CHAPTER SEVEN: SUMMARY

Background: Increased oxidative stress and low-grade vascular inflammation are hallmarks of endothelial dysfunction in diabetes mellitus. 3-hydroxy-methyl-3-glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) exert beneficial effects by lipid lowering and pleiotropic effects. In the present study, we evalulated *in vivo* the influence of a treatment with low-dose atorvastatin on endothelial function, oxidative stress, inflammation and underlying critical molecular pathways in a rat model of diabetes mellitus.

Methods: Diabetes mellitus was induced in 8-week-old male Sprague Dawley rats by single administration of streptozotocin (STZ, 70 mg kg^{-1}, i.p.). SD-STZ diabetic rats were treated with atorvastatin (STZ-Ator, 50 mg kg^{-1}, p.o.) or with vehicle for 48 days. Age-matched non-diabetic SD rats were used as controls (SD-Con). At the end of the experimental protocol, serum triglycerides, total cholesterol, LDL- and HDL- cholesterol were determined. Endothelium-dependent vasodilatation was assessed by resistance changes induced by flow-mediated dilatation, after incremental doses of Krebs-Henseleit solution (80, 200 and 600 µl kg^{-1}) in the *in vivo* autoperfused hindlimb. Sodium nitroprusside (40 µg kg^{-1}, i.a.) was used to estimate endothelium-independent vascular responsiveness to exogenous nitric oxide (NO). Quadriceps muscle NAD(P)H oxidase activity was measured by superoxide dismutase (SOD)-inhibitable cytochrome *c* reduction using NADH or NADPH as substrate. Gene expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM-1), tumor necrosis factor (TNF-α), interleukin (IL)-1β, endothelial NO synthase (eNOS) was assessed by TaqMan quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR). Western blot was performed to analyze expression of eNOS and underlying signaling transduction pathways: phosphorylated and total extracellular-regulating kinase (ERK) 1/2 and nuclear factor (NF)-κB p65. The expression of VCAM-1 and ICAM-1 in quadriceps was visualized and quantified by immunohistochemistry and image analysis.

Results: The SD-STZ diabetic group displayed severe hyperglycemia and hyperlipidemia. Diabetes was associated with severe impairment of endothelium-dependent and -independent vasodilatation. There were significant increases in NAD(P)H oxidase activity and expression of eNOS compared to the SD-Con group, displaying enhanced oxidative stress in diabetic skeletal muscle. These results were paralleled by increased expression of the pro-inflammatory markers of TNF-α, IL-1β, ICAM-1 and VCAM-1, enhanced phosphorylation of ERK 1/2 and upregulated expression of NF-κB p65 in SD-STZ diabetic rats. Low-dose therapy by atorvastatin did not alter the lipid profile but led to a substantial improvement of endothelium-dependent vasodilatation, reduced NAD(P)H oxidase activity and expression of eNOS as well as pro-inflammatory markers (TNF-α, IL-1β, ICAM-1, VCAM-1), suggesting an attenuation of endothelial activation by atorvastatin in SD-STZ diabetic rats, independently of lipid lowering. These effects were associated with inhibtion of phosphorylation of ERK 1/2 and decreased expression of NF-κB p65 pathway.

Conclusions: STZ-induced diabetes increased oxidative stress, producing inflammatory responses and endothelial dysfunction in the rat hindlimb. Ator-treatment reduced diabetes-associated oxidative excess and inflammation, which involved the ERK1/2 kinases and NF-κB-pathway, resulting in improved endothelial function, independently of cholesterol-lowering.