

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 diameter⁷⁻⁹.

In type I diabetic animals, acetylcholine-induced relaxations are potentiated in the rat aorta and canine coronary arteries¹⁰⁻¹², normal in the aorta¹³⁻¹⁶ and the mesenteric arteries¹⁴ of the rat, and/or depending on the authors, blunted in the same blood vessels¹⁷⁻¹⁸. Moreover, NO-mediated relaxation is augmented at the early stage of diabetes followed by an impaired relaxation as the disease progresses¹⁹⁻²⁰. Likewise, in type I diabetes patients, endothelium-dependent relaxations are impaired^{4, 21-23}, normal²⁴⁻²⁸ or enhanced²⁹.

In type II diabetic animals, NO-mediated relaxation is increased³⁰, preserved³¹ or reduced³² in the aorta of obese Zucker fatty rats. Blunted NO-mediated relaxations are observed in carotid and mesenteric arteries from Zucker rats³³⁻³⁴, mesenteric arteries from db/db mice³⁵⁻³⁶ and coronary arteries of db/db mice³⁷⁻³⁸. In type II diabetes patients, blunted dilatations are observed consistently³⁹⁻⁴⁰. 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 functions⁴¹. 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 present⁴²⁻⁴³. Thus it remains unclear whether or not diabetes type II *per se* affects endothelial function.

Prostacyclin (PGI₂) is not only an endothelium-derived relaxing factor⁴⁴⁻⁴⁶ but also is an important inhibitor of platelet aggregation⁴⁷.

In type I diabetes, prostacyclin contributes to the enhanced acetylcholine-induced and endothelium-dependent relaxation in the coronary artery of dogs¹⁰ and the aorta of the mouse⁴⁸. In addition, the oral administration of prostacyclin analogues restores the blunted

relaxation to acetylcholine in the rat aorta⁴⁹. 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 cell⁵⁰⁻⁵⁵. EDHFs play a more pronounced role in the smaller arteries⁵⁶⁻⁵⁷. They are also regarded as an important compensatory mechanism in the absence or blockade of the production of nitric oxide⁵⁸⁻⁶². 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 preserved⁶³⁻⁶⁶. Nonetheless in the mesenteric artery of the same rat model, an augmented contribution of EDHF compensates for the blunted nitric oxide response¹⁸.

In type II diabetes, EDHF-mediated relaxations are reduced in the mesenteric artery of Goto-Kakizaki rats⁶⁷, Zucker obese rats⁶⁸, and Otsuka-Long-Evans-Tokushima Fatty (OLETF) rats⁶⁹ 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 reduced⁷⁰.

Endothelium-derived and cyclooxygenase-dependent contracting factor (EDCF) is a hallmark of endothelial dysfunction. The product of cyclooxygenase activates thromboxane-prostanoid (TP-) receptors⁷¹⁻⁷⁵. The nature of endothelium-derived contracting factor depends

on the experimental model studied and the stimulating agents used. It can be endoperoxide⁷⁶⁻⁷⁷, thromboxane A₂⁷⁸⁻⁷⁹, prostaglandin E₂⁷⁹, or prostacyclin⁸⁰⁻⁸².

In type I diabetes, the EDCF-mediated response is potentiated in the aorta of rabbits⁸³, the aorta of mice⁸⁴ 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_{2α}, reduces the arterial diameter⁸⁵.

In the renal artery of a type II diabetes model, the OLETF rat, indomethacin restores the blunted relaxation to acetylcholine⁸⁶. Similar results were obtained in the aorta from db/db mice⁸⁷. An augmented EDCF-mediated contraction is observed in the mesenteric artery of OLETF rats⁸⁸⁻⁸⁹.

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 pathology⁹⁰.

An ET-1 antagonist, J-104132 normalizes the up-regulation of p22phox subunit of NAD(P)H oxidase in streptozotocin-treated rats⁹¹. Moreover, the dual blockade of ETA and ETB receptors enhances the endothelium-dependent vasodilatation to acetylcholine in insulin-resistant patients⁹². These observations suggest that ET-1 may play a role in the endothelial dysfunction of diabetes.

Reactive Oxygen Species in Diabetes

Reactive oxygen species (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 aging⁹³.

Oxygen-derived free radical species originate from molecular oxygen. Superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\cdot) 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 oxidases⁹⁴, mitochondria⁹⁵. In addition, xanthine oxidase, cytochrome P450(s), cyclooxygenases and NO synthase are capable of producing reactive oxygen species⁹⁶.

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 species⁹⁶⁻¹⁰². 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 methylglyoxal and 3-deoxyglucosone, intermediate products of glycation, are typical examples. The serum concentrations of both methylglyoxal and 3-deoxyglucosone are increased in patients with diabetes mellitus¹¹².

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 peroxidase¹¹⁹⁻¹²⁰, 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 1980⁷. 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 concentration¹²⁴. 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 enhanced¹²⁸⁻¹²⁹. Since nitric oxide has a half-life of only a few seconds¹³⁰ and is rapidly inactivated by hemoglobin and superoxide^{8, 130}, the concept of loss of nitric oxide bioavailability is more precise and

important¹³¹. The blunted nitric oxide mediated response can be restored by the L-arginine, the substitute of nitric oxide synthase¹³²⁻¹³³ or BH4, an essential cofactor of nitric oxide synthase^{20, 36}. Treatment with antioxidants also prevents the blunted relaxation³⁶.

Uncoupled eNOS is an important source of reactive oxygen species under pathological condition¹³⁴⁻¹³⁵. 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 vasoconstriction¹³¹.

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 mice¹³⁷⁻¹³⁸, the mesenteric artery of humans¹³⁹ and the porcine coronary artery¹⁴⁰.

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 rats¹⁴⁴ 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 rat¹⁴⁴ 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 rats¹⁴⁴ 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 contracting 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 H₂O₂ 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 artery¹⁰⁰. A similar conclusion has been reached in the aorta of spontaneously hypertensive rats¹⁴⁴.

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 dysfunction¹¹⁶. Folic acid restores the impaired endothelial function as well⁴¹. Likewise, in diabetes patients, vitamin E, vitamin C, or folic acid improve

endothelial dysfunction¹⁴⁷⁻¹⁵¹. 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 events¹⁵²⁻¹⁵⁴. 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.

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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, N ω -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).

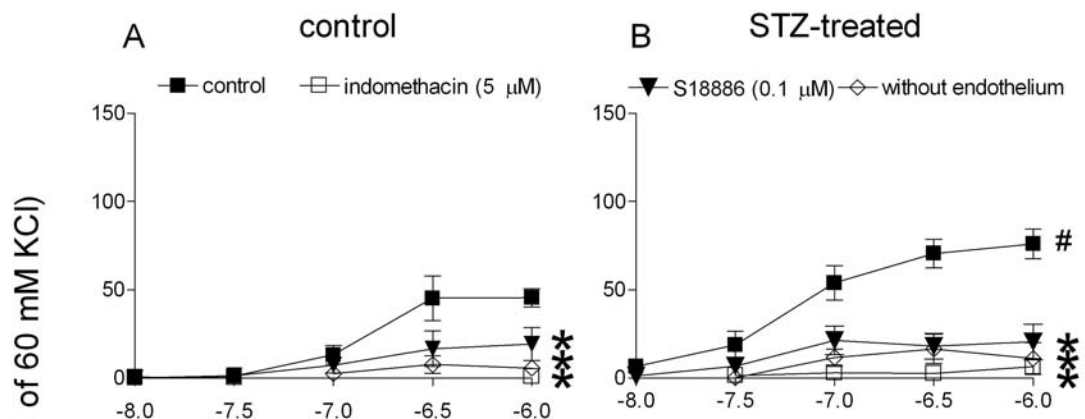
Figure 5. The presence of COX-1 (a and b) in intact femoral artery from control and streptozotocin (STZ)-treated rats, 4 weeks and 12 weeks after the injection of streptozotocin (STZ). Data presented are a percentage of control and shown as means \pm SEM; n=6. *Statistically significant difference from 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 \pm SEM; *Significant difference (P<0.05) from the controls (rings with endothelium). DETCA, diethyldithiocarbamic acid; L-NAME, N ω -nitro-L-arginine methyl ester hydrochloride; SOD, superoxide dismutase; STZ, streptozotocin (From Shi et al., 2007, with permission ¹⁰⁰).

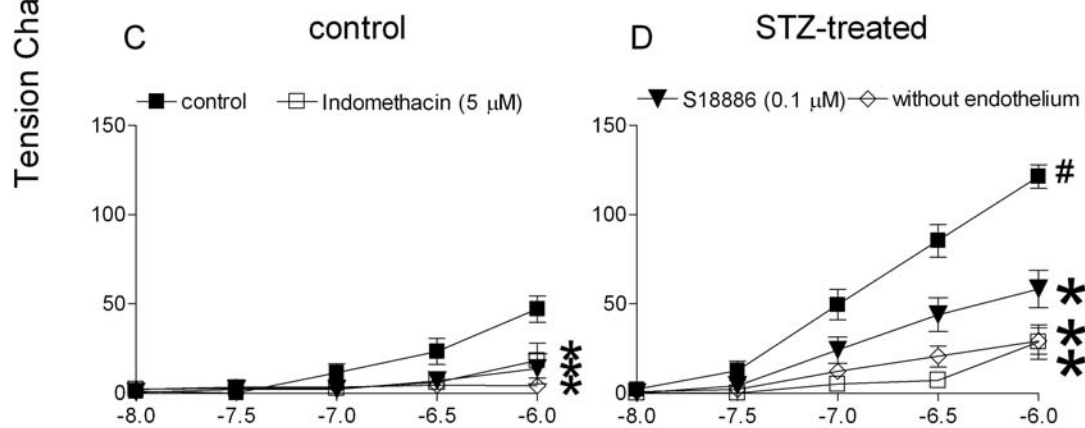
Figure 7. Proposed mechanisms of endothelial dysfunction in diabetes. Oxidative stress is an important contributor to this dysfunction. Oxygen-derive free radicals inactivate NO. Increased basal oxidative stress in the endothelium potentiates endothelium-dependent contractions. Thus, the balance tips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

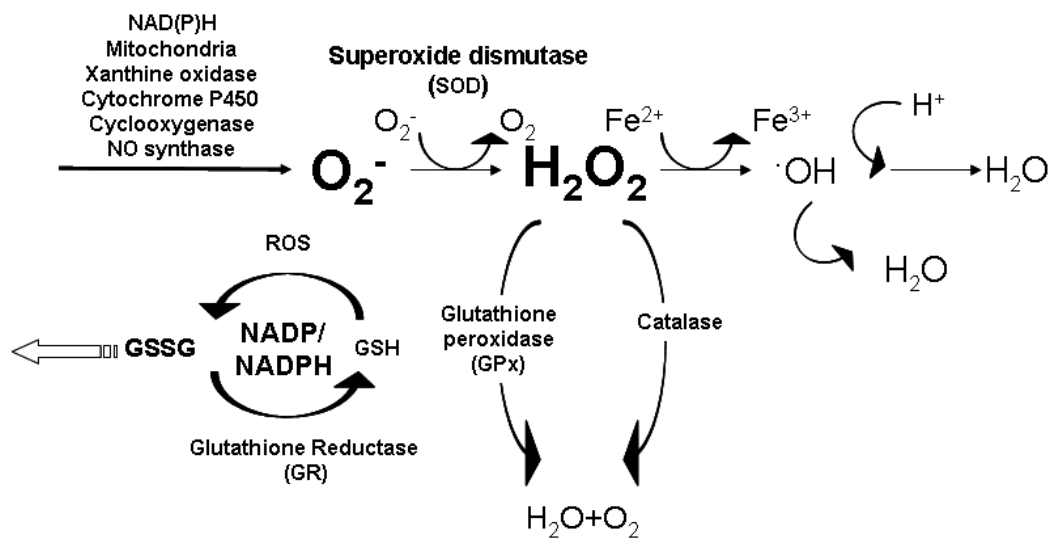
Diabetes, 4 weeks

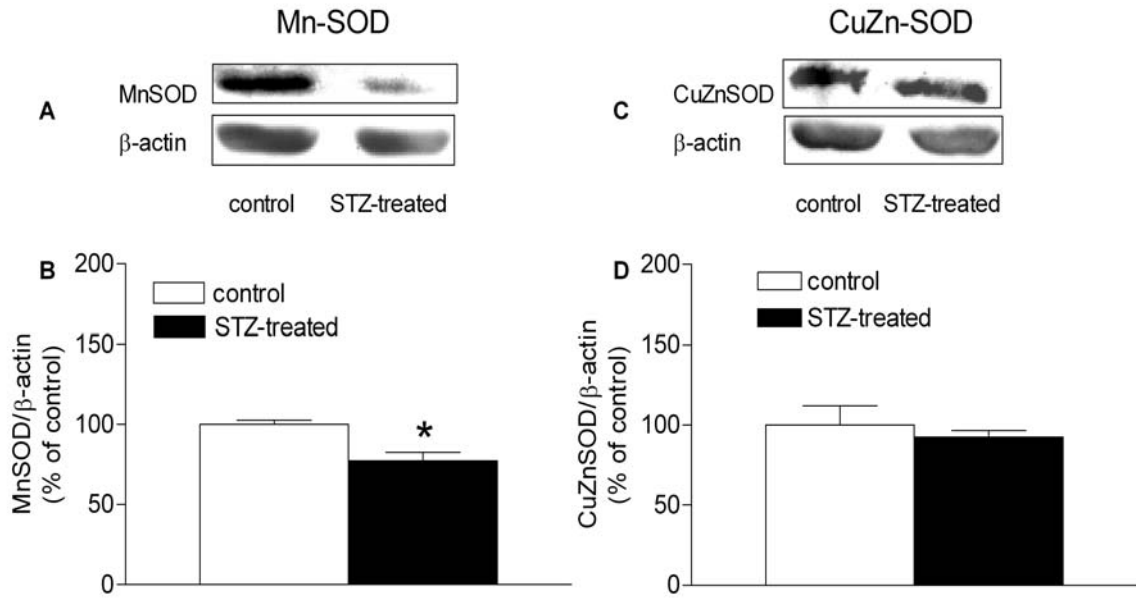


Diabetes, 12 weeks

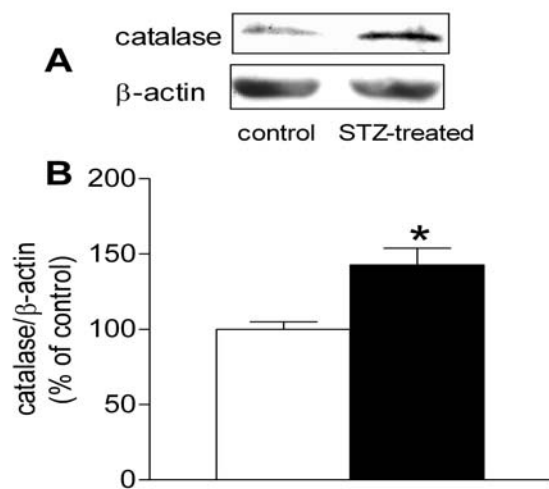


A23187 (log M)

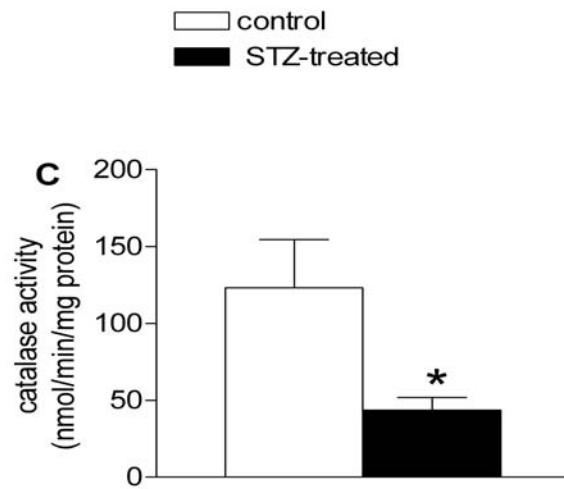




Catalase presence



Catalase Activity



COX-1

