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**Dissertations-Kurzfassung**

**Iron Mediated Toxicity in Hyperglycemia: *in vitro* studies on Cultured Endothelial - and Renal Tubular Epithelial Cells to assess the protective properties of L-carnosine**

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During the last years became apparent that diabetes is not just a condition of high blood sugar but underlies a much more complicated multifactorial metabolic imbalance of the body. Excessive oxidative stress has been recognized as a key factor in the pathogenesis for most of the chronic complications of diabetes. Radicals produced in the course of several cellular metabolic pathways, seem to over accumulate already in the early stages of diabetes. In addition Hyperglycemia increases non-enzymatic glycation, characterized by the binding of reactive dicarbonyls to amino groups of proteins. This reaction leads to production of advanced glycation end products. Following a viscous circle, advanced glycation end products formation induces oxidative stress, further increases the production of reactive oxygen species and acts as intermediates in the cross-linking of proteins. This feedback loop of reactions consequently lead to an accelerated formation of reactive oxygen species and advanced glycation end products, compounds which are now directly associated with the pathogenesis of the most chronic complications of diabetes. Lately a number of studies showed that transition metals and particularly iron may have a large impact on the risk to develop diabetes and diabetic complications. The role of iron as contributing factor in the pathogenesis of a variety of chronic diseases has been discussed already for more than 2 decades. Its contribution to chronic disease is believed to be due to increased oxidative stress, lipid peroxidation and a resulting chronic inflammation state. Indeed iron overload has been associated with such as atherosclerosis, neurodegeneration, and even carcinogenesis. In keeping with the protective effect of carnosine in diabetic models and the relevance of increased iron stores in type 2 diabetes mellitus patients we sought to address in this study : 1) if high glucose conditions as occurs in hyperglycemia or glycosuria makes cultured human endothelial cells and proximal tubular epithelial cells more susceptible to iron toxicity, 2) if these conditions influence the expression of the different iron transporters in these cells and 3) if iron toxicity is mitigated in the presence of carnosine.

Results: Human umbilical vein endothelial cells and proximal tubular epithelial cells, cultured under normal glucose (5 mM, normal glucose) or high glucose (30mM), were challenged for 24 h with FeCl<sub>3</sub> in the presence or absence of 20mM L-carnosine. Both cell types showed a dose dependent susceptibility to Iron (III) chloride which was not increased under high glucose HG conditions. HG did not change the expression of divalent metal transporter1, ferroportin, and transferrin receptor protein 1. L-Carnosine showed a strong protective effect regardless if the cells were cultured under normal glucose or high glucose conditions. The protective effect only occurred if carnosine was present during Iron (III) chloride challenge but was not observed if carnosine was only applied as pre-treatment.

Conclusions: In our *in vitro* studies no clear enhancement of the toxic effect of iron could be demonstrated under hyperglycemic conditions. Proximal tubular epithelial cells showed more tolerant to iron toxicity as compared to human umbilical vein endothelial cells. High glucose culture conditions did not change the expression of divalent metal transporter1, ferroportin, and transferrin receptor protein 1. Irrespective of glucose concentrations L-carnosine prevented toxicity in a dose-dependent manner, only if it was present during the Iron (III) chloride challenge. It was clearly demonstrated the ability of carnosine to ameliorate iron mediated toxicity in cultured endothelial and proximal tubular cells, most likely by extracellular chelation of iron.