Reactive oxygen-derived free radicals are key to the endothelial dysfunction of diabetes

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Abstract

Vascular complications are an important pathological issue in diabetes, which lead to further functional deterioration of several organs. The balance between endothelium-dependent relaxing (EDRF) and endothelium-dependent contracting (EDCF) factors is crucial in controlling local vascular tone and function under normal conditions. Diabetic endothelial dysfunction is characterized by reduced endothelium-dependent relaxations and/or enhanced endothelium-dependent contractions. Elevated levels of oxygen-derived free radicals are the initial source of endothelial dysfunction in diabetes. Oxygen-derived free radicals not only reduce nitric oxide bioavailability, but also facilitate the production and/or the action of EDCFs. Thus, the endothelial balance tips towards vasoconstrictor responses in the course of diabetes.

Key words:

Diabetes mellitus, type 1

Diabetes mellitus, type 2

Endothelium-dependent contraction

Endothelium-derived hyperpolarizing factor

Endothelial dysfunction

Nitric oxide

Oxidative stress

Reactive oxygen-derived free radicals

Prostanoids

Thromboxane-prostanoid (TP-) receptors

Diabetes mellitus groups heterogeneous etiologies, but shares abnormalities in carbohydrate, fat and protein metabolisms. Long-term diabetes affects many organ systems. The vascular consequences of the disease can be subdivided into microvascular (retinopathy and nephropathy) and macrovascular complications (coronary and peripheral arterial disease). Whereas the former lesions are relatively unique for diabetes, the latter are similar both morphologically and functionally with atherosclerotic lesions due to other causes. Since diabetes is such an important risk factor for the occurrence of atherosclerosis and coronary heart disease, not surprisingly cardiovascular disease is the leading cause of morbidity and mortality in diabetic patients [1].

A large body of evidence demonstrates the impairment of endothelium-dependent vasodilatation in cardiovascular diseases such as hypertension, hypercholesterolemia, atherosclerosis, and heart failure [2-5]. However the available data are not so conclusive as regards to diabetes [6]. Thus, the present non-exhaustive review will focus on the impact of diabetes on vascular dysfunction, in particular on endothelium-derived vasoactive factors.

Endothelial Dysfunction in Diabetes

Endothelium-derived Nitric oxide is essential for the local control of vascular diameter [7-9].

In type I diabetic animals, acetylcholine-induced relaxations are potentiated in the rat aorta and canine coronary arteries [10-12], normal in the aorta [13-16] and the mesenteric arteries [14] of the rat, and/or depending on the authors, blunted in the same blood vessels[17-18]. Moreover, NO-mediated relaxation is augmented at the early stage of diabetes followed by an impaired relaxation as the disease progresses [19-20]. Likewise, in type I diabetes patients, endothelium-dependent relaxations are impaired [4, 21-23], normal [24-28] or enhanced [29].

In type II diabetic animals, NO-mediated relaxation is increased [30], preserved [31] or reduced [32] in the aorta of obese Zucker fatty rats. Blunted NO-mediated relaxations are observed in carotid and mesenteric arteries from Zucker rats [33-34], mesenteric arteries from db/db mice [35-36] and coronary arteries of db/db mice [37-38]. In type II diabetes patients, blunted dilatations are observed consistently [39-40]. The evidence suggesting an impairment of endothelium-dependent vasodilatation in type II diabetes is more obvious than in type I diabetes, since the former is almost inevitably confounded by the high prevalence of other cardiovascular risk factors that are known to affect endothelial functions [41]. Several studies demonstrated impaired endothelium-dependent vasodilatation in patients with type II diabetes, but even after rigorous patient selection, mild dyslipidaemia or hypertension were often present [42-43]. Thus it remains unclear whether or not diabetes type II *per se* affects endothelial function.

Prostacyclin (PGI_2) is not only an endothelium-derived relaxing factor [44-46] but also is an important inhibitor of platelet aggregation [47].

In type I diabetes, prostacyclin contributes to the enhanced acetylcholine-induced and endothelium-dependent relaxation in the coronary artery of dogs [10] and the aorta of the mouse [48]. In addition, the oral administration of prostacyclin analogues restores the blunted

relaxation to acetylcholine in the rat aorta [49]. These results indicate that prostacyclin takes part in the preservation of endothelium-dependent relaxation in diabetes, especial in type I.

Endothelium-derived hyperpolarizing factors (EDHF) represent the third endothelium-dependent vasodilator pathway(s) [50].

EDHF-mediated responses are observed in certain blood vessels in the presence of inhibitors of cyclooxygenases and NO synthases and are associated with hyperpolarization of the vascular smooth muscle cell [50-55]. EDHFs play a more pronounced role in the smaller arteries [56-57]. They are also regarded as an important compensatory mechanism in the absence or blockade of the production of nitric oxide [58-62]. However the correlation between diabetes and endothelium-dependent hyperpolarization remains unclear.

In type I diabetes, the endothelium-dependent response to acetylcholine attributed to EDHF is depressed in isolated rings of mesenteric arteries from streptozotocin-treated rats and mice, while the nitric oxide component is preserved [63-66]. Nonetheless in the mesenteric artery of the same rat model, an augmented contribution of EDHF compensates for the blunted nitric oxide response [18].

In type II diabetes, EDHF-mediated relaxations are reduced in the mesenteric artery of Goto-Kakizaki rats [67], Zucker obese rats [68], and Otsuka-Long-Evans-Tokushima Fatty (OLETF) rats [69] when the nitric oxide mediated responses are not affected. However, the EDHF component is augmented in genetic diabetic (db/db) mice when nitric oxide bioavailability is reduced [70].

Endothelium-derived and cyclooxygenase-dependent contracting factor (EDCF) is a hallmark of endothelial dysfunction. The product of cyclooxygenase activates thromboxane-prostanoid (TP-) receptors [71-75]. The nature of endothelium-derived contracting factor depends

on the experimental model studied and the stimulating agents used. It can be endoperoxide [76-77], thromboxane A_2 [78-79], prostaglandin E_2 [79], or prostacyclin [80-82].

In type I diabetes, the EDCF-mediated response is potentiated in the aorta of rabbits [83], the aorta of mice [84] and the femoral artery of rats (Figure 1) [79]. In the mesenteric artery from rats with alloxan-induced diabetes, an increased production of prostanoids, presumable prostaglandin $F_{2\alpha}$, reduces the arterial diameter [85].

In the renal artery of a type II diabetes model, the OLETF rat, indomethacin restores the blunted relaxation to acetylcholine [86]. Similar results were obtained in the aorta from db/db mice [87]. An augmented EDCF-mediated contraction is observed in the mesenteric artery of OLETF rats [88-89].

Endothelin (ET) is also a hallmark of endothelial dysfunction. The contribution of ET-1 is not obvious in the early stages of diseases, indicating that the production of endothelin reflects an advanced or even terminal pathology [90].

An ET-1 antagonist, J-104132 normalizes the up-regulation of p22phox subunit of NAD(P)H oxidase in streptozotocin-treated rats [91]. Moreover, the dual blockade of ETA and ETB receptors enhances the endothelium-dependent vasodilatation to acetylcholine in insulin-resistant patients [92]. These observations suggest that ET-1 may play a role in the endothelial dysfunction of diabetes.

Reactive Oxygen Specials in Diabetes

Reactive oxygen specials (ROS) are generated during normal metabolic processes, but at supra physiological levels they cause DNA mutation, protein oxidation and lipid peroxidation. Elevated production of reactive oxygen species is implicated in atherosclerosis, hypertension, cancer, diabetes and premature aging [93].

Oxygen-derived free radical species originate from molecular oxygen. Superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and peroxynitrite ($ONOO^-$) fall into this group. The special feature of these agents is their high oxidative reactivity, creating a significant threat for redox-sensitive components. Superoxide anions are the primary oxygen-derived free radicals. They have a short half-life and are intracellular-restrained molecules. Unlike superoxide anion, hydrogen peroxide diffuses freely within tissues since it is not charged. The metal catalyzed interaction between hydrogen peroxide and superoxide anion produces hydroxyl radical. Reactive oxygen species can either be generated exogenously or produced intracellularly from several different sources. Cytosolic enzyme systems contributing to oxidative stress include the family of NADPH oxidases [94], mitochondria [95]. In addition, xanthine oxidase, cytochrome P450(s), cyclooxygenases and NO synthase are capable of producing reactive oxygen species [96].

The burden of free radical production is largely counteracted by the antioxidant defense system that includes the enzymatic scavenger superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). Superoxide dismutase speeds up the conversion of superoxide anions to hydrogen peroxide, whereas catalase and glutathione peroxidase convert hydrogen peroxide to water. A variety of other non-enzymatic, low molecular mass molecules are important in scavenging reactive oxygen species. These include ascorbate, pyruvate, flavonoids, and carotenoids, and perhaps most importantly, glutathione, which is present in millimolar concentrations within cells (Figure 2).

Imbalance of the oxygen-derived free radicals and antioxidant defense

Diabetes increases the level of reactive oxygen species [96-102]. These radicals play a crucial role in the pathogenesis of diabetes-associated vascular complications [96, 103-105]. However, neither the exact source of the free radicals initiating the cascade leading to cell dysfunction in diabetes nor their exact chemical nature is fully understood. Under hyperglycemic and diabetic conditions, glycation reactions produce intermediate compounds that also increase the levels of reactive oxygen species [6, 103, 106-111]. Dicarbonyl compounds such as methyglyoxal and 3-deoxyglucosone, intermediate products of glycation, are typical examples. The serum concentrations of both methyglyoxal and 3-deoxyglucosone are increased in patients with diabetes mellitus [112].

In diabetes, it is also reported that the augmented production of oxidative specials results from a reduced activity of superoxide dismutase (Figure 3) [34, 100, 113-116] and catalase (Figure 4) [100, 117], from reduced total glutathione level [100, 118], from increased activity of glutathione peroxidase [119-120], and from up-regulation of NADPH oxidase [34, 38, 115, 121-122] and cyclooxygenase (Figure 5) [79, 88-89, 123].

NO and ROS

Obviously nitric oxide is not the sole answer to the endothelial dysfunction of diabetes. The obligatory role of endothelium-derived nitric oxide was first reported in 1980 [7]. It is generated through the oxidation of L-arginine to L-citrulline by NO synthase (NOS). In blood vessels, endothelial NO synthase (eNOS) is constitutively active in the endothelium but the activity of the enzyme can be augmented further by stimuli that increase the intracellular calcium concentration[124]. The impact of diabetes on the presence of nitric oxide synthase is controversial. It is reduced [34, 37, 88, 116, 125], normal [79, 123, 126-127], or enhanced [128-129]. Since nitric oxide has a half-life of only a few seconds [130] and is rapidly inactivated by hemoglobin and superoxide [8, 130], the concept of loss of nitric oxide bioavailability is more precise and

important[131]. The blunted nitric oxide mediated response can be restored by the L-arginine, the substitute of nitric oxide synthase [132-133] or BH4, an essential cofactor of nitric oxide synthase [20, 36]. Treatment with antioxidants also prevents the blunted relaxation [36].

Uncoupled eNOS is an important source of reactive oxygen species under pathological condition [134-135]. The production of peroxynitrite, a strong cytotoxic oxidant which is formed by the combination of nitric oxide and superoxide anions, also contributes to endothelial dysfunction [129, 136]. When prostacyclin synthase in isolated arteries is inactivated by peroxynitrition, prostacyclin-dependent vasodilatation is replaced by prostaglandin-dependent vasoconstriction [131].

EDHF and ROS

Since the correlation between diabetes and endothelium-dependent hyperpolarization is inconclusive yet, it is difficult to link EDHF and oxidative stress. Interestingly, hydrogen peroxide is proposed to be an endothelium-derived hyperpolarizing factor in the mesenteric artery of type II diabetic mice [137-138], the mesenteric artery of humans [139] and the porcine coronary artery [140].

EDCF and ROS

Oxygen-derived free radicals play an important role in the occurrence of endothelium-dependent contractions in the basilar artery of the dog and the aorta of the spontaneously hypertensive rat [74, 81, 141-144]. ROS potentiates endothelium-derived contracting factor in the aorta of the spontaneously hypertensive rats [144] and the femoral artery of streptozotocin-induced type I diabetic rats (unpublished observation). The potentiating effect was not observed in the artery from control rats, suggesting that the redox abnormality is seen only under certain disease conditions.

Oxygen-derive free radicals are also directly involved in the occurrence of the endothelium-

derived contractions, since an increased oxidative state, measured by DCF fluorescence intensity using confocal microscopy, accompanies the genesis of endothelium-dependent contractions to acetylcholine or the calcium ionophore A23187 [81, 100]. Furthermore, tiron (an intracellular scavenger of superoxide anion) reduces endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat [144] and the femoral artery of rats with streptozotocin-induced diabetes (Figure 6a) [100], indicating that superoxide anions inside endothelial cells are a primary oxidative contributor to endothelium-dependent contractions [72, 100, 144]. Catalase and deferoxamine reduce the endothelium-dependent contraction in the aorta of hypertensive rats [144] and in the femoral artery of diabetic rats (Figure 6b) [100], suggesting that hydrogen peroxide could be, at least the precursor of endothelium-derived contracting factor.

In addition, hydroxyl radicals *per se* have been proposed as candidates for endothelium-derived contacting factors. In the femoral artery of streptozotocin-induced type I diabetes, hydrogen peroxide produces moderate contractions in rings without endothelium which are prevented by catalase and deferoxamine, indicating that the contraction generated by exogenous H_2O_2 is presumably due to hydroxyl radicals, rather than hydrogen peroxide itself. The hydroxyl radicals-mediated contraction is comparable in amplitude to the deferoxamine-sensitive part of the endothelium-dependent contraction in the same artery [100]. A similar conclusion has been reached in the aorta of spontaneously hypertensive rats [144].

Antioxidant treatment

It seems reasonable to hypothesize that antioxidant treatment would prevent endothelial dysfunction in diabetes. In diabetic animals, vitamin E alone or combined with insulin prevents endothelial dysfunction [97, 145]. Exercise which is assumed to reduce oxidative stress *in vivo* improves endothelial dysfunction [116]. Folic acid restores the impaired endothelial function as well [41]. Likewise, in diabetes patients, vitamin E, vitamin C, or folic acid improve

endothelial dysfunction [147-151]. However, trials with these beneficial results are carried out in small-groups of patients whereas in large, long-term clinical trials, neither vitamin E nor vitamin C reduces the risk of cardiovascular events [152-154]. Therefore, caution is required before drawing a conclusion as to the effectiveness of antioxidant treatments *in vivo* for the prevention of endothelial dysfunction in diabetes.

Conclusion

The endothelial dysfunction of diabetes includes reduced nitric oxide bioavailability and/or enhanced endothelium-dependent contractions which can be largely attributed to increased oxidative stress (Figure 7). Since the results of antioxidants in large clinic trials are not consistent with those in experimental animals, further investigation is critical to assess the exact role of ROS on the genesis of endothelial dysfunction, in particular in diabetes.

Disclosure

The authors declare no conflict of interest.

References

1. Goff DC Jr, Gerstein HC, Ginsberg HN et al., Prevention of cardiovascular disease in persons with type 2 diabetes mellitus: current knowledge and rationale for the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Am J Cardiol.* 2007;**99**:4i-20i

2. Lüscher TF, Yang ZH, Diederich D, Buhler FR. Endothelium-derived vasoactive substances: potential role in hypertension, atherosclerosis, and vascular occlusion. *J Cardiovasc Pharmacol.* 1989; **14** Suppl 6:S63-9.

3. Raij L. Hypertension, endothelium, and cardiovascular risk factors. *Am J Med*. 1991; **90**:S13-18.

4. Makimattila S, Virkamaki A, Groop PH et al., Chronic hyperglycemia impairs endothelial function and insulin sensitivity via different mechanisms in insulin-dependent diabetes mellitus. *Circulation*. 1996; **94**:1276-1282.

5. Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. *Acta Pharmacol Sin* 2009 **(in press).**

6. De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. *Br J Pharmacol*. 2000;**130**:963-974.

7. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980; **288**:373-376.

8. Martin W, Furchgott RF, Villani GM, Jothianandan D. Phosphodiesterase inhibitors induce endothelium-dependent relaxation of rat and rabbit aorta by potentiating the effects of spontaneously released endothelium-derived relaxing factor. *J Pharmacol Exp Ther.* 1986; **237**:539-547.

9. Moncada S, Higgs A. The L-arginine-nitric oxide pathway *N Engl J Med*. 1993; **329**:2002-2012.

10. Gebremedhin D, Koltai MZ, Pogatsa G, Magyar K, Hadhazy P. Influence of experimental diabetes on the mechanical responses of canine coronary arteries: role of endothelium. *Cardiovasc Res.* 1988; **22**:537-544

11. Altan VM, Karasu C, Ozuari A. The effects of type-1 and type-2 diabetes on endothelium-dependent relaxation in rat aorta. *Pharmacol Biochem Behav*.1989;**33**:519-522.

12. Shen WL, Zhong MF, Ding WL et al., Elevated catalase and heme oxygenase-1 may contribute to improved postischaemic cardiac function in long-term type 1 diabetes. *Clin Exp Pharmacol Physiol.* 2008;**35**:820-826.

13. Head RJ, Longhurst PA, Panek RL, Stitzel RE. A contrasting effect of the diabetic state upon the contractile responses of aortic preparations from the rat and rabbit. *Br J Pharmacol*.1987; **91**:275-286.

14. Harris KH, MacLeod KM. Influence of the endothelium on contractile responses of arteries from diabetic rats. *Eur J Pharmacol*. 1988; **153**:55-64.

15. Mulhern M, Docherty JR. Effects of experimental diabetes on the responsiveness of rat aorta. *Br J Pharmacol.*1989; **97**:1007-1012.

16. Glocker S, Quast U. Binding and effects of P1075, an opener of ATP-sensitive K+ channels, in the aorta from streptozotocin-treated diabetic rats. *Naunyn Schidedbergs Arch Pharmacol* 1997, **356**:210-215.

17. Vallejo S, Angulo J, Peiro C et al., Prevention of endothelial dysfunction in streptozotocin-induced diabetic rats by gliclazide treatment. *J Diabetes Complications*. 2000; **14**: 224-233.

18. Shi Y, Ku DD, Man RY, Vanhoutte PM. Augmented endothelium-derived hyperpolarizing factor-mediated relaxations attenuate endothelial dysfunction in femoral and mesenteric, but not in carotid arteries from type I diabetic rats. *J Pharmacol Exp Ther*. 2006;**318**:276-281

19. Heygate KM, Daviese J, Holmes M, James RF, Thurston H. The effect of insulin treatment and of islet transplantation on the resistance artery function in the STZ-induced diabetic rat. *Br J Pharmacol* 1996, **119**:495-504

20. Pieper GM. Acute amelioration of diabetic endothelial dysfunction with a derivated of the nitric oxide synthase cofactor, tetrahydrobiopterin. *J Cardiovasc Pharmacol*.1997, **29**:8-15

21. Smits P, Kapma JA, Jacobs MC, Lutterman J, Thien T. Endothelium-dependent vascular relaxation in patients with type I diabetes *Diabetes*. 1993; **42**:148-153.

22. Lambert J, Aarsen M, Donker AJ, Stehouwer CD. Endothelium-dependent and -independent vasodilation of large arteries in normoalbuminuric insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 1996; **1**6:705-711

23. Enderle MD, Benda N, Schmuelling RM, Haering HU, Pfohl M. Preserved endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy subjects. *Diabetes Care*. 1998; **21**:271-277.

24. Zenere BM, Arcaro G, Saggiani F, Rossi L, Muggeo M, Lechi A. Noninvasive detection of functional alterations of the arterial wall in IDDM patients with and without microalbuminuria. *Diabetes Care.* 1995; **18**:975-982.

25. Clarkson P, Celermajer DS, Donald AE et al., Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. *J Am Coll Cardiol*. 1996; **28**:573-579.

26. Lekakis J, Papamichael C, Anastasiou H et al., Endothelial dysfunction of conduit arteries in insulin-dependent diabetes mellitus without microalbuminuria. Cardiovasc Res. 1997; **34**:164-168.

27. Huvers FC, De Leeuw PW, Houben AJ et al., Endothelium-dependent vasodilatation, plasma markers of endothelial function, and adrenergic vasoconstrictor responses in type 1 diabetes under near-normoglycemic conditions. *Diabetes*. 1999; **48**:1300-1307.

28. Arcaro G, Zenere BM, Saggiani F et al., ACE inhibitors improve endothelial function in type 1 diabetic patients with normal arterial pressure and microalbuminuria. *Diabetes Care*. 1999; **22**:1536-1542.

29. Makimattila S, Mantysaari M, Groop PH et al. Hyperreactivity to nitrovasodilators in forearm vasculature is related to autonomic dysfunction in insulin-dependent diabetes mellitus. *Circulation*. 1997; **95**:618-625.

30. Turner NC, White P. Effects of streptozotocin-induced diabetes on vascular reactivity in genetically hyperinsulinaemic obese Zucker rats. *Cardiovasc Pharmacol*. 1996;**27**: 884-890

31. Bohlen HG, Lash JM. Endothelial-dependent vasodilation is preserved in non-insulin-dependent Zucker fatty diabetic rats. *Am J Physiol*. 1995; **268:**H2366-2374

32. Walker AB, Chattington PD, Buckingham RE, Williams G. The thiazolidinedione rosiglitazone (BRL-49653) lowers blood pressure and protects against impairment of endothelial function in Zucker fatty rats. *Diabetes.* 1999; **48**:1448-1453.

33. Arvola P, Wu X, Kahonen M et al., Exercise enhances vasorelaxation in experimental obesity associated hypertension. *Cardiovasc Res*. 1999; **43**:992-1002.

34. Su J, Lucchesi PA, Gonzalez-Villalobos RA et al., Role of advanced glycation end products with oxidative stress in resistant artery dysfunction in type 2 diabetic mice. *Arterioscler Thromb Vasc Biol* 2008, **28**:1432-1438.

35. Lagaud GJ, Masih-Khan E, Kai S, van Breemen C, Dube GP. Influence of type II diabetes on arterial tone and endothelial function in murine mesenteric resistance arteries. *J Vasc Res*. 2001; **38**:578-589.

36. Pannirselvam M, Verma S, Anderson TJ, Triggle CR. Cellular basis of endothelial dysfunction in small mesenteric arteries from spontaneously diabetic (db/db -/-) mice: role of decreased tetrahydrobiopterin bioavailability. *Br J Pharmacol*. 2002; **136**: 255-263

37. Belmadani S, Palen DI, Gonzaley-Villalobos RA, Boulares HA, Matrougui K. Elevated epidermal growth factor receptor phosphorylation induces resistance artery dysfunction in diabetic db/db mice. *Diabetes* 2008, **57**:1629-1637.

38. Gao X, Zhang H, Schemidt AM, Zhang C. AGE/RAGE produces endothelial dysfunction in coronary arterioles in type 2 diabetic mice. *Am J Physiol Heart Circ Physiol* 2008, **295**: H491-498.

39. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus Circulation. 1993; 88:2510-2516.

40. Huszka M, Kaplar M, Rejto L et al., The association of reduced endothelium derived relaxing factor-NO production with endothelial damage and increased in vivo platelet activation in patients with diabetes mellitus. *Thromb Res*. 1997; **86**:173-180.

41. De Vriess AS, Van De Voorde J, Blom HJ, Vanhoutte PM, Verbeke M, Lameire NH. The impaired renal vasodilator response attributed to endotheium-derived hyperpolarizing factor in streptozotocin-induced diabetic rats is restored by 5-methyltetrahydrofolate. *Diabetologia* 2000; **43**:1116-1125.

42. McVeigh GE, Brennan GM, Johnston GD et al., Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*. 1992; **35**:771-776.

43. Nitenberg A, Valensi P, Sachs R, Dali M, Aptecar E, Attali JR. Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. *Diabetes.* 1993;**42**:1017-1025

44. Moncada S, Vane JR. The role of prostacyclin in vascular tissue. *Fed Proc*. 1979; **38**:66-71.

45. Busse R, Forstermann U, Matsuda H, Pohl U. The role of prostaglandins in the endothelium-mediated vasodilatory response to hypoxia *Pflugers Arch*. 1984; **401**:77-83.

46. Trachte GJ. Prostacyclin mediates arachidonic acid-induced relaxation of rabbit isolated mesenteric arteries. *J Cardiovasc Pharmacol*. 1986; **8**:758-764.

47. Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature. 1976; 263:663-665.

48. Shen B, Ye CL, Ye KH, Liu JJ. Mechanism underlying enhanced endothelium-dependent vasodilatation in thoracic aorta of early stage streptozotocin-induced diabetic mice. *Acta Pharmacol Sin* 2003, **24**:422-428.

49. Matsumoto K, Morishita R, Tomita N et al., Impaired endothelial dysfunction in diabetes mellitus rats was restored by oral administration of prostaglandin I2 analogue. *J Endocrinol*. 2002; **175**:217-223.

50. Feletou M, Vanhoutte PM. *EDHF: The complete story.* Taylor and Francis. Boca Raton FL. 2006

51. Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol*. 1988; **93**:515-524.

52. Chen G, Suzuki H, Weston AH. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. *Br J Pharmacol*. 1988; **95**:1165-1174.

53. Rand VE, Garland CJ. Endothelium-dependent relaxation to acetylcholine in the rabbit basilar artery: importance of membrane hyperpolarization. *Br J Pharmacol*. 1992; **106**:143-150.

54. Garland CJ, McPherson GAD. Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. *Br J Pharmacol*. 1992; **105**: 429-435.

55. Waldron GJ, Garland CJ. Contribution of both nitric oxide and a change in membrane potential to acetylcholine-induced relaxation in the rat small mesenteric artery. *Br J Pharmacol*. 1994; **112**:831-836

56. Nagao T, Vanhoutte PM. Endothelium-derived hyperpolarizing factor and endothelium-dependent relaxations. *Am J Respir Cell Mol Biol.* 1993;**8**:1-6.

57. Shimokawa H, Yasutake H, Fujii K et al., The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. *J Cardiovasc Pharmacol.* 1996;**28**:703-11

58. Olmos L, Mombouli JV, Illiano S, Vanhoutte PM. cGMP mediates the desensitization to bradykinin in isolated canine coronary arteries. *Am J Physiol*. 1995; **268**: H865-870.

59. Waldron GJ, Ding H, Lovren F, Kubes P, Triggle CR. Acetylcholine-induced relaxation of peripheral arteries isolated from mice lacking endothelial nitric oxide synthase. *Br J Pharmacol.* 1999; **128**:653-658.

60. Ding H, Hubes P, Tiggle C. Potassium- and acetylcholine-induced vasorelaxation in mice lacking endothelial nitric oxide synthase. *Br J Pharmacol* 2000, **129**:1194-1200

61. Thollon C, Fournet-Bourguignon MP, Saboureau D et al., Consequences of reduced production of NO on vascular reactivity of porcine coronary arteries after angioplasty: importance of EDHF. *Br J Pharmacol*. 2002; **136**:1153-1161.

62. Shimokwa H, Morikawa K Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in animals and humans. *J Mol Cell Cardiol* 2005, **39**:725-732

63. Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A. Alterations in endothelium-dependent hyperpolarization and relaxation in mesenteric arteries from streptozotocin-induced diabetic rats. *Br J Pharmacol.* 1997; **121**:1383-1391.

64. 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.* 2000; **130**:549-556.

65. Wigg ST, Tare M, Tonta MA, O'Brien RC, Meredith IT, Parkington HC. Comparison of effects of diabetes mellitus on an EDHF-dependent and an EDHF-independent artery. *Am J Physiol Heart Circ Physiol.* 2001;**281**:H232-240

66. Morikawa K, Matoba T, Kubota H et al., Influence of diabetes mellitus, hypercholesterolemia, and their combination on EDHF-mediated responses in mice. *J Cardiovasc Pharmacol*. 2005;**45**:485-90.

67. Oniki H, Fujii K, Kansui Y, Iida M. Effects of angiotensin II receptor antagonist on impaired endothelium-dependent and endothelium-independent relaxation in type II diabetic rats. *J Hyperten* 2006, **24**:331-338.

68. Burnham MP, Johnson IT, Weston AH. Impaired small-conductance Ca2+-activated K+ channel-dependent EDHF response in type II diabetic ZDF rats. *Br J Pharmacol* 2006, **148**:434-414.

69. Matsumoto T, Kobayashi T, Kamata K. Mechanisms underlying the impaired EDHF-type relaxation response in mesenteric arteries from Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Eur J Pharmacol* 2006, **538**:132-140.

70. Triggle CR, Ding H, Anderson TJ, Pannirselvam M. The endothelium in health and disease: A discussion of the contribution of non-nitric oxide endothelium-derived vasoactive mediators to vascular homestasis in normal vessels and in type II diabetes. *Molecular and Cellular Biochemistry*.2004;**263**:21-27

71. Lüscher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension*.1986; **8**:344-348.

72. Auch-Schwelk W, Katusic ZS, Vanhoutte PM. Thromboxane A2 receptor antagonists inhibit endothelium-dependent contractions Hypertension. 1990; 15: 699-703.

73. Yang D, Gluais P, Zhang JN, Vanhoutte PM, Feletou M. Endothelium-dependent contractions to acetylcholine, ATP and the calcium ionophore A 23187 in aortas from

spontaneously hypertensive and normotensive rats. *Fundam Clin Pharmacol*. 2004; **18**:321-326.

74. Vanhoutte PM, Feletou M, Taddei S. Endothelium-dependent contractions in hypertension. *Br J Pharmacol*. 2005;**144**:449-458

75. Vanhoutte PM, Tang EH. Endothelium-dependent contractions: when a good guy turns bad! *J Physiol.* 2008;**586**:5295-304

76. Tesfamariam B, Jakubowski JA, Cohen RA. Contraction of diabetic rabbit aorta caused by endothelium-derived PGH2-TxA2. *Am J Physiol*. 1989; **257**:H1327-1333.

77. Ge T, Hughes H, Junquero DC, Wu KK, Vanhoutte PM, Boulanger CM. Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H synthase-1 and hypersensitivity to prostaglandin H2 in the SHR aorta. *Circ Res.* 1995;**76**:1003-10.

78. Gluais P, Paysant J, Badier-Commander C, Verbeuren T, Vanhoutte PM, Feletou M. In the SHR aorta, the calcium ionophore A 23187 releases prostacyclin and thromboxane A2 as endothelium-derived contracting factors (EDCF). *Am J Physiol Heart Circ Physiol.* 2006;**291**:H2255–H2264.

79. Shi Y, Feletou M, Ku DD, Man RY, Vanhoutte PM. The calcium ionophore A23187 induces endothelium-dependent contractions in femoral arteries from rats with streptozotocin-induced diabetes. *Br J Pharmacol*. 2007;**150**:624-632

80. Gluais P, Lonchampt M, Morrow JD, Vanhoutte PM, Feletou M. Acetylcholine-induced endothelium-dependent contractions in the SHR aorta: the Janus face of prostacyclin. *Br J Pharmacol.* 2005;**146**:834–845.

81. Tang EH, Leung FP, Huang Y, Feletou M, So KF, Man RY, Vanhoutte PM. Calcium and reactive oxygen species increase in endothelial cells in response to releasers of endothelium-derived contracting factor. *Br J Pharmacol*. 2007;**151**:15-23.

82. Feletou M, Tang EH, Vanhoutte PM. Nitric oxide the gatekeeper of endothelial vasomotor control. *Front Biosci*. 2008;**13**:4198-4217

83. Tesfamariam B, Cohen RA. Contractions to oxygen-derived free radicals are augmented in aorta of the spontaneously hypertensive rat. *Hypertension.* 1989; **13**:859-864.

84. Ling X, Cota-Gomez A, Flores NC et al., Alterations in redox homeostasis and prostaglandins impair endothelial-dependent vasodilation in euglycemic autoimmune nonobese diabetic mice. *Free Radic Biol Med*. 2005;**39**:1089-1098

85. Akamine EH, Urakawa TA, de Oliveira MA et al., Decreased Endothelium-Dependent Vasodilation in Diabetic Female Rats: Role of Prostanoids. *J Vasc Res.* 2006; **43**: 401-410.

86. Kagota S, Yamaguchi Y, Nakamura K, Kunitomo M. Altered endothelium-dependent responsiveness in the aortas and renal arteries of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of non-insulin-dependent diabetes mellitus. *Gen Pharmacol.* 2000; **34**:201-209.

87. Okon EB, Szado T, Laher I, McManus B, van Breemen C. Augmented contractile response of vascular smooth muscle in a diabetic mouse model *J Vasc Res*. 2003; **40**:520-530.

88. Matsumoto T, Kakami M, Noguchi E, Kobayashi T, Kamata K. Imbalance between endothelium-derived relaxing and contracting factors in mesenteric arteries from aged OLETF rats, a model of Type 2 diabetes. *Am J Physiol Heart Circ Physiol.* 2007;**293**:H1480-1490.

89. Matsumoto T, Noguchi E, Ishida K, Kobayashi T, Yamada N, Kamata K. Metformin normalizes endothelial function by suppressing vasoconstrictor prostanoids in mesenteric artery from OLETF rats, a model of type 2 diabetes. *Am J Physiol Heart Circ Physiol* 2008, **295**: H1165-1176.

90. Vanhoutte PM. Say NO to ET. *J Auton Nerv Syst*. 2000; **81**:271-277

91. Kanie N, Kamata K. Effects of chronic administration of the novel endothelin antagonist J-104132 on endothelial dysfunction in streptozotocin-induced diabetic rat. *Br J Pharmacol.* 2002; **135**: 1935-1942.

92. Shemyakin A, Bohm F, Wagner H, Efendic S, Bavenholm P, Pernow J. Enhanced endothelium-dependent vasodilatation by dual endothelin receptor blockade in individuals with insulin resistance. *J Cardiovasc Pharmacol.* 2006; **47**: 385-390.

93. Liu H, Colavitti R, Rovira II, Finkel T. Redox-dependent transcriptional regulation. *Circ Res*. 2005; **97**:967-74.

94. Lassegue B, Sorescu D, Szocs K et al., Novel gp91(phox) homologues in vascular smooth muscle cells : nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. *Circ Res.* 2001; **88**:888-894.

95. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem J*. 1973**; 134**:707-716.

96. Spitaler MM, Graier WF. Vascular targets of redox signalling in diabetes mellitus. *Diabetologia.* 2002; **45**:476-494.

97. Keegan A, Walbank H, Cotter MA, Cameron NE. Chronic vitamin E treatment prevents defective endothelium-dependent relaxation in diabetic rat aorta. *Diabetologia* 1995, **38**: 1475-1478.

98. Karasu C. Increased activity of H2O2 in aorta isolated from chronically streptozotocin-diabetic rats: effects of antioxidant enzymes and enzymes inhibitors. *Free Radic Biol Med.* 1999; **27**:16-27.

99. Rodriguez-Mänas L, Angulo J, Vallejo S et al., Early and intermediate Amadori glycosylation adducts, oxidative stress, and endothelial dysfunction in the streptozotocin-induced diabetic rats vasculature. *Diabetologia.* 2003; **46**:556-566.

100. Shi Y, So KF, Man RY, Vanhoutte PM. Oxygen-derived free radicals mediate endothelium-dependent contractions in femoral arteries of rats with streptozotocin-induced diabetes. *Br J Pharmacol.* 2007;**152**:1033-1041.

101. Inkster ME, Cotter MA, Cameron NE. Effects of trientine, a metal chelator, on defective endothelium-dependent relaxation in the mesenteric vasculature of diabetic rats. *Free Radic Res* 2002, **36**:1091-1099.

102. Romero MJ, Platt DH, Tawfik HE et al., Diabetes-induced coronary vascular dysfunction involves increase arginase activity. *Cir Res* 2008, **102**:95-102

103. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care. 1996; 19: 257-267

104. Frustaci A, Kajstura J, Chimenti C et al., Myocardial cell death in human diabetes. Circ Res. 2000; 87:1123-1132.

105. Rota M, LeCapitaine N, Hosoda T et al., Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66shc gene. *Circ Res.* 2006; **99**: 42-52.

106. Kontos HA. Oxygen radicals from arachidonate metabolism in abnormal vascular responses. *Am Rev Respir Dis.* 1987; **136**:474-477.

107. Tesfamariam B, Brown ML, Deykin D, Cohen RA. Elevated glucose promotes generation of endothelium-derived vasoconstrictor prostanoids in rabbit aorta *J Clin Invest*. 1990; **85**:929-932.

108. Ceriello A. Hyperglycaemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. *Diabetes Nutr Metab*. 1999; **12**:42-46.

109. Inoguchi T, Nawata H. NAD(P)H oxidase activation: a potential target mechanism for diabetic vascular complications, progressive beta-cell dysfunction and metabolic syndrome. *Curr Drug Targets*. 2005; **6**:495-501.

110. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proc Natl Acad Sci U S A.* 2006; **103**:2653-2658.

111. Zhang M, Kho AL, Anilkumar N et al., Glycated proteins stimulate reactive oxygen species production in cardiac myocytes: involvement of Nox2 (gp91phox)-containing NADPH oxidase. *Circulation.* 2006; **113**:1235-1243.

112. Taniguchi N, Takahashi M, Sakiyama H et al., A common pathway for intracellular reactive oxygen species production by glycoxidative and nitroxidative stress in vascular endothelial cells and smooth muscle cells. *Ann N Y Acad Sci*. 2005; **1043**:521-528.

113. Kamata K, Kobayashi T. Change in superoxide dismutase mRNA expression by streptozotocin-induced diabetes. *Br J Pharmacol* 1996, **119**:583-589.

114. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol*. 2003;**17**:24-38.

115. Matsumoto T, Yoshiyama S, Wakabayashi K, Kobayashi T, Kamata K. Effects of chronic insulin on endothelial dysfunction of basilar arteries from established streptozotocin-diabetic rats. *Eur J Pharmacol.* 2004;**504**:119-127

116. Moien-Afshari F, Ghosh S, Khazaei M, Kieffer TJ, Brownsey RW, Laher I. Exercise restores endothelial function independently of weight loss or hyperglycaemic status in db/db mice. *Diabetologia* 2008, **51**:1327-1337.

117. Kedziora-Kornatowska KZ, Luciak M, Paszkowski J. Lipid peroxidation and activities of antioxidant enzymes in the diabetic kidney: effect of treatment with angiotensin convertase inhibitors. *IUBMB Life.*2000; **49**: 303-307.

118. Aragno M, Tamagno E, Gatto V et al., Dehydroepiandrosterone protects tissues of streptozotocin-treated rats against oxidative stress. *Free Radic Biol Med.*1999*;* **26**: 1467-1474.

119. Kocak G, Aktan F, Canbolat O et al., ADIC Study Group--Antioxidants in Diabetes-Induced Complications. Alpha-lipoic acid treatment ameliorates metabolic parameters, blood pressure, vascular reactivity and morphology of vessels already damaged by streptozotocin-diabetes. *Diabetes Nutr Metab.* 2000;**13**: 308-318.

120. Hamaty M, Guzmàn CB, Walsh MF, Bode AM, Levy J, Sowers JR. High glucose-enhance acetylcholinestimulated cGMP masks impaired vascular reactivity in tail arteries from short-term hyperglycemic rats. *Int J Exp Diabetes Res* 2000, **1**:69-79.

121. San Martin A, Foncea R, Laurindo FR, Ebensperger R, Griendling KK, Leighton F. Nox1-based NADPH oxidase-derived superoxide is required for VSMC activation by advanced glycation end-products. *Free Radic Biol Med.*2007; **42**: 1671-1679.

122. Wei Y, Whaley-Connell AT, Chen K et al., NADPH oxidase contributes to vascular inflammation, insulin resistance, and remodeling in the transgenic (mRen2) rat. *Hypertension*.2007; **50**: 384-391.

123. Pannirselvam M, Wiehler WB, Anderson T, Triggle CR. Enhanced vascular reactivity of small mesenteric arteries from diabetic mice is associated with enhanced oxidative stress and cyclooxygenase products. *Br J Pharmacol*. 2005;**144**:953-960

124. Long CJ, Stone TW. The release of endothelium-derived relaxant factor is calcium dependent. *Blood Vessels.* 1985; **22**:205-208.

125. Fu GS, Huang H, Chen F et al., Carvedilol ameliorates endothelial dysfunction in streptozotocin-induced diabetic rats. *Eur J Pharmacol*. 2007;**567**:223-230

126. Matsumoto T, Miyamori K, Kobayashi T, Kamata K. Specific impairment of endothelium-derived hyperpolarizing factor-type relaxation in mesentric arteries from streptozotocin-induced diabetic mice. *Vascul Pharmacol* 2006, **44**:450-460.

127. Shinbori C, Saito M, Kinoshita Y et al., Cyclohexenonic long-chain fatty alcohol has therapeutic effects on diabetes-induced angiopathy in the rat aorta *Eur J Pharmacol.* 2007;**567**:139-144.

128. Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation*. 1997; **96**:25-28.

129. Rabini RA, Vignini A, Salvolini E et al., Activation of human aortic endothelial cells by LDL from Type 1 diabetic patients: an in vitro study. *Atherosclerosis.* 2002; **165**:69-77.

130. Rubanyi GM, Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor *Am J Physiol*. 1986; **250**:H822-827.

131. Cohen RA. Does DECF contribute to diabetic endothelial cell dysfunction? *Dialogues in cardiovascular medicine.* 2002; **7**: 225-231.

132. Pieper GM, Siebeneich W, Moore-Hilton G, Roza AM. Reversal by L-arginine of a dysfunctional arginine/nitric oxide pathway in the endothelium of the genetic diabetic BB rat. *Diabetologia.* 1997;**40**:910-915.

133. West MB, Ramana KV, Kaiserova K, Srivastava SK, Bhatnagar A. L-arginine prevents metabolic effects of high glucose in diabetic mice. *FEBS Lett* 2008, **528**:2609-2014.

134. Landmesser U, Harrison DG. Oxidant stress as a marker for cardiovascular events: Ox marks the spot. *Circulation*. 2001;**104**:2638-2640

135. Crabtree MJ, Smith CL, Lam G, Goligorsky MS, Gross SS. Ratio of 5,6,7,8-tetrahydrobiopterin to 7,8-dihydrobiopterin in endothelial cells determines glucose-elicited changes in NO vs. superoxide production by eNOS. *Am J Physiol Heart Circ Physiol* 2008, **294**:H1530-1540.

136. Qian LB, Wang HP, Qiu WL, Huang H, Bruce IC, Xia Q. Interleukin-2 protects against endothelial dysfunction induced by high glucose levels in rats. *Vascul Pharmacol.* 2006;**45**:374-382.

137. Matoba T, Shimokawa H, Kubota H et al., Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries, *Biochem. Biophys. Res. Commun*. 2002; **290**: 909–913.

138. Park Y, Capobianco S, Gao X, Falck JR, Dellsperger KC, Zhang C. Role of EDHF in type 2 diabetes-induced endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2008. **295**:H1982-1988.

139. Matoba T, Shimokawa H, Morikawa K, Kubota H, Kunihiro I, Urakami-Harasawa L., Electron spin resonance detection of hydrogen peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary microvessels, *Arterioscler. Thromb. Vasc. Biol*. 2003; **23**:1224–1230.

140. Yada T, Shimokawa H, Hiramatsu O et al., Hydrogen peroxide, an endogenous endothelium-derived hyperpolarizing factor, plays an important role in coronary autoregulation in vivo, *Circulation* 2003;**107**:1040–1045

141. Katusic ZS, Vanhoutte PM. Superoxide anion is an endothelium-derived contracting factor *Am J Physiol.* 1989; **257**:H33-37.

142. Katusic ZS, Schugel J, Cosentino F, Vanhoutte PM. Endothelium-dependent contractions to oxygen-derived free radicals in the canine basilar artery. *Am J Physiol*. 1993; **64**:H859-864.

143. Yang D, Feletou M, Boulanger CM et al., Oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in aortas from spontaneously hypertensive rats. *Br J Pharmacol*. 2002; **136**:104-110.

144. Yang D, Levens N, Zhang JN, Vanhoutte PM, Feletou M. Specific potentiation of endothelium-dependent contractions in SHR by tetrahydrobiopterin. *Hypertension*.2003; **41**:136-142.

145. Haidara MA, Mikhailidis DP, Rateb MA et al., Evaluateion of the effect of oxidative stress and vitamin E suppplementation on renal function in rats with streptozotocin-induced type 1 diabetes. *J Diabetes Complication 2008* (**in press**)

146. MacKenzie KE, Wiltshire EJ, Gent R, Hirte C, Piotto L, Couper JJ. Folate and vitamin B6 rapidly normalize endothelial dysfunction in children with type 1 diabetes mellitus. *Pediatrics*. 2006;**118**:242-253

147. Title LM, Ur E, Giddens K, McQueen MJ, Nassar BA. Folic acid improves endothelial dysfunction in type 2 diabetes--an effect independent of homocysteine-lowering. *Vasc Med*. 2006;**11**:101-109

148. MacKenzie KE, Wiltshire EJ, Gent R, Hirte C, Piotto L, Couper JJ. Folate and vitamin B6 rapidly normalize endothelial dysfunction in children with type 1 diabetes mellitus. Pediatrics. 2006;118:242-253

149. Rizzo MR, Abbatecola AM, Barbieri M et al., Evidence for anti-inflammatory effects of combined administration of vitamin E and C in older persons with impaired fasting glucose: impact on insulin action. *J Am Coll Nutr*. 2008;**27**:505-511

150. Vignini A, Nanetti L, Moroni C et al., A study on the action of vitamin E supplementation on plasminogen activator inhibitor type 1 and platelet nitric oxide production in type 2 diabetic patients. *Nutr Metab Cardiovasc Dis.* 2008;**18**:15-22.

151. Afkhami-Ardekani M, Shojaoddiny-Ardekani A. Effect of vitamin C on blood glucose, serum lipids & serum insulin in type 2 diabetes patients. *Indian J Med Res*. 2007;**126**:471-474.

152. Liu S, Lee IM, Song Y et al., Vitamin E and risk of type 2 diabetes in the women's health study randomized controlled trial. *Diabetes.* 2006;**55**:2856-2862.

153. Ward NC, Wu JH, Clarke MW et al., The effect of vitamin E on blood pressure in individuals with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *J Hypertens.* 2007;**25**:227-234

154. Sesso HD, Buring JE, Christen WG et al., Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA.* 2008;**300**:2123-33.

Legends

Figure 1. Contractions to A23187 in femoral arteries from control and streptozotocin-treated rats in the presence of L-NAME (0.3 mM) after 4 (**a** and **b**) and 12 (**c** and **d**) weeks. Data shown are means±SEM, (vertical lines); *n*=7. *Significant difference from control rings (*P*<0.05). #Significant difference from rings of control rats under identical conditions (*P*<0.05). L-NAME, Nω-nitro-L-arginine methyl ester hydrochloride. (From Shi et al., 2007, with permission [79])

Figure 2. The generation of reactive oxygen species. ROS: reactive oxygen species; GSH: glutathione; GSSG: glutathione disulfide; NAD(P)H: nicotinamide adenine dinucleotide phosphate-oxidase; NO: nitric oxide (Modified from Shi et al., 2007, with permission [100]).

Figure 3. Presence of Mn-SOD (a, b) and copper–zinc-SOD (c, d) in femoral arteries with endothelium from control and STZ-treated rats. Representative western blots demonstrating the presence of proteins (a and c) and graphic representation of the data (b and d), normalized to β-actin, presented as percentage of control and shown as means±SEM, n=6. *Significant difference from controls (P<0.05). STZ, streptozotocin. CuZn-SOD, copper–zinc-SOD; Mn-SOD, manganese-superoxide dismutase (From Shi et al., 2007, with permission [100]).

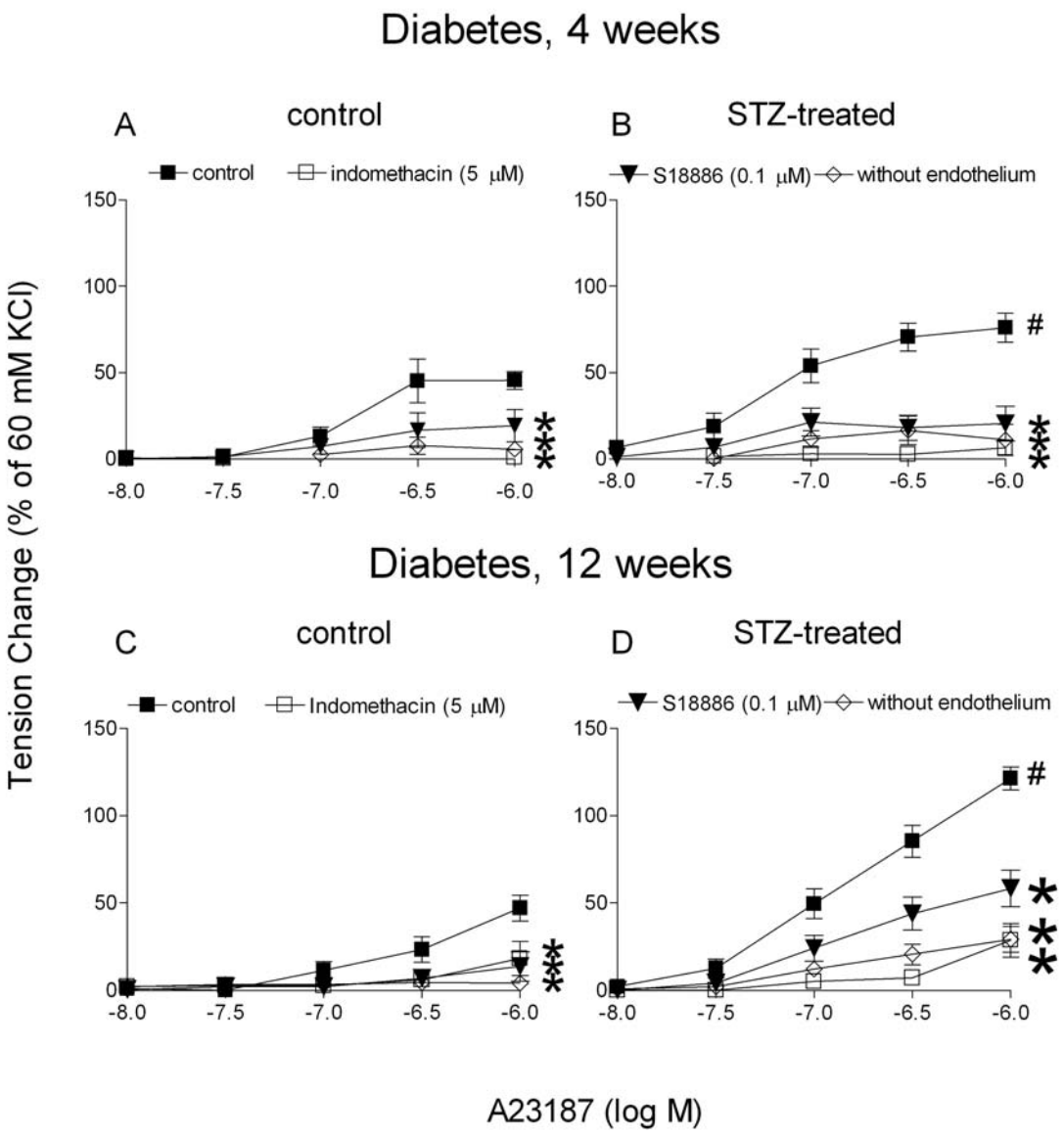
Figure 4. Catalase protein presence and activity in femoral arteries with endothelium from control and STZ-treated rats. Western blot demonstrating the presence of catalase (a). Graphic representation of the data (b), normalized to β-actin, presented as percentage of control and shown as means±SEM; n=6. Catalase activity in femoral arteries with endothelium from control and STZ-treated rats (c). Data are normalized to the protein level and are shown as means±SEM; n=6. *Significant difference from controls (P<0.05). STZ, streptozotocin (From Shi et al., 2007, with permission [100]).

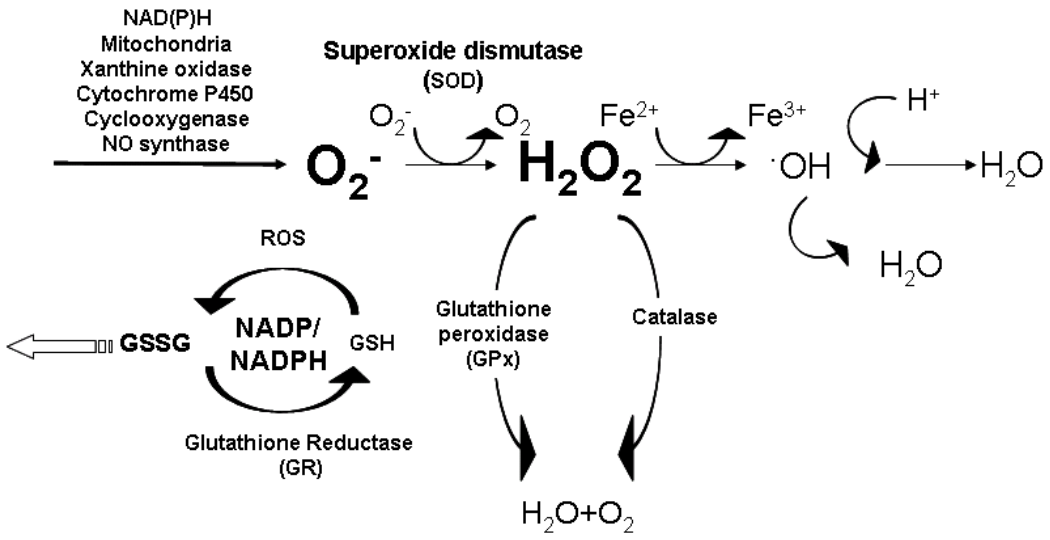
Figure 5. The presence of COX-1 (a and b) in intact femoral artery from control and streptozotocin (STZ)-treated rats, 4 weeks and 12 weeks after the injection of streptozotocin (STZ). Data presented are a percentage of control and shown as means±SEM; n=6. *Statistically significant difference from control rats ($P<0.05$) (from Shi et al., 2007 with permission [79]).

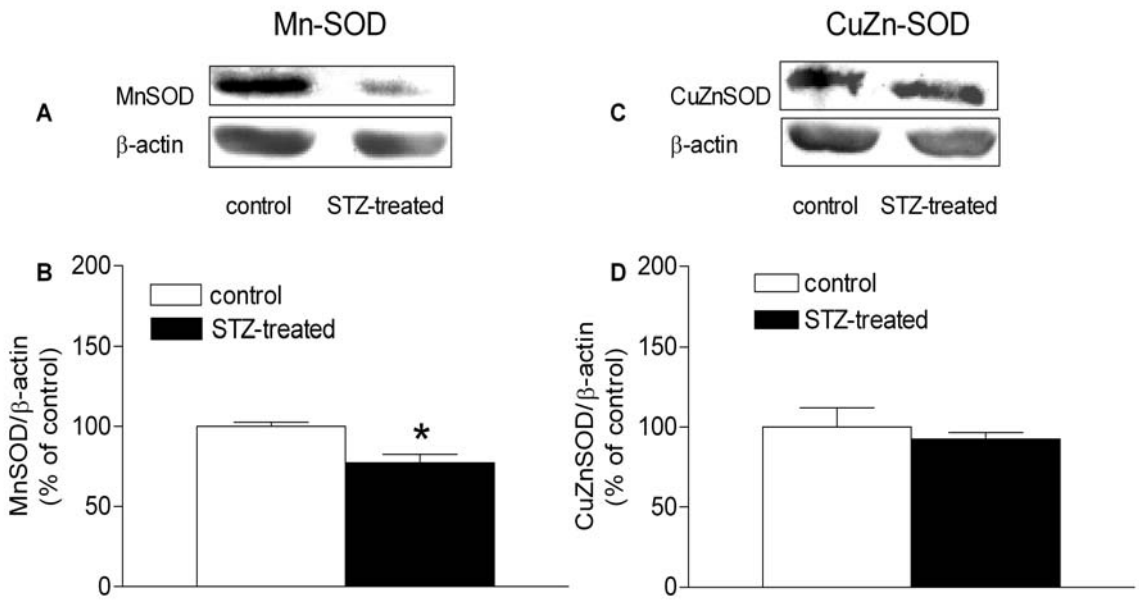
Figure 6. Effect of antioxidants on concentration-dependent contractions to A23187 in femoral arteries with endothelium from STZ-treated rats in the presence of L-NAME. (a) Tiron and SOD, n=8–10, (b) deferoxamine, catalase and DETCA; n=12–20. Data are expressed as percentage of the reference contraction to 60 mM KCl and are shown as means±SEM; *Significant difference ($P<0.05$) from the controls (rings with endothelium). DETCA, diethyldithiocarbamic acid; L-NAME, Nω-nitro-L-arginine methyl ester hydrochloride; SOD, superoxide dismutase; STZ, streptozotocin (From Shi et al., 2007, with permission [100]).

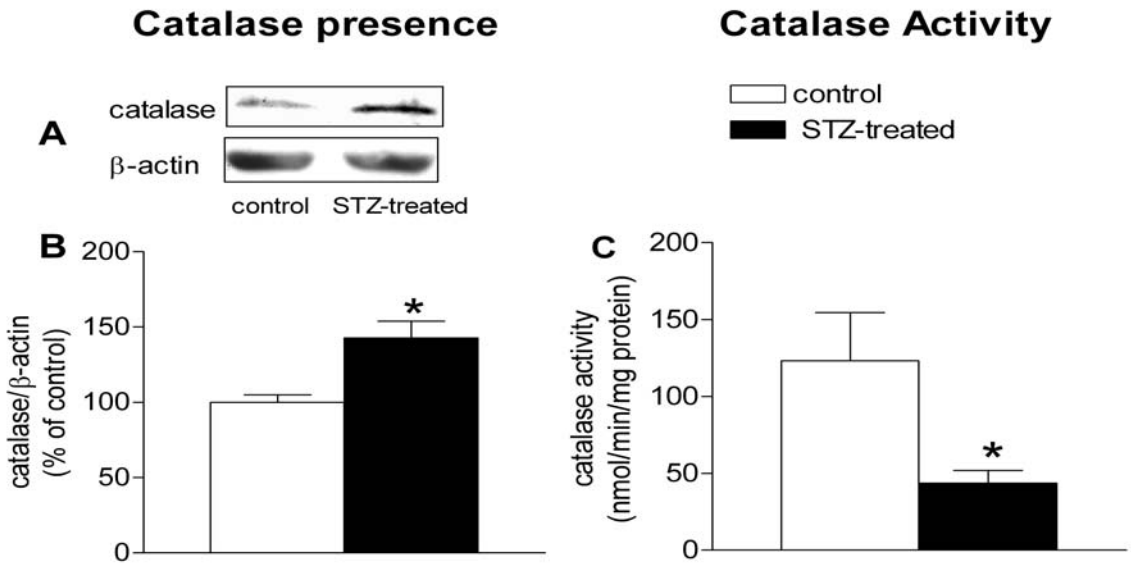
Figure 7. Proposed mechanisms of endothelial dysfunction in diabetes. Oxidative stress is an important contributor to this dysfunction. Oxygen-derive free radicals inactivate NO. Increased basal oxidative stress in the endothelium potentiates endothelium-dependent contractions. Thus, the balance tips towards vasoconstrictor responses.

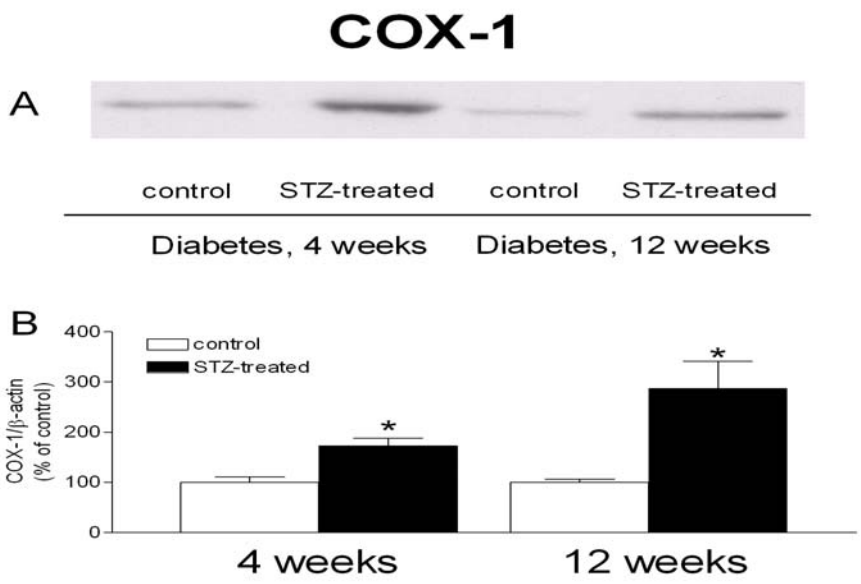
cAMP: cyclic AMP; cGMP: cyclic GMP; COX: Cyclooxygenase; EDHF: Endothelium-Derived Hyperpolarizing Factor; EP1: Prostanoid Receptor E type I ; NOS: Nitric Oxide Synthase; ONOO-: Peroxynitrite; PGH2 : Endoperoxide ; PGI2: Prostacyclin; R: Receptor; ROS: Ractive Oxygen Species; TXA2: Thromboxane A2; TP: Thromboxane prostanoid receptor

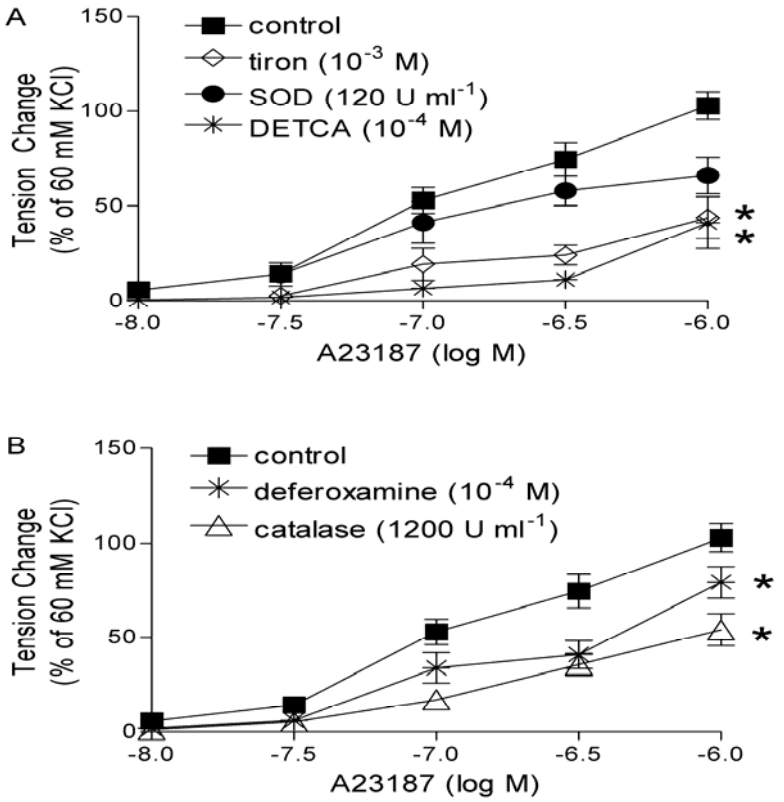

Diabetes, 4 weeks
A
control
control
indomethacin (5 μM)
B
STZ-treated
S18886 (0.1 μM)
without endothelium
Diabetes, 12 weeks
C
control
control
Indomethacin (5 μM)
D
STZ-treated
S18886 (0.1 μM)
without endothelium
Tension Change (% of 60 mM KCl)
A23187 (log M)
150
100
50
0
-8.0
-7.5
-7.0
-6.5
-6.0
#
***
*
**

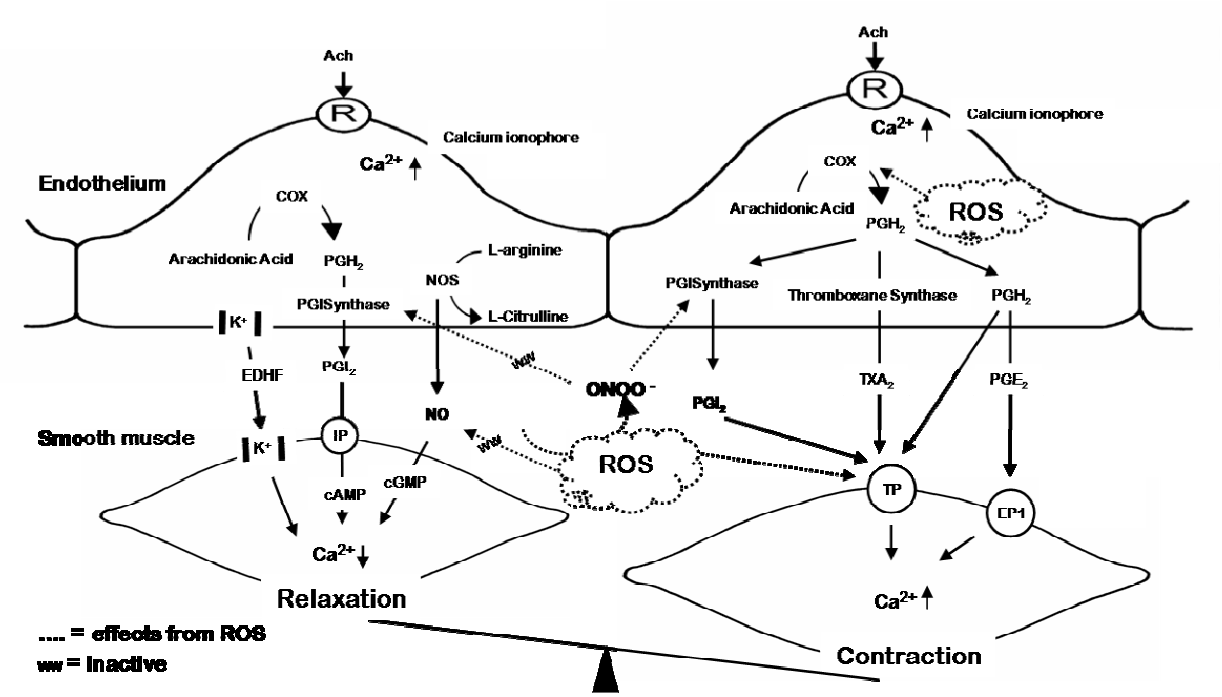

NAD(P)H
Mitochondria
Xanthine oxidase
Cytochrome P450
Cyclooxygenase
NO synthase
Superoxide dismutase
(SOD)
O_2^-
O_2
Fe^{2+}
Fe^{3+}
H^+
H_2O
O_2^-
H_2O_2
·OH
H_2O
ROS
GSSG
NADP/
NADPH
GSH
Glutathione
peroxidase
(GPx)
Catalase
Glutathione Reductase
(GR)
H_2O+O_2


Mn-SOD
A
MnSOD
β-actin
control
STZ-treated
CuZn-SOD
C
CuZnSOD
β-actin
control
STZ-treated
B
MnSOD/β-actin
(% of control)
200
150
100
50
0
control
STZ-treated
*
D
CuZnSOD/β-actin
(% of control)
200
150
100
50
0
control
STZ-treated


Catalase presence
Catalase Activity
A
catalase
β-actin
control
STZ-treated
control
STZ-treated
B
catalase/β-actin
(% of control)
200
150
100
50
0
*
C
catalase activity
(nmol/min/mg protein)
200
150
100
50
0
*


COX-1
A
control
STZ-treated
control
STZ-treated
Diabetes, 4 weeks
Diabetes, 12 weeks
B
COX-1/β-actin
(% of control)
400
300
200
100
0
control
STZ-treated
*
*
4 weeks
12 weeks


A
Tension Change
(% of 60 mM KCl)
150
100
50
0
control
tiron (10-3 M)
SOD (120 U ml-1)
DETCA (10-4 M)
*
*
-8.0
-7.5
-7.0
-6.5
-6.0
A23187 (log M)
B
Tension Change
(% of 60 mM KCl)
150
100
50
0
control
deferoxamine (10-4 M)
catalase (1200 U ml-1)
*
*
-8.0
-7.5
-7.0
-6.5
-6.0
A23187 (log M)


Ach
R
Calcium ionophore
Endothelium
COX
Arachidonic Acid
PGH2
L-arginine
NOS
PGISynthase
L-Citrulline
K+
EDHF
PGI2
NO
ONOO-
Smooth muscle
IP
cAMP
cGMP
Ca2+
Relaxation
ROS
PGISynthase
Thromboxane Synthase
PGH2
TXA2
PGE2
TP
EP1
Contraction
.... = effects from ROS
ww = inactive