

# Regulation of Oxidative Stress and Cardioprotection in Diabetes Mellitus

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**Abstract:** Analysis of the Framingham data has shown that the risk of heart failure is increased substantially among diabetic patients, while persons with the metabolic syndrome have an increased risk of both atherosclerosis and diabetes mellitus. Sleep apnea may be related to the metabolic syndrome and systemic inflammation through hypoxia, which might also cause the cardiac remodeling by increased oxidative stress. On the other hand, the renin-angiotensin system is activated in diabetes, and local angiotensin II production may lead to oxidative damage *via* the angiotensin II type 1 receptor. Basic and clinical data indicate that angiotensin II receptor blockers have the potential to preserve left ventricular function and prevent cardiac remodeling that is exaggerated by oxidative stress in patients with diabetes. Thus, alleviation of oxidative stress might be one possible strategy in the treatment of diabetic patients associated with sleep apnea.

**Key Words:** Oxidative stress, diabetes mellitus, sleep apnea, hypoxia, remodeling.

## INTRODUCTION

Diabetes mellitus is a leading cause of morbidity and mortality because of its vascular complications [1,2]. Although type 1 diabetes is an important clinical problem with numerous long-term complications [3], the vast majority of diabetic patients with vascular complications have type 2 diabetes [4,5]. Diabetes mellitus currently affects 171 million persons worldwide, and there are predicted to be 366 million diabetic patients by the year 2030. In fact, the prevalence of type 2 diabetes is projected to double, especially in developing countries [6].

The most common cause of death among patients with diabetes is atherosclerotic cardiovascular disease. Current theories suggest that the initial event in atherogenesis is endothelial cell dysfunction, which can be induced by various insults, including diabetes, hyperlipidemia, hypertension, and smoking [7-10]. It has been suggested that hyperglycemia, hyperinsulinemia and insulin resistance, glycation of proteins, oxidative stress, inflammation, and many other factors may be related to atherogenesis in diabetes [11].

Recently, obstructive sleep apnea syndrome (OSAS), which is often found in obese people, has been identified as an independent risk factor for cardiovascular disease [12,13]. We have reported that intermittent hypoxia increases oxidative stress and induces left ventricular remodeling in an experimental model of sleep apnea [14]. The present review focuses on the role of oxidative stress in diabetes mellitus and its regulation from the viewpoint of cardioprotection.

## OXIDATIVE STRESS AND CARDIOVASCULAR DISEASE IN DIABETES

### Increased Oxidative Stress in Diabetics

Hyperglycemia seems to promote an imbalance between the generation and elimination of reactive oxygen species

(ROS). Oxidative stress in diabetes could arise from a variety of mechanisms, such as excessive production of ROS from the auto-oxidation of glucose, glycation of proteins, and glycation of antioxidant enzymes (limiting their capacity to detoxify ROS). These changes could result in damage to cellular organelles and membranes, which may lead to diabetic complications [15].

Hyperglycemia is a key clinical manifestation of diabetes mellitus, and it stimulates several pathways. The polyol pathway is one of the pathways by which ROS increase in hyperglycemia: it involves conversion of glucose to sorbitol by aldose reductase and consumes NADPH, which acts as a coenzyme in the production of reduced glutathione, with the resulting depletion of NADPH causing an increase of oxidative stress through inadequate catalysis of H<sub>2</sub>O<sub>2</sub> [16]. Also, increased conversion of sorbitol to fructose by sorbitol dehydrogenase leads to an increase of diacylglycerol (DAG), and activates protein kinase C (PKC), which might in turn induce the activation of NADPH oxidase and increase oxidative stress by decreasing the NAD<sup>+</sup>/NADH ratio [17].

Increased production of fructose, the end production of the polyol pathway, leads to an increase of advanced glycation end-products (AGEs), and ROS might also be produced during the creation of AGEs [18]. AGEs could generate ROS directly or *via* the receptors for AGEs (RAGE) [19,20]. In addition, AGEs promote the migration, proliferation, and differentiation of smooth muscle cells, the production of several cytokines, induction of adhesion molecule expression, and production of extracellular matrix (ECM) through RAGE or the scavenger receptor.

Recently, it has been shown that vascular smooth muscle cells and endothelial cells can produce ROS through activation of NADPH oxidase, which seems to be the most important source of ROS in intact arteries rather than enzymes involved with arachidonic acid (xanthine oxidase) or release from mitochondrial sources [21,22]. Inoguchi *et al.* [23] have shown that a high glucose level stimulates ROS production through activation of PKC-dependent NADPH oxidase in

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both vascular smooth muscle cells and endothelial cells, and they have also shown that the increase of ROS production by high glucose is completely reversed by diphenylene iodonium, an NADPH oxidase inhibitor. The increase of free radical production by exposure to high glucose was also completely blocked by a specific PKC inhibitor, suggesting that there was PKC-dependent activation of NADPH oxidase. On the other hand, Nishikawa *et al.* [24] reported that normalizing mitochondrial superoxide production blocks glucose-induced activation of PKC, increases the formation of AGEs, and promotes the polyol pathway. Thus, oxidative stress is increased in patients with diabetes mellitus through many pathways, and which of these should become the therapeutic target remains controversial.

#### **Vascular Damage by Oxidative Stress**

Oxidative stress in diabetics induces thrombogenesis, endothelial dysfunction, and vascular inflammation [25]. Nitric oxide (NO) has an important role in protecting the vasculature against atherosclerosis, and endothelial NO synthase (eNOS) is responsible for most vascular NO production. Superoxide reacts with vascular NO to form peroxynitrite, and the cofactor tetrahydro-L-biopterin (BH4) is highly sensitive to oxidation by peroxynitrite. A decrease of the BH4 level promotes superoxide production by eNOS [26]. Both mechanisms lead to the loss of NO bioactivity, which might induce endothelial dysfunction and atherosclerosis [27].

ROS are reported to induce the expression of various growth-related genes, including c-fos, c-myc, and c-jun [28-30]. Furthermore, ROS production *via* NADPH oxidase has been implicated in the pathogenesis of angiotensin II-induced hypertension and vascular smooth muscle hypertrophy.

In endothelial cells, cytokine-induced expression of vascular cell adhesion molecule-1 (VCAM-1) has been reported to involve mobilization of nuclear factor-kappa B (NF- $\kappa$ B) through ROS and can be blocked by an antioxidant. Expression of VCAM-1 promotes the adhesion of monocytes to endothelial cells and may be important in the development of atherosclerosis. These findings suggest that an increase of ROS production *via* NADPH oxidase in vascular cells may contribute to the acceleration atherosclerosis in patients with diabetes.

#### **Myocardial Damage by Oxidative Stress**

Oxidative stress related to hyperglycemia has been implicated as a major factor in the pathogenesis of cardiac hypertrophy and diabetic cardiomyopathy [15], which is not accompanied by either hypertension or coronary artery disease [31]. Diabetes is a well-known risk factor for the development of heart failure. Indeed, the Framingham Heart Study showed that the frequency of heart failure is twice as high in diabetic men and five times as high in diabetic women compared with age-matched control subjects [32]. Gonzalez-Vlilchez *et al.* [33] reported that diabetics developed concentric left ventricular hypertrophy and with impaired systolic and diastolic function. Diabetic cardiomyopathy is a major reason for the high morbidity and mortality of diabetics. Factors that are involved in the development of diabetic cardio-

myopathy include impaired calcium homeostasis, upregulation of the renin-angiotensin system (RAS), increased oxidative stress, altered substrate metabolism, and mitochondrial dysfunction [31]. Several groups have shown that overproduction of ROS occurs in both type 1 and type 2 diabetes [31].

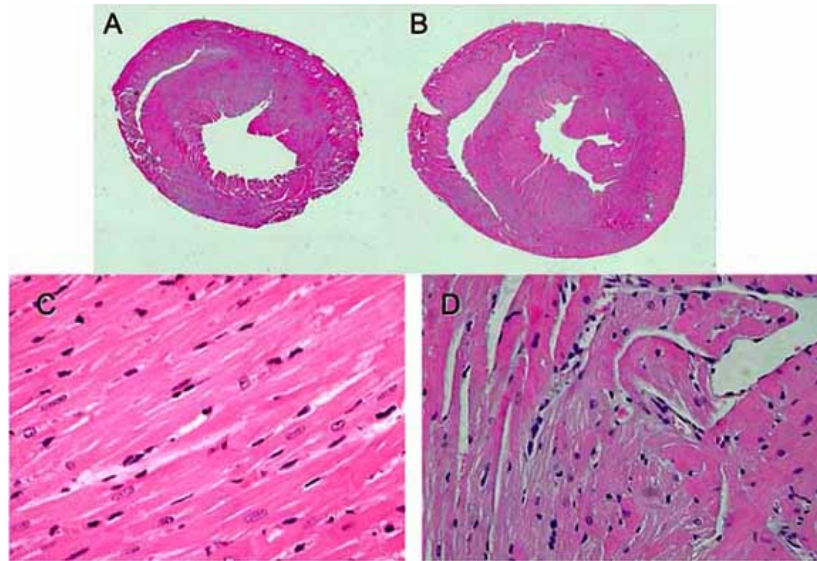
Recently, it was suggested that myocardial dysfunction may play an important role in the pathogenesis of impaired cardiac contractility in diabetics [34]. Boudina *et al.* [35] reported that decreased mitochondrial respiration and reduced expression of proteins involved in oxidative phosphorylation were observed in obese type 2 diabetic mice, and stated that such changes might contribute to cardiac dysfunction *via* reduced ATP production. Under physiological conditions, most of the ROS generated within a cell come from the mitochondria. Increased mitochondrial generation of ROS has been demonstrated in various tissues exposed to hyperglycemia [36]. Nitration of mitochondrial proteins (an index of oxidative damage) is increased in the hearts of diabetic mice [37]. Because mitochondrial hydrogen peroxide production is increased and glutathione levels are reduced in diabetic hearts, the source of ROS has been suggested to be the mitochondria [38]. Non-mitochondrial sources of ROS, including increased AGE formation, increased PKC isoform expression, and increased hexosamine pathway flux, have also been suggested to play a role in the diabetic heart [39]. Increased ROS generation activates maladaptive signaling pathways, which might lead to cell death and thus contribute to the development of diabetic cardiomyopathy.

Increased ROS generation activates maladaptive signaling pathways, which might lead to cell death and thus contribute to the development of diabetic cardiomyopathy. An increase of apoptosis, an increase of DNA damage, and reduced activity of the DNA repair pathway have been reported in diabetic animals [40]. ROS activate NF- $\kappa$ B, which plays a crucial role in mediating the immune and inflammatory responses, as well as apoptosis. The c-jun NH(2)-terminal kinases (JNK) and p38 MAPKs, which are members of the complex superfamily of MAP serine/threonine protein kinases, are stimulated by ROS. The pathways mediated by NF- $\kappa$ B, JNK, and p38 MAPK are potential stress-signaling systems that could have a role in the late complications of diabetes [39].

#### **SLEEP APNEA SYNDROME AND DIABETES**

Obstructive sleep apnea syndrome (OSAS) is characterized by recurrent episodes of upper airway obstruction during sleep that induce hypoxia. Coughlin *et al.* [41] reported that OSAS was closely associated with an increased prevalence of metabolic syndrome. Metabolic syndrome is a cluster of risk factors for atherosclerotic cardiovascular disease, and this syndrome contributes to the development of diabetes mellitus [2]. In addition, we previously reported that continuous exposure to hypoxia causes the acceleration of myocardial degeneration in diabetic rats [42] (Figs. 1 and 2). These findings suggest that a strong relationship may exist between OSAS and diabetes.

OSAS patients have significantly higher fasting blood glucose and insulin levels compared with obese controls [43]. Polotsky *et al.* [44] have shown that intermittent hy-



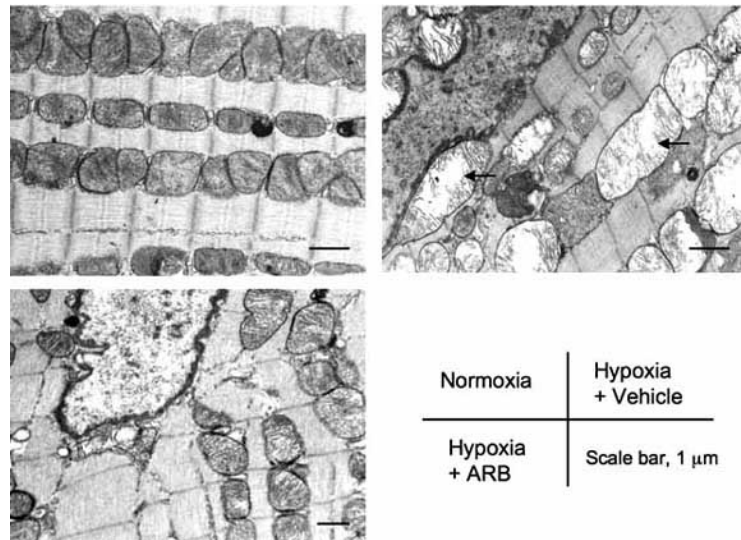
**Fig. (1).** Representative macrographs (A, B) and light micrographs (C, D) of hearts from the diabetic rats. The diabetic rats kept under normoxia exhibited nearly normal morphology (A, C). Hypoxia caused cardiac hypertrophy, disarrangement of myofibers, and increased interstitial fibrosis (B, D). original magnification; x 100.

poxia due to OSAS exacerbates insulin resistance and glucose intolerance associated with obesity in the presence of leptin deficiency. On the other hand, several studies have demonstrated that OSAS is associated with insulin resistance and glucose intolerance independently of obesity [45-47]. Intermittent hypoxia due to OSAS rather than obesity might play an important role in the development of diabetes by inducing insulin resistance and glucose intolerance. Patients with OSAS have elevated plasma levels of TNF- $\alpha$  and IL-6 [48,49], and such inflammatory cytokines may be responsible for the development of diabetes. In particular, TNF- $\alpha$  has been reported to inhibit insulin signaling [50,51]. Therefore,

TNF- $\alpha$  is likely to be crucial for the pathogenesis of diabetes in patients with OSAS. Further studies are needed to better clarify the role of inflammatory cytokines in both OSAS and diabetes.

**HYPOXIA AND REACTIVE OXYGEN SPECIES**

The hyperglycemic state contributes to cardiovascular complications in patients with OSAS. Intermittent hypoxia due to OSAS is known to be an independent risk factor for cardiovascular disease, including hypertension, congestive heart failure, and stroke [52]. We previously reported that hypoxia induced LV remodeling in diabetic rats and athero-



**Fig. (2).** Electron micrographs of the left ventricular (LV) myocardium in diabetic rats. In diabetic rats kept under normoxia, mild deformity of mitochondria was observed. Hypoxia induced ballooning and loss of cristae in many mitochondria (arrows). Treatment with angiotensin-II receptor blocker (ARB) preserved the fine structure of the LV myocardium. Reproduced from Inamoto S, Hayashi T, Tazawa N, *et al.* Angiotensin-II receptor blocker exerts cardioprotection in diabetic rats exposed to hypoxia. *Circ J* 2006; 70: 787-792.

genic mice [42, 53]. In addition, hypoxia accelerates the progression of atherosclerosis in atherogenic mice [54].

Overproduction of ROS causes oxidative stress, and has been implicated in the pathophysiology of cardiovascular disease. Recent studies have revealed that intermittent hypoxia increases ROS production [55], lipid peroxidation, and isoprostane levels [56] in the brains of experimental animals. In addition, Chen *et al.* [57] demonstrated an increase of oxidative stress in the hearts of rats exposed to intermittent hypoxia. These results suggest that oxidative stress may play a crucial role in the development of cardiovascular disease among patients with OSAS.

NADPH oxidase is a major producer of ROS. This enzyme is composed of two membrane-bound subunits (gp91phox and p22phox), as well as four cytosolic subunits (p40phox, p47phox, p67phox, and rac-1). Both angiotensin II and inflammatory cytokines have already been shown to stimulate NADPH oxidase, while hypoxic stress may be similarly important for its activation. In fact, Zhan *et al.* [58] reported that NADPH oxidase-derived ROS contribute to oxidative injury in the brains of mice exposed to intermittent hypoxia. Moreover, we have shown that hypoxia increases ROS production by NADPH oxidase in the aorta and LV myocardium, and consequently accelerates both atherosclerosis and LV remodeling [53,54]. Thus, intermittent hypoxia might enhance oxidative stress at least partly through activation of NADPH oxidase. Oxidative stress is also responsible for the activation of NF- $\kappa$ B [59,60], which regulates the expression of inflammatory cytokines and mediates monocyte-endothelial cell adhesion. We observed that hypoxia activates NF- $\kappa$ B in the LV myocardium of atherogenic mice [53]. This raises the possibility that NF- $\kappa$ B is an essential factor for the development of cardiovascular disease associated with hypoxic states. Taken together, these findings suggest that cardiovascular disease might be promoted by oxidative stress related to intermittent hypoxia (Fig. 3). In addition, NADPH oxidase might be a useful target for therapeutic intervention to prevent cardiovascular disease in patients with OSAS.

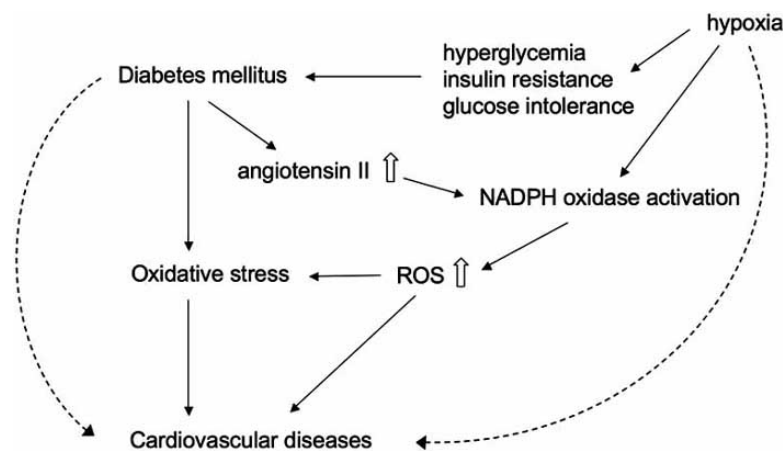
## NOVEL THERAPEUTIC STRATEGIES FOR OXIDATIVE STRESS

Oxidative stress has now been proved to play an important role in the development and progression of myocardial remodeling in patients with diabetes [61-63] (Fig. 3). There is a growing body of evidence suggesting that antioxidants exert a protective effect in experimental model of heart failure [63-65]. Studies on myocardial ischemia-reperfusion injury have demonstrated the potential therapeutic value of radical scavengers, antioxidant extracts from a variety of plants, and polyphenols from food and wine, as well as vitamin E, vitamin C, and beta-carotene [66-69].

Haidara *et al.* [63] reviewed this area and concluded that administration of antioxidants might have a cardioprotective effect in the experimental setting and might protect against endothelial dysfunction associated with atherosclerosis, thus providing an effective means of reducing cardiovascular complications in diabetics.

In the clinical trials performed so far, however, the efficacy of treatment with antioxidants has been variable, probably due to inadequate doses and incorrect protocols, so these agents might still be promising [70-73]. Achieving the same beneficial outcome in the clinical setting might require a different approach that targets more specific intracellular pathways, in addition to the scavenging of excess oxygen radicals.

It has been shown that intensified multifactorial intervention with tight glucose regulation, renin-angiotensin system blockers, aspirin, and lipid-lowering agents can reduce the risk of nonfatal cardiovascular disease in patients with type 2 diabetes [74-76]. Recently, Gæde *et al.* [77] reported that intensive intervention with multiple drugs and behavior modification had a sustained beneficial effect on vascular complications, as well as reducing the all cause death rate and the cardiovascular death rate. Thus, it is obvious that more research is required to evaluate the efficacy of antioxidants in patients with diabetes.



**Fig. (3).** Role of oxidative stress in the progression of cardiovascular diseases accompanied by diabetes mellitus. Diabetes mellitus induced oxidative stress at least partly through NADPH oxidase activation, and consequently accelerated the progression of cardiovascular diseases. In addition, hypoxia might be implicated in the development of diabetes mellitus and production of reactive oxygen species (ROS), associated with the insult to mitochondria in cardiomyocytes by hypoxia itself.

### Angiotensin-converting Enzyme (ACE) Inhibitors and Angiotensin-II Receptor Blockers (ARB)

Activation of RAS and the subsequent increase of angiotensin II and aldosterone levels contribute to changes of the insulin/IGF-1 signaling pathway and promote the formation of ROS that induce endothelial dysfunction and cardiovascular disease [78]. Angiotensin-II is known to increase the expression of adhesion molecules, cytokines, and chemokines and it exerts a proinflammatory effect on leucocytes, endothelial cells, and vascular smooth muscle cells. The inflammatory cascade is initiated by angiotensin-II *via* its type 1 receptor, followed by increased production of ROS and activation of NF- $\kappa$ B, which mediates the transcription and expression of various genes [79].

RAS activation is important for the progression of cardiovascular pathology along the continuum from the existence of hypertension and other risk factors to end-stage cardiovascular disease [80]. Many studies have shown that blockade of angiotensin-II significantly reduces the levels of proinflammatory mediators and oxidative stress products in various models of inflammation. We previously reported that administration of the ARB candesartan intraperitoneally *via* an osmotic minipump prevented microangiopathy and preserved diastolic function in diabetic rats [81]. Candesartan was also effective for improving cardiomyocyte diameter and decreasing the levels of inflammatory cytokines, such as IL-1 $\beta$  and IL-6. Transmission and scanning electron microscopy clearly showed the cardioprotective effect of ARB therapy (Fig. 4).

Recently, we reported that ARBs could reduce oxidative stress and ameliorate hypoxia-induced left ventricular re-

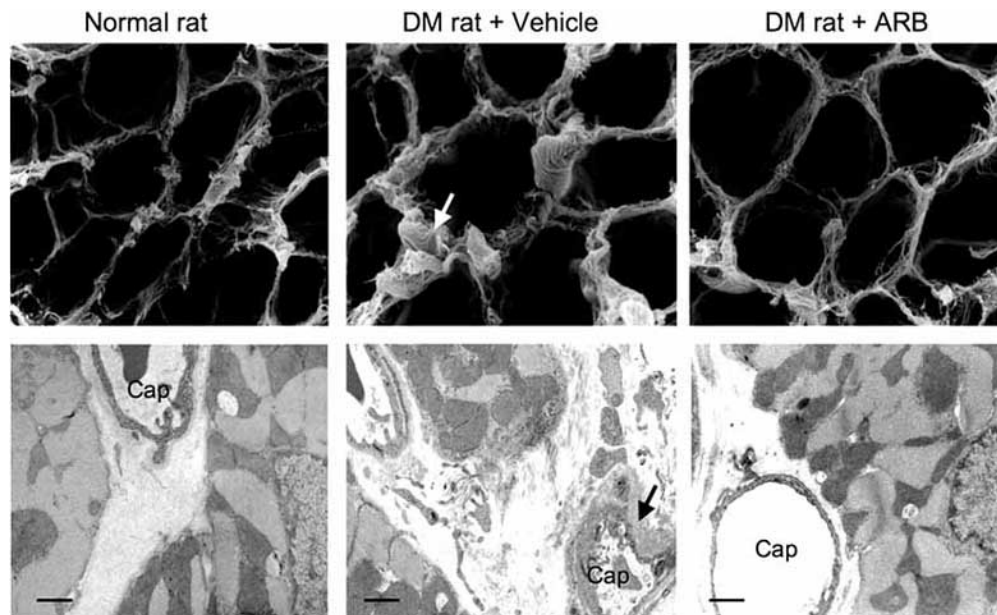
modeling, partly through the inhibition of NF- $\kappa$ B and matrix metalloproteinase (MMP)-9, in diabetic rats and atherogenic mice [42,53]. Thus, ARBs might provide effective cardio-protection even under hypoxic conditions, such as in diabetic patients with sleep apnea.

In clinical trials, blocking angiotensin-II by treatment with ACE inhibitors or ARBs has been found to be beneficial for patients with various inflammatory diseases [82]. RAS blockade delays or avoids the onset of type 2 diabetes, and also prevents cardiovascular or renal events in diabetic patients [83-85]. Furthermore, recent studies have shown that ARB therapy reduces the frequency of atrial fibrillation and stroke [86-88]. Thus, inhibition of the RAS by administration of ACE inhibitors or ARBs represents first-line treatment for hypertensive target organ damage and progressive cardiovascular disease [89].

### Statin Therapy

Statins reduce the plasma cholesterol level by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Besides lowering cholesterol, statins are known to modify endothelial function and atherogenesis, stabilize atherosclerotic plaques, and reduce inflammation and thrombosis [90,91]. Recent studies have shown that intermediate products of the mevalonate pathway cause the activation of Rac1, a subunit of NADPH oxidase, leading to the production of ROS [92]. Accordingly, statins reduce oxidative stress by inhibiting NADPH oxidase.

In animal models, statins have been shown to ameliorate oxidative stress, prevent the progression of cardiac hypertrophy, and improve left ventricular function, which are all actions that might be beneficial in patients with heart failure



**Fig. (4).** Representative scanning (above) and transmission (below) electron micrographs. Compared with normal rats, increased interstitial fibrosis (white arrow) and thickened basement membrane (black arrow) of capillary (Cap) were observed in diabetic (DM) rats. Treatment with angiotensin-II receptor blocker (ARB) suppressed the interstitial fibrosis and preserved the capillary basement membrane thickness. Scale bar=1  $\mu$ m. Reproduced from Hayashi T, Sohmiya K, Ukimura A, *et al.* Angiotensin II receptor blockade prevents microangiopathy and preserves diastolic function in the diabetic rat heart. HEART 2003; 89: 1236-42.

[93]. Horiuchi *et al.* [94] reported that a combination of low-dose ARB and low-dose statin therapy acted synergistically to block neointimal growth. These “pleiotropic” properties of statins may have important clinical implications in addition to their use for lowering cholesterol levels.

Several clinical studies have demonstrated an increase of AT1 receptor expression in hypercholesterolemic men [95]. Subgroup analysis of the Heart Outcomes Prevention Evaluation (HOPE) study indicated that a beneficial effect of ACE inhibition was more evident in patients with concomitant statin therapy [96]. Nickenig [91] has suggested that a combination of ARB and statin could be beneficial for patients with type 2 diabetes. Thus, further studies are warranted to confirm the beneficial impact of ARBs and statins through potentially synergistic modes of action, since these drugs could be used for potent and effective combination therapy in a variety of patient populations.

#### **Other Agents (Acarbose, Edaravone, and Calcium Channel Blockers)**

Hyperglycemia may induce the generation of free radicals such as superoxide ( $O_2^-$ ) and the hydroxyl radical. The interaction of glycosylated proteins with their cell surface binding sites may also lead to oxidative stress. Treatment with an  $\alpha$ -glucosidase inhibitor might not only be useful for preventing postprandial hyperglycemia but also for suppressing oxidative stress in diabetics. Large-scale clinical trials have shown an impressive vasculoprotective effect of acarbose [97,98]. Recently, Rösen *et al.* [99] reported that treatment of insulin-resistant rats with acarbose prevented an excessive rise of the plasma glucose level and the initiation of a reaction starting with the generation of ROS and spreading to affect the whole cell.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a free radical scavenger that traps hydroxyl radicals, as indicated by its inhibition of the formation of hydroxylated salicylate. Edaravone has been reported to exert a protective effect against cerebral and myocardial ischemia in rats. We have also reported that edaravone effectively decreased the activity of inducible nitric oxide synthase in the left ventricular myocardium of type 2 diabetic rats and preserved the ultrastructure of the mitochondria [100]. Thus, edaravone has a modest cardioprotective effect on the hearts of diabetic animals.

Both clinical studies and basic research have revealed that calcium antagonists not only protect the endothelium through their hypotensive action, but also improve endothelial function through stimulation of NO production [101]. Although the precise vasoprotective mechanisms of calcium channel blockers are still obscure, recent studies have suggested that nifedipine might stimulate SOD expression in endothelial cells *via* enhancement of VEGF expression by vascular smooth muscle cells, and thus might reduce oxidative stress and increase NO production [102].

Therefore,  $\alpha$ -glucosidase inhibitors, free radical scavengers, and calcium channel blockers should also be included as candidates for antioxidant therapy in the treatment of patients with diabetes.

#### **CONCLUSION**

Of course, meticulous glycemic control is important to prevent cardiovascular remodeling in diabetes. As reviewed above, oxidative stress has also been proved to play an important role in the development and progression of cardiovascular remodeling. New insights into the mechanisms that increase oxidative stress in diabetes might lead to novel treatment strategies. In conclusion, alleviation of oxidative stress should be taken into consideration as one of the possible strategies for the treatment of patients with diabetes, especially when they are exposed to intermittent hypoxia due to the presence of sleep apnea.

#### **REFERENCES**

- [1] Everhart JE, Pettitt DJ, Knowler WC, *et al.* Medial arterial calcification and its association with mortality and complications of diabetes. *Diabetologia* 1988; 31: 16-23.
- [2] Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 2002; 287: 2570-81.
- [3] Klein R, Klein BE, Moss SE, Cruickshanks KJ, Brazy PC. The 10-year incidence of renal insufficiency in people with type 1 diabetes. *Diabetes Care* 1999; 22: 743-51.
- [4] Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1996; 27: 567-74.
- [5] Tooke JE. Microvascular function in human diabetes. A physiological perspective. *Diabetes* 1995; 44: 721-6.
- [6] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27: 1047-53.
- [7] Feener EP, King GL. Vascular dysfunction in diabetes mellitus. *Lancet* 1997; 350: S19-13.
- [8] Cohen RA, Zitny KM, Haudenschild CC, Cunningham LD. Loss of selective endothelial cell vasoactive functions caused by hypercholesterolemia in pig coronary arteries. *Circ Res* 1988; 63: 903-10.
- [9] Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol* 2002; 40: 505-10.
- [10] Celemajer DS, Sorensen KE, Georgakopoulos, D, *et al.* Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 1993; 88: 2149-55.
- [11] Klein BE, Klein R, Lee KE. Components of the metabolic syndrome and risk of cardiovascular disease and diabetes in Beaver Dam. *Diabetes Care* 2002; 25: 1790-94.
- [12] Levinson PD, McGarvey ST, Carlisle CC, *et al.* Adiposity and cardiovascular risk factors in men with obstructive sleep apnea. *Chest* 1993; 103: 1336-42.
- [13] Shahar E, Whitney CW, Redline S, *et al.* Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med* 2001; 163: 19-25.
- [14] Hayashi T, Yamashita C, Matsumoto C, *et al.* Role of gp91phox-containing NADPH Oxidase in Left Ventricular Remodeling Induced by Intermittent Hypoxic Stress. *Am J Physiol Heart Circ Physiol* 2008; 294: H2197-203.
- [15] Tappia PS, Dent MR, Dhalla NS. Oxidative stress and redox regulation of phospholipase D in myocardial disease. *Free Radic Biol Med* 2006; 41: 349-61.
- [16] Lee AY, Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J* 1999; 13: 23-30.
- [17] Ramana KV, Friedrich B, Tammali R, West MB, Bhatnagar A, Srivastava SK. Requirement of aldose reductase for the hyperglycemic activation of protein kinase C and formation of diacylglycerol in vascular smooth muscle cells. *Diabetes* 2005; 54: 818-29.
- [18] Hamada Y, Araki N, Horiuchi S, Hotta N. Role of polyol pathway in nonenzymatic glycation. *Nephrol Dial Transplant* 1996; 11: 95-8.



- [19] Hi Bahl Lee, Hunjoo Ha, and George L. King Reactive Oxygen Species and Diabetic Nephropathy. *J Am Soc Nephrol* 2003; 14: S209-10.
- [20] Kislinger T, Fu C, Huber B, *et al.* N(epsilon)-(carboxymethyl) lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. *J Biol Chem* 1999; 274: 31740-9.
- [21] Mohazzab KM, Kaminski PM, Wolin MS. NADH oxidoreductase is a major source of superoxide anion in bovine coronary artery endothelium. *Am J Physiol* 1994; 266: H2568-72.
- [22] Rajagopalan S, Kurz S, Münzel T, *et al.* Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 1996; 97: 1916-23.
- [23] Inoguchi T, Li P, Umeda F, *et al.* High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* 2000; 49: 1939-45.
- [24] Nishikawa T, Edelstein D, Du XL, *et al.* Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; 404: 787-90.
- [25] Yamagishi SI, Nakamura K, Matsui T, Ueda SI, Imaizumi T. Role of postprandial hyperglycaemia in cardiovascular disease in diabetes. *Int J Clin Pract* 2007; 61: 83-7.
- [26] Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 2006; 113: 1708-14.
- [27] Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 2000; 87: 840-4.
- [28] Rao GN, Berk BC. Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression. *Circ Res* 1992; 70: 593-9.
- [29] Rao GN, Lassègue B, Griendling KK, Alexander RW. Hydrogen peroxide stimulates transcription of c-jun in vascular smooth muscle cells: role of arachidonic acid. *Oncogene* 1993; 8: 2759-64.
- [30] Rao GN, Lassègue B, Griendling KK, Alexander RW, Berk BC. Hydrogen peroxide-induced c-fos expression is mediated by arachidonic acid release: role of protein kinase C. *Nucleic Acids Res* 1993; 21: 1259-63.
- [31] Boudina S, Abel ED. Diabetic cardiomyopathy revisited. *Circulation* 2007; 115: 3213-23.
- [32] Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *JAMA* 1979; 241: 2035-8.
- [33] González-Vílchez F, Ayuela J, Ares M, Pi J, Castillo L, Martín-Durán R. Oxidative stress and fibrosis in incipient myocardial dysfunction in type 2 diabetic patients. *Int J Cardiol* 2005; 101: 53-8.
- [34] Bugger H, Abel ED. Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome. *Clin Sci (Lond)* 2008; 114: 195-210.
- [35] Vest JA, Wehrens XH, Reiken SR, *et al.* Defective cardiac ryanodine receptor regulation during atrial fibrillation. *Circulation* 2005; 111: 2025-32.
- [36] Brownlee M. Advanced protein glycosylation in diabetes and aging. *Annu Rev Med* 1995; 46: 223-34.
- [37] Turko IV, Li L, Aulak KS, Stuehr DJ, Chang JY, Murad F. Protein tyrosine nitration in the mitochondria from diabetic mouse heart. Implications to dysfunctional mitochondria in diabetes. *J Biol Chem* 2003; 278: 33972-7.
- [38] Nishio Y, Kanazawa A, Nagai Y, Inagaki H, Kashiwagi A. Regulation and role of the mitochondrial transcription factor in the diabetic rat heart. *Ann N Y Acad Sci* 2004; 1011: 78-85.
- [39] Wold LE, Ceylan-Isik AF, Ren J. Oxidative stress and stress signaling: menace of diabetic cardiomyopathy. *Acta Pharmacol Sin* 2005; 26: 908-17.
- [40] Barouch LA, Berkowitz DE, Harrison RW, O'Donnell CP, Hare JM. Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. *Circulation* 2003; 108: 754-9.
- [41] Coughlin SR, Mawdsley L, Mugarza JA, Calverley PM, Wilding JP. Obstructive sleep apnoea is independently associated with an increased prevalence of metabolic syndrome. *Eur Heart J* 2004; 25: 735-41.
- [42] Inamoto S, Hayashi T, Tazawa N, *et al.* Angiotensin-II receptor blocker exerts cardioprotection in diabetic rats exposed to hypoxia. *Circ J* 2006; 70: 787-92.
- [43] Vgontzas AN, Papanicolaou DA, Bixler EO, *et al.* Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J Clin Endocrinol Metab* 2000; 85: 1151-8.
- [44] Polotsky VY, Li J, Punjabi NM, *et al.* Intermittent hypoxia increases insulin resistance in genetically obese mice. *J Physiol* 2003; 552: 253-64.
- [45] Strohl KP, Novak RD, Singer W, *et al.* Insulin levels, blood pressure and sleep apnea. *Sleep* 1994; 17: 614-8.
- [46] Ip MS, Lam B, Ng MM, Lam WK, Tsang KW, Lam KS. Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med* 2002; 165: 670-6.
- [47] Punjabi NM, Sorkin JD, Katzel LI, Goldberg AP, Schwartz AR, Smith PL. Sleep-disordered breathing and insulin resistance in middle-aged and overweight men. *Am J Respir Crit Care Med* 2002; 165: 677-82.
- [48] Yokoe T, Minoguchi K, Matsuo H, *et al.* Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. *Circulation* 2003; 107: 1129-34.
- [49] Minoguchi K, Tazaki T, Yokoe T, *et al.* Elevated production of tumor necrosis factor-alpha by monocytes in patients with obstructive sleep apnea syndrome. *Chest* 2004; 126: 1473-9.
- [50] Engelman JA, Berg AH, Lewis RY, Lisanti MP, Scherer PE. Tumor necrosis factor alpha-mediated insulin resistance, but not differentiation, is abrogated by MEK1/2 inhibitors in 3T3-L1 adipocytes. *Mol Endocrinol* 2000; 14: 1557-69.
- [51] Qin B, Qiu W, Avramoglu RK, Adeli K. Tumor necrosis factor-alpha induces intestinal insulin resistance and stimulates the overproduction of intestinal apolipoprotein B48-containing lipoproteins. *Diabetes* 2007; 56: 450-61.
- [52] Lattimore JD, Celermajer DS, Wilcox I. Obstructive sleep apnea and cardiovascular disease. *J Am Coll Cardiol* 2003; 41: 1429-37.
- [53] Yamashita C, Hayashi T, Mori T, *et al.* Angiotensin II receptor blocker reduces oxidative stress and attenuates hypoxia-induced left ventricular remodeling in apolipoprotein E-knockout mice. *Hypertens Res* 2007; 30: 1219-30.
- [54] Nakano D, Hayashi T, Tazawa N, *et al.* Chronic hypoxia accelerates the progression of atherosclerosis in apolipoprotein E-knockout mice. *Hypertens Res* 2005; 28: 837-45.
- [55] Xu W, Chi L, Row BW, *et al.* Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. *Neuroscience* 2004; 126: 313-23.
- [56] Row BW, Liu R, Xu W, Kheirandish L, Gozal D. Intermittent hypoxia is associated with oxidative stress and spatial learning deficits in the rat. *Am J Respir Crit Care Med* 2003; 167: 1548-53.
- [57] Chen L, Einbinder E, Zhang Q, *et al.* Oxidative stress and left ventricular function with chronic intermittent hypoxia in rats. *Am J Respir Crit Care Med* 2005; 172: 915-20.
- [58] Zhan G, Serrano F, Fenik P, *et al.* NADPH oxidase mediates hypersomnolence and brain oxidative injury in a murine model of sleep apnea. *Am J Respir Crit Care Med* 2005; 172: 921-9.
- [59] Baldwin AS Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* 1996; 14: 649-83.
- [60] Li Y, Ha T, Gao X, *et al.* NF-kappa B activation is required for the development of cardiac hypertrophy *in vivo*. *Am J Physiol Heart Circ Physiol* 2004; 287: H1712-20.
- [61] Opara EC, Abdel-Rahman E, Soliman S, *et al.* depletion of total antioxidant capacity in type 2 diabetes. *Metabolism* 1999; 48: 1414-7.
- [62] Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb Vasc Biol* 1994; 14: 1521-8.
- [63] 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.
- [64] Karasu C, Ozansoy G, Bozkurt O, Erdogan D, Omeroglu S. Antioxidant and TG-lowering effects of vitamin E associated with the prevention of abnormalities in the reactivity and morphology of aorta from STZ-diabetic rats. *Metabolism* 1997; 46: 872-9.

- [65] Baydas G, Cantan H, Turkoglu A. Comparative analysis of the protective effects of melatonin and Vit E on STZ-induced diabetes mellitus. *J Pineal Res* 2002; 32: 225-30.
- [66] Ghatak A, Brar MJ, Agarwal A, Goel N. Oxygen free radical system in heart failure and therapeutic role of oral vitamins. *Int J Cardiol* 1996; 57: 119-27.
- [67] Devaraj S, Jialal I. The effect of  $\alpha$ -tocopherol supplementation on monocyte function. *J Clin Invest* 1996; 98: 756-63.
- [68] Motoyama T, Kawano H, Kugiyama, *et al.* Vitamin E administration improves impairment of endothelium-dependent vasodilatation in patients with coronary spastic angina. *J Am Coll Cardiol* 1998; 32: 1672-9.
- [69] Devaraj S, Jialal I.  $\alpha$ -tocopherol decreases interleukin-1 $\beta$  release by inhibition of 5-lipoxygenase. *Arterioscler Thromb Vasc Biol* 1999; 19: 1125-33.
- [70] Willett W, Stampfer M. What vitamins should I be taking doctor? *N Eng J Med* 2001; 25: 1819-24.
- [71] Vivekananthan D, Penn M, Sepp S, Hsu A, Topol E. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta analysis of randomized trials. *Lancet* 2003; 361: 2017-23.
- [72] Lonn E, Bosch J, Yusuf S, *et al.* HOPE and HOPE-TOO trial investigators. Effect of long term vitamin E supplementation on cardiovascular events and cancer, a randomized controlled trial. *JAMA* 2005; 293: 1338-46.
- [73] Milman U, Blum S, Shapira C, *et al.* Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2-2 genotype: a prospective double-blinded clinical trial. *Arterioscler Thromb Vasc Biol* 2008; 28: 341-7.
- [74] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; 352: 837-53.
- [75] Gæde P, Vedel P, Larsen N, Jensen GVH, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Eng J Med* 2003; 348: 383-93.
- [76] Keech A, Simes RJ, Barter P, *et al.* Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomized controlled trial. *Lancet* 2005; 366: 1849-61.
- [77] Gæde P, Lund-Andersen H, Parving H-H, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Eng J Med* 2008; 358: 580-91.
- [78] Cooper SA, Whaley-Connell A, Habibi J, *et al.* Renin-angiotensin-aldosterone and oxidative stress in cardiovascular insulin resistance. *Am J Physiol Heart Circ Physiol* 2007; 293: H2009-23.
- [79] Dandona P, Dhindsa S, Ghanim H, Chaudhuri A. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. *J Hum Hypertens* 2007; 21: 20-7.
- [80] Dzau VJ, Antman EM, Black HR, *et al.* The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes: part I: pathophysiology and clinical evidence (risk factors through stable coronary artery disease). *Circulation* 2006; 114: 2850-70.
- [81] Hayashi T, Sohmiya K, Ukimura A, *et al.* Angiotensin II receptor blockade prevents microangiopathy and preserves diastolic function in the diabetic rat heart. *Heart* 2003; 89: 1236-42.
- [82] Dagenais NJ, Jamali F. Protective effects of angiotensin II interruption: evidence for anti-inflammatory actions. *Pharmacotherapy* 2005; 25: 1213-29.
- [83] Kjeldsen SE, Julius S, Mancina G, *et al.* Effects of valsartan compared to amlodipine on preventing type 2 diabetes in high-risk hypertensive patients: the VALUE trial. *J Hypertens* 2006; 24: 1405-12.
- [84] McCall KL, Craddock D, Edwards K. Effect of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers on the rate of new-onset diabetes mellitus: a review and pooled analysis. *Pharmacotherapy* 2006; 26: 1297-306.
- [85] Andraws R, Brown DL. Effect of inhibition of the renin-angiotensin system on development of type 2 diabetes mellitus (meta-analysis of randomized trials). *Am J Cardiol* 2007; 99:1006-12.
- [86] Healey JS, Baranchuk A, Crystal E, *et al.* prevention of atrial fibrillation with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: a meta-analysis. *J Am Coll Cardiol* 2005; 45: 1832-9.
- [87] Fogari R, Mugellini A, Destro M, *et al.* Losartan and prevention of atrial fibrillation recurrence in hypertensive patients. *J Cardiovasc Pharmacol* 2006; 47: 46-50.
- [88] Schrader J, Kulschewski A, Dendorfer A. Inhibition of the renin-angiotensin system and the prevention of stroke. *Am J Cardiovasc Drugs* 2007; 7: 25-37.
- [89] Schmieder RE, Hilgers KF, Schlaich MP, Schmidt BM. Renin-angiotensin system and cardiovascular risk. *Lancet* 2007; 369: 1208-19.
- [90] Wassmann S, Nickenig G. Interrelationship of free oxygen radicals and endothelial dysfunction: modulation by statins. *Endothelium* 2003; 10: 23-33.
- [91] Nickenig G. Should angiotensin II receptor blockers and statins be combined? *Circulation* 2004; 110: 1013-20.
- [92] Brown JH, Del Re DP, Sussman MA. The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. *Circ Res* 2006; 98: 730-42.
- [93] Ramasubbu K, Estep J, White DL, Deswal A, Mann DL. Experimental and clinical basis for the use of statins in patients with ischemic and nonischemic cardiomyopathy. *J Am Coll Cardiol* 2008; 51: 415-26.
- [94] Horiuchi M, Cui TX, Li Z, Li JM, Nakagami H, Iwai M. Fluvastatin enhances the inhibitory effects of a selective angiotensin II type 1 receptor blocker, valsartan, on vascular neointimal formation. *Circulation* 2003; 107: 106-12.
- [95] Nickenig G, Bäumer AT, Temur Y, Kebben D, Jockenhövel F, Böhm M. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation* 1999; 100: 2131-4.
- [96] The Heart Outcomes Prevention Evaluation Study Investigators. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Eng J Med* 2000; 342: 145-53.
- [97] Chiasson JL, Josse RG, Gomis R, *et al.* Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomized trial. *Lancet* 2002; 359: 2072-7.
- [98] Chiasson JL, Josse RG, Gomis R, *et al.* Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* 2003; 290: 486-94.
- [99] Rösen P, Osmers A. Oxidative stress in young Zucker rats with impaired glucose tolerance is diminished by acarbose. *Horm Metab Res* 2006; 38: 575-80.
- [100] Hayashi T, Mori T, Sohmiya K, *et al.* Efficacy of edaravone, a free radical scavenger, on left ventricular function and structure in diabetes mellitus. *J Cardiovasc Pharmacol* 2003; 41: 923-9.
- [101] Brovkovych V, Kalinowski L, Muller-Peddinghaus R, Malinski T. Synergistic antihypertensive effects of nifedipine on endothelium: concurrent release of NO and scavenging of superoxide. *Hypertension* 2001; 37: 34-9.
- [102] Fukuo K, Yang J, Yasuda O, *et al.* Nifedipine indirectly upregulates superoxide dismutase expression in endothelial cells *via* vascular smooth muscle cell-dependent pathways. *Circulation* 2002; 106: 356-61.