Glycoxidative Stress and Cardiovascular Complications in Experimentally-Induced Diabetes: Effects of Antioxidant Treatment

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Abstract: Diabetes mellitus (DM) is a common metabolic disease, representing a serious risk factor for the development of cardiovascular complications, such as coronary heart disease, peripheral arterial disease and hypertension. Oxidative stress (OS), a feature of DM, is defined as an increase in the steady-state levels of reactive oxygen species (ROS) and may occur as a result of increased free radical generation and/or decreased anti-oxidant defense mechanisms. Increasing evidence indicates that hyperglycemia is the initiating cause of the tissue damage in DM, either through repeated acute changes in cellular glucose metabolism, or through long-term accumulation of glycated biomolecules and advanced glycation end products (AGEs). AGEs are formed by the Maillard process, a non-enzymatic reaction between ketone group of the glucose molecule or aldehydes and the amino groups of proteins that contributes to the aging of proteins and to the pathological complications of DM. In the presence of uncontrolled hyperglycemia, the increased formation of AGEs and lipid peroxidation products exacerbate intracellular OS and results in a loss of molecular integrity, disruption in cellular signaling and homeostasis, followed by inflammation and tissue injury such as endothelium dysfunction, arterial stiffening and microvascular complications. In addition to increased AGE production, there is also evidence of multiple pathways elevating ROS generation in DM, including; enhanced glucose auto-oxidation, increased mitochondrial superoxide production, protein kinase C-dependent activation of NADPH oxidase, uncoupled endothelial nitric oxide synthase (eNOS) activity, increased substrate flux through the polyol pathway and stimulation of eicosanoid metabolism. It is, therefore, not surprising that the correction of these variables can result in amelioration of diabetic cardiovascular abnormalities. A linking element between these phenomena is cellular redox imbalance due to glycoxidative stress (GOS). Thus, recent interest has focused on strategies to prevent, reverse or retard GOS in order to modify the natural history of diabetic cardiovascular abnormalities. This review will discuss the links between GOS and diabetes-induced cardiovascular disorders and the effect of antioxidant therapy on altering the development of cardiovascular complications in diabetic animal models.

Keywords: Glycoxidative stress, glycation, diabetes mellitus, antioxidant, cardiovascular.

INTRODUCTION

In the past few decades, diabetes mellitus (DM) has rapidly increased in the world. It has been estimated that the number of diabetic patients will more than double within 20 years. DM is mainly characterized by the development of increased morbidity and mortality due to cardiovascular disease (CVD) [1-3]. Diabetic cardiovascular complications appear to be multifactorial in origin, but in particular, glycoxidative stress (GOS) has been suggested to be the unifying link between the various molecular disorders in DM. The biochemical process of advanced glycation end products (AGEs), is accelerated in DM due to chronic hyperglycemia-induced oxidative stress (OS), which has been postulated to play a central role in this disorder [4-6]. DM has been suggested to be a major source of AGEs *in vivo* [7]. The consequences of GOS include; damage to DNA, lipids, proteins, disruption in cellular homeostasis and accumulation of damaged molecules. These damaged molecules disrupt endothelial cells and cardiomyocytes and impair cardiovascular reactivity [7]. Nitric oxide (NO) regulates vasodilatation, anticoagulation, leukocyte adhesion, smooth muscle proliferation and the antioxidative capacity of endothelial cells [8]. Hyperglycemia has been reported to decrease the availability of NO in animals and humans, which may contribute to the hemodynamic and physiological changes occurring in DM [9]. G protein-adenylyl cyclase signaling, diacylglycerol (DAG)/ protein kinase C (PKC) pathway and calcium current/ entry, which play an important role in the regulation of cardiovascular functions has also been reported to be impaired in diabetes-induced GOS [10]. The harmful effects of hyperglycemia-induced GOS on cardiovascular function and the link to pathophysiological mechanisms underlying diabetic complications have lead to the development of new pharmacotherapeutic strategies. Although, aldose reductase inhibitors and PKC inhibitors represent a potential therapeutic approach to prevent the onset or the progression of these complications, recent interest has

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1874-1924/10

focused on strategies to prevent firstly GOS and then its harmful effects [11, 12]. Indeed, there is accumulating evidence that an interaction between AGE and its receptor (RAGE) generates OS and subsequently evokes vascular inflammation and thrombosis, thereby playing a central role in diabetic vascular complications [13]. This review presents evidence of the pathophysiology and cellular mechanisms of cardiovascular complications in experimental DM; discuss the therapeutic strategies with antioxidant compounds and evaluates the pyridoindole derivatives as potential remedy for diabetes-induced metabolic, structural and functional abnormalities in the cardiovascular system.

EXPERIMENTAL DIABETES-INDUCED CARDIOVASCULAR COMPLICATIONS

The Mechanisms of Abnormal Cardiac Contractility in DM

Patients with DM are more prone to develop cardiomyopathy than nondiabetic subjects [14]. This complication is serious and is a major cause of mortality due to DM. According to the Framingham study, the incidence of cardiovascular morbidity per year is 39.1% in diabetic males and 17.2% in diabetic females; chronic heart failure afflicts 7.6% of diabetic males and 11.4% of diabetic females. Diabetic cardiomyopathy is a specific syndrome, consisting of cardiomegaly, left ventricular dysfunction, electrical remodeling of the ventricle, and symptoms of congestive heart failure [15, 16]. Hypertension is recognized as an important cofactor in the development of fatal congestive heart failure in diabetics [17]. The pathophysiology of diabetic cardiomyopathy is incompletely understood and several mechanistic approaches are under debate. Decreased left ventricular compliance and increased interstitial connective tissue have been observed in chronically diabetic animals [17]. In contrast, ventricular myocardium from diabetic rats exhibit a reversible decrease in the speed of contraction, prolongation of contraction, and a delay in relaxation [17, 18]. These mechanical changes are associated with changes in the responses to alpha- and beta-adrenergic and cholinergic stimulation [18]. We have shown that the changes in myocardial beta-adrenergic responsiveness may contribute to heart failure in streptozotocin (STZ)-diabetic rats [19]). Both insulin-dependent diabetes mellitus (IDDM) [20] and non-insulin-dependent diabetes mellitus (NIDDM) [21] causes significant alterations in myocardial contractility induced by adrenergic agonists. In our laboratory, it has been showed that experimental diabetes produces a marked decrease in the rate of spontaneously beating right atria and a significant increase in basal contractile force of left ventricular papillary muscle. In diabetic rat atria, the positive chronotropic effect of noradrenaline was markedly increased, while the positive chronotropic effect of isoprenaline was significantly decreased. STZ-diabetes also induced an increased positive inotropic response of papillary muscle to both noradrenaline and isoprenaline [19, 21]. In addition, the ultrastructural degeneration of the diabetic myocardium (i.e. intracellular oedema, myofibrillar fragmentation, condensed pleomorphic mitochondria, thick capillary irregular basement membrane and swollen endothelial cells) was associated with mechanical alterations caused by adrenergic agonists [21]. Long-term experimental IDDM, characterized by ventricular remodeling, overt resting left ventricular systolic dysfunction and decreased left ventricular responsiveness to beta-adrenergic receptor stimulation is associated with histopathological changes indicating dilatational remodeling as increased capillary tortuosity and type IV collagen in capillaries and cardiomyocytes [22]. Insulin replacement therapy in STZ-diabetic rats can prevent these changes [19, 20], and thyroid hormones mediate the effect of insulin therapy [19]. These findings were recently confirmed in a study using conscious diabetic rats, which showed a decrease in basal mean arterial pressure, heart rate, +dP/dtmax and -dP/dtmax. In that study, insulin prevented the hemodynamic and myocardial function alterations observed in STZ-diabetic rats [23]. Another study indicated that male and female rats are affected differently by DM in terms of left ventricular developed pressure (LVDP) responses to beta-adrenergic receptors (beta-ARs) stimulation, probably due to the difference between their beta-ARs induced cyclic adenosine monophosphate (cAMP) responses [24]. Alterations in the myocardial beta-AR signaling pathway in two different models of rat NIDDM, has also been addressed; it showed that the beta-AR signaling system of the insulin deficient model is altered more than the hyperinsulinemic model and the exaggerated cAMP response of NIDDM [25, 26]. Hearts from diabetic db/db mice, a model of NIDDM, exhibited left ventricular failure and altered metabolism of exogenous substrates, was prevented by PPAR-alpha and -gamma agonists [27]. Diabetic cardiomyopathy was expressed by a decreased beta1-AR mRNA levels, and an increased beta2- and beta3-ARs mRNA levels in STZ-treated diabetic rat hearts [28, 29]. Insulin-deficient diabetes was associated with a reduced expression and concomitant functional loss of G inhibitor protein in ventricular cardiomyocytes [30]. Long-term DM results in a marked decrease in the positive inotropic response of left atria to electrical field stimulation due to the impairment of noradrenaline release from sympathetic nerve terminals [31]. On the other hand, it has been shown that an alpha-adrenergic receptors (alpha-ARs)-mediated electrophysiological response is unmasked when the beta-AR-mediated response is desensitized in the papillary muscle of STZ-induced diabetic rats [32]. Alpha1-adrenergic responsiveness of rat myocardium was reported to be higher in experimental diabetes and partly reversed by insulin treatment [32]. This increase has been suggested to serve as a compensatory mechanism for the lower beta-adrenergic responsiveness in this organ [32]. The results showed that diabetes-induced impairment in cardiac alpha 1-AR signaling is closely linked with the abnormal activation of cardiac PKC [33, 34]. Other studies also indicated that presynaptic alpha 2-ARs and muscarinic receptors that are linked to the inhibition of noradrenaline release during nerve stimulation are functionally impaired in diabetic animals [30].

The altered cholineacetyltransferase, cholinesterase activities and choline concentration has been suggested to be the reasons for the supersensitivity to muscarinic agonists in the myocardium of STZ-diabetic rats [35]. In addition, cardiac M2-muscarinic receptor (M2-MR) gene expression was increased in STZ-diabetic rats [35, 36]. Alterations in the atrial muscarinic system in STZ-diabetic rats have been suggested to be a consequence of impaired functional muscarinic receptor-G protein coupling [37, 38]. In fact, aldose reductase inhibitors have been successfully used to treat and prevent muscarinic receptor-mediated cardiac complications

in experimental DM [35]. In the light of previous reports, diabetes-induced functional abnormalities in the heart may be attributed to altered adenosine action. The purinergic response of rat atria was increased in STZ-diabetes [39]. It has been demonstrated that the changes in adenosine receptor gene expression and protein content correlated with some of the abnormalities characterized in the diabetic heart [39]. An increase in adenosine A1 receptor protein content, without a change in mRNA level was observed in isolated cardiac myocytes. DM caused an increase of adenosine A3 receptor mRNA and protein content in the heart and cardiac myocytes. The level of adenosine A2a receptor mRNA was increased in the whole diabetic heart, but it decreased in cardiac myocytes with undetectable changes in protein content. Interestingly, the administration of insulin to diabetic rats for four days resulted in the adenosine receptors, mRNA and protein content returning to levels observed in the heart of normal rats [39, 40].

Hyperglycemia-induced up-regulation of the endothelin (ET)-system is another important event in the pathogenesis of diabetic CVD. The measurement of ET-1, ET (A) and ET (B) receptors and mRNAs showed a significant increase in mRNA levels in the hearts of diabetic rats. ET-1 was detected in the cardiomyocytes, endothelium and smooth muscle cells of the larger blood vessels by immunohistochemistry, which diabetes increased. Autoradiographic localization of ET-1 receptors showed increased binding in the endothelium and myocardium of diabetic animals [41]. ET-1 was also elevated in atherosclerotic plaques. Diabetic patients have accelerated atherosclerosis and also show elevated plasma levels of ET-1. Inhibiting the actions of ET-1 and the signaling pathways are thought to represent potential therapeutic targets for the prevention of atherosclerotic diabetic disease [42].

Metabolic impairment such as hyperglycemia, hyperlipidemia, hypoinsulinemia, and alterations in cardiac metabolism are the main reasons for vascular structural and functional changes, which instigate the cellular effects leading to redox imbalance, interstitial fibrosis, myocyte death, and disturbances in ion transport and homeostasis [43]. A decrease in myosin ATPase, a shift in myosin isoenzyme distribution, alterations in a variety of Ca^{2+} fluxes associated with cardiac mechanical alterations are observed in experimental DM [44]. A deficiency in sarcoplasmic reticulum Ca^{2+} uptake contributes to a lower beta-ARs-stimulated inotropic response of diabetic myocardium [45]. Chronic STZ-diabetes leads to Ca^{2+} accumulation in cardiac myocytes [46]. In accordance with this, the changes in intracellular Ca^{2+} mobilization has been suggested to account for the depressed IGF-I-induced inotropic response in the diabetic heart [47]. Indeed, alterations in intracellular Ca^{2+} handling play a pivotal role in the impairment of cardiac function in diabetes. The reduced function of the sarcoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+}-release in response to beta-AR challenge, is thought to have a causative role in the depressed hemodynamic performance of the challenged heart in the early stage of DM [47]. The observed changes in the contractility, together with Ca^{2+} handling of the diabetic heart are most likely attributable to functional disturbances of SERCA2a, RyR2 and NO/ cyclic guanosine monophosphate (cGMP) systems in response to beta-adrenergic activation [48,49]. The changes in cardiac mechanical parameters correlate with decreased ATP and increased lactate in the inner layer of the left ventricle in diabetic rats. But, of course, this cannot be explained by the rate of decrease in total ATP and lactate accumulation alone [50]. Recent evidence implicates disturbances in cardiac energy metabolism; in the uncontrolled diabetic state, cardiac myocytes use fatty acids almost exclusively to support ATP synthesis [51].

IMPAIRED VASCULAR REACTIVITY IN DM: ROLE OF ENDOTHELIAL CELLS

Vascular disease, a complicating feature of DM, is characterized mainly by atherosclerotic and coronary artery abnormalities [52]. Diabetic patients have a 2- to 4-fold increase in the incidence for coronary artery disease and 10-fold increase in peripheral vascular diseases, due in part, to accelerated atherogenesis [53]. Alterations of vascular smooth muscle function have been widely implicated in the development of vascular complications and circulatory dysfunction in DM [54]. However, the influence of DM on catecholamine-induced vasopressor activity under *in vitro* conditions is contradictory. Although, a decreased responsiveness of the rat aorta to noradrenaline and phenylephrine has been noted in STZ-diabetes [55-57], many investigators have demonstrated an increased responsiveness of the rat aorta to alpha-AR stimulation in the same model [58, 59]. Similarly, we found an increased responsiveness of isolated aorta to alpha-AR agonists in rats with at least six weeks diabetes, which was reversed by insulin treatment [60-64]. The duration of DM might be the main reason for this discrepancy [65, 66]. Our observations using diabetic rat aorta were subsequently confirmed by the finding of an increase in the contractility of the internal mammary artery and saphenous vein obtained from diabetic patients undergoing coronary artery bypass surgery [67]. The increased responsiveness of the diabetic aorta to vasoconstrictor agents was associated with the abnormal structure of the smooth muscle and endothelial cells [62, 63].

We demonstrated that the entry of Ca^{+2} into the cytosolic membrane of diabetic rat aorta was augmented by the increased activation of voltage (L-type) and receptor-operated channels, since there was an increased contraction to L-type Ca^{2+} channel activator BayK-8644 and AR-receptor agonist phenylephrine [68, 69]. Moreover, Ca^{2+} overload and/or impaired Ca^{2+},Mg^{2+}-ATPase activity might also been responsible for these abnormalities [45]. Indeed, other investigators suggested that changes in functional Ca^{2+} store sizes and the Ca^{2+} entry seems to be responsible for the alterations in contractile responses to phenylephrine following DM [65, 70]. In fact, diabetes-induced contractile disturbances are not only related to the activity of Ca^{2+} channels or Ca^{2+} current but also the involvement of K^+ channels. A diminished relaxation response to cromakalim, a K_{ATP} channel opener, has been reported in diabetic vessels by us, as well as others [71-73].

In 1980, Furchgott discovered that vascular endothelial cells produce an endothelium-derived relaxing factor (EDRF) in response to stimulation by ACh [74]. Vascular relaxation to ACh and a number of other agonists was found to be dependent on the presence of an intact endothelium. In 1987, Moncada *et al.* and Ignarro *et al.* separately, proved

that EDRF is NO [75, 76]. A year later, Moncada also demonstrated that NO is synthesized from the amino acid L-arginine [77]. Earlier, Murad had shown that the relaxing effect of nitrovasodilators depended on the activation of soluble guanylate cyclase to increase cGMP levels in the arteries [78]. All these works have contributed to the establishment of NO as a signaling molecule in the cardiovascular system. In 1992, NO was described as the molecule of the year [79] and the importance of the NO discovery was recognized with the Nobel Prize in Physiology and Medicine awarded to Furchgott, Ignarro and Murad. Subsequently, NO had been implicated in the pathogenesis of diseases such as DM, atherosclerosis and hypertension. Today, it is hard to find a disease which is not associated with altered NO homeostasis. In 1989 it was reported that endothelial cells contain a cytosolic enzyme that is directly or indirectly regulated by Ca^{2+}, which converts L-arginine into a compound that stimulates guanylate cyclase and behaves similarly to EDRF [80]. In the same year we reported, for the first time, that endothelium-dependent relaxations produced by ACh and histamine in aortic rings precontracted with noradrenaline was significantly increased in 6 weeks STZ-induced, IDDM [60]. In contrast, the relaxations elicited by those agents were significantly attenuated in aorta of NIDDM rats. [60]. Previously, an attenuated response of isolated aorta to endothelium-dependent vasodilators had been reported by Oyama *et al*. in STZ-diabetic rats [81] and by Durante *et al*. in Bio Bred (BB) rats [82]. However, we found that the abnormal endothelial function in diabetic aorta was especially dependent on hyperglycemic control and the duration of DM [61, 62].

Over the years, many investigators have demonstrated some abnormalities in endothelium-dependent relaxation in arteries from a number of experimental animal models of DM [83-87], while others have found no changes in response to endothelium-dependent vasodilators [88]. In this context, the endothelium of diabetic aorta exhibits decreased, increased or unchanged responsiveness to ACh depending on the model, severity and/or duration of DM [88]. Now, endothelium-dependent vasodilatation is recognized as a reproducible and accessible parameter to probe endothelial function in different pathophysiological conditions, including DM, aging and CVD. Similarly, we confirmed endothelial dysfunction in studies of human NIDDM [67]. The impaired endothelium-dependent vasodilatation might arise from decreased NO production, enhanced inactivation of NO, impaired diffusion of NO to the underlying smooth muscle cells, decreased responsiveness of the smooth muscle to NO and enhanced generation of endothelium-derived constricting factors. As a common observation, we and many others reported that the relaxation of diabetic aorta induced by sodium nitroprusside (an endothelium-independent and cGMP-mediated relaxant agent) was comparable to control tissue, suggesting that vascular smooth muscle exhibit a normal responsiveness to a guanylate cyclase stimulator such as NO, in the diabetic state [60-62, 84, 85]. Despite a growing understanding of the mechanisms by which NO is released from the endothelium, the precise molecular mechanisms by which NO relaxes vascular smooth muscle are not fully understood. Previous studies have provided evidence that a major action of NO in normal vascular smooth muscle is to reduce intracellular free Ca^{2+} levels *via* cGMP-dependent and-independent mechanisms. It was also proposed that NO can activate SERCA by a cGMP-independent process [89, 90]. NO influences vascular homeostasis in many ways beyond modulation of vasomotion, such as inhibition of smooth muscle cell proliferation, platelet aggregation, platelet and monocyte adhesion to the endothelium, LDL oxidation, expression of adhesion molecules and endothelin production [91].

Besides cyclooxygenase and nitric oxide synthase (NOS), another distinct endothelial pathway is endothelium-dependent hyperpolarization (EDHF), which is also involved in the relaxation of the vascular smooth muscle cells. EDHF has been demonstrated unequivocally in various blood vessels from different species, including human and is likely to play an important role in cardiovascular physiology. This alternative pathway involves the activation of two populations of endothelial K^+ channels, the small conductance and intermediate conductance Ca^{2+}-activated K^+ channels, which are altered in DM [92, 93]. On the other hand, the interaction between endothelial cells and monocytes is thought to play an important role in normal vascular biology, while an increased/prolonged interaction is implicated in the pathogenesis of vascular disease [91]. Monocyte adhesion to the endothelium suppresses the release of biologically active NO in monocyte in a concentration-and time-dependent manner, *via* an IL-1B dependent pathway [91, 94]. This event would predispose the vessel to develop atherosclerotic lesions due to the potential consequence of increased macromolecular permeability and intimal proliferation *via* the development of vasospasm, thrombosis and recruitment of platelets and leukocytes [91, 94]. In this respect, we confirmed the monocyte adhesion to the endothelial cells and impaired morphology of diabetic aorta by electron microscopy [63, 64, 68, 95]. Beside monocytes, there are many other inhibitors of the biological activity of NO, such as decreased L-arginine uptake, decreased co-factors (Ca^{2+}, calmodulin, BH4), inhibition of electron flow (nicotinamide adenine dinucleotide phosphate; NADPH, flavins), inhibition of NOS expression, inhibition of substrate binding to NOS, and NO scavengers [97, 98].

GLYCOXIDATIVE STRESS IN THE NATURAL HISTORY OF DIABETES-INDUCED CARDIOVASCULAR COMPLICATIONS

Although the mechanisms responsible for mediating endothelial dysfunction have not been completely defined, elevated OS plays a key role in the development of endothelial dysfunction, as well as, atherosclerosis in diabetes-associated vascular disease. Increased basal production of superoxide (O_2^-), and hydrogen peroxide (H_2O_2) in the aorta has been shown in early studies using diabetic animals [98, 99]. The main reasons for the diabetes- induced increase in OS are; an increased generation of oxygen-derived free radicals through autoxidation of glucose, AGE-formation, increased substrate flux through the polyol pathway, and stimulation of eicosanoid metabolism, sorbitol-DAG metabolism and NOS [4-7, 11, 13, 92, 96, 100, 101]. Glucose-induced changes in the activity of NADPH oxidase and endothelial nitric oxide synthase (eNOS) can cause vascular endothelial cell dysfunction *via* disregulation of eNOS and/or changes in the expression of the subunits of NADPH oxidase [102]. Generally, the development of OS is due to the excessive formation and/or

insufficient removal of highly reactive molecules such as, reactive oxygen species (ROS) and reactive nitrogen species (RNS) [100-103]. ROS includes; free radicals such as O_2^-, hydroxyl (OH), peroxyl (RO_2), hydroperoxyl (HRO_2^-), as well as, non-radical species such as H_2O_2 and hydrochlorous acid (HOCl). RNS include free radicals such as; NO and nitrogen dioxide (NO_2^-), as well as non-radicals such as peroxynitrite ($ONOO^-$), nitrous oxide (HNO_2) and alkyl peroxynitrates (RONOO). There are multiple sources of OS in DM, which include non-enzymatic, enzymatic and mitochondrial pathways [4-7, 11, 101, 103]. Non-enzymatic sources of OS originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause an increase in ROS generation. Glucose can undergo autoxidation and generate OH radicals [103]. In addition, glucose can react with proteins in a non-enzymatic manner leading to the development of Amadori products followed by the formation of advanced glycation end-products AGEs; ROS is generated at multiple steps during this process. The formation of AGEs progressively increases with normal aging, even in the absence of disease. However, they are formed at an accelerated rate in DM due to uncontrolled hyperglycemia. DM is a major source of AGEs production *in vivo* and is not only a marker but also an important causative factor for the pathogenesis of the disease. AGE modification of proteins leads to alterations in their normal function, abnormal signal transduction pathways and tissue injury all contribute to the development of serious complications, which is responsible for the morbidity and mortality observed in diabetic patients [6, 7, 12, 13, 104-106]. Binding and activation of cellular RAGE by AGEs or similar ligands can also promote OS and AGE formation *via* the NAD(P)H oxidase and the myeloperoxidase pathways. Another potential mechanism of AGE formation is the polyol pathway. Glucose entering the polyol pathway may form AGEs *via* reactive intermediates, i.e. glyoxal, methylglyoxal or 3-deoxyglucosone, as well as, *via* depletion of NAD(P)H or glutathione raising intracellular OS, all of which indirectly result in the increased formation of AGEs [104-106]. There are two types of cell surface AGE receptors, those that bind AGEs and initiate cell activation and those that bind and degrade AGEs. RAGE is the best studied receptor of the first category; it recognizes AGEs and initiates OS. The second group of receptors includes; AGER1, AGER3 and CD36. The best evaluated receptor in this category is AGER1, which has been found to have marked anti-oxidant properties [12, 13, 106, 107]. In the presence of chronic hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of O_2^- [106]. Enzymatic sources for increased generation of ROS in DM include mainly NOS, NAD(P)H oxidase and xanthine oxidase [13,104,108,109]. All isoforms of NOS require five cofactors/prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH4 and Ca^{2+}-calmodulin. If NOS lacks its substrate L-arginine or one of its cofactors, NOS may produce O_2^-instead of NO and this is referred to as the uncoupled state of NOS [109, 110]. NAD(P)H oxidase is a membrane associated enzyme that consists of five subunits and is a major source of O_2^- production [109]. It has been demonstrated that the enhanced production of O_2^- in the diabetic vasculature, predominantly mediated by the increased levels of NAD(P)H oxidize also stimulates PKC activity [109]. It has been suggested that mitochondrial O_2^- is the initiating "snowball" that turns OS into an avalanche in DM by stimulating more ROS and RNS production *via* downstream activation of nuclear factor-kappaB (NF-KB)-mediated cytokine production, PKC and NAD(P)H oxidase [111-113].

In light of the OS hypothesis, we previously investigated the time course for changes in endothelium-dependent relaxations using isolated aorta rings. It was found that endothelium-dependent and -independent relaxant responses of diabetic aorta are more transient than those of non-diabetic control vessels and the time required to reach peak relaxation after the addition of ACh was shorter in aortic rings isolated from 6-month STZ-diabetic rats compared to non-diabetic rats [114]. In our study, pretreatment of diabetic vessels with superoxide dismutase (SOD) normalized the recovery phases of endothelium-dependent and -independent relaxations, but had no effect on the peak responses to ACh and sodium nitroprusside. In the presence of diethyldithiocarbamate, an inhibitor of SOD, the transient nature of the relaxant response to ACh or sodium nitroprusside was found to be more marked and the peak relaxations were inhibited; these effects of diethyldithiocarbamate were more pronounced in diabetic than in control rings. Catalase caused a decrease in the peak relaxant response to ACh and accelerated the fading of the relaxation in diabetic aorta. While, pretreatment with 3-amino-1,2,4 triazole, (a catalase inhibitor), inhibited the peak relaxant response to ACh in diabetic rings. The combination of SOD plus 3-amino-1,2,4 triazole produced an increase in the transient nature of endothelium-dependent relaxation of diabetic aorta, which was greater than that with 3-amino-1,2,4 triazole alone. Neither catalase nor 3-amino-1,2,4 triazole affected the characteristics of sodium nitroprusside-induced relaxation. Desferrioxamine, an inhibitor of OH radical production, or mannitol, a OH radical scavenger, had no effect on the characteristics of either ACh- or sodium nitroprusside-induced relaxation in control and diabetic aorta [114]. Similarly, others have reported that the rapid fade of the endothelium-dependent responses of diabetic vessels is significantly suppressed by pretreatment with SOD but not with catalase, deferoxamine, allopurinol, or indomethacin [115]. The OS-related rapid inactivation of NO has been recognized as an important factor leading to diabetes-induced endothelial dysfunction [92, 114-117].

We, as well as, others have found that the diabetic aorta is more sensitive to ROS exposure when compared to non-diabetic, healthy aorta [118, 119]. H_2O_2 induced a brief contraction before relaxation of aorta rings with an intact endothelium that was more pronounced in diabetic vessels. Removal of the endothelium or pretreatment of the rings with L-nitro-arginine methyl ester (L-NAME), a NO synthase inhibitor, immediately abolished the H_2O_2-induced response with a transient increase in tone, while pre-incubation with indomethacin had no significant effect on aortic contractions induced by H_2O_2 in long-term diabetic and healthy control animals. In addition, long-term STZ-diabetes caused a significant increase in the maximum relaxation induced by H_2O_2 in aorta already preconstricted with alpha-AR agonists. Preincubation with SOD had no effect on the maximum relaxation induced by H_2O_2, in both non-diabetic and diabetic animals but led to an increase in H_2O_2-induced contractions

in non-diabetic rats. Aortic rings preincubated with diethyldithiocarbamate; H_2O_2 produced only contractions in non-diabetic rats, while H_2O_2-induced relaxations were markedly depressed in diabetic animals. Catalase did not affect the smooth muscle tone of intact or endothelium-denuded aortic rings, stimulated by H_2O_2 and aminotriazole failed to affect H_2O_2-induced contractions or relaxations of diabetic and non-diabetic vessels. These series of experiments suggest that long-term DM causes a decrease in vessel SOD activity that may lead to a change in the dismutation reaction of O_2^- to H_2O_2, causing an increase in endogenous O_2^- anions. The diabetes-induced increase in catalase activity could account for the increased production of harmful OH in the aorta. The enhanced endogenous formation of OH might be an important factor in the development of vascular disorders associated with chronic DM [118].

The excessive production of O_2^- in DM plays a significant role in the inhibition of EDRF/NO bioavailability under basal physiological conditions. The increased vasorelaxation caused by exogenous stimulants of NOS in the early stages of experimental DM [60-62] might compensate for an environment where the basal endothelial relaxation is decreased. Moreover, the involvement of endogenous H_2O_2 during ACh-stimulated endothelium-dependent relaxation might be increased, which is likely to account for the maintenance of the vasorelaxant effect of ACh in the later stage of DM. This phenomenon of an enhanced contribution of H_2O_2 in the establishment of stimulated endothelium-dependent relaxation could account for the maintenance of NOS-mediated relaxation in diabetic arteries [114, 118, 119], is also supported by Matoba *et al.* These authors reported that in some pathological conditions eNOS generates O_2^-, which is converted by SOD to H_2O_2 facilitating endothelium-dependent relaxation [120]. It has also been reported that acute administration of scavengers of O_2^-, including SOD [113, 120], the combination of SOD with catalase [121] or OH radical scavengers, restored or normalized the abnormal endothelium-dependent responses in different models of DM [115, 121-123]. Nevertheless, an increased production of OH might also mediate the increased vasoconstriction tendency of diabetic vessels under basal condition. This is supported by the observation that OH can catalyze the production of vasoconstrictor prostanoids [122-124]. We subsequently revealed an increased SOD-induced relaxation of diabetic aorta when compared with non-diabetic aorta. Pretreatment of aortic rings with L-NAME inhibited SOD-induced relaxation, which was greater in diabetic rings compared to control aorta. While, catalase inhibited SOD-induced relaxation only in diabetic rings. Pretreatment with the cyclooxygenase inhibitor, indomethacin, or the catalase inhibitor, aminotriazole, had no effect on SOD-induced relaxation in both control and diabetic vessels. These findings suggested that: (i) under normal physiological conditions, the relaxant effect of SOD is related to the inhibition of O_2^--induced EDRF/NO destruction in the rat aorta; (ii) in the diabetic state, an excess of O_2^- increasingly inhibits basal EDRF/NO, and exogenously added SOD causing an enhancement of the dismutation reaction resulting in the excess O_2^- forming H_2O_2. The production of H_2O_2, a vasorelaxant molecule, probably accounts for the increased responsiveness of diabetic rings to exogenous SOD [119]. On the other hand, the responsiveness of the diabetic aorta may reflect an important facet of H_2O_2 linked to its specific modulatory role that might compensate for an increase in vascular reactivity in response to alpha-AR stimulation due to OS. It is well known that the vasorelaxation of alpha-AR agonist-precontracted artery rings by H_2O_2 requires the formation of cGMP, and is mediated by NO [125]. H_2O_2 also augments the secretion of other endothelium-derived relaxing peptides, e.g., adrenomodulin and C-type natriuretic peptide, which are thought to compensate for vasoconstriction due to increased OS, in conditions such as, hypertension, atherosclerosis as well as DM [126]. In fact, under hyperglycemic conditions the excessive production of O_2^- interacts with the basal production of EDRF/NO, leading to an increase in the generation of the highly reactive molecule $ONOO^-$. In this situation, the chemical environment of NO (i.e. an increase in O_2^-), may be a determining factor whether NO exerts protective or harmful effects [127]. Accordingly, it has been reported that a greater production of O_2^- and a greater formation of nitrate (NO is metabolized by O_2^- to NO_3^-) occurs in aortic rings from diabetic rats [128]. It is also known that $ONOO^-$ can modify tyrosine residues in various proteins to form nitrotyrosine, which can lead to damage that alters protein function and stability [128, 129]. Thus, nitrotyrosine is a marker of OS [129, 130], in diseases such as atherosclerosis, heart disease and DM [129]. Several studies have shown that the formation of nitrotyrosine and $ONOO^-$ are factors in NO responsiveness and NO production in blood vessels [129, 130]. In this regard, we compared the vasodilatory effect of exogenous $ONOO^-$ with sodium nitrite in precontracted aorta isolated from STZ-induced diabetic and age-matched control rats [131]. $ONOO^-$ produced a concentration-dependent relaxation of aortic rings with/without endothelium. Vasorelaxation occurred with higher concentrations of the decomposition product $ONOO^-$ or sodium nitrite, although these relaxations were considerably slower with reduced sensitivity. Endothelium intact rings were less sensitive to the vasorelaxant effect of $ONOO^-$ than endothelium-denuded rings, in control but not in diabetic aorta. The maximum relaxation to $ONOO^-$ was also increased in diabetic vessels, which was unaffected by the removal of the endothelium either in diabetic or control rings. DM did not alter the relaxations elicited by both decomposed $ONOO^-$ and sodium nitrite. $ONOO^-$-induced relaxation was not inhibited by diethylenetriaminepentaacetic acid, an inhibitor of OH formation. While, pretreatment with $ONOO^-$ significantly suppressed the phenylephrine-induced tone and ACh-stimulated endothelium-dependent relaxation; both effects were more pronounced in diabetic than in control aorta. Taken together, these findings suggest that there is a relationship between the increased responsiveness to exogenous $ONOO^-$ and the depressed basal NO bioavailability in diabetic aorta [131]. The reported inhibitory effect of $ONOO^-$ on NO bioavailability and the elevation in endothelial dysfunction by angiotensin II in diabetic animals is consistent with the findings. Angiotensin II has been found to increase the generation of ROS within vascular smooth muscle cells, primarily through activation of membrane-bound NADPH/NADH oxidase, which promotes a progressive deterioration of the cardiovascular system in STZ-induced diabetic rats [132, 133]. Diabetes-induced over production of angiotensin II may also in turn, trigger the dysfunction of vascular smooth muscle SERCA by the generation of $ONOO^-$ [134].

In fact, the physiological actions of NO and O_2^- may depend crucially on their local concentrations and their intracellular site of formation. In order to accumulate as H_2O_2, endothelial O_2^- must first escape interacting with NO molecules; under normal conditions, at least in some blood vessels, it appears that a small amount of O_2^- survives long enough to be acted upon by SOD to generate H_2O_2. However, under pathological conditions, the production of O_2^- (largely from sources other than eNOS) may overwhelm the production of NO in many blood vessels. Under these circumstances, H_2O_2 production will be limited by the availability of SOD, and endothelium derived $ONOO^-$, prostanoids, and possibly isoprostanes may tip the balance toward vasoconstriction [92, 124, 135].

Although the mechanisms underlying diabetes-induced endothelial dysfunction are likely to be multi-factorial, *in-vivo* and *in-vitro* studies indicate a crucial role for the DAG-PKC pathway in mediating this phenomenon. PKC may have adverse effects on vascular function, including the activation of O_2^--producing enzymes such as NADPH oxidase, as well as, increased expression of a dysfunctional O_2^- producing uncoupled (eNOS III). PKC-mediated O_2^- production may inactivate NO derived from eNOS III, and inhibit the activity and/or expression of the NO downstream target; soluble guanylyl cyclase [124, 135]. During the development of DM a number of biochemical and mechanical factors converge on the endothelium, resulting in endothelial dysfunction and vascular inflammation. Hyperglycaemia leads to increased formation of AGE, which quenches NO and impair endothelial function. AGEs act directly to induce cross-linking of proteins such as collagen to promote vascular stiffness, and, thus, alter vascular structure and function. Receptor-mediated actions of AGEs can induce intracellular signaling that leads to enhanced OS and increase key proinflammatory and prosclerotic cytokines [6, 7, 12, 104]. Increased vascular inflammation, including enhanced expression of interleukin-6 (IL-6), vascular cellular adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein (MCP-1) are observed, as is a marked decrease in NO bioavailability [136].

GLYCOXIDATIVE STRESS: A KEY THERAPEUTIC TARGET?

The Role of Antioxidants in Prevention and Delaying Cardiovascular Complications Associated with Experimental DM

A possible target for the management of the multiple risk factors in diabetic CVD could be the treatment of endothelial dysfunction, since all the risk factors are related to endothelial dysfunction [135-139]. As mentioned above, various interventions have proven effective in restoring impaired endothelium-dependent vasodilatation in certain vascular beds in animal and human DM. In this regard, optimizing eNOS function is a reasonable approach; this can be realized by using co-factors, e.g., BH4, L-arginine, or by increasing cGMP availability *via* phosphodiesterase type 5 (PDE5) inhibitors, or by enhancing eNOS expression. [138]. Several studies have reported on the modulating effect L-arginine supplementation has on diabetic *in vivo* NO production, under conditions known to be associated with endothelial dysfunction [139]. Angiotensin-converting enzyme (ACE) inhibition, which is conventionally used for the management of CVD, has been shown to; decrease angiotensin II-induced NADH oxidase activity and vascular production of O_2^-, to stimulate basal NO production by suppression of bradykinin breakdown or perhaps by potentiating the vascular effects of insulin [140]. In addition to ACE inhibitors, physical exercise, calcium channel blockers and angiotensin II receptor antagonists have been found to ameliorate flow-evoked endothelium-dependent vasodilatation in diabetic patients and animals [141]. We and others, showed an improvement in endothelium-dependent vasodilation, as well as, myocardial structure and function following the administration of lipid-lowering statins to diabetic rats [142, 143]. More recently, endothelin A receptor antagonists, atresantan or 1-methylnicotinamide, a primary metabolite of nicotinamide preserved endothelium-dependent relaxation in diabetic vessels [144, 145]. Interestingly, a considerable body of evidence suggests that increasing NO bioavailability *via* antioxidant therapy is an important intervention not only for endothelial dysfunction but also other cardiovascular abnormalities induced by DM [22, 63, 68, 119].

The inhibition of intracellular free radical formation would provide a therapeutic strategy to prevent OS and the related diabetic vascular complications. In this regard, the determination of ROS production is an important evaluation of the intracellular and extracellular redox balance and homeostasis. It would be possible determine the level of ROS generating enzymes in the extracellular and intracellular compartments depending on location, e.g., NAD(P)H/NADH oxidase in the cell membrane, xanthine oxidase, myeloperoxidase, cytochrome P450 in cytoplasm, and mitochondrial oxidation and antioxidant enzymes such as, SODs, glutathione peroxidase, heme oxygenase, thioredoxin peroxidase/peroxiredoxin, catalase, and paraoxonase [112, 146]. Although there is controversy about the antioxidant status in DM, our biochemical measurements revealed that SOD activity is inhibited in diabetic aorta together with the activation of catalase [114]. In addition to this, hearts from 12 weeks diabetic animals showed about a four-fold increase in the level of thiobarbituric acid reactive substances (TBARS), indicative of increased lipid peroxidation. This was accompanied by approximately 100 % increase in both catalase and glutathione peroxidase (GSHPx) enzyme activities [147]. We have also found; increased GSHPx and catalase activity, decreased glutathione reductase (GR) activity and SOD activity unchanged in the diabetic heart. The activity of pentose phosphate pathway enzymes, glucose-6-phosphate dehydrogenase (G-6PD) and 6-phosphogluconate dehydrogenase (6-PGD) were significantly decreased in diabetic aorta [148]. In an attempt to neutralise OS, cells utilize antioxidant defenses comprised of enzymatic and non-enzymatic compounds that determine redox balance. Enzymes include; SOD, catalase, thioredoxin, and GSHPx, while, non-enzymatic compounds include; glutathione, ascorbate, tocopherol and polyphenolic compounds [109, 149]. SOD immediately converts O_2^- to H_2O_2, which is then detoxified to water either by catalase in the lysosomes or GSHPx, in the mitochondria. GR, which regenerates glutathione, is used as a hydrogen donor by GSHPx during the elimination of H_2O_2. Similarly, SOD and GSHPx expression/activities were decreased whereas; catalase was increased in experimental models of DM [101, 150, 151]. The modulation of these enzymes in target organs prone to diabetic complications such as the heart may prove

beneficial in the prevention and management of heart failure. Accordingly, the use of gene transfer of extracellular SOD to study the endothelial effects of OS has recently been reported. Gene transfer of normal extracellular SOD improved endothelial dysfunction in several disease states, thus, this technique may be useful to study the mechanism by which gene variants predispose vessels to endothelial dysfunction and vascular disease [152]. More recently, Tempol, a cell permeable SOD mimetic, was found to increase endothelium-dependent vasodilatation in arteries from hypertensive animals, most likely through the lowering of ROS, making it a potential therapeutic tool for a disease characterized by decreased NO bioavailability [141].

Several reports using experimental diabetic models have shown that improving glycemic control does not sufficiently improve the accompanying OS [153]. We found that *in vivo* insulin replacement therapy alone was unable to prevent or restore metabolic, functional and/or structural abnormalities in the heart and aorta of STZ-diabetic rats [19-21, 61, 62]. However, the combination of insulin with non-enzymatic antioxidants showed more effectiveness in the prevention and amelioration of vascular [154] and cardiac [147] abnormalities in STZ-diabetic rats. In this context, to avoid interference from glucose toxicity, the increased availability of AGE detoxification systems, which include; enzymatic mechanisms such as the glyoxalase-I and II system, antioxidant defenses, circulating proteins that trap AGEs, receptor-dependent intracellular uptake and destruction, as well as, urinary excretion have stimulated great interest. Thus, methylglyoxal, a highly reactive AGE precursor molecule, is detoxified by glyoxalase I and II. Its reduction can be catalyzed by aldehyde reductase, aldose reductase and carbonyl reductase. While, a group of circulating proteins such as lysozyme, defensins and lactoferrin can bind AGEs, preventing them from causing cellular toxicity or from binding to other molecules [12, 104, 106]. Consequently, the use of antiglycation agents, AGEs crosslink breakers, including alagebrium, benfotiamin, aminoguanidine, carnosine may be considered as future therapeutic approaches to improve endothelial function, vascular reactivity and cardiac hemodynamics in diabetic patients [106, 155-157]. Another treatment strategy has been recently suggested that involves the increase in AGER1 function (the chief endocytic AGE receptor), which inhibits the function of RAGEs and may provide protection against the destructive effect of a chronically high OS state such as in DM [12, 13, 106].

Over the past three decades, many *in vivo* studies have been performed utilizing exogenous antioxidants in experimental diabetic models. The beneficial effects of this form of treatment on OS can be measured by certain observable biomarkers. These markers include the enzymatic activities of catalase, SOD, GSH-Px, GR and TBARS levels. The indirect measurement of free-radical production can also be undertaken, i.e., malondialdehyde (MDA) and F_2-Isoprostanes, and more recently 4-hydroxynonenal, which has been shown to be consistently elevated in DM [11, 108, 117, 158]. Normalization of the activity of any of these markers, and ultimately, the balance of free-radical production/removal, would be an effective method to reduce ROS-induced damage. Many animal studies have been conducted with this aim in mind and indeed have shown that diabetes-induced alterations of OS indicators can be reversed when the animals are treated with various antioxidants.

Experiments from "the ADIC Study Group", observed the effectiveness of exogenous antioxidant treatments in the recovery of OS markers and endogenous antioxidant enzyme activities in STZ-diabetic rats [22, 63, 64, 154, 159-161]. These recoveries were associated with the amelioration in vascular and cardiac metabolism/function [22, 46, 63, 64, 69, 95, 119, 142, 147, 148, 154, 159-161]. We have previously focused on investigating the effects of dietary supplementation of the antioxidants vitamin E, alpha-lipoic acid and vitamin A. 0.5% dietary vitamin E (DL-alpha tocopheryl acetate) supplementation given to STZ-diabetic rats eliminated the accumulation of lipid peroxides and returned plasma triglycerides toward normal levels. In addition, diabetes-induced abnormal aortic contractility and endothelial dysfunction were significantly but not completely prevented by vitamin E treatment. It also protected the morphological integrity of the diabetic aorta [63]. Vitamin E treatment (400-500 IU/kg/day) prevented protein glycation, and Ca^{2+}accumulation in STZ-diabetic rats [46] and gluthation-dependent antioxidant defense in the aorta and/or heart and did not affect the decreased activity of G-6PD, 6-PGD in diabetic aorta [148]. It also restored isoprenaline-stimulated relaxation, as well as, structural and metabolic alterations in the diabetic aorta [95]. Other actions of this antioxidant include; the prevention of (1) capillary basement membrane thickening, vascular endothelial growth factor expression and aldose reductase enzyme activity in retina of diabetic rats [161], (2) Ca^{2+}-ATPase activity, protein glycosylation and/or Ca^{2+} levels in the brain [162] and in the kidney [163], ameliorates impaired sympathetic neurotransmission and the contractility of isolated vas deferens [160], protects nerve blood flow and conduction velocity [164, 165]. In general, studies with vitamin E showed that it protects vascular endothelium and cardiac function in experimental models of DM [166, 167]. Studies on the effect of vitamin E supplementation and deprivation in diabetic patients have also been undertaken [168]. Although plasma vitamin E levels was significantly lower in poorly controlled NIDDM patients with coronary heart disease, small clinical trials revealed the beneficial cardiovascular effect of vitamin E supplementation [169-172]. In a double-blind, placebo-controlled, randomized study, vitamin E supplementation (1000 IU/day) given to IDDM patients for three months significantly improved endothelium-dependent vasorelaxation [170]. Similarly, in another study the administration of vitamin E (800 IU/day) and C (1000 mg/day) in combination for six months had a positive effect on endothelium-dependent vasorelaxation in IDDM patients but had no effect in NIDDM [171]. Results from the HOPE trial, investigating myocardial infarction, stroke and death from CVD in diabetic patients 55 years of age or older was published in 2000, it found no significant difference in the primary outcome between vitamin E and placebo treated groups [172]. A recent randomized trial reported that there was no significant overall effects of treatment with vitamin C + vitamin E + beta-carotene on the risk of developing NIDDM in patients at high risk of CVD [173]. However, another recent clinical study suggest that supplementation with vitamin E accompanied by a change in lifestyle might help to reduce the damage brought about by free radical toxicity in DM [174]. Another randomized clinical trial comparing the effectiveness

of alpha and gamma isomers of vitamin E demonstrated that at every dose of alpha vitamin E, gamma vitamin E concentration was significantly suppressed. It was thought that if a standardized preparation of gamma vitamin E (without the alpha isomer) became available it may be useful in the prevention of atherosclerosis in NIDDM patients [175].

Alpha lipoic acid (ALA; thioctic acid) is another important chemical with antioxidant properties that can be used in DM studies. ALA scavenges OH radical, HOCl and singlet oxygen. It can also exert antioxidant effects in biological systems through transitional metal chelation. In its reduced form dihydrolipoic acid (DHLA) it is not only an antioxidant but also has pro-oxidant properties in systems where OH radicals are generated. Thus, the ALA/DHLA ratio helps to recycle endogenous antioxidants such as vitamin E [176, 177]. We found that, ALA treatment (50 mg/kg/day) effectively reversed body weight, blood glucose, plasma insulin, cholesterol, triglycerides and lipid peroxidation levels of 12 weeks STZ-diabetic rats. In addition, it partially improves blood pressure, catalase activity and tissue morphology and completely reversed the contractile effect of phenylephrine in diabetic aorta [64]. In another study, we showed that ALA treatment also partially reverse SOD-induced relaxation, which is probably related to the elimination of O_2^- and H_2O_2, mediating the recovery of basal EDRF/NO availability [119]. The potency of ALA in reversing hypertension due to an effect on vascular reactivity and tissue morphology, as well as, its general metabolic actions in diabetic rats, confirms the importance of hyperglycemia-induced OS in the development of diabetes-induced vascular complications [178, 179]. The metabolic and hemodynamic sequela insulin-deficiency in the heart is also corrected by ALA [180]. ALA reduces OS in STZ-induced diabetic rats by modulating the thiol status of the cells [177, 180], thus, preventing high glucose-induced receptor death and mitochondriondependent cardiac apoptosis [181], it also increases HSP60 and decreases 4-HNE in diabetic heart [182]. Clinical trials with ALA have focused on the treatment of diabetic neuropathy, the results obtained are more promising than those with vitamin E. Pharmacodynamic studies have shown that ALA favorably influences the vascular abnormalities of diabetic polyneuropathy, such as impaired microcirculation, the increased indices of OS and increased levels of markers for vascular dysfunction, such as thrombomodulin, albuminuria, and NF-KB [183, 184]. Thus far, seven controlled randomized clinical trials giving ALA to patients with diabetic peripheral and cardiac autonomic neuropathy have been completed; (Alpha-Lipoic Acid in Diabetic Neuropathy [ALADIN I-III] [185, 186], Deutsche Kardiale Autonome Neuropathie [DEKAN] [187], Oral Pilot [ORPIL] [188], Symptomatic Diabetic Neuropathy [SYDNEY] [189], Neurological Assessment of Thioctic Acid in Neuropathy [NATHAN I- II] [190]. These studies have used different; study designs, treatment duration, doses, sample sizes, and patient population. They have revealed the efficacy and safety of thioctic acid (600 mg intravenously for 3 weeks) in diabetic patients with symptomatic polyneuropathy [184, 191].

We demonstrated that 12 weeks STZ-diabetes caused a reduction in plasma retinol and retinol-binding protein (RBP), which was significantly improved by insulin therapy (8-10 IU/rat/day s.c), whereas, vitamin A (retinol acetate, 30 mg/kg/day i.o) treatment failed to increase plasma retinol and RBP levels. These findings suggest that the combination of insulin with vitamin A provides more benefit than either agent alone in the treatment of the general characteristics of DM and that the maintenance of the antioxidant defense of the diabetic heart can reduce peroxidative stress-induced cardiac injury [147]. In addition, our findings suggest that the maintenance of ACh-stimulated endothelium-dependent vasorelaxant tone within normal physiological levels depends largely on the prevention and/or inhibition of peroxidative stress, and that the use of vitamin A together with insulin provides better metabolic control with a reduction in diabetes-induced vascular complications than when insulin is used alone [154]. Vitamin A is also an ingredient of the Cod Liver Oil (CLO). CLO is mainly comprised of; omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as vitamin A. CLO treatment caused significant improvements in hyperlypidemia and glucose metabolism, allowing the recovery of the normal growth rate of STZ-diabetic rats. It also ameliorated OS/peroxidative stress and endogenous antioxidant enzyme activities in the heart and blood vessels [22, 159]. In addition, CLO treatment also completely prevented endothelial deficiency and partially corrected the phenylephrine-induced vasoconstriction of diabetic aorta. The impaired responsiveness of the right atria to the positive chronotropic effect of isoprenaline and noradrenaline and the positive inotropic effect of the papillary muscle to both agents, and abnormal ultrastructure of cardiomyoctes were also significantly ameliorated by CLO [22, 159]. Several reports have shown that a combination of antioxidants may be superior to the use of monotherapy.

The Steno-2 trial compared the effect of a multifactorial intensive therapy (n=80) with that of conventional treatment (n=80) on modifiable risk factors for CVD in patients with NIDDM. The patients received pharmacotherapy that targeted hyperglycemia, dyslipidemia, hypertension and microalbuminuria including daily supplementation of vitamin C (250 mg), E (100 mg), folic acid (400 mg) and chromium picolinate (100 mg), as well as, behavioral modification including; low-fat diet, exercise and smoking cessation. The control group received conventional therapy as recommended by national guidelines. The intensive therapy resulted in almost a 50% decrease in the risk of cardiovascular events, providing evidence that a multifactorial approach is superior to conventional therapy for the prevention of OS-induced vascular complications in DM [192]. However, a more recent trial (POPADAD) does not provide evidence to support the use of aspirin or antioxidants in the primary prevention of cardiovascular events and mortality in the diabetic population studied [193]. Treatment with Zycose, a new compound, provided therapeutic benefit believed to be due to its additive effects with other drugs; lowering homocysteine levels (folic acid), reducing the production of advanced glycation end products (benfotiamine), improving endothelial function (folic acid, benfotiamine, ALA), reducing oxidative stress (ALA, vitamin E) and reducing carbonyl stress (benzamine) [194].

Probucol is another antioxidant compound used in experimental DM to determine its effect on cardiovascular function. Treatment with this antioxidant prevented lipid abnormalities, sorbitol pathway enzymes and nerve blood flow and conduction velocity deficits in diabetic rats. The

effectiveness of probucol was more apparent when used in combination with essential fatty acids (evening primrose oil) [164]. Short term probucol treatment (300 mg/kg/day, i.o.) also resulted in partial restoration of endothelial function and noradrenaline-induced contraction in aorta without any alterations in morphological abnormalities in STZ-diabetic rats [68]. Essentially, probucol is a diphenolic compound that reduces atherosclerosis and restenosis probably through anti-hyperlypidemic, anti-inflammatory and anti-oxidant properties [150]. It also preserves pancreatic beta-cell function through a reduction of OS in diabetic patients [195, 196].

In addition to the antioxidants mentioned above, a number of commonly used drugs have been reported to have antioxidant activity, in addition to their primary pharmacological property. For example, gemfibrozil, a lipid lowering fibrate, was previously reported to have antioxidant actions [197]. However, although it reduced lipid peroxidation, it did not alter blood glucose or tissue antioxidant enzymes in chronically diabetic rats [198]. This suggests that the beneficial effect of short-term gemfibrozil treatment in reducing lipid peroxidation, endothelial dysfunction and vascular reactivity are attributed to its effect on circulating lipids [198, 199]. Antihyperlipidemic statins are thought to exert an antioxidant effects *via* a variety of mechanisms. Statin therapy (10 mg/kg/day,orally) markedly reduces OS markers and ultra structural impairments in diabetic rat myocardium [142]. It also ameliorated diabetes-induced vascular abnormal vasoconstriction and endothelial dysfunction *via* an affect on; oxidizing metabolism, NO bioavailability and intracellular Ca^{2+} mobilization [143]. In addition, we have determined the vascular effect of thiazolidinediones, using human internal mammary artery, human saphenous vein and diabetic rat aorta *in vitro* [73, 200, 201], the beneficial effect were, at least in part linked with their antioxidant properties, similar to those shown in diabetic animals [202].

BENEFICIAL EFFECTS OF PYRIDOINDOL ANTIOXIDANTS IN DM

Numerous studies have proven that stobadine (STB), a novel synthetic pyridoindole, is an efficient antioxidant [203]. During the last decade, in collaboration with the Slovak Academy of Sciences, we examined the antioxidant properties of STB and related pyridoindole derivatives. The protective effect of STB against glycoxidative damage using an *in vitro* experimental glycation model has been reported [204]. STB also attenuated diabetes-induced pathological changes in various organs, decreased albuminuria, enzymuria, lipid peroxidation and matrix collagen cross-linking [205]. In addition, it reduced plasma cholesterol and triglyceride levels in STZ-diabetic rats [46, 206] Recently, we showed that STB caused a significant decrease in protein carbonylation, advanced oxidation protein products and normalized protein thiol, total thiol, non-protein thiol groups in the liver of diabetic animals [207]. The combination of STB with vitamin E was beneficial in reducing hyperglycemia and lipid peroxidation in diabetic animals. The anti-hyperglyceamic effect of these antioxidants may be due to the early stabilization of the pancreas beta cell membrane. These antioxidants may partially protect insulin-secreting cells against the detrimental effects of free radicals when given at the onset of diabetes or promptly after STZ injection [46]. STB was also able to control the glycation of hemoglobine, determined by HbA1c levels, which was significantly decreased by long-term STB treatment of chronically STZ-diabetic rats [160]. STZ-diabetic rats, orally treated with a low dose of STB (24.7 mg/kg/day), showed an inhibition in cardiac protein glycation and Ca^{2+} accumulation. These effects were associated with decreased plasma lipid peroxidation. However, STB treatment did not influence the diabetes-induced inhibition of cardiac Ca^{2+},Mg^{2+}-ATPase activity [46]. This is supported by the finding that long term STB treatment prevents diabetes-induced deterioration of cardiac Na^{+},K^{+}-ATPase [208] and reduces oxidative damage of myocardial tissue, as measured by conjugated dienes. It also reverses myocardial levels of alpha-tocopherol and coenzyme Q9 to near control values. Moreover, it attenuates the elevated activity of SOD and diminishes angiopathic and atherogenic processes in diabetic myocardium [206-208]. Diabetes-induced stimulation of cardiac GSH-Px activity and inhibition of cardiac GR activity was prevented by STB treatment [148]. These findings suggest that the cardioprotective effect of STB is more complex than its preservation of mitochondrial function [209].

The combination of STB and vitamin E provided more benefits than each individual therapy alone in the reduction of lipid peroxidation and cardiac abnormalities in diabetic animals [46]. This was evident in the; kidney, brain [148], liver [46], peripheral nerves [160, 164] and retina of diabetic rats [161]. Interestingly, the increase in retinal capillary basement membrane thickness (RCBMT) was 12.34% in diabetic rats treated with STB and 23.07% in diabetic rats treated with vitamin E. While, the increase in RCBMT was just 4.38% following combination therapy [161]. However, combination therapy did not protect against leukocyte free radical production, while STB alone had a significant protective effect on leukocyte function [210]. Although STB and vitamin E produces parallel effects on the preservation/ restoration of the pro-oxidant feature of DM and generally has similar characteristics in the protection of tissue function; some differences have also been observed. For instance, while the *in vivo* administration of vitamin E alone had no effect, STB treatment completely returned noradrenaline-induced contractions to basal levels in diabetic vas deferens [160]. The effect of long term treatment with STB or vitamin E in the development of cataracts in diabetic animals has also been investigated. Interestingly, at the end of the experimental period, the visual cataract score was significantly decreased in the diabetic groups treated with STB, while vitamin E had no significant effect [211]. It is conceivable that an antioxidant given alone *in vivo* would be more effective when other antioxidants are present. It is well known that alpha-tocopherol can recycle stobadine [212], thus the antioxidant potency of stobadine *in vivo* may be increased by its interaction with other antioxidants [213]. We anticipated that stobadine given in a mixture with vitamin E would have enhanced antioxidant and anticataract action when given to diabetic rats. However, contrary to our expectation, combined treatment with STB + vitamin E accelerated the progression of cataract, without affecting the overall visual cataract score. Moreover, biochemical analysis showed a further decrease of lens protein free sylfhydryls in diabetic rats treated with the mixture of STB + vitamin E compared to the

untreated diabetic group, indicating a prooxidant effect of the STB + vitamin E cocktail [212, 213]. Similar observations are in the literature showing that individually taken vitamin E, C, or A was effective in reducing cataract risk, while the combination of the three supplements potentiated cataract formation [214].

The effects of STB on vascular function have been largely studied at different stages of STZ-diabetes. Satnikova *et al*. reported that 8-month STB supplementation to diabetic rats resulted in the protection of aortic function and ultrastructure; evaluated by aortic reactivity to noradrenaline, ACh and H_2O_2 under isometric conditions and transmission electron microscopy, respectively [215]. We found that STB treatment significantly reduced phenylephrine and BayK 8644-induced contractions in diabetic rats compared to control and untreated-diabetic rats. Neither DM nor STB treatment changed the vascular reactivity to KCl and $CaCl_2$. Diabetes-induced decrease in vascular relaxation to ACh was ameliorated by STB treatment and STB partially controlled mean arterial blood pressure in diabetic rats. These observations support the findings that increased entry of Ca^{+2} mediates elevated contraction to BayK 8644 and phenylephrine in diabetic aorta and the inhibitory effect of *in vivo* STB treatment on Ca^{+2} entry through cytosolic membrane-bound Ca^{+2} channels may account for its reducing effectiveness on the vasoconstriction and blood pressure. A23187-induced vasorelaxation was markedly inhibited in STB-treated diabetic rats compared with control and untreated-diabetic rats. The inhibitory effect of STB on Ca^{+2} influxes in endothelial cells may account for its reduced effect on A23187-stimulated relaxations [69]. These results are also in agreement with the Ca^{+2} mobilization regulating effect of STB on membranes [216-218] and support our previous findings, which demonstrated an inhibitory effect of STB on intracellular Ca^{+2} accumulation in diabetic heart and liver [56]. STB also exerts a protective effect through free radical-scavenging and anti-oxidant activities in the ischemia/reperfusion injury model, as previously reported [218-220]. More recently, we have focused on evaluating the effects of STB and other antioxidant pyridoindoles [221] using transformed and non-transformed cells. Pre-treating cells with STB significantly increased cell viability and decreased apoptosis rate; determined by flow cytometric analysis and by measuring caspase-3 and caspase-9 activities in doxorubicin-induced, free radical-mediated apoptosis model of P815 cells [222]. We have also suggested that STB and its analogues protect insulin release in the INS 1E-cell line exposed to H_2O_2 or cytokine cocktail and that the antioxidant effects of pyridoindoles also mediate antiapoptotic processes *via* intrinsic as well as extrinsic pathway of programmed cell death in insulin releasing cells. This may provide an appropriate explanation for the decrease in severity of hyperglycemia caused by STB, which might have therapeutic implications in the prevention of autoimmune DM [223]. Interestingly, a derivative of STB, suppressed the intracellular generation of ROS, reduced cell swelling, improved the reduced cell proliferation and viability was not able to suppress protein carbonyl formation in HT22 neuronal cells under glucose toxicity [224].

CONCLUSION

Several mechanisms have been proposed to explain the harmful effects of glucose. Glucose could be directly toxic to cell components because it can promote non-enzymatic glycosylation and the accumulation of AGEs, which impair cellular functions and mediate an increase in the production of ROS. In the presence of elevated glucose metabolism, the excessive production of electrons can promote the generation of ROS; if there is not a matching increase in the efficiency of electron transport system, this can lead to GOS and cellular injury in the long term. Although, multiple biochemical pathways are likely to be responsible for diabetic cardiovascular complications, substantial evidence suggests a key role for GOS. Therefore, recent interest has focused on strategies to prevent, reverse or retard GOS in order to modify the natural history of diabetic CVD. Even though, there is an increasing amount of experimental evidence showing the beneficial effects of antiglycoxidative (antioxidant) agents in the prevention/treatment of diabetic CV abnormalities, some clinical trials conducted to date, failed to provide adequate support for the use of antioxidant therapy in DM. However, it is still too early to reach a definitive conclusion on the clinical benefits of antioxidants. It is possible that antioxidants would be more demonstrably effective in a patient population chosen on the basis of elevated levels of GOS. Unfortunately, none of the studies to date effectively assessed the baseline GOS at the time of patient recruitment. The clinical trials generally used endpoints that were not directly related to GOS, but rather gross markers of overall cardiovascular health, such as effect on mortality. In the evaluation of GOS/OS and the effects of antioxidants thereon, specific markers should be measured. We strongly believe that more research on the use of antioxidants (antiglycoxidative agents) in the prevention of diabetic cardiovascular complications is necessary and should be encouraged. Pharmacological intervention using different antioxidants may have significant implications in the prevention of the pro-oxidant feature of DM and protect the redox status of the cardiovascular cells. Finally, the search for novel antioxidant/antiglycoxidative agents with enhanced efficiency, optimized bioavailability, reduced adverse effects and decreased toxicity should be continued, with the prospect of preventing, or delaying long-term cardiovascular diabetic complications.

ABBREVIATIONS

Ach	=	Acetylcholine
AR	=	Adrenergic receptor
AGEs	=	Advanced glycation end products
ACE	=	Angiotensin-converting enzyme
alpha AR	=	Alpha-adrenergic receptor
ALA	=	Alpha lipoic acid
RONOO	=	Alkyl peroxynitrates
beta-Ars	=	Beta-adrenergic receptors
CLO	=	Cod Liver Oil
cAMP	=	Cyclic adenosine monophosphate
cGMP	=	Cyclic guanosine monophosphate
DM	=	Diabetes mellitus
DAG	=	Diacylglycerol

DHLA = Dihydrolipoic acid
DHA = ocosahexaenoic acid
EPA = Eicosapentaenoic acid
eNOS = Endothelial nitric oxide synthase
ET = Endothelin
EDHF = Endothelium-dependent hyperpolarization
EDRF = Endothelium-derived relaxing factor
FAD = Flavin adenine dinucleotide
FMN = Flavin mononucleotide
G-6PD = Glucose-6-phosphate dehydrogenase
GOS = Glycoxidative stress
GSH-Px = Glutathione peroxidase
GR = Glutathione reductase
HOCl = hydrochlorous acid
H_2O_2 = Hydrogen peroxide
HRO_2 = Hydroperoxyl
HNO_2 = Nitrous oxide
IDDM = Insulin-dependent diabetes mellitus
IL-6 = interleukin- 6
LVDP = Left ventricular developed pressure
L-NAME = L-nitro-arginine methyl ester
MDA = Malondialdehyde
MCP-1 = Monocyte chemoattractant protein
NADPH = Nicotinamide adenine dinucleotide phosphate
NO = Nitric oxide
NOS = Nitric oxide synthase
NO_2 = Nitrogen dioxide
NIDDM = Non-insulin-dependent diabetes mellitus
NF-KB = Nuclear factor-kappaB
OS = Oxidative stress
ONOO = Peroxynitrite
OH = Hydroxyl
PDE5 = Phosphodiesterase type 5
PUFAs = Omega-3 polyunsaturated fatty acids
PKC = Protein kinase C
ROS = Reactive oxygen species
RNS = Reactive nitrogen species
RAGE = Receptor advanced glycation end products
RCBMT = Retinal capillary basement membrane thickness
RBP = Retinol-binding protein
RO_2 = Peroxyl
STB = Stobadine
STZ = Streptozotocin
SOD = Superoxide dismutase
O_2 = Superoxide
TBARS = Thiobarbituric acid reactive substances
VCAM-1 = Vascular cellular adhesion molecule-1
6-PGD = 6-phosphogluconate dehydrogenase

REFERENCES

[1] Amos A, McCarthy D, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabet Med 1997; 14: S1-S85.
[2] Howard BV, Magee MF. Diabetes and cardiovascular disease. Curr Atheroscler Rep 2000; 2: 476-81.
[3] Brownlee M. Biochemistry and molecular biology of diabetic complications. Nature 2001; 414: 813-20.
[4] Giugliano D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? Metabolism 1995; 44: 363-8.
[5] Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signalling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002; 23: 599-622.
[6] Goh SY, Cooper ME. Clinical review: The role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab 2008; 93: 1143-52.
[7] Negre-Salvayre A, Salvayre R, Augé N, Pamplona R, Portero-Otín M. Hyperglycemia and glycation in diabetic complications. Antioxid Redox Signal 2009; 11: 3071-109.
[8] Vanhoutte PM. Endothelial dysfunction and vascular pathology. Bull Mem Acad R Med Belg 2006; 161: 529-36.
[9] Potenza MA, Gagliardi S, Nacci C, Carratu' MR, Montagnani M. Endothelial dysfunction in diabetes: from mechanisms to therapeutic targets. Curr Med Chem 2009; 16: 94-112.
[10] Aronson D. Hyperglycemia and the pathobiology of diabetic complications. Adv Cardiol 2008; 45: 1-16.
[11] Jakus V. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. Bratisl Lek Listy 2000; 101: 541-51.
[12] Yan SF, Ramasamy R, Schmidt AM. The receptor for advanced glycation endproducts (RAGE) and cardiovascular disease. Expert Rev Mol Med 2009; 11: e9.
[13] Yamagishi S. Advanced glycation end products and receptor-oxidative stress system in diabetic vascular complications. Ther Apher Dial 2009; 13: 534-9.
[14] Bell DS. Heart failure: the frequent, forgotten, and often fatal complication of diabetes. Diabetes Care 2003; 26: 2433-41.
[15] Ahmed SS, Jakeri GA, Narang RM, Regan TJ. Preclinical abnormality of left ventricular function in diabetes mellitus. Am Heart J 1975; 89: 153-58.
[16] Fumelli P, Romagnoli F, Carlino G, Fumelli C, Boemi M. Diabetes mellitus and chronic heart failure. Arch Gerontol Geriatr 1996; 23: 277-81.
[17] Fein FS, Sonnenblick EH. Diabetic cardiomyopathy. Cardiovasc Drugs Ther 1994; 8: 65-73.
[18] Asghar O, Al-Sunni A, Khavandi K, *et al*. Diabetic cardiomyopathy. Clin Sci 2009; 116: 741-60.
[19] Karasu C, Oztürk Y, Altan N, Yildizoglu-Ari N, Ikizler C, Altan VM. Thyroid hormones mediated effect of insulin on alloxan diabetic rat atria. Gen Pharmacol 1990; 21: 735-40.
[20] Öztürk Y, Yildizoglu-Ari N, Altan VM, Özçelikay AT. Effect of insulin on the decreased b-adrenergic responses of duodenum and atrium isolated from streptozotocin diabetic rats. Gen Pharmacol 1993; 24: 217-23.
[21] Özüari A, Öztürk Y, Yldizoglu-Ari N, Özçelikay AT, Altan VM. The effects of glyburide and insulin on the cardiac performance in rats with non-insulin dependent diabetes mellitus. Gen Pharmacol 1993; 24: 165-9
[22] Ceylan-Isik A, Hünkar T, Asan E, *et al*. (Antioxidants in Diabetes-Induced Complications) The ADIC Study Group. Cod liver oil supplementation improves cardiovascular and metabolic abnormalities in streptozotocin diabetic rats. J Pharm Pharmacol 2007; 59: 1629-41.

[23] Nemoto O, Kawaguchi M, Yaoita H, Miyake K, Maehara K, Maruyama Y. Left ventricular dysfunction and remodeling in streptozotocin-induced diabetic rats. Circ J 2006; 70: 327-34.

[24] Borges GR, de Oliveira M, Salgado HC, Fazan R Jr. Myocardial performance in conscious streptozotocin diabetic rats. Cardiovasc Diabetol 2006; 5: 26.

[25] Bilginoglu A, Cicek FA, Ugur M, Gurdal H, Turan B. The role of gender differences in beta-adrenergic receptor responsiveness of diabetic rat heart. Mol Cell Biochem 2007; 305: 63-9.

[26] Huisamen B, Marais E, Genade S, Lochner A. Serial changes in the myocardial beta-adrenergic signalling system in two models of non-insulin dependent diabetes mellitus. Mol Cell Biochem 2001; 219: 73-82.

[27] Kim SK, Zhao ZS, Lee YJ, *et al.* Left-ventricular diastolic dysfunction may be prevented by chronic treatment with PPAR-alpha or -gamma agonists in a type 2 diabetic animal model. Diabetes Metab Res Rev 2003; 19: 487-93.

[28] Gando S. Pharmacological studies on alterations in myocardial beta-adrenoceptors and their intracellular signal transduction in experimental diabetic rats. Hokkaido Igaku Zasshi 1994; 69: 1140-53.

[29] Dinçer UD, Bidasee KR, Güner S, Tay A, Ozçelikay AT, Altan VM. The effect of diabetes on expression of beta1-, beta2-, and beta3-adrenoreceptors in rat hearts. 1: Diabetes 2001; 50: 455-61.

[30] Wichelhaus A, Russ M, Petersen S, Eckel J. G protein expression and adenylate cyclase regulation in ventricular cardiomyocytes from STZ-diabetic rats. Am J Physiol 1994; 267: H548-55.

[31] Gando S, Hattori Y, Kanno M. Altered cardiac adrenergic neurotransmission in streptozotocin-induced diabetic rats. Br J Pharmacol 1993; 109: 1276-81.

[32] Kamata K, Satoh T, Tanaka H, Shigenobu K. Changes in electrophysiological and mechanical responses of the rat papillary muscle to alpha- and beta-agonist in streptozotocin-induced diabetes. Can J Physiol Pharmacol 1997; 75: 781-8.

[33] Lafçi-Erol D, Altan VM, Oztürk Y. Increased alpha 1-adrenergic responsiveness of alloxan diabetic rat atria: effects of insulin therapy and thyroidectomy. Gen Pharmacol 1994; 25: 559-64.

[34] Tanaka Y, Kashiwagi A, Saeki Y, Shigeta Y. Abnormalities in cardiac alpha 1-adrenoceptor and its signal transduction in streptozocin-induced diabetic rats. Am J Physiol 1992; 263: E425-9.

[35] Carrier GO, Aronstam RS. Altered muscarinic receptor properties and function in the heart in diabetes. J Pharmacol Exp Ther 1987; 242: 531-5.

[36] Liu TP, Juang SW, Cheng JT, Tong YC. The role of sorbitol pathway and treatment effect of aldose reductase inhibitor ONO2235 in the up-regulation of cardiac M2-muscarinic receptors in streptozotocin-induced diabetic rats. Neurosci Lett 2005; 383: 131-5.

[37] Lee LM, Chang CK, Cheng KC, Kou DH, Liu IM, Cheng JT. Increase of cardiac M_2-muscarinic receptor gene expression in type-1 but not in type-2 diabetic rats. Neurosci Lett 2008; 441: 201-4.

[38] Aronstam RS, Carrier GO. Insulin prevention of altered muscarinic receptor-G protein coupling in diabetic rat atria. Diabetes 1989; 38: 1611-6.

[39] Cox BF, Clark KL, Perrone MH, *et al.* Cardiovascular and metabolic effects of adenosine A1-receptor agonists in streptozotocin-treated rats. J Cardiovasc Pharmacol 1997; 29: 417-26.

[40] Grdeñ M, Podgórska M, Szutowicz A, Pawe³czyk T. Altered expression of adenosine receptors in heart of diabetic rat. J Physiol Pharmacol 2005; 56: 587-97.

[41] Chen S, Evans T, Mukherjee K, Karmazyn M, Chakrabarti S. Diabetes-induced myocardial structural changes: role of endothelin-1 and its receptors. J Mol Cell Cardiol 2000; 32: 1621-9.

[42] Little PJ, Ivey ME, Osman N. Endothelin-1 actions on vascular smooth muscle cell functions as a target for the prevention of atherosclerosis. Curr Vasc Pharmacol 2008; 6: 195-203.

[43] Stratmann B, Stork I, Tschoepe D. Diabetic cardiomyopathy--a type of coronary artery disease? Internist (Berl). 2008; 49: 436-40.

[44] Zhao XY, Hu SJ, Li J, Mou Y, Chen BP, Xia Q. Decreased cardiac sarcoplasmic reticulum Ca^{2+} -ATPase activity contributes to cardiac dysfunction in streptozotocin-induced diabetic rats. Physiol Biochem 2006; 62: 1-8.

[45] Tamada A, Hattori Y, Houzen H, *et al.* Effects of beta-adrenoceptor stimulation on contractility, $[Ca^{2+}]i$, and Ca^{2+} current in diabetic rat cardiomyocytes. Am J Physiol 1998; 274: H1849-57.

[46] Pekiner B, Ulusu NN, Das-Evcimen N, *et al.* Antioxidants in Diabetes-Induced Complications Study Group. *In vivo* treatment with stobadine prevents lipid peroxidation, protein glycation and calcium overload but does not ameliorate Ca^{2+}-ATPase activity in heart and liver of streptozotocin-diabetic rats: comparison with vitamin E. Biochim Biophys Acta 2002; 1588: 71-8.

[47] Ren J, Walsh MF, Hamaty M, Sowers JR, Brown RA. Altered inotropic response to IGF-I in diabetic rat heart: influence of intracellular Ca^{2+} and NO. Am J Physiol 1998; 275: H823-30.

[48] Ligeti L, Szenczi O, Prestia CM, *et al.* Altered calcium handling is an early sign of streptozotocin-induced diabetic cardiomyopathy.Int J Mol Med 2006; 17 : 1035-43.

[49] Smith JM, Sondgeroth KB, Wahler GM. Inhibition of nitric oxide synthase enhances contractile response of ventricular myocytes from streptozotocin-diabetic rats. Mol Cell Biochem 2007; 300: 129-37.

[50] Higuchi M, Ikema S, Sakanashi M. Correlation of contractile dysfunction and abnormal tissue energy metabolism during hypoperfusion with norepinephrine in isolated rat hearts: differences between normal and diabetic hearts. J Mol Cell Cardiol 1992; 24: 1125-41.

[51] Feuvray D, Darmellah A. Diabetes-related metabolic perturbations in cardiac myocyte.Diabetes Metab 2008; 34 Suppl 1: S3-9.

[52] Wilson PW. Diabetes mellitus and coronary heart disease. Am J Kidney Dis 1998; 32: S89-100.

[53] Ding H, Triggle CR. Endothelial cell dysfunction and the vascular complications associated with type 2 diabetes: assessing the health of the endothelium.Vasc Health Risk Manage 2005; 1: 55-71.

[54] Fleischhacker E, Esenabhalu VE, Spitaler M, *et al.* Human diabetes is associated with hyperreactivity of vascular smooth muscle cells due to altered subcellular Ca^{2+} distribution. Diabetes 1999; 48: 1323-30.

[55] Pfaffman MA, Ball CR, Darby A, Hilman R. Incsulin reversal of diabetes-induced inhibition of vascular contractility in the rat. Am J Physiol 1982; 242: H490-95.

[56] Head PJ, Longhurst PA, Panek RL, Stitzel RE. A contrasting effect of the diabetic state upon the contractile responses of aortic preparations from therat and rabbit. Br J Pharmacol 1987; 91: 275-86.

[57] Reyes-Toso CF, Rosón MI, Albornoz LE, Damiano PF, Linares LM, Cardinali DP. Vascular reactivity in diabetic rats: effect of melatonin. J Pineal Res 2002; 33: 81-6.

[58] Owen MP, Carrier GO. Alteration in vascular smooth muscle sensitivity to vasoconstrictor agents by streptozotocin induced diabetes. Proc West Pharmacol Soc 1979; 22: 363-66.

[59] Harris KH, MacLeod KM. Infuence of the endothelium on contractile responses of arteries from diabetic rats. Eur J Pharmacol 1988; 153: 55-64.

[60] Altan VM, Karasu C, Ozüari A. The effects of type-1 and type-2 diabetes on endothelium-dependent relaxation in rat aorta. Pharmacol Biochem Behav 1989; 33: 519-22.

[61] Karasu Ç, Altan VM. The role of endothelial cells on the alterations in vascular reactivity induced by insulin-dependent diabetes mellitus: Effects of insulin treatment. Gen Pharmacol 1993; 24: 743-57.

[62] Karasu C, Altan VM. The role of the endothelium on enhanced contractile response of non-insulin-dependent diabetic rat aortae: effects of insulin treatment.Gen Pharmacol 1994; 25: 795-802.

[63] Karasu C, Ozansoy G, Bozkurt O, Erdogan D, Omeroglu S. Antioxidant and triglyceride-lowering effects of vitamin E associated with the prevention of abnormalities in the reactivity and morphology of aorta from streptozotocin-diabetic rats. Antioxidants in Diabetes-Induced Complications (ADIC) Study Group. Metabolism 1997; 46: 872-9.

[64] Koçak 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: 308-18.

[65] Abboud K, Bassila JC, Ghali-El-Ghoul R, Sabra R. Temporal changes in vascular reactivity in early diabetes mellitus in rats: role of changes in endothelial factors and in phosphodiesterase acitvity. Am J Physiol Heart Circ Physiol 2009; 297: H836-45.

[66] Zhu BH, Guan YY, Min J, He H. Contractile responses of diabetic rat aorta to phenylephrine at different stages of diabetic duration. Acta Pharmacol Sin 2001; 22: 445-9.

[67] Karasu C, Soncul H, Altan VM. Effects of non-insulin dependent diabetes mellitus on the reactivity of human internal mammary artery and human saphenous vein. Life Sci 1995; 57: 103-12.

[68] Karasu C. Acute probucol treatment partially restores vasomotor activity and abnormal lipid metabolism whereas morphological

changes are not affected in aorta from long-term STZ-diabetic rats. Exp Clin Endocrinol Diabetes 1998; 106: 189-96.

[69] Ceylan A, Karasu C, Ari N, Stefek M. Stobadine regulates vascular reactivity and blood pressure via the effects on calcium channels in chronically diabetic rat aorta. 12th International Congress on Cardiovascular Pharmacotherapy; Vascular Biology Section, May 7-10, 2003; P15

[70] Ma L, Zhu B, Chen X, Liu J, Guan Y, Ren J. Abnormalities of sarcoplasmic reticulum Ca^{2+} mobilization in aortic smooth muscle cells from streptozotocin-induced diabetic rats. Clin Exp Pharmacol Physiol 2008; 35: 568-73.

[71] Kamata K, Miyata N, Kasuya Y. Functional changes in potassium channels in aortas from rats with streptozotocin-induced diabetes. Eur J Pharmacol 1989; 166: 319-23.

[72] Irat AM, Aslamaci S, Karasu C, Ari N. Alteration of vascular reactivity in diabetic human mammary artery and the effects of thiazolidinediones. J Pharm Pharmacol 2006; 58: 1647-53.

[73] Yöntem O, Sahilli M, Karasu C, Ozçelikay AT, Altan VM, Ari N. Troglitazone has no effect on K(ATP) channel opener induced-relaxations in rat aorta and in human saphenous veins from patients with type 2 diabetes. Life Sci 2000; 68: 557-68.

[74] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-6.

[75] Ignarro LJ, Byrns RE, Buga GM, Wood KS. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. Circ Res 1987; 61: 866-79.

[76] Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524-6.

[77] Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988; 333: 664-6.

[78] Katsuki S, Arnold W, Mittal C, Murad F. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. J Cyclic Nucleotide Res 1977; 3: 23-35.

[79] Koshland DE Jr. The molecule of the year. Science 1992; 258: 1861.

[80] Mayer B, Schmidt K, Humbert P, Böhme E. Biosynthesis of endothelium-derived relaxing factor: a cytosolic enzyme in porcine aortic endothelial cells Ca^{2+}-dependently converts L-arginine into an activator of soluble guanylyl cyclase. Biochem Biophys Res Commun 1989; 164: 678-85.

[81] Oyama Y, Kawasaki H, Hattori Y, Kanno M. Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. Eur J Pharmacol 1986; 132: 75-8.

[82] Durante W, Sen AK, Sunahara FA. Impairment of endothelium-dependent relaxation in aortae from spontaneously diabetic rats. Br J Pharmacol 1988; 94: 463-8.

[83] Taylor PD, McCarthy AL, Thomas CR, Poston L. Endothelium-dependent relaxation and noradrenaline sensitivity in mesenteric resistance arteries of streptozotocin-induced diabetic rats. Br J Pharmacol 1992; 107: 393-9.

[84] Pieper GM, Mei DA, Langenstroer P, O'Rourke ST. Bioassay of endothelium-derived relaxing factor in diabetic rat aorta. Am J Physiol 1992; 263: H676-80.

[85] Pieper GM. Review of alterations in endothelial nitric oxide production in diabetes: protective role of arginine on endothelial dysfunction. Hypertension 1998; 31: 1047-60.

[86] Abiru T, Watanabe Y, Kamata K, Miyata N, Kasuya Y. Decrease in endothelium-dependent relaxation and levels of cyclic nucleotides in aorta from rabbits with alloxan-induced diabetes. Res Commun Chem Pathol Pharmacol 1990; 68: 13-25

[87] Mulhern M, Docherty JR. Effects of experimental diabetes on the responsiveness of rat aorta. Br J Pharmacol 1989; 97: 1007-12

[88] Pieper, G.M. Enhanced, unaltered and impaired nitric oxide-mediated endothelium-dependent relaxation in experimental diabetes mellitus: importance of disease duration. Diabetologia 1999; 42: 204-13.

[89] Trepakova ES, Cohen RA, Bolotina VM. Nitric oxide inhibits capacitative cation influx in human platelets by promoting sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase-dependent refilling of Ca^{2+} stores. Circ Res 1999; 84: 201-9.

[90] Cohen RA, Weisbrod RM, Gericke M, Yaghoubi M, Bierl C, Bolotina VM. Mechanism of nitric oxide-induced vasodilatation: refilling of intracellular stores by sarcoplasmic reticulum Ca2+ ATPase and inhibition of store-operated Ca^{2+} influx. Circ Res 1999; 84 : 210-9

[91] Yetik-Anacak G, Catravas JD. Nitric oxide and the endothelium: History and impact on cardiovascular disease. Vasc Pharmacol 2006; 45: 268-76.

[92] De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. Br J Pharmacol 2000; 130: 963-74.

[93] Félétou M, Vanhoutte PM. EDHF: new therapeutic targets? Pharmacol Res 2004; 49: 565-80.

[94] Marczin N, Antonov A, Papapetropoulos A, *et al.* Monocyte-induced downregulation of nitric oxide synthase in cultured aortic endothelial cells. Arterioscler Thromb Vasc Biol 1996; 16: 1095-103.

[95] Karasu C, Ozansoy G, Bozkurt O, Erdogan D, Omeroglu S. Changes in isoprenaline-induced endothelium-dependent and -independent relaxations of aorta in long-term STZ-diabetic rats: reversal effect of dietary vitamin E. Gen Pharmacol 1997; 29: 561-7.

[96] Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004; 24: 816-23.

[97] Chatterjee A, Catravas JD. Endothelial nitric oxide (NO) and its pathophysiologic regulation.Vasc Pharmacol 2008; 49: 134-40.

[98] 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 1993; 266: 992-1000.

[99] Heinzel B, John M, Klatt P, Böhme E, Mayer B. Ca2+/calmodulin-dependent formation of hydrogen peroxide by brain nitric oxide synthase. Biochem J 1992; 281: 627-30.

[100] Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care1996; 19: 257-67.

[101] Maritim AC, Sanders RA,Watkins JB 3rd: Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003; 17: 24-38.

[102] Turko IV, Marcondes S, Murad F: Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA: 3-oxoacid CoA transferase. Am J Physiol Heart Circ Physiol 2001; 281 : H2289-94

[103] Piconi L, Quagliaro L, Ceriello A Oxidative stress in diabetes. Clin Chem Lab Med 2003; 41: 1144-9.

[104] Jakus V, Rietbrock N. Advanced glycation end-products and the progress of diabetic vascular complications. Physiol Res 2004; 53: 131-42.

[105] Ceriello A. Hyperglycaemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. Diabetes Nutr Metab 1999; 12: 42-6.

[106] Peppa M, Uribarri J, Vlassara H. Aging and glycoxidant stress. Hormones 2008; 7: 123-32.

[107] Cai W, He JC, Zhu L, Lu C, Vlassara H. Advanced glycation end product (AGE) receptor 1 suppresses cell oxidant stress and activation signaling via EGF receptor. Proc Natl Acad Sci USA 2006; 103: 13801-6.

[108] Haidara MA, Yassin HZ, Rateb M, Ammar H, Zorkani MA. Role of oxidative stress in development of cardiovascular complications in diabetes mellitus. Curr Vasc Pharmacol 2006; 4: 215-27

[109] Guzik TJ, Mussa S, Gastaldi D, *et al.* Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 2002; 105 : 1656-62

[110] Simonsen U, Rodriguez-Rodriguez R, Dalsgaard T, Buus NH, Stankevicius E. Novel approaches to improving endothelium-dependent nitric oxide-mediated vasodilatation. Pharmacol Rep 2009; 61: 105-15.

[111] Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol 2005; 4: 5.

[112] Gao L, Mann GE. Vascular NAD(P)H oxidase activation in diabetes: a double-edged sword in redox signalling. Cardiovasc Res 2009; 82: 9-20

[113] Ding H, Hashem M, Triggle C. Increased oxidative stress in the streptozotocin-induced diabetic apoE-deficient mouse: changes in expression of NADPH oxidase subunits and eNOS. Eur J Pharmacol 2007; 561: 121-8.

[114] Karasu C. Time course of changes in endothelium-dependent and -independent relaxation of chronically diabetic aorta: role of reactive oxygen species. Eur J Pharmacol 2000; 392: 163-73.
[115] Hattori Y, Kawasaki H, Abe K, Kanno M. Superoxide dismutase recovers altered endothelium-dependent relaxation in diabetic rat aorta. Am J Physiol 1991; 261: H1086-94.
[116] Cohen RA. The role of nitric oxide and other endothelium-derived vasoactive substances in vascular disease. Prog Cardiovasc Dis 1995; 38: 105-28.
[117] Kamata K, Kobayashi T. Changes in superoxide dismutase mRNA expression by streptozotocin-induced diabetes. Br J Pharmacol 1996; 119: 583-9.
[118] Karasu C. Increased activity of H_2O_2 in aorta isolated from chronically streptozotocin-diabetic rats: effects of antioxidant enzymes and enzymes inhibitors. Free Radic Biol Med 1999; 27: 16-27.
[119] Koçak G, Karasu C. Elimination of $O_2^{\cdot}/H_2O_2$ by alpha-lipoic acid mediates the recovery of basal EDRF/NO availability and the reversal of superoxide dismutase-induced relaxation in diabetic rat aorta. Diabetes Obes Metab 2002; 4: 69-74.
[120] Matoba, T, Shimokawa H, Nakashima M, *et al*. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J Clin Invest 2000; 106: 1521-30.
[121] Pieper GM, Gross GJ. Oxygen free radicals abolish endothelium-dependent relaxation in diabetic rat aorta. Am J Physiol 1988; 255: H825-33.
[122] Tesfamariam B, Cohen RA. Free radicals mediate endothelial cell dysfunction caused by elevated glucose. Am J Physiol 1992; 263: H321-6.
[123] Pieper GM, Siebeneich W, Roza AM, Jordan M, Adams MB. Chronic treatment *in vivo* with dimethylthiourea, a hydroxyl radical scavenger, prevents diabetes-induced endothelial dysfunction. J Cardiovasc Pharmacol 1996; 28 : 741-5
[124] Vanhoutte PM. Endothelium-derived free radicals: for worse and for beter. J Clin Invest 2001; 107: 23-25.
[125] Yang Z, Zhang A, Altura BT, Altura BM. Hydrogen peroxide-induced endothelium-dependent relaxation of rat aorta involvement of Ca^{2+} and other cellular metabolites. Gen Pharmacol 1999; 33: 325-36.
[126] Chun TH, Itoh H, Saito T, *et al*. Oxidative stress augments secretion of endothelium-derived relaxing peptides, C-type natriuretic peptide and adrenomedulin. J Hyperten 2000; 18: 575-80.
[127] Kobayashi T, Kamata K. Effect of chronic insulin treatment on NO production and endothelium-dependent relaxation in aortae from established STZ-induced diabetic rats. Atherosclerosis 2001; 155: 313-20.
[128] Ceriello A. Nitrotyrosine: new findings as a marker of postprandial oxidative stress. Int J Clin Pract Suppl 2002; 129: 51-8.
[129] Szabo C. Role of nitrosative stress in the pathogenesis of diabetic vascular dysfunction. Br J Pharmacol 2009; 156: 713-27
[130] Zou MH, Cohen R, Ullrich V. Peroxynitrite and vascular endothelial dysfunction in diabetes mellitus. Endothelium 2004; 11: 89-97.
[131] Zobali F, Cakici I, Karasu C. Effects of peroxynitrite on the reactivity of diabetic rat aorta. Pharmacology 2001; 63: 58-64.
[132] Sowers JR. Hypertension, angiotensin II, and oxidative stress. N Engl J Med 2002; 346: 1999-2001.
[133] Crespo MJ, Moreta S, González J. Cardiovascular deterioration in STZ-diabetic rats: possible role of vascular RAS. Pharmacology 2003; 68: 1-8.
[134] Taguchi K, Kobayashi T, Hayashi Y, Matsumoto T, Kamata K. Enalapril improves impairment of SERCA-derived relaxation and enhancement of tyrosine nitration in diabetic rat aorta. Eur J Pharmacol 2007; 556: 121-8.
[135] Hadi HA, Suwaidi JA. Endothelial dysfunction in diabetes mellitus. Vasc Health Risk Manag 2007; 3: 853-76.
[136] Hartge MM, Unger T, Kintscher U. The endothelium and vascular inflammation in diabetes. Diab Vasc Dis Res 2007; 4: 84-8.
[137] Kamata K, Miyata N, Abiru T, Kasuya Y. Functional changes in vascular smooth muscle and endothelium of arteries during diabetes mellitus. Life Sci 1992; 50: 1379-87.
[138] Gewaltig MT, Kojda G. Vasoprotection by nitric oxide: mechanisms and therapeutic potential. Cardiovasc Res 2002; 55: 250-60.
[139] Pieper GM, Peltier BA. Amelioration by L-arginine of a dysfunctional arginine/nitric oxide pathway in diabetic endothelium. J Cardiovasc Pharmacol 1995; 25 : 397-403
[140] Ribeiro-Oliveira A Jr, Nogueira AI, Pereira RM, Boas WW, Dos Santos RA, Simões e Silva AC. The renin-angiotensin system and diabetes: an update. Vasc Health Risk Manage 2008; 4: 787-803.
[141] Simonsen U, Rodriguez-Rodriguez R, Dalsgaard T, Buus NH, Stankevicius E. Novel approaches to improving endothelium-dependent nitric oxide-mediated vasodilatation. Pharmacol Rep 2009; 61: 105-15.
[142] Ceylan A, Karasu C, Aktan F, Güven C, Can B, Ozansoy G. Effects of simvastatin treatment on oxidant/antioxidant state and ultrastructure of diabetic rat myocardium. Gen Physiol Biophys 2003; 22: 535-47.
[143] Ceylan A, Karasu C, Aktan F, Ozansoy G. Simvastatin treatment restores vasoconstriction and the inhibitory effect of LPC on endothelial relaxation via affecting oxidizing metabolism in diabetic rats. Diabetes Nutr Metab 2004; 17: 203-10.
[144] Wölkart G, Stessel H, Saad Z, Kirchengast M, Brunner F.Cardioprotective effects of atrasentan, an endothelin-A receptor antagonist, but not of nitric oxide in diabetic mice with myocyte-specific overexpression of endothelial nitric oxide synthase. Br J Pharmacol 2006; 148: 671-81.
[145] Bartuœ M, £omnicka M, Kostogrys RB, *et al*. 1-Methylnicotinamide (MNA) prevents endothelial dysfunction in hypertriglyceridemic and diabetic rats. Pharmacol Rep 2008; 60: 127-38.
[146] Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and *in vivo* monitoring of ROS. Circulation 2003; 108: 1912-16.
[147] Zobali F, Avci A, Canbolat O, Karasu C. Effects of vitamin A and insulin on the antioxidative state of diabetic rat heart: a comparison study with combination treatment. Cell Biochem Funct 2002; 20: 75-80.
[148] Ulusu NN, Sahilli M, Avci A, *et al*. Pentose phosphate pathway, glutathione-dependent enzymes and antioxidant defense during oxidative stress in diabetic rodent brain and peripheral organs: effects of stobadine and vitamin E. Neurochem Res 2003; 28: 815-23.
[149] Guzik TJ, Harrison DG. Vascular NADPH oxidases as drug targets for novel antioxidant strategies. Drug Discov Today 2006; 11: 524-33.
[150] Kaul N, Siveski-Iliskovic N, Hill M, Khaper N, Seneviratne C, Singal PK. Probucol treatment reverses antioxidant and functional deficit in diabetic cardiomyopathy. Mol Cell Biochem 1996; 160-61: 283-8.
[151] Baluchnejadmojarad T, Roghani M. Chronic administration of genistein improves aortic reactivity of streptozotocin-diabetic rats: mode of action. Vascul Pharmacol 2008; 49: 1-5.
[152] Heistad DD. Endothelial function in the time of the giants. J Cardiovasc Pharmacol 2008; 52: 385-92.
[153] Wiernsperger NF. Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. Diabetes Metab 2003; 29: 579-85.
[154] Zobali F, Besler T, Ari N, Karasu C. (Antioxidants in Diabetes-Induced Complications) The ADIC Study Group. Hydrogen peroxide-induced inhibition of vasomotor activity: evaluation of single and combined treatments with vitamin A and insulin in streptozotocin-diabetic rats. Int J Exp Diabetes Res 2002; 3: 119-30.
[155] Zieman SJ, Melenovsky V, Clattenburg L, *et al*. Advanced glycation endproduct crosslink breaker (alagebrium) improves endothelial function in patients with isolated systolic hypertension. J Hypertens 2007; 25: 577-83.
[156] Stirban A, Negrean M, Stratmann B, *et al*. Benfotiamine prevents macro- and microvascular endothelial dysfunction and oxidative stress following a meal rich in advanced glycation end products in individuals with type 2 diabetes. Diabetes Care 2006; 29: 2064-71.
[157] Moreau R, Nguyen BT, Doneanu CE, Hagen TM. Reversal by aminoguanidine of the age-related increase in glycoxidation and lipoxidation in the cardiovascular system of Fischer 344 rats. Biochem Pharmacol 2005; 69: 29-40.
[158] Mattson MP. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. Exp Gerontol 2009; 44: 625-34.
[159] Hünkar T, Aktan F, Ceylan A, Karasu C. Antioxidants in Diabetes-Induced Complications (ADIC) Study Group. Effects of cod liver oil on tissue antioxidant pathways in normal and streptozotocin-diabetic rats. Cell Biochem Funct 2002; 20: 297-302.
[160] Günes A, Ceylan A, Sarioglu Y, Stefek M, Bauer V, Karasu C. Antioxidants in Diabetes-induced Complications (ADIC) Study

Group. Reactive oxygen species mediate abnormal contractile response to sympathetic nerve stimulation and noradrenaline in the vas deferens of chronically diabetic rats: effects of *in vivo* treatment with antioxidants. Fundam Clin Pharmacol 2005; 19: 73-9

[161] Yülek F, Or M, Ozoğul C, *et al*. Effects of stobadine and vitamin E in diabetes-induced retinal abnormalities: involvement of oxidative stress. Arch Med Res 2007; 38: 503-11.

[162] Das Evcimen N, Ulusu NN, Karasu C, Doğru B. Adenosine triphosphatase activity of streptozotocin-induced diabetic rat brain microsomes: Effect of vitamin E. Gen Physiol Biophys 2004; 23: 347-55.

[163] Dogru Pekiner B, Das Evcimen N, Ulusu NN, Bali M, Karasu C. Effects of vitamin E on microsomal Ca^{2+}-ATPase activity and calcium levels in streptozotocin-induced diabetic rat kidney. Cell Biochem Funct 2003; 21: 177-82.

[164] Karasu C, Dewhurst M, Stevens EJ, Tomlinson DR. Effects of antioxidant treatment on sciatic nerve dysfunction in streptozotocin-diabetic rats; comparison with essential fatty acids. Diabetologia 1995; 38: 129-34.

[165] Skalska S, Kyselova Z, Gajdosikova A, Karasu C, Stefek M, Stolc S. Protective effect of stobadine on NCV in streptozotocin-diabetic rats: augmentation by vitamin E. Gen Physiol Biophys 2008; 27: 106-14.

[166] Keegan A, Walbank H, Cotter MA, Cameron NE. Chronic vitamin E treatment prevents defective endothelium-dependent relaxation in diabetic rat aorta. Diabetologia 1995; 38: 1475-8.

[167] Hamblin M, Smith HM, Hill MF.Dietary supplementation with vitamin E ameliorates cardiac failure in type I diabetic cardiomyopathy by suppressing myocardial generation of 8-iso-prostaglandin F2alpha and oxidized glutathione. J Card Fail 2007; 13: 884-92.

[168] Peerapatdit T, Patchanans N, Likidlilid A, Poldee S, Sriratanasathavorn C. Plasma lipid peroxidation and antioxidiant nutrients in type 2 diabetic patients. J Med Assoc Thai 2006; 89(5): S147-55.

[169] Skyrme-Jones RA, O'Brien RC, Berry KL, Meredith IT. Vitamin E supplementation improves endothelial function in type I diabetes mellitus: a randomized, placebo-controlled study. J Am Coll Cardiol 2000; 36: 94-102.

[170] Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF Jr, Creager MA. Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. Am J Physiol 2003; 285: H2392-98.

[171] Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000; 342: 154-60.

[172] Lonn E, Bosch J, Yusuf S, *et al*. HOPE and HOPE-TOO Trial Investigators. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. JAMA 2005; 293: 1338-47.

[173] Song Y, Cook NR, Albert CM, Van Denburgh M, Manson JE. Effects of vitamins C and E and beta-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. Am J Clin Nutr 2009; 90: 429-37.

[174] Srivatsan R, Das S, Gadde R, *et al*. Antioxidants and lipid peroxidation status in diabetic patients with and without complications. Arch Iran Med 2009; 12: 121-7

[175] Gutierrez AD, de Serna DG, Robinson I, Schade DS. The response of gamma vitamin E to varying dosages of alpha vitamin E plus vitamin C. Metabolism 2009; 58: 469-78.

[176] Packer L, Witt EH, Tritschler HJ. Alpha-Lipoic acid as a biological antioxidant. Free Radic Biol Med 1995; 19: 227-50.

[177] Nohl H, Gille L. Evaluation of the antioxidant capacity of ubiquinol and dihydrolipoic acid. Z Naturforsch C 1998; 53: 250-3.

[178] Balkis Budin S, Othman F, Louis SR, *et al*. Effect of alpha lipoic acid on oxidative stress and vascular wall of diabetic rats. Rom J Morphol Embryol 2009; 50: 23-30.

[179] Strödter D, Lehmann E, Lehmann U, Tritschler HJ, Bretzel RG, Federlin K. The influence of thioctic acid on metabolism and function of the diabetic heart. Diabetes Res Clin Pract 1995; 29: 19-26.

[180] Maritim AC, Sanders RA, Watkins JB 3rd. Effects of alpha-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. J Nutr Biochem 2003; 14: 288-94

[181] Bojunga J, Nowak D, Mitrou PS, Hoelzer D, Zeuzem S, Chow KU. Antioxidative treatment prevents activation of death-receptor- and mitochondrion-dependent apoptosis in the hearts of diabetic rats. Diabetologia 2004; 47: 2072-80.

[182] Oksala NK, Laaksonen DE, Lappalainen J, *et al*. Heat shock protein 60 response to exercise in diabetes: effects of alpha-lipoic acid supplementation. J Diabetes Complicat 2006; 20: 257-61.

[183] Ziegler D. Thioctic acid for patients with symptomatic diabetic polyneuropathy: a critical review. Treat Endocrinol 2004; 3: 173-89.

[184] Ziegler D, Hanefeld M, Ruhnau KJ, *et al*. Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant alpha-lipoic acid. A 3-week multicentre randomized controlled trial (ALADIN Study). Diabetologia 1995; 38: 1425-33.

[185] Reljanovic M, Reichel G, Rett K, *et al*. Treatment of diabetic polyneuropathy with the antioxidant thioctic acid (alpha-lipoic acid): a two year multicenter randomized double-blind placebo-controlled trial (ALADIN II). Alpha Lipoic Acid in Diabetic Neuropathy. Free Radic Res 1999; 31: 171-9.

[186] Ziegler D, Hanefeld M, Ruhnau K, *et al*. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a 7-month multicenter randomized controlled trial (ALADIN III Study). ALADIN III Study Group. Alpha-Lipoic Acid in Diabetic Neuropathy. Diabetes Care 1999; 22: 1296-301

[187] Ziegler D, Schatz H, Conrad F, Gries FA, Ulrich H, Reichel G. Effectsof treatment with the antioxidant alpha-lipoic acid on cardiac autonomic neuropathy in NIDDM patients. A 4-month randomized controlled multicenter trial (DEKAN Study). Deutsche Kardiale Autonome Neuropathie. Diabetes Care 1997; 20: 369-73.

[188] Ziegler D. Painful diabetic neuropathy: advantage of novel drugs over old drugs? Diabetes Care 2009; 32 (Suppl 2): S414-9.

[189] Low PA, Nehrdich D, Novosadova M, O'Brien PC, et al. The sensory symptoms of diabetic polyneuropathy are improved with {alpha}-Lipoic Acid: The SYDNEY Trial. Diabetes Care 2003; 26: 770-6.

[190] Ziegler D, Nowak H, Kempler P, Vargha P, Low PA. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. Diabet Med 2004; 21: 114-21.

[191] El-Swefy S, Schaefer EJ, Seman LJ, *et al*. The effect of vitamin E, probucol, and lovastatin on oxidative status and aortic fatty lesions in hyperlipidemic-diabetic hamsters. Atherosclerosis 2000; 149: 277-86.

[192] Gaede P, Vedel P, Larsen N, Jensen GVH, Parving H-H, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with Type 2 diabetes. N Engl J Med 2003; 348: 383-93.

[193] Belch J, MacCuish A, Campbell I, *et al*. Prevention of Progression of Arterial Disease and Diabetes Study Group; Diabetes Registry Group; Royal College of Physicians Edinburgh. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. Ann Intern Med 2009; 150: JC1-8.

[194] Stirban A. Drugs for the treatment of diabetes complications. Zycose: a new player in the field? Drugs Today (Barc) 2008; 44: 783-96.

[195] Tanous D, Hime N, Stocker R. Anti-atherosclerotic and anti-diabetic properties of probucol and related compounds. Redox Rep 2008; 13: 48-59.

[196] Gorogawa S, Kajimoto Y, Umayahara Y, *et al*. Probucol preserves pancreatic beta-cell function through reduction of oxidative stress in type 2 diabetes. Diabetes Res Clin Pract 2002; 57: 1-10.

[197] Aviram M, Rosenblat M, Bisgaier CL, Newton RS. Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. Atherosclerosis 1998; 138: 271-80.

[198] Ozansoy G, Akin B, Aktan F, Karasu C. Short-term gemfibrozil treatment reverses lipid profile and peroxidation but does not alter blood glucose and tissue antioxidant enzymes in chronically diabetic rats. Mol Cell Biochem 2001; 216: 59-63.

[199] Ozansoy G, Akin FB. Effects of gemfibrozil treatment on vascular reactivity of streptozotocin-diabetic rat aorta. J Pharm Pharmacol 2004; 56: 241-6.

[200] Sahilli M, Irat AM, Isik AC, Karasu C, Ozansoy G, Ari N. A lack of synergistic interaction between insulin and pioglitazone on reactivity of rat aorta from chronically high dose insulin-treated diabetic rats. Gen Physiol Biophys 2007; 26: 48-55.

[201] Fukui T, Noma T, Mizushige K, Aki Y, Kimura S, Abe Y. Dietary troglitazone decreases oxidative stress in early stage type II diabetic rats. Life Sci 2000; 66: 2043-9.

[202] Lida KT, Kawakami Y, Suzuki M, *et al*. Effect of thiazolidinediones and metformin on LDL oxidation and aortic endothelium relaxation in diabetic GK rats. Am J Physiol 2003; 284: E1125-30.

[203] Horakova L, Stolc S. Antioxidant and pharmacodynamic effects of pyridoindole stobadine. Gen Pharmacol 1998; 30: 627-38.

[204] Stefek M, Krizanova L, Trnkova Z. Oxidative modification of serum albumin in an experimental glycation model of diabetes mellitus *in vitro*: effect of the pyridoindole antioxidant stobadine. Life Sci 1999; 65: 1995-7.

[205] Stefek M, Gajdosik A, Tribulova N, *et al*. The pyridoindole antioxidant stobadine attenuates albuminuria, enzymuria, kidney lipid peroxidation and matrix collagen cross-linking in streptozotocin-induced diabetic rats. Methods Find Exp Clin Pharmacol 2002; 24: 565-71.

[206] Stefek M, Sotnikova R, Okruhlicova L, *et al*. Effect of dietary supplementation with the pyridoindole antioxidant stobadine on antioxidant state and ultrastructure of diabetic rat myocardium. Acta Diabetol 2000; 37: 111-7.

[207] Cumaoglu A, Cevik C, Rackova L, Ari N, Karasu C. Effects of antioxidant stobadine on protein carbonylation, advanced oxidation protein products and reductive capacity of liver in streptozotocin-diabetic rats: role of oxidative/nitrosative stress. Biofactors 2007; 30: 171-8.

[208] Vlkovicová J, Javorková V, Stefek M, Kysel'ová Z, Gajdosíková A, Vrbjar N. Effect of the pyridoindole antioxidant stobadine on the cardiac Na^+,K^+-ATPase in rats with streptozotocin-induced diabetes. Gen Physiol Biophys 2006; 25: 111-24.

[209] Guzy J, Barnová E, Benes L. Stobadine and heart mitochondria. Life Sci 1999; 65: 1935-7.

[210] Demiryürek AT, Karasu C, Stefek M, Stolc S. Effect of stobadine on leukocyte free radical generation in streptozotocin-diabetic rats: comparison with vitamin E. Pharmacology 2004; 70: 1-4.

[211] Kyselova Z, Gajdosik A, Gajdosikova A, *et al*. Effect of the pyridoindole antioxidant stobadine on development of experimental diabetic cataract and on lens protein oxidation in rats: comparison with vitamin E and BHT. Mol Vis 2005; 11: 56-65.

[212] Steenken S, Sundquist AR, Jovanovic SV, Crockett R, Sies H. Antioxidant activity of the pyridoindole stobadine. Pulse radiolytic characterization of one-electron-oxidized stobadine and quenching of singlet molecular oxygen. Chem Res Toxicol 1992; 5: 355-60.

[213] Kagan VE, Tsuchiya M, Serbinova E, Packer L, Sies H. Interaction of the pyridoindole stobadine with peroxyl, superoxide and chromanoxyl radicals. Biochem Pharmacol 1993; 45: 393-400.

[214] Clarke IS, Dzialoszynski T, Sanford SE, Chevendra V, Trevithick JR. Dietary strategies to prevent retinopathy in RCS rats. In: Lerman S, Tripathi RC, eds. Ocular toxicology: proceedings of the First Congress of the International Society of Ocular Toxicology. New York: Dekker 1989; pp. 395-413.

[215] Sotnikova R, Stefek M, Okruhlicova L, *et al*. Dietary supplementation of the pyridoindole antioxidant stobadine reduces vascular impairment in streptozotocin-diabetic rats. Methods Find Exp Clin Pharmacol 2001; 23: 121-9.

[216] Misík V, Ondrias K, Gergel D. Antioxidative properties of cardioactive agents. Bratisl Lek Listy 1993; 94: 66-70.

[217] Stolc S. Pyridoindole stobadine is a nonselective inhibitor of voltage-operated ion channels in rat sensory neurons. Gen Physiol Biophys 1994; 13: 259-66.

[218] Sotníková R, Okruhlicová L, Noskovic P. Endothelial protective effect of stobadine on ischaemia/reperfusion-induced injury. Gen Physiol Biophys 1998; 17: 253-64.

[219] Nosál'ová V, Navarová J, Mihalová D, Sotníková R.Mesenteric ischemia/reperfusion-induced intestinal and vascular damage: effect of stobadine. Methods Find Exp Clin Pharmacol 2007; 29: 39-45.

[220] Guz G, Demirogullari B, Ulusu NN, *et al*. Stobadine protects rat kidney against ischaemia/reperfusion injury.Clin Exp Pharmacol Physiol. 2007; 34: 210-6.

[221] Stefek M, Snirc V, Djoubissie PO, *et al*. Carboxymethylated pyridoindole antioxidants as aldose reductase inhibitors: synthesis, activity, partitioning, and molecular modeling. Bioorg Med Chem 2008; 16: 4908-20.

[222] Umit Bağriaçik E, Uslu K, Yurtçu E, Stefek M, Karasu C. Stobadine inhibits doxorubicin-induced apoptosis through a caspase-9 dependent pathway in P815 mastocytoma cells. Cell Biol Int 2007; 31: 979-84.

[223] Rackova L, Cumaoglu A, Maechler P, Stefek M, Karasu C. (±)-8-Methoxy-1,3,4,4,A,5,9B-Hexahydro-pyrido [4,3-B] indole2-carboxylic acid ethyl ester as a protective agent against H_2O_2 induced oxidative damage in pancreas βcell lines. Türkiye Klinikleri J Med Sci 2008; 29 (Suppl 1): S129.

[224] Rackova L, Snirc V, Jung T, Stefek M, Karasu C, Grune T. Metabolism-induced oxidative stress is a mediator of glucose toxicity in HT22 neuronal cells. Free Radic Res 2009; 43: 876-86.

Received: September 03, 2010 Revised: September 24, 2010 Accepted: October 04, 2010