PROJECT REVIEW

"Screening of Designer Steroids by NMR/Pattern Recognition"

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The overall aim of the proposed project is to apply NMR spectroscopy and pattern recognition (PR) methods as innovative method for anti-doping screening and the elucidation of the overall biochemical effects of different doping methods.

This project will test the hypothesis that there are quantifiable variations in the urinary excretion of low molecular weight metabolites, induced by physiological response to doping, and that these changes could be related to different doping methods. By this means doping could be monitored without searching a specific compound but evidenced as a whole, in a fingerprinting approach, through NMR spectra of biofluids and subsequent multivariate analysis. As a first approach, this project will focus the detection of the use of designer steroids, by examining athlete's positive samples for synthetic steroids like stanozolol.

The project includes the use of the large sample databank of Doping Control Laboratory of Athens, the generation of NMR spectral database and the consecutive analysis as well as the discovery of novel relevant biomarkers. The specific objectives are to apply biofluid NMR spectroscopy and Pattern Recognition in order to build multivariate biochemical models based on the use of prohibited formulations by athletes and to create databases of NMR spectra of characterised biofluids in selected human states. The derived models will be further applied to classify unknown samples according to primary and secondary metabolite variations induced by doping. Data will be analysed using a variety of standard methods to investigate multivariate analysis mapping of endogenous biochemical profiles. The new NMR-based metabonomic approaches will be compared with conventional methods, based on clinical chemistry and mass spectrometry and their relative usefulness in the field of anti-doping control will be evaluated.

To our knowledge the multiparametric approach proposed in this project has not been done before in humans. Such studies could lead to new strategies to complement antidoping control based on the Metabonomic approach maximizing the reliability of screening practices in use.

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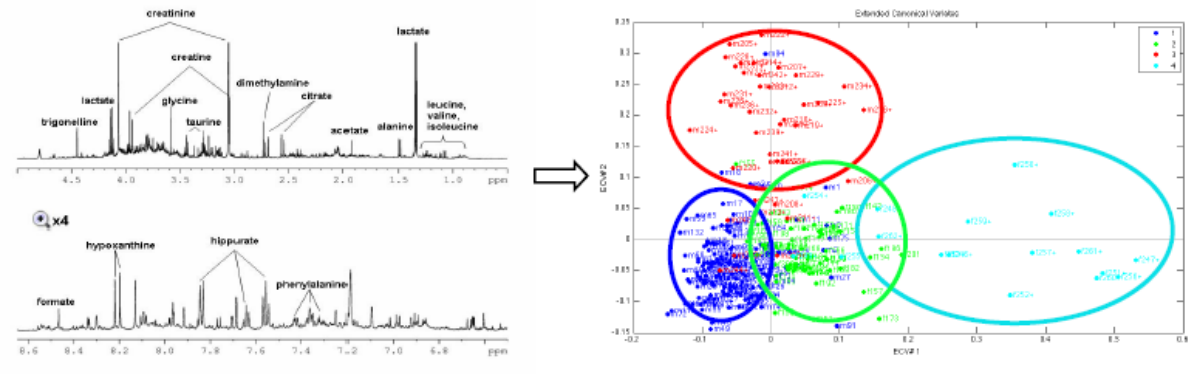
Results and Conclusion

Metabonomics is well established as a powerful method for the evaluation of the metabolic profile of an organism, proffering a holistic view of an organism's response to different exogenous factors. The aim of the project was to examine whether the NMR based metabonomics approach could be used as complementary tool to the existing methods on the basis of a non-targeted profiling analysis that is able to capture the metabolic signature of athletes who have utilized exogenous steroids. The applicability of the method was tested in a cohort of 263 human urine samples of both men and women athletes targeted to doping controls. Among them, 59 had been reported as positive to the official doping controls for the application of exogenous anabolic steroids.

The study included the NMR measurements of all samples and the chemometric analysis of the resulted spectroscopic data set. The latter consisted of complex 1H NMR spectra containing hundreds of signals from both endogenous and exogenous metabolites and was analyzed by multivariate statistical tools in two ways:
i) using data reduction of the original matrix to a lower dimension, followed by Principal Components Analysis (PCA), Partial Least Square–Discriminant Analysis (PLS-DA) and Orthogonal PLS-DA (OPLS-DA) and ii) using a pre-processed full spectroscopic matrix with peak alignment followed by PCA and interval based Extended Canonical Variate Analysis (iECVA).



The method i) resulted in models with partial grouping betweenn the originally classified groups, while method ii) provided a clearer classification that highlighted significant differences of metabolites, such as creatine, creatinine, hippurate, and

acetate among the groups of athletes. It is worth noticing that the samples derived from positive athletes displayed a significant higher variance comparing to the "negative" ones in all developed statistical models. This depicts one limitation of the present study, which arises from the low homogeneity of the sample pool and the lack of sufficient metadata that could be added to the statistical models development.

It is concluded that the NMR based metabonomics approach could be used as an ultra fast and cost effective predictive tool in anti-doping control that could highlight those samples originated from doped athletes among a larger cohort of samples on the basis of their metabolic fingerprint. In order to be fully applicable, the samples collection should be accompanied with extended metadata information that could be utilized in the multivariate statistical analysis.