

## ***“Metabolism of exogenous testosterone in UGT2B17 del/del genotype”***

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### **Project overview**

Testosterone misuse still remains a challenging task for doping control laboratories as the steroid is a naturally occurring hormone. It is detectable in all doping control urine samples, and concentration-based thresholds have been established to indicate illicit testosterone administrations.

To facilitate testosterone excretion into urine it is metabolically converted to testosterone-glucuronide, a conjugate with substantially increased solubility in water. Depending on the genotype of an athlete, this transformation is impaired, resulting in very low concentrations of testosterone-glucuronide in urine even after administration of significant amounts of the drug. Routine strategies to disclose testosterone doping are therefore not applicable to athletes with this particular genetic constitution, and depending on the geographic/ethnic origin, up to 70 % of individuals produce these very low urinary concentrations. Despite this limitation to detect testosterone misuse by routine screening methods in so-called 'del/del' steroid profiles, the confirmatory analysis based on carbon isotope ratio determinations is a viable option for such samples.

The aim of the present study will be to investigate and compare the metabolism of testosterone in both genotypes (athletes with high and very low urinary excretion of testosterone-glucuronide), i.e. to determine the metabolic fate and alternative routes of elimination. This will support the identification of potential markers and strategies to enhance sports drug testing for testosterone misuse independent from an athlete's genotype and the corresponding individual metabolism. Deuterium-labeled testosterone will be administered, and all metabolites tagged by deuterium atom(s) will be identified unambiguously by hydrogen isotope ratio mass spectrometry. All analytes of interest will be quantified and identified by means of high-resolution and high-accuracy mass spectrometry to provide new insights into the metabolism and elimination of testosterone particularly in 'del/del' genotypes.

### **Results and Conclusions:**

Testosterone (T) misuse still represents a major problem in sports drug testing. Many strategies have been developed and applied to routine doping controls within the recent years to enable both identifying suspicious samples in initial testing procedures and to confirm the exogenous origin of urinary T by means of carbon isotope ratio (CIR) determinations. Depending on the tested individual's genotype of UGT2B17, significantly different amounts of T

are glucuronidated and excreted, which results in unaffected T/epitestosterone ratios after T misuse in those subjects with the deletion/deletion polymorphism (del/del).

The aim of this study was to investigate differences in metabolic pathways of orally administered T between persons of del/del and insertion/insertion (ins/ins) genetic polymorphism. Therefore, a recently established method using hydrogen isotope ratios together with high-resolution and high-accuracy mass spectrometry was applied after administration of deuterated T to n = 4 subjects including both genotypes. Participants collected urine specimens directly before and for up to 8 days after the application. Urine aliquots were prepared to yield unconjugated, glucuronidated, and sulphoconjugated fractions of urinary steroids.

Besides the significant difference in the excretion of T-glucuronide, all measured metabolites varied rather on an individual basis than due to a genotype difference. New T metabolites (both methylated and demethylated) were detected and investigated regarding their potential to enhance the screening for T misuse. Sulphoconjugated epiandrosterone was further identified as the biomarker allowing for a significant prolonged retrospective detection of T misuse by means of CIR determinations when compared to the currently applied sports drug testing procedures.