PROJECT REVIEW

"Fast analysis of insulin/c-peptide ratios in plasma as possible indicators for insulin misuse employing ELISA methods"

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

Insulin, a potentially performance enhancing agent, is prohibited for non-diabetic athletes according to the WADA list of banned substances since 1999. Despite the already existing methods for the mass spectrometric determination of the chemically modified synthetic insulin analogues in regular doping control samples, an approach to uncover the misuse of recombinant human insulin is missing. Insulin is a peptide consisting of an A-chain (21 AA) and a B-chain (30 AA), which are connected by two disulfide bonds. It is endogenously produced in the pancreas from the single chain precursor proinsulin after cleavage into insulin and C-peptide (31 AA). In healthy individuals insulin and C-Peptide is secreted in equimolar amounts from the vesicles of the Langerhans` islets cells into the bloodstream. Thus, the ratio of these peptides in human plasma is supposed to be constant and a significant shift towards higher insulin amounts should provide a reliable hint for a surreptitious insulin application. Existing ELISA diagnosis kits for determination of insulin and C-peptide will be utilized to assess the physiological ranges of the ratio in regular plasma specimens and doping control samples. Moreover, specimens obtained from patients being treated with recombinant human insulin will be measured and serve as "positive control" samples. The planned methodology, which has commonly been used for clinical or forensic purposes, could also serve as a screening procedure for plasma doping control specimens to indicate the misuse of any kind of exogenous insulin supplement that cross-reacts with the employed ELISA.

Development of an ELISA-based insulin and C-peptide assay in human plasma to uncover the misuse of recombinant human insulin

Results and Conclusions

The determination of insulin/C-peptide ratio in doping control specimens is not a promising approach to uncover the misuse of insulin(s) in sport due to insignificant changes upon insulin administration and a great influence of concurrently provided carbohydrates. Future studies that may provide a more helpful tool are planned and include the examination of the autoimmune status of the volunteers and the determination of potentially formed anti-Insulin-antibodies occurring in plasma from patients that were treated with exogenous human insulin. It is known that these antibodies are produced endogenously and may enable the detection of insulin applications in an 'indirect' fashion.