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**Protein carbonylation in endothelial cells in the course of diabetes:
Effect on redox enzyme expression and function**

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Diabetes mellitus is a metabolic disease characterized by elevated blood glucose levels among others. Hyperglycemia as well as enhanced levels of the glucose metabolite methylglyoxal contribute to the development of diabetic complications partly via increased generation of reactive oxygen and nitrogen species (ROS / RNS). ROS are not only part of signalling pathways themselves but also lead to specific carbonylation of certain amino acid side chains. This might alter expression and activity of the affected enzymes. Thus, mild oxidative stress is likely to be cell protective due to signalling functions that can increase antioxidant defence mechanisms whereas severe oxidative stress might lead to cell damage.
This study analyzed the role of protein carbonylation in endothelial dysfunction in the course of diabetes. It especially focused on the impact of glutathione peroxidase 1 (GPx1) carbony-lation in human macro- (HUVECs) and glomerular microvascular endothelial cells (hgmECs) as oxidative modification of an important antioxidant enzyme.
Both high glucose and methylglyoxal were taken up into endothelial cells where they enhanced intracellular oxidative stress. Protein carbonylation was hardly augmented at all pointing to a generally effective antioxidant defence in endothelial cells. In HUVECs, increased GPx1 activity seemed to compensate for decreased protein levels. Mass spectrometry data proposed that enhanced GPx1 activity might be due to carbonylation that could lead to conformational changes and subsequently an augmented substrate affinity. Expression of other antioxidant enzymes, such as superoxide dismutase, was raised only in hgmECs but not in HUVECs. Endothelial nitric oxide synthase (eNOS) was regulated antagonistically: Whereas high glucose decreased its expression in HUVECs, methylglyoxal transiently increased it in both micro- and macrovascular endothelial cells.
Initial analyses of hyperglycemic effects on transcription factors revealed decreased cytosolic FoxO1/3a and by trend increased nuclear FoxO1/3a levels. This suggests an increased shuttle of the transcription factor into the nucleus or a decreased nuclear export. Nuclear Nrf2, in contrast, was not affected by high glucose. The carbonylation pattern of nuclear proteins was altered following hyperglycemic treatment. This could affect epigenetics and thus play a role in the formation of metabolic memory.
The results from this study contribute to a deeper understanding of redox homeostasis and its role in the development of diabetes and diabetic complications. Mild oxidative stress, as during the onset of diabetes, seems to activate protective cellular pathways whereas excess ROS, as during the progression of diabetes, overwhelm cellular antioxidant defence mechanisms and thus lead to cell damage.