In human red blood cells, the rate of methylglyoxal (MG) formation is ca. 120 µM per day (approx. 0.1% of the triosephosphate flux). The concentration of MG is increased five- to six-fold in blood samples of type 1 and two- to three-fold in type 2 diabetic patients. To minimize the production of MG and therefore the extent of glycation, the body has evolved a number of enzymatic and non-enzymatic defenses. The system that handles the most of the cellular MG is the glyoxalase system, which is present in the cytosol of all human cells and comprises two enzymes: glyoxalase 1 (GLO 1) and glyoxalase 2 (GLO 2), as well as a catalytic amount of glutathione (GSH). It has been shown that the induction of diabetes in wildtype mice decreased expression of GLO 1 and furthermore, preliminary clinical surveys have revealed that the concentration of MG is increased in blood samples from diabetic patients. So far, it has not been studied to which extent detection of MG levels and GLO1 activity in the blood of diabetic patients could reflect the pathobiology observed \textit{in vitro} and in animal models.

In this study, GLO 1 activity and MG levels were measured in red blood cells and plasma in a well-defined group of patients affected by type 2 diabetes to study whether the observed decrease in GLO 1 activity in animal models of diabetes, and the associated increase in MG, is also observed in patients with type 2 diabetes, and to determine the relationship between methylglyoxal (MG), glyoxal (Gx), 3-deoxyglucosone (3-DG) and GLO 1 as novel prognostic markers for the development of diabetic complications.

The activity of GLO 1 in type 2 diabetic patients was determined by using the spectrometric method, which monitors the initial rate of change in absorbance at 235 nm caused by the formation of S-D-lactoylglutathione. Activity was determined in hemolysates, which were obtained from packed red blood cells, and was normalized to the hemoglobin concentration. Concentrations of MG, Gx and 3-DG in plasma samples of diabetic patients were determined by derivatization of the dicarbonyl of interest with 1,2-diamino-4,5-dimethoxybenzene. Each dicarbonyl generates a specific fluorescent quinoxaline derivative
which is easily separated and quantified by reverse-phase high performance liquid chromatography using a fluorescence detector.

After measurement of GLO 1 activity in hemolysates of diabetic patients, three distinct patient groups were identified. Furthermore, a negative correlation between storage time and GLO 1 activity could be observed, which led to the conclusion that storage conditions may have a detrimental effect on GLO 1 activity. The mean concentration of MG, Gx and 3-DG in the plasma samples of diabetic patients was 238 nM, 326 nM and 210 nM. MG and Gx levels correlated positively with age using bivariate analysis, and furthermore, MG correlated positively with age when using multivariate analysis. Bivariate analysis was also performed with MG, Gx and 3-DG and the glucose parameters (fasting glucose and HbA1c), but no correlation was found.

In summary, this study showed that storage conditions, as well as storage time might have significant impacts on enzyme activity. Furthermore, the study provided indications that poly-medication of diabetic patients may have detrimental effects on MG levels.