***"Application of Metabolomic to Doping Controls- Investigating alternative markers for Testosterone and Growth Hormone abuse using mass Spectrometry and Biostatistics"***

**Dr. M. Thevis, Dr. A. Thomas** (German Sport University Cologne, Germany)

## Project Summary

Metabolomics has become a major tool in systems biology and biomarker discovery studies and possesses great potential also concerning modern anti-doping efforts. Most assays currently applied in doping controls are designed to sensitively and specifically detect the administered drug or its diagnostic metabolite to provide unambiguous evidence for the presence of a banned substance. A few marker-based methods have also been established in the doping control arena such as the steroid profile and the hematological module of the Athlete Biological Passport.

The influence of 'external circumstances' on the metabolome has been recognized decades ago, particularly concerning the diagnosis of diseases. Analogously, the search for biomarkers towards the detection of drug (ab)use in sport will be of great utility for a modern anti-doping fight as demonstrated in pilot studies concerning animal sport and livestock production. Upon drug administration, shifts in metabolomic signatures occur; these are largely unknown but, once identified, potentially easier and/or longer monitored than the administered compound(s). Hence, analysis, quantitation, or profiling of (bio)markers rather than the detection of the drug itself will indicate and possibly provide evidence for a previous illicit intervention on the basis e.g. of growth promoting hormones. This is especially helpful for those therapeutics and designer substances that are difficult to determine (due to short half-life or structures identical with endogenously produced hormones). Modern analytical tools, particularly mass spectrometry-based methods, have proved useful for the identification of parameters that provide information on metabolome alterations. In combination with biostatistics, multivariate statistical analyses are enabled to highlight the value of identified compounds as potential (bio)markers. Upon characterization of marker candidates, these analytes can be implemented in anti-doping methods. Therefore, a comprehensive screening of the urinary metabolome of samples collected from different (administration) studies is planned to provide new insights into new targets for future doping control programs.

## Results and Conclusions

The urinary metabolome, i.e. the entire set of low molecular mass substances of the human organism eliminated into urine, is composed of the

endogenous and the exogenous metabolome. This reflects the metabolic products naturally produced by the individual and metabolites resulting from xenobiotics (e.g. drugs, toxins, food additives, etc.), respectively. It is consequently an extraordinary complex and dynamic object, which is significantly influenced (amongst others) by drug (mis)use. In order to assess whether –omics strategies such as urine metabolomics can indicate the misuse of drugs relevant in a doping control context, a pilot study was conducted, probing for the potential of this analytical strategy of identifying samples collected during and after applications of anabolic-androgenic steroids. Three scenarios were pursued with controlled administration studies including (1) oral, and (2) transdermal testosterone, where both intra- and interindividual variations of the metabolome were measured. In addition, the metabolome of a reference population of healthy and active individuals was compared to the urinary metabolome of athletes tested positive for the misuse of anabolic-androgenic steroids in routine doping controls (3). The urinary metabolomic 'signatures' were recorded using high resolution/high accuracy mass spectrometry with both gas chromatographic and liquid chromatographic separation of analytes to provide utmost comprehensiveness. Following sample analyses, non-targeted data evaluation was conducted to enable pattern recognition as supported by statistical methods such as principal component analysis (PCA) and orthogonal projection to latent structures (OPLS).

Comparing the samples of the reference population with those resulting from adverse analytical findings (i.e. scenario 3), extreme interindividual variations were observed. Nevertheless, distribution patterns of typical non-suspicious and atypical urine samples were also recognized that potentially contribute information supporting the identification of unusual anti-doping analyses. Within both testosterone administration studies (1 and 2) it was readily possible to distinguish between pre- and post-administration samples on an individual basis. By comparing the undisturbed metabolome of each volunteer with the effects induced by testosterone, many entities or metabolites could be detected that were either up- or down-regulated. Even for the application of low doses of testosterone via transdermal testosterone gel, the metabolome was significantly influenced in all 4 volunteers on an individual basis. However, the interindividual variability was extensive and the effects of testosterone administrations on the metabolome became more or less negligible compared to the large biological differences. These individual variations are composed of many factors such as the different regulation of enzymes and metabolic pathways due to physical activity or simply the individual´s diet. Consequently, metabolome analyses seem to have potential to indicate drug (mis)use based on pattern positioning when compared to a reference population. Moreover, supporting information on anabolic-androgenic steroid misuse (here: testosterone) is obtained when intraindividual metabolomics data are available.

On a sideline, another unexpected result was observed. The UDP-glucuronosyltransferase 2B17 genotype obviously has a large impact not only on selected glucuronidated (steroid) metabolites but seems to significantly influence the entire urinary metabolome. Since only 2 participants with this polymorphism were included in this study it remains speculative if identifying

the genotype can be accomplished by a distinctive urinary metabolome pattern; however, the preliminary dataset suggests this possibility.