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Pyruvate Kinase Expression and Activity in Hearts, Kidneys and Livers of Mice with Streptozotocin-Induced Diabetes

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Oxidative stress plays an important role in the development of diabetic complications. In diabetes, the formation of reactive oxygen species is increased, and additionally, the anti-oxidant defense system is impaired. The resulting oxidative stress leads to a series of harmful effects in several organs.

Biochemical pathways in diabetic patients are altered in a complex manner, which is still only partially understood. Several bottlenecks occur in glycolysis, one of which is the inhibition of the glycolytic enzyme GAPDH as a consequence of oxidative stress. Hence, glycolytic intermediates accumulate and are shifted into alternative pathways that are responsible for the development of diabetic complications. Another glycolytic enzyme that may be affected in diabetes is pyruvate kinase.

Pyruvate kinase activity is known to be impaired in many forms of cancer because expression is shifted from the tissue-specific pyruvate kinase isoform to the less active isoform M2. This may be relevant to diabetes because there are some links between diabetes and cancer: The two diseases have some common predisposing risk factors, both feature high intracellular glucose levels and possible pro-malignant effects of diabetes-related pathways are subject of current research and discussion.

Pyruvate kinase expression and activity in diabetes have previously been studied in different species, organs and disease conditions, with partially contradictory results. The most studies are available about pyruvate kinase activity in the liver of rats with experimental diabetes, and all of those studies have found a lower activity in the liver of diabetic animals compared with healthy controls. The results of genetic studies also suggest that pyruvate kinase may play a role in diabetes. Additionally, pyruvate kinase has been implicated with Alzheimer's disease, which is considered to belong to the group of diabetes mellitus diseases by some researchers.

The reported findings about pyruvate kinase alterations in different forms of diabetes and the association of diabetes and cancer have lead the author to the hypothesis that, like in cancer, pyruvate kinase activity may be impaired in diabetes — due to inhibition or reduced activation, due to reduced expression or due to a shift of expression to isoform M2 (or another isoform with similar properties). In order to test that hypothesis, pyruvate kinase expression and activity was investigated in different organs under long-term experimental diabetes in mice.

The hearts, kidneys and livers from mice with streptozotocin-induced diabetes and from untreated mice (n = 7 control + 7 diabetic) were examined 3 months after the disease induction. Pyruvate kinase mRNA transcription was analysed by real-time PCR, pyruvate kinase protein content by Western blot and pyruvate kinase activity by a photometric assay.

Specific primers and antibodies were used for detection of the different pyruvate kinase isoforms M1, M2 and L.

The results of this study show that in long-term experimental diabetes of mice, the heart and kidney exhibit a PKM expression that is different from healthy animals. In the heart, overall PKM expression is reduced, and in both the heart and kidney, expression is shifted from isoform M1 to isoform M2. Despite those changes in expression, activity remains unchanged. The liver of diabetic mice does not exhibit altered PKL expression or activity compared with healthy controls.

The Western blot results of the heart and kidney samples only partially match the respective PCR results, which is most likely due to technical limitations of the Western blot assay. It was not possible to analyse the pyruvate kinase protein content of the liver samples at all because of unspecific binding of the tested antibodies.

The fact that pyruvate kinase activity in the heart and kidney is not different between the diabetic and control mice despite the changed expression indicates that a mechanism enhancing the specific pyruvate kinase activity may be in effect to compensate for the impaired enzyme expression. Accordingly, it may be that the risk to develop diabetic complications depends on the individual ability to activate and maintain such compensatory mechanisms to cope with deranged metabolic pathways. This notion is supported by another study showing that pyruvate kinase expression of fibroblasts in human type~1 diabetic patients with and without nephropathy differs from healthy controls, but pyruvate kinase activity differs from healthy controls only in patients with nephropathy, not in patients without nephropathy.

The present finding that pyruvate kinase expression and activity in the liver of diabetic mice are not different from healthy animals contradicts the aforementioned reports about a reduced activity in the liver of diabetic rats. That difference may be due to the different species, the longer duration of the disease in the present study or technical differences between the activity assays.

This project was conceived as a pilot study, and the results from mice with experimental diabetes must be confirmed with data from human patients.

Current treatment strategies focussing on glycæmic control are not sufficient to prevent diabetic complications. Pathomechanisms triggered by transient hyperglycæmia persist even when blood glucose levels are normalized again. It is crucial to better understand those mechanisms to develop better treatment strategies. This study introduces changes in pyruvate kinase expression as one part of the metabolic alterations in diabetes.