CHAPTER ONE: INTRODUCTION

Diabetes mellitus has been recognized as an independent major cardiovascular risk factor since the publication of the first large-scale epidemiologic investigations in the 1970s [1]. There are over 100 million people worldwide with diabetes (5% to 8% of overall population), and this number is likely to increase significantly in the near future. According to data from clinical studies, macro- and microvascular disease are currently the principal causes of morbidity and mortality in patients with type I and type II diabetes mellitus. Loss of the modulatory role of the endothelium may be a critical and initiating factor in the development of diabetic vascular disease. Impaired endothelium-dependent vasodilatation has been demonstrated in various vascular beds of different animal models of diabetes [2-6] and in humans with type 1 [7] and type 2 diabetes [8].

1.1   Structure and functions of endothelium

The endothelium is a continuous single layer of cells located between the wall of blood vessels and the blood stream. The luminal surface of the endothelial cell is smooth, non-thrombogenic and the albuminal surface is adhesive to inner wall as well as platelets. In different regions, the structural features vary with specificity [9]. For instance, cell junctions are mainly tight in the brain, while intracellular cleft is wide open in the liver to facilitate protein biotransport.

The functions of endothelium are numerous and vary according to size and distribution of blood vessels. The endothelium controls many important functions, including maintenance of blood circulation and fluidity as well as regulation of vascular tone, coagulation, and inflammatory responses [10, 11]. It senses mechanical stimuli, such as pressure and shear stress, and hormonal stimuli, such as vasoactive substances. In response, it releases various chemical mediators that regulate vasomotor function, trigger inflammatory processes, and affect hemostasis (Table 1). Many endothelium-derived substances are functionally active, mutually antagonistic and some are apparently redundant [12]. Generally, the endothelium is rather an active and dynamic organ than an inert single-cell lining just separating blood from the vessel wall.

Table 1   The products of endothelium.

Vasoconstrictors:	Endothelins (ET) 1, 2 and 3, angiotensin II (Ang II), thromboxane A_2 (TXA_2) , reactive oxygen species (ROS), superoxide radical (O_2), endothelium-derived constriction factor (EDCF).
Vasodilators:	NO, prostacyclin (PGI_2), PGE_2, endothelium derived hyperpolarization factor (EDHF), C-type natriuretic peptide.
Growth factors:	Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β).
Inflammatory modulators:	NO, interleukins (ILs), intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin, and nuclear factor (NF)-κB.
Other proteins:	B-natriuretic peptide, adrenomedulin, endoadenosine diphosphatase, thrombomodulin, tissue factor, α-urokinase, tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI), protein S.

Modifed from Ref. [13].

The vasodilatory capacity (endothelium-dependent vasodilation, EDV) of the endothelium is one measure of its function. Several substances, e.g. hormones, neurotransmittors, platelet-derived substances, shear stress, etc., can stimulate EDV. The main mediator of EDV is the free radical Nitric Oxide (NO) [10], which is formed from the amino acid L-arginine by the calcium/calmodulin-dependent enzyme nitric oxide synthase (NOS) in the site of vascular endothelium [14]. Directly after synthesis, NO diffuses out of the endothelial cells and traverses membranes of circulating blood cells

and vascular smooth muscle cells (VSMC). NO acts via stimulation of guanylate cyclase activity in the target cell, leading to formation of cyclic guanosine monophosphate (cGMP) from guanosine-triphosphate (GTP) which results in muscle relaxation chiefly via decreased intracellular ionized calcium (Ca^{2+}) [15]. Inhibition of NOS causes vasoconstriction indicating the presence of an active, tonic vasodilation is maintained by NO. Vasodilator mechanisms, physiological as well as pharmacological, may therefore be characterized as endothelium-dependent (i.e. NO-mediated), or endothelium-independent (i.e. not mediated by NO). Shear stress and acetylcholine are usually used to induce nitric oxide–mediated, endothelium-dependent vasodilation, whereas sodium nitroprusside (SNP) is used to evaluate endothelium-independent vasodilation (EIDV) by donating NO to VSMC. Physiologically, mixed mechanisms occur.

1.2   Endothelial dysfunction

Endothelial cells maintain basal vascular tone and actively regulate vascular reactivity in physiological and pathological conditions, by responding to mechanical forces and neurohumoral mediators with the release of a variety of relaxing and contricting factors [11]. Injury to the endothelium causes dysfunction. Endothelial dysfunction was initially identified as impaired vasodilation to specific stimuli such as acetylcholine or bradykinin. Failure of the vascular endothelium to elicit NO-mediated vasodilatation may be due to decreased formation, increased degradation, decreased sensitivity to the NO formed, or a mixture of these factors. Irrespective of the mechanism behind, this is referred to as endothelial dysfunction. A broader understanding of the term would include not only reduced vasodilation but also a proinflammatory and prothrombic state associated with dysfunction of the endothelium. The clinical and pathological manifestations of endothelial injury differ according to the type of insult, blood vessel, blood flow and shear stress [16].

1.2.1   Causes and consequences of endothelial dysfunction

Endothelium encounters blood borne insults incessantly. The causes of endothelial injury include lipids, immune complexes, angioplasty, micro-organisms and their elaborated toxins. Hypertension, shear stress, hypoxia, acidosis, smoking, aging, diabetes mellitus,

trauma and surgery do inflict injury to endothelium. Reperfusion injury is also regarded as a common factor that cause endothelial dysfunction [17]. Furthermore, inflammatory diseases exacerbate endothelial dysfunction and the converse is also true.

These vascular risk factors promote the occurrence of endothelial dysfunction, resulting in a proinflammatory and prothrombotic phenotype of the endothelium. Endothelial dysfunction leads to further abnormalities, pushing the cadiovascular system into a vicious cycle of disease, resulting in physiological and structural changes that can lead to cardiovascular disease. Dysfunction of the endothelium has been implicated in the pathophysiology of different forms of cardiovascular disease, including hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure. The frequent outcome of endothelial dysfunction is atherosclerosis which, in turn, results in further disruption of the endothelium.

1.2.2   Mechanism of endothelial dysfunction

The pathophysiology of endothelial dysfunction is complex and involves mutiple mechanisms. However, some of these seem to be common to most conditions.

1.2.2.1   *Bioavailability of NO*

NO is one of the most important vasodilating substances released by the endothelium. NO acts as a vasodilator, inhibits growth and inflammation, and has anti-aggregant effects on platelets. It is a chemically unstable radical formed by enzymatic conversion of L-arginine in the presence of molecular oxygen by NOS. NOSs include two constitutively expressed isoforms, namely, endothelial NOS (eNOS) and neuronal NOS (nNOS), and an inducible NOS (iNOS) isoform [14]. eNOS–derived NO is the predominant regulator of vascular tone and the importance of nNOS- and iNOS-derived NO seems to be negligible [11, 15]. The availability of NO *in vivo* is regulated by a combination of synthesis and breakdown of NO. NO is continuously released from endothelial cells in response to stimulation of eNOS by both receptor-independent stimuli, such as shear stress, as well as receptor-dependent agonists, such as acetylcholine, thrombin, serotonin and bradykinin. A number of models of endothelial

dysfunction in experimental animals together with clinical data provide evidence that NO bioavailability is reduced by increased production of ROS in the vessel wall [18, 19]. A depletion of NO production in endothelium-impaired function disorders may result from a decreased amount of eNOS protein, a deficiency of substrate or cofactors for eNOS, and changes in cellular signaling that finally lead to inappropriate eNOS activation. In contrast to the decreased eNOS expression in atherosclerosis [20], the expression of eNOS is up-regulated in hypertension [21], diabetes [5] and aging [22], indicating that NO degradation process is mostly responsible for a loss of NO bioavailability in the vessel wall.

1.2.2.2 *Oxidative excess*

There is considerable evidence that increased oxidative stress is associated with endothelial dysfunction. Elevated concentrations of ROS such as superoxide anions and lipid peroxides may account for the impaired endothelium-dependent vasodilatation in response to agonists or flow by scavenging endothelium-released NO [23]. Oxidative excess in hypertensive patients leads to diminished NO bioavailability [24] and correlates with the degree of impairment of endothelium-dependent vasodilatation and with cardiovascular events [25]. In animal models of hypertension, oxidative excess leads to endothelial dysfunction as evidenced by improvement of the impaired endothelium-dependent relaxation after use of anti-oxidants [26]. In patients with chronic renal failure, markers of oxidative excess also correlated with endothelial dysfunction [27], and administration of vitamin C improved endothelial dysfunction of resistance arteries [28]. Findings in animal models of chronic renal failure suggest that enhanced generation of ROS leads to decreased NO bioavailability and endothelial dysfunction, which may be improved by antioxidant pretreatment [29]. In experimental diabetic models, increased oxidative excess also leads to endothelial dysfunction [30, 31]. Moreover, oxidative excess is linked to a proinflammatory state of the vessel wall. For example, ROS upregulate adhesion (VCAM-1 and ICAM-1) and chemotactic molecules (macrophage chemoattractant protein-1 (MCP-1) [32], both of which are involved in early steps in atherogenesis. In addition, ROS seems to be involved in the mediation of endothelial injury leading to programmed cell death or apoptosis [33]. The main source for oxidative

excess in the vasculature is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [19, 34]. Other sources include xanthine oxidase [35], the mitochondria [36], and uncoupled NOS [37].

1.2.2.3   Ang II

Ang II has been implicated in the pathophysiology of vascular diseases. Production of Ang II begins with conversion of angiotensinogen to angiotensin I by renin, a selective enzyme, in the liver. Angiotensin I is transformed to Ang II, a peptide hormone, primarily by the catalyzing action of the ACE. Ang II infusion induces endothelial dysfunction in rats [38, 39], decreases NO bioavailability by promoting oxidative stress, increases ROS by stimulating NAD(P)H oxidase [34], and promotes vascular inflammation [40]. In hypertensive patients, interruption of the renin-angiotensin system with ACEI or ang II receptor blockers restores endothelial function [40, 41].

1.2.3   Prognostic implications of endothelial dysfunction

Endothelial dysfunction, as assessed in terms of vasomotor dysfunction, can occur well before the structural manifestation of atherosclerosis and the degree of impairment of endothelium-dependent vasomotion has profound and independent prognostic implications. Thus, endothelial dysfunction can serve as an independent predictor of future cardiovascular events and may be of prognostic value. Non-invasive endothelial function testing predicted cardiovascular events in patients with coronary artery disease [25], peripheral artery disease [42], and hypertension [43] and in patients who underwent vascular surgery [42]. Moreover, there is increasing evidence that markers of endothelial dysfunction and vascular inflammation are linked with cardiovascular events. Soluble VCAM-1 [44] and interleukin-18 [44] predicted cardiovascular death in patients with coronary artery disease independently from other risk factors. Plasminogen activator inhibitor (PAI-1) activity has been shown to predict coronary artery disease in hemodialysis patients [45]. Soluble VCAM and C-reactive protein (CRP) predicted risk of death in patients with type 2 diabetes [46]. Even in healthy men, soluble ICAM was a predictor of peripheral artery disease [47] and myocardial infarction [48].

1.2.4   Therapeutic target of endothelial dysfunction

It is possible to improve endothelial dysfunction by several means and methods to achieve benefits in many clinical conditions. Clear understanding of the role played by various molecules synthesized by endothelium is essential to rationalize the drug therapy. Both non-pharmacological and pharmacological treatments are useful to reduce the incidence of endothelial dysfunction related cardiovascular disorders. The non-pharmacological approach includes regular exercise, low fat diet rich in monounsaturated fatty acids, cessation of smoking and food rich in vegetables and fruits. The pharmacological treatment includes a wide variety of drugs (Table 2) modulating endothelial function that

Table 2   List of drugs known to modulate endothelial cell function

1. NO, NO donors, NO antagonists

2. PGI_2 analogs and PGI_2 synthesis inhibitors

3. Ang II synthesis inhibitors and receptor blockers

4. Adenosine function modulators

5. Thrombomodulin antagonists

6. Bradykinin and bradykinin antagonists

7. Endothelins and their antagonists

8. tPA substitutes and inhibitors

9. Lipid lowering agents

10. K^+ channel openers and blockers

Others: Estrogen, mibefrandil, folic acid, nebivolol, thapsigargin, lebeluzole and antioxidants

Derived from Ref. [13]

are of great therapeutic interest. Among commonly employed anti-hypertensive drugs, ACEI and calcium channel blockers appear to have more effects on endothelium to improve its function. ACEIs reduce angiotensin II levels which in turn, decrease

endothelin activation, interrupt degradation of NO by superoxide anions and inhibit bradykinin breakdown, which augments NO release. These actions of ACEI undoubtedly offer benefit in many clinical settings by improving endothelial function. However, much remains to be known about long term benefit. The dihydropyridine calcium channel blocker nifedipine also enhances the availability of endothelial NO, attenuates vascular effects of endothelins [49] and restores endothelial permeability, resulting in the anti-atherosclerotic effect. Recently, there is increasing evidences that 3-hydroxy-methyl-3-glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have beneficial effects on endothelial dysfunction, which may be the result in part of lipid lowering but also of their pleiotropic anti-oxidant and anti-inflammatory effects [50-52].

1.3   Statin therapy beyond lipid-lowering effects in cardiovascular diseases

The HMG-CoA reductase inhibitors have been employed as therapeutic agents in the treatment of cardiovascular diseases associated with hypercholesterolaemia. These compounds, collectively referred to as statins, exert their biological effects by blocking the conversion of HMG-CoA to mevalonate in the hepatic cholesterol biosynthesis pathway [53]. The subsequent lipid-lowering effect is correlated with a decreased risk of coronary and cerebrovascular events, and results in increased survival rates in patients with coronary artery disease [50]. Though the products of mevalonate metabolism are critical for many cellular processes in eukaryotic cells, recent studies have shown that statins may also exert pleiotropic effects beyond their lipid-lowering properties (Table 3).

1.3.1   Improvement of endothelial dysfunction

Evidence suggests that elevations in total cholesterol and LDL-C adversely affect endothelial function, including impaired endothelium-dependent vasodilation [54]. Several studies have examined the impact of statin therapy on endothelial function in hypercholesterolemic patients with and without established coronary heart disease [50-52]. In each study, significant improvements in endothelial function were demonstrated by measuring acetylcholine-induced coronary vasodilation, methacholine brachial artery

Table 3   Effects of statins on the vascular wall

Endothelial function	↑eNOS expression and activity
	↑Angiogenesis
	↑Circulating endothelial progenitor cells
	↓ET-1 synthesis and expression
	↓ROS
Oxidative stress	↓ROS
	↓Oxidised LDL (ox-LDL)
	↓NAD(P)H
Inflammation	↓High-sensitivity CRP level
	↓Angiotensin type-1 receptor expression
	↓Adhesionmolecule expression
	↓Leukocyte-endothelial cell adhension
	↓iNOS expression
	↓Proinflammatory cytokines (TNF-α, IL-1, IL-6) expression
	↓MCP-1 secretion
	↓IL-8 secretion
Plaque stabilization	↓Macrophage growth
	↓Matrix metalloproteinase (MMP) expression and secretion
	↓Tissue factor expression and activity
	↓Major histocompatibility class II antigen expression
	↓Scavenger receptor expression
Thrombosis	↑tPA expression
	↓PAI-1 expression
	↓Platelet reactivity
	↓TXA$_2$ biosynthesis
Apoptosis	↑Caspase-3
	↑Caspase-9
	↓Bcl-2
	↓prenylation of p21-RhoB

Arrows indicate enhanced (↑) or diminished (↓) activation /expression of the pathway and/or mediatore after stain administration. Modified from Schonbeck U and Libby P [55].

plethysmography, or *in vitro* vascular ring vasodilation of excised vessels. Statin therapy has been shown to limit acetylcholine-induced vasoconstriction and improve peripheral NO-mediated vascular relaxation [50, 51]. The mechanism is caused, in part, by statins' ability to increase endothelial NO production by stimulating and upregulating eNOS [56, 57]. Furthermore, statins have been shown to restore eNOS activity in the presence of hypoxia [57] and oxidised LDL (ox-LDL) [57], conditions that lead to endothelial dysfunction. Statins also increase the expression of t-PA [58] and inhibit the expression of ET-1, a potent vasoconstrictor and mitogen [59]. Statins, therefore, exert many favorable effects on the endothelium and attenuate endothelial dysfunction in the presence of atherosclerotic risk factors.

1.3.2 Anti-oxidant effects of statins

Another potential mechanism accounting for statins' pleiotropic effects is through their antioxidant properties. Free radicals and reactive oxygen are involved in the degradation of NO and impair endothelial function [60]. Moreover, the susceptibility of LDL-C to oxidation plays a key role in the pathogenesis of atherosclerosis [61]. Lipid peroxidation products interrupt G protein-dependent NO release and block the physiological action of NO. ox-LDL contributes to cholesterol accumulation in the macrophages, foam cell formation, thrombosis and inflammation.

Research has shown that statins have antioxidant properties that prevent LDL-C modification [62-65]. For example, Rikitake *et al*. recently reported that fluvastatin given to cholesterol-fed rabbits at a dose insufficient to reduce plasma cholesterol levels (2 mg/kg per day) reduced the susceptibility of LDL to *ex vivo* copper-induced oxidation, decreased vascular superoxide generation and atheromatous plaque formation [65]. Metabolites of statins have also been shown to possess anti-oxidant activity. Indeed, the hydroxy metabolites of atorvastatin have more potent anti-oxidative potential than the parent compound [66].

There appear to be several mechanisms whereby statins may reduce oxidative stress. For example, statins downregulate macrophage scavenger receptors, thereby reducing oxidized LDL-C uptake and subsequent foam cell formation [67-69]. Statins attenuate

Ang II–induced free radical production in vascular smooth muscle cells by inhibiting Rac1-mediated NAD(P)H oxidase activity and downregulating angiotensin AT_1-receptor expression [70]. NAD(P)H oxidases have been suggested as one of the most important sources of superoxide in human coronary arteries and their activities are increased in patients with coronary heart disease [71]. More recently, Wassmann *et al* reported, that atorvastatin reduced vascular mRNA expression of essential NAD(P)H oxidase subunits p22phox and nox1 by a mechanism that might involve the translocation of Rac1 from the cytosol to the cell membrane [70]. Because NO is scavenged by ROS, these findings indicate that the antioxidant properties of statins may also contribute to their ability to improve endothelial function. Furthermore, statins are demonstrated to increase levels of natural antioxidants (eg. Vitamin C and E, ubiquinone, and glutathione) [63, 72, 73] and withdrawal of statin treatment in mice has been shown to impair endothelium-dependent relaxation by increasing vascular O_2^- generation via a pathway involving the Rac-dependent activation of the gp91phox-containing vascular NAD(P)H oxidase [74].

1.3.3   Anti-inflammatory properties

Although restoration of endothelial function is one of the earliest responses to statin treatment—often occurring before any significant reduction in plasma LDL-C levels [50, 51, 75]—these agents have been reported to positively influence a number of other atherogenic processes, including the inflammatory response, plaque stability, thrombosis and apoptosis.

Research has shown that statins have anti-inflammatory properties, including inhibition of leukocyte-endothelium interactions and the reduction of inflammatory cell numbers within atherosclerotic plaques [76]. Although the mechanisms involved are largely unknown, a study conducted in mice suggests the inhibition of nonsterol mevalonate-derived compounds may play a key role [77]. Diomede *et al*. found that short-term lovastatin treatment did not reduce plasma cholesterol in mice, but did produce a significant reduction in HMG-CoA reductase activity and the nonsterol intermediates of cholesterol synthesis [77]. Lovastatin treatment was also associated with a reduction in leukocyte recruitment due to an inhibition of IL-6 and MCP-1. In comparison, administration of the selective inhibitor of sterol derivatives, squalestatin did not lead to

anti-inflammatory activity. In a separate study, the early anti-inflammatory activity of short-term statin therapy has been attributed to enhanced NO release from the vasculature during severe hypercholesterolemia [78]. A recent study demonstrated that simvastatin effectively reduces serum levels of CRP, TNF-α and IL-6 in subjects with hypercholesterolemia [79]. Futhermore, statins can reduce the expression of adhesion molecules such as ICAM-1, which are involved in the recruitment of circulating monocytes [80]. In addition, Weitz-Schmidt *et al.* have shown that statins can inhibit the interaction between β_2 integrin leukocyte function antigen-1 (LFA-1) and ICAM-1 [81]. LFA-1 is expressed solely on the surface of leukocytes where it interacts with ICAM-1, -2 and -3 to promote the adhesion events involved in both normal and pathologic functions of the immune systems. Statins could have anti-inflammatory effects by disrupting these interactions. Reduced expression of the adhesion molecule P-selectin may also account for the inhibition of leukocyte-endothelial interactions by statins [82].

1.3.4 Stablization of plaques

Statins may influence plaque stability by preventing macrophage activation, the process that has been implicated in the pathophysiology of most acute coronary syndromes [83]. Preventing activation is crucial to plaque stabilization, as it reduces the uptake and endogenous synthesis of cholesterol and the production of metalloproteinases by macrophages. It is known that metalloproteinases are the enzymes responsible for weakening the plaque's fibrous cap thereby increasing the risk of rupture [84]. In addition, lipid lowering with statins may contribute to stability by reducing plaque size or by modifying the physiochemical properties of the lipid core [85]. More recent data suggest the plaque stabilizing properties of pravastatin may be mediated through a combined reduction in lipids, lipid oxidation, inflammation, matrix metalloproteinase-2 (MMP-2) and cell death, and increase in tissue inhibitor of metalloproteinase-1 (TIMP-1) and collagen content [86].

1.3.5 Anti-thrombotic effects

Increased platelet activity is a known risk factor for the development of acute coronary syndromes [87]. Platelet function is also influenced by statins, although the precise

mechanisms involved are not fully understood [88, 89]. Potential mechanisms include a reduction in the production of TXA_2, increased synthesis of PGI_2 and modifications in the cholesterol content of platelet membranes [76]. TXA_2 participates in platelet aggregation and vascular contraction, whilst PGI_2 is an anti-aggregant and vasodilator. A recent study has shown mevastatin and lovastatin upregulate COX-2 expression and subsequent prostacyclin synthesis in smooth muscle cells [90]. Reductions in the cholesterol content of both platelet and erythrocyte membranes have been reported in patients treated with pravastatin, and these changes may ultimately decrease the thrombogenic potential of these cells [76]. Indeed, animal studies suggest statin therapy inhibits platelet deposition on damaged vessels and reduces platelet thrombus formation [88, 91]. Statins have been shown to reduce the levels of PAI-1 and increase the levels of t-PA within smooth muscle and endothelial cells, thereby promoting fibrinolytic activity within the vessel wall [92]. Furthermore, *in vitro* experiments have demonstrated that statins inhibit tissue factor expression by macrophages with a subsequent reduction in thrombotic events [93].

1.3.6   Pro-apoptotic function

Statins is reported to induce apoptosis in a range of tumor cell lines and in cell types implicated in atherogenesis, originally smooth muscle cell (SMC)s, and endothelium and macrophages [94, 95]. Concentrations of statins as low as 10 nM induce the expression of the proapoptotic enzymes caspase-3 and -9, and furthermore, limit expression of Bcl-2, an inhibitor of apoptosis [95]. In addition, HMG-CoA reductase inhibitors sensitize SMCs to FasL (CD95)-induced apoptosis by yet-undetermined mechanisms [96]. Although the role of statins in apoptosis remains uncertain, mevalonic acid, the product of HMG-CoA reductase, serves as the precursor of isoprenoids required for the activation of the G-protein Ras, a promoter of cell survival. Indeed, statins might promote programmed cell death by interfering with Ras prenylation. Exposure of SMCs to atorvastatin, simvastatin, or lovastatin diminished prenylation of p21-RhoB, a central regulator of apoptosis [94]. Consideration of the relevance of statin-induced cell death should take into account multiple roles for this process in atherogenesis: it probably promotes formation of the lipid core by the death of foam cells and also diminishes SMC content in the fibrous cap [97]. Of note, induction of apoptosis by statins appears

restricted to the lipophilic members, such as simvastatin, lovastatin, fluvastatin, and atorvastatin, because the hydrophilic statin pravastatin lacks this function *in vitro* and reportedly diminished cell death in human carotid plaques [86, 94].

1.4   Aim of the study

Endothelial dysfunction has been shown to play a key role in the pathophysiology of macrovascular and microvascular complications of diabetes. Multiple pathways contribute to endothelial cell dysfunction in diabetes mellitus, including increased oxidative stress and inflammatory burden. HMG-CoA reductase inhibitors, statins, have cholesterol-independent or pleiotropic effects that may affect on endothelial dysfunction, plaque stabilization, apoptosis and inflammatory and thrombogenic responses. The mechanisms by which statin ameliorates endothelial dysfunction in diabetes are not well understood. Using a well-established rat model of type I diabetes, the present study is designed to investigate the lipid-independent effects of atorvastatin on endothelial function *in vivo*, and underlying mechanisms, including signaling transduction pathways involved during the process. The major points of this study are the following:

1. To evaluate the role of atorvastatin on diabetes-induced endothelial dysfunction *in vivo*.
2. To investigate the effect of atorvastatin on diabetes-associated oxidative excess.
3. To assess the effect of atorvastatin on diabetes-associated inflammation.
4. To examine signaling cascade involved in the regulation of atorvastatin on endothelial function.