36.4% ± 10.6% ($P < .05$, Fig 1), while NO production showed only an increasing trend without statistical significance (Table 1 and Fig 2). Flow is expressed against 1 g myocardium, dividing the coronary flow by the weight of the wet myocardium of each rat.

Sole application of insulin at 1 μU/mL did not cause any significant change at the limits of our system in either flow or NO production in both diabetic and normal rats (see Table 1). NO production is expressed against 1 g myocardium, dividing the amount of NO production per minute by the weight of the wet myocardium of each rat.

C-peptide of 10 nmol/L showed an increasing tendency of coronary flow (9.0 ± 0.7 *v* 9.7 ± 0.7 mL/g heart weight/min) in diabetic rats compared with that of basal conditions ($P \cong .2$), but the low dose of insulin alone did not. However, it is noteworthy that concomitant perfusion of C-peptide and insulin to diabetic rats significantly decreased the flow increment in diabetic rats to 7.5 ± 0.6 mL/g heart weight/min ($P < .05$ *v* DM basal conditions and sole C-peptide, Fig 1), showing no significant flow difference compared with that of normal basal.

C-peptide of 10 nmol/L increased NO production significantly from 1.71 ± 0.18 to 2.85 ± 0.42 nmol/g heart weight/min in diabetic rats, showing significantly higher values compared with that of diabetic basal conditions. Concomitant perfusion of C-peptide and insulin (1 μU/mL) significantly decreased NO production to 1.28 ± 0.18 nmol/g heart weight/min in diabetic rats ($P < .05$, Fig 2) from the increased NO production induced by C-peptide, resulting in the simi-

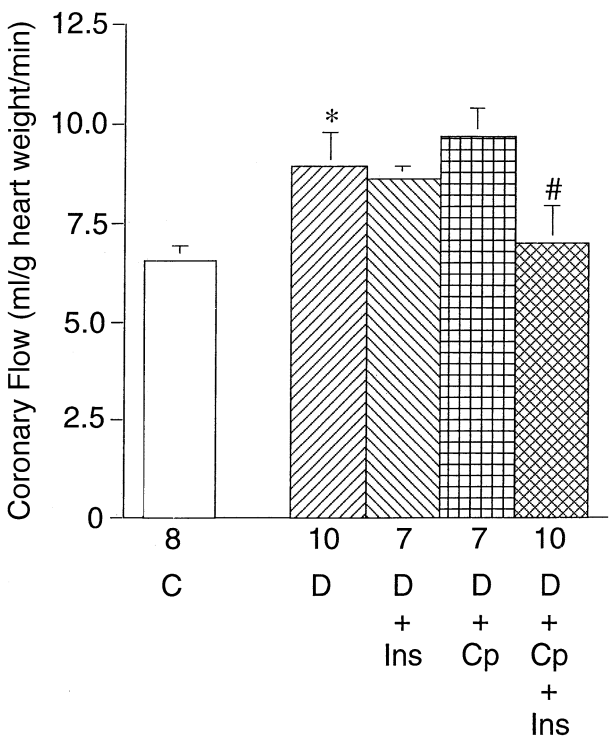

Fig 1. Coronary flow (derived from pulmonary arterial effluent volume (mL/g heart weight/min)) at basal states and after C-peptide (10 nmol/L) and/or insulin (1 μU/mL) administration into Langendorff heart preparation of normal and diabetic rats. Significant differences from normal basal flow were found against the flows of diabetic basal and sole C-peptide administration groups. A significant difference was found against diabetic basal flow after concomitant administration of C-peptide and insulin to diabetic rats. The difference of flow between normal control rats and diabetic rats with both C-peptide and insulin was not significant. Sole insulin or C-peptide administration did not cause any flow change in diabetic rats. Numbers under the bars indicate the number of samples adopted for analysis. *$P \leq .05$ (C *v* D); #$P \leq .05$ (D + Cp *v* D + Cp + Ins). C, normal; D, diabetic; Ins, insulin; Cp, C-peptide.

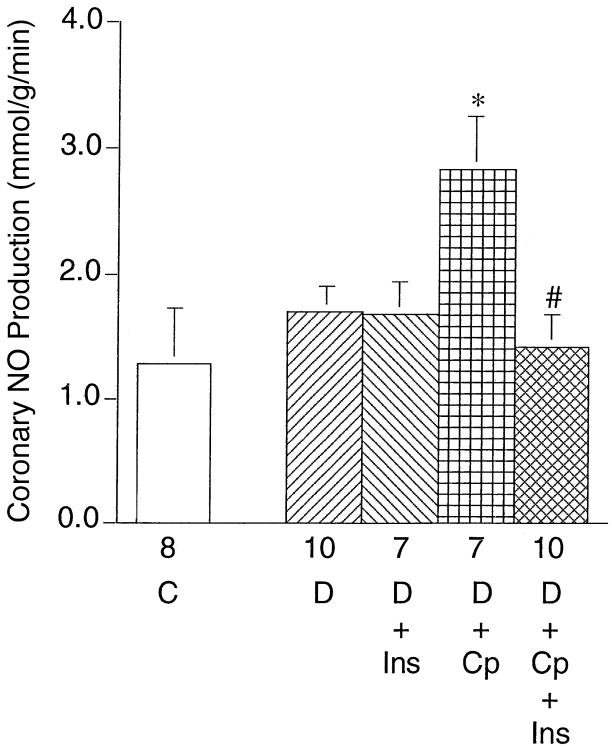

Fig 2. Coronary NO production (pulmonary arterial effluent rate multiplied by NOx concentration (nmol/g heart weight/min)) at basal states and after C-peptide (10 nmol/L) and/or insulin (1 μU/mL) administration into Langendorff heart preparation of normal and diabetic rats. There was an increasing trend of NO production in diabetic control rats compared with that of normal control rats without statistical significance. A significant difference was found after sole C-peptide administration in diabetic rats compared with normal control rats. The difference in NO production between normal control rats and diabetic rats with both C-peptide and insulin was not significant. Sole insulin administration did not cause any NO production change in diabetic rats. Numbers under the bars indicate the number of samples adopted for analysis. *$P \leq .05$ (D *v* D + Cp); #$P \leq .05$ (D + Cp *v* D + Cp + Ins). C, normal; D, diabetic; Ins, insulin; Cp, C-peptide.

lar NO production value to normal basal without significant difference.

DISCUSSION

Increase of Coronary Flow

Basal coronary flow was significantly increased in diabetic rats compared with the normal basal flow (Figs 1 and 2). Diabetes, as reported by Pieper[20] with much attention on disease duration, affects the tissue and the vasculature biphasically both at the early stage and at the later stage. He hypothesized that an early increase in blood flow would be followed by a transition state to impaired relaxation. Diabetes mellitus is a major cause of ischemic coronary artery disease. Cardiac complications in diabetes mellitus are frequently ascribed to microangiopathy. Although there have been several studies on coronary flow in diabetes,[21-23] there are few that deal with the early diabetes, clinically or experimentally. Under these circumstances, the role of NO in the control of coronary flow during development is still unknown.[24] Functional abnormalities of the coronary microcirculation have been reported in experimental diabetes mellitus. Recently, the role of the endothelium in the regulation of coronary arterial dimensions has gained a lot of attention.[25] We have focused on coronary flow during the early stages of diabetes, in particular, in the present study. We adopted a 4-week model of moderate diabetes in the present study as the early stage of experimental diabetes following the study by Ido and others.[12,20]

There have been few studies focused on early diabetes that deal with the relation of coronary flow and NO production quantitatively in rat hearts. In long-lasting diabetes, endothelium-dependent vascular relaxation is impaired and blood flow is reduced.[4-8] However, blood flow alteration during the early stages of diabetes in various organs has been debated among studies.[6,7,20] In the early stages of diabetes, renal plasma flow was reported to be increased.[26] Recently, it was hypothesized that the balance between enhancement and suppression of NO bioavailability is shifted towards NO enhancement during the early phases of nephropathy.[9] Supportively, we observed afferent and efferent arteriolar vasodilatation by our intravital-microscopy.[27] The mechanism of coronary flow increases in the early stage of diabetes will be considered next.

The increase of coronary flow during the early stages of diabetes can be explained by the following hypotheses, pseudohypoxia, or activation of protein kinase C due to hyperglycemia.[4,6,7,28] Increased flow may be viewed as a normal compensatory reaction to hypoxia.[6,7,20] Pseudohypoxia is a characteristic state for diabetes mellitus in which the cytosolic ratio of free NADH/NAD^+ is increased mimicking the effects of true hypoxia.[5] The effects of pseudohypoxia are mediated by a branching cascade of imbalances in lipid metabolism, increased production of superoxide anion, and possibly, increased NO formation. In the other hypothesis, protein kinase C (PKC) activation, mediated by elevated glucose, may lead to an alteration in endothelial prostaglandin synthesis and play a role in the development of diabetic vascular complications.[28,29] The interaction of PKC and NOS has also been indicated.[30] Therefore, an increase of NO production may be related to flow increase during the early stages of diabetes.[31] The elevated coronary flow causes high shear stress and consequently NO is produced further to counteract high shear stress.[31] A recent study by Milsom et al[32] has reported that there is increased nitrosyl hemoglobin (HbNO) in type 1 diabetic patients compared with blood isolated from control subjects. If blood is perfused in the present study, their finding partly contributes to the reason why NO is not significantly increased in a manner matching flow increase in STZ-induced diabetic rats, which implies entrapment of increased NO by hemoglobin (Hb). Because a buffer solution was perfused instead, NO may not be the sole factor for enhanced coronary flow. Rosen et al[33] reported that the basal release of NO was not changed by diabetes lasting from 5 to 26 weeks, stressing the role of accelerated inactivation of NO by superoxide anions. Thus, it remains to be resolved whether the enhanced flow during the early stages might be due to combination effects of other vasoactive substances with NO, such as prostaglandins, endothelin, endothelium-derived hyperpolarizing factor (EDHF), and so on.[7] We speculate that the mismatched excessive coronary flow causes enhanced mechanical stress by both micro-

vascular hypertension and high wall shear stress and may initiate and develop microangiopathy of diabetes leading to endothelial dysfunction. From the literature, renal cortical expression of all NOS isoforms, including eNOS, is markedly increased in STZ-diabetic rats.[9] We speculate that eNOS expression is elevated. It requires further study with NOS inhibition to determine the effect of NO on the increased coronary flow in diabetic rats.

C-Peptide Action

C-peptide administration did not change coronary flow, but significantly increased coronary NO production in diabetic rats, and concomitant administration of C-peptide and insulin decreased coronary flow and NO production in diabetic rats significantly from the levels when C-peptide was administered solely, obtaining the values of basal flow conditions in control rats (Figs 1 and 2).

It is known that in healthy human subjects no significant influence was exerted by C-peptide on blood flow or oxygen uptake.[3] The concentration of 10 nmol/L C-peptide is reported to show effects[15,17] in diabetic patients, unlike in healthy subjects, while 1 μU/mL insulin is reported to exert no physiologically vasoactive effect in both healthy and diseased subjects.[17] Our confirming additional experiment using 1 μU/mL insulin did not show any flow change or NO production change. Thus, this dose of insulin is considered to be below the physiologic vasodilating concentration.

It is now understood that activation of the C-peptide receptor is coupled to a G protein.[34] Subsequently, Na^+-K^+ adenosine triphosphatase (ATPase), and Ca^{2+}-dependent intracellular pathways are activated resulting in stimulation of endothelial NOS activity.[35] This mechanism may be the reason for NO production increase in the present study when C-peptide is administered solely. Although NO production was increased by C-peptide, a matching flow increase was not observed, only a slight flow increase. One reason is that blood vessels may be dilated to some extent in this preparation by crystalloid buffer perfusate (see Table 1), resulting in decreased vasodilatory capacity. Although this is the limitation of the Langendolff experiment, the preparation was necessary and appropriate to keep perfusion pressure strictly constant and to keep the contents of perfusate the same without any influence from the systemic neurohumoral effects. Another reason may be due to NO inhibitory agents and/or vasoconstrictive substances produced in diabetes, such as superoxide anion, endothelin, and vasoconstrictive prostaglandins, counteracting the vasodilatory effects of NO.[7,28,36-39]

The results in the present study demonstrated the synergistic effect of C-peptide and insulin. Concomitant administration with C-peptide and insulin caused a reduction of coronary flow, as well as NO production, compared with the results for C-peptide alone. It has been demonstrated that C-peptide may act in synergism with some hormones. Recently, synergism of C-peptide and neuropeptide Y has been reported[40] with C-peptide augmenting the vasoconstricting effects of neuropeptide Y. The present study also indicates synergistic action of C-peptide and insulin, presumed to be the correction of exaggerated coronary flow in early diabetes. The mechanisms underlying the synergistic effects are not clear and need further investigation. Schroeder et al[41] reported that insulin has bidirectional vasoactive properties, ie, insulin indirectly evokes an arteriolar dilatation that is endothelium-dependent and NO-mediated, while through a direct effect on the vascular smooth muscle, evokes arteriolar constriction. On the other hand, Jensen and Messina[17] reported that a higher dose of C-peptide induces dilation of skeletal muscle arterioles in the presence of insulin. The possibility that increased oxidative inactivation of NO by enhanced vascular superoxide production and/or a reduced superoxide-scavenging capacity may have contributed to impair or prevent any responsiveness of vascular smooth muscle cell to C-peptide. Alternatively, the stimulating effect of insulin on endothelin production in diabetic rats, added to the attenuating effect observed on the NO production stimulated by C-Peptide, may have contributed to the reduction in coronary flow observed during concomitant administration of C-peptide and insulin. Although the difference in species, organs, disease model, and drug concentrations might be involved in the variation of reported outcome, the synergistic action of C-peptide and insulin on endothelium and/or smooth muscle may play a crucial role in restoring the excessive coronary flow in early diabetes in the present study.

Conclusions

In conclusion, coronary flow was increased during the early stages of diabetes mellitus in rats. C-peptide alone increased coronary NO production without affecting flow. In the presence of insulin, C-peptide restored flow and NO production, reducing an increase of flow induced by diabetes and an increase of NO production caused by C-peptide.

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REFERENCES

1. Kannel WB, McGee DL: Diabetes and cardiovascular disease: The Framingham Study. JAMA 241:2035-2038, 1979

2. Wahren J, Johansson BL: New aspects of C-peptide physiology. Horm Metab Res 30:A2-5, 1998

3. Kunt T, Forst T, Pfutzner A, et al: The physiological impact of proinsulin C-peptide. Pathophysiology 5:257-262, 1999

4. Rodriguez-Manas L, Angulo J, Peiro C, et al: Endothelial dysfunction and metabolic control in streptozotocin-induced diabetic rats. Br J Pharmacol 123:1495-1502, 1998

5. Williamson JR, Chang K, Frangos M, et al: Perspectives in diabetes hyperglycemic pseudohypoxia and diabetic complications. Diabetes 42:801-813, 1993

6. Pieper GM: Review of alterations in endothelial nitric oxide reduction in diabetes protective role of arginine on endothelial dysfunction. Hypertension 31:1047-1060, 1998

7. King GL, Kunisaki M, Nishio Y, et al: Biochemical and molecular mechanisms in the development of diabetic vascular complications. Diabetes 45:S105-S108, 1996 (suppl 3)

8. Johannson BL, Kernell A, Sjoberg S, et al: Influence of combined C-peptide and insulin administration on renal function and

metabolic control in diabetes type 1. J Clin Endocrinol Metab 77:976-981, 1993

9. Komers R, Anderson S: Paradoxes of nitric oxide in the diabetic kidney. Am J Physiol 284:F1121-1137, 2003 (review)

10. Sjoquist M, Huang W, Johannson BL: Effects of C-peptide on renal function at the early stage of experimental diabetes. Kidney Int 54:758-764, 1998

11. Johansson BL, Sjoberg S, Wahren J: The influence of human C-peptide on renal function and glucose utilization in type 1 diabetic patients. Diabetologia 35:121-128, 1992

12. Ido Y, Vindigni A, Chang K, et al: Prevention of vascular and neural dysfunction in diabetic rats by C-peptide. Science 277:563-566, 1997

13. Johansson BL, Linde B, Wahren J: Effects of C-peptide on blood flow, capillary diffusion capacity, and glucose utilization in the exercising forearm of type 1 diabetic patients. Diabetologia 35:1151-1158, 1992

14. Winegrad AI: Does a common mechanism induce the diverse complications of diabetes? Diabetes 36:396-406, 1987

15. Forst T, Kunt T, Pohlmann T, et al: C-peptide on the skin microcirculation in patients with insulin-dependent diabetes mellitus. J Clin Invest 101:2036-2041, 1998

16. Lindstrom K, Johansson C, Johnsson E, et al: Acute effects of C-peptide on the microvasculature on isolated perfused skeletal muscles and kidneys in rat. Acta Physiol Scand 156:19-25, 1996

17. Jensen ME, Messina EJ: C-peptide induces a concentration-dependent dilation of skeletal muscle arterioles only in presence of insulin. Am J Physiol 276:H1223-1228, 1999

18. Ishibashi T, Matsubara T, Ida T, et al: Negative NO_3- difference in human coronary circulation with severe atherosclerotic stenosis. Life Sci 66:173-184, 2000

19. Green LC, Wagner DA, Glogowski J, et al: Analysis of nitrate, nitrite, and (15N) nitrate in biological fluids. Anal Biochem 126:131-138, 1982

20. Pieper GM: Enhanced, unaltered and impaired nitric-mediated endothelium-dependent relaxation in experimental diabetes mellitus: Importance of disease duration. Diabetologia 42:204-213, 1999

21. Gattullo D, Pagliaro P, Linden RJ, et al: The role of nitric oxide in the initiation and in the duration of some vasodilator responses in the coronary circulation. Pflugers Arch 430:96-104, 1995

22. Drexler H, Hornig B: Endothelial dysfunction in human disease. J Mol Cell Cardiol 31:51-60, 1999 (review)

23. Graier WF, Wascher TC, Lackner L, et al: Exposure to elevated D-glucose concentrations modulates vascular endothelial cell vasodilatory response. Diabetes 42:1497-1505, 1993

24. Zhao G, Zhang X, Smith CJ, et al: Reduced coronary NO production in conscious dogs after the development of alloxan-induced diabetes. Am J Physiol 277:H268-278, 1999

25. Mandinov L, Kaufmann P, Maier W, et al: Flow-dependent vasodilation in the coronary circulation: Alterations in diseased states. Semin Interv Cardiol 3:5-12, 1998 (review)

26. Tooke JE: Microcirculation and diabetes. Br Med Bull 45:206-223, 1989

27. Yamamoto T, Tomura Y, Tanaka H, et al: In vivo visualization of characteristics of renal microcirculation in hypertensive and diabetic rats. Am J Physiol 281:F571-F577, 2001

28. Tesfamariam B, Brown ML, Cohen RA: Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase C. J Clin Invest 87:1643-1648, 1991

29. Heygate KM, Lawrence I-G, Bennett MA, et al: Impaired endothelium-dependent relaxation in isolated resistance arteries of spontaneously diabetic rats. Br J Pharmacol 116:3251-3259, 1995

30. Huang Q, Yuan Y: Interaction of PKC and NOS in signal transduction of microvascular hyperpermeability. Am J Physiol 273: H2442-2451, 1997

31. Joannides R, Haefeli WE, Linder L, et al: Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 91:1314-1319, 1995

32. Milsom AB, Jones CJ, Goodfellow J, et al: Abnormal metabolic fate of nitric oxide in type I diabetes mellitus. Diabetologia 45:1515-1522, 2002

33. Rosen P, Ballhausen T, Bloch W, et al: Endothelial relaxation is disturbed by oxidative stress in the diabetic rat heart: Influence of tocopherol as antioxidant. Diabetologia 38:1157-1168, 1995

34. Forst T, Kunt T, Pfutzner A, et al: New aspects on biological activity of C-peptide in IDDM patients. Exp Clin Endocrinol Diabetes 106:270-276, 1998

35. De La Tour DD, Jannot DR, Coste T, et al: Erythorocyte Na/K ATPase activity and diabetes: Relationship with C-peptide level. Diabetologia 41:1080-1084, 1998

36. Baynes JW: Role of oxidative stress in development of complications in diabetes. Diabetes 40:405-412, 1991

37. Chang KC, Chung SY, Chong WS, et al: Possible superoxide radical-induced alteration of vascular reactivity in aortas from streptozotocin-treated rats. J Pharmacol Exp Ther 266:992-1000, 1993

38. Loke KE, Laycock SK, Mital S, et al: Endogenous endothelial nitric oxide synthase-derived nitric oxide is a physiological regulator of myocardial oxygen consumption. Circ Res 84:840-845, 1999

39. Ammar RF Jr, Gutterman DD, Brooks LA, et al: Free radicals mediate endothelial dysfunction of coronary arterioles in diabetes. Cardiovasc Res 47:595-601, 2000

40. Johansson BL, Pernow J: C-peptide potentiates the vasoconstrictor effect of neuropeptide Y in insulin-dependent diabetic patients. Acta Physiol Scand 165:39-44, 1999

41. Schroeder CA, Chen YL, Messina EJ: Inhibition of NO synthesis or endothelium removal reveals a vasoconstrictor effect of insulin on isolated arterioles. Am J Physiol 276:H815-H820, 1999