“Characterization of the urinary metabolite profile of human insulin by LC-MS/MS: a possible means to uncover insulin abuse.”

Pr. M. Thevis, Dr. A. Thomas, Pr. W. Schänzer (German Sport University, Germany)

Project Overview

The misuse of recombinant human insulin in sport has frequently been mentioned by confessing athletes but is not detectable with currently available methodologies. This is mainly due to the fact that there are no measurable differences to the endogenously produced hormone. Several attempts to establish diagnostic marker ratios to other endogenously produced hormones (C-peptide) failed due to unstable or highly variable conditions in the living organism. With the present study we aim to establish potential marker metabolites, which will uncover a surreptitious insulin application. Pilot studies demonstrated that there are substantial differences in the degradation/metabolism of insulin between the endogenously secreted and the recombinant and injected insulin. It is assumed, that these differences are due to the exposition of insulin to specific enzymes after subcutaneous or intramuscular administration.
Several urinary metabolites were identified in earlier studies by this research group and an extension of this approach with most modern analytical instruments is planned, potentially revealing new indicative and diagnostic metabolites. Therefore, purification of target compounds from urine followed by the determination of insulin metabolite profiles by LC-MS/MS is planned, which might serve as diagnostic tool to uncover the misuse of recombinant human insulin.

Results and Discussion:

This study was conducted to explore the metabolic fate of subcutaneously administered recombinant human insulin. Due to the exposure to endogenous proteases in the subcutaneous tissue, a minor amount of the bioactive peptide hormone is cleaved to its truncated metabolite DesB30 human insulin, which still owns the full biological activity. In addition to that, DesB30 is a known by-product of recombinant insulin preparations (at ~ 1%) and, thus, it can enter the circulation from the injection site. Post administration of recombinant human insulin, circulating DesB30 was identified in plasma samples of diabetic patients (type II). No specimen obtained from healthy volunteers with endogenous insulin analyzed in the context of this study contained DesB30, not even when the production of insulin

was stimulated by ingestion of a concentrated glucose solution (OGTT). The analysis of athletes' routine doping control samples yielded analytical findings of trace amounts (=LOD) of DesB30 HI in 3 out of 64 samples. The additional monitoring of the respective C-peptide levels yielded inconspicuous results for all athlete samples but, conversely, suppressed C-peptide levels in diabetic individuals after HI administration. Thus, the detection of DesB30 in athlete blood samples in combination with C-peptide levels represents a potential combination of markers for the misuse of recombinant human insulin in sports. Due to degradation processes, urine specimens were not found suitable for this approach. Furthermore, special care regarding the storage and sampling conditions are crucial for the collected plasma samples and degradation processes must be avoided by applying appropriate conditions during the whole post-sampling period until analysis. This is important for both inhibiting the degradation of HI to DesB30 HI on the one hand and stabilizing the C-peptide of the other hand.