

"Improving Detection of Endogenous Anabolic Steroids misuse by measuring endogenous Sulfate Metabolite (IDEASS)"

Rosa Ventura, Núria Monfort, Élide Alechaga Silva, Xavier Matabosch Geronés, Argitxu Esquivel López, (Fundacio IMIM, Barcelona, Spain)

Project Overview:

The detection of endogenous anabolic steroids (EAS) abuse is currently performed using the steroid profile. The steroid profile is composed of concentrations of testosterone and related endogenous metabolites excreted as glucurono-conjugates, and ratios between them, being Testosterone/Epitestosterone ratio the most important one. These parameters are monitored for each individual to define the individual basal ranges, and changes on them reveal the use of EAS. Suspicious samples are confirmed by carbon isotope ratio mass spectrometry to demonstrate the exogenous origin of testosterone and metabolites. The steroid profile is a powerful tool to detect EAS misuse, however improvements are needed to prolong detection windows.

Testosterone and metabolites are also excreted as sulfates in urine. The sulfate fraction has not been comprehensively evaluated for the detection of EAS misuse. The objective of the project will be to evaluate the sulfate fraction of testosterone metabolites to look for new biomarkers to prolong the detectability of the misuse of EAS.

First, a comprehensive methodology to quantify endogenous steroid sulfates based on their direct analysis by liquid chromatography-tandem mass spectrometry will be optimized and validated. Second, steroid sulfates will be quantified in urines obtained from healthy population to define normal population ranges. Finally, steroid sulfates will be quantified in urines collected after administration of testosterone to healthy volunteers by different routes. Evaluation of sulfate metabolites as markers of EAS administration will be performed by comparison of the excretion profiles of testosterone and metabolites excreted as sulfates with the excretion profiles of metabolites included in the conventional steroid profile. The successful outcome of the project will be directly applicable to sports drug testing by improving the detection of EAS misuse.

Results and Conclusions:

The objective of the project was a comprehensive evaluation of the sulfate fraction of testosterone (T) metabolites to look for new biomarkers to prolong the detectability of the misuse of endogenous anabolic steroids. First of all, an analytical method was developed and validated to quantify fourteen T related metabolites conjugated with sulfate, based on a mixed-mode solid phase extraction and the direct measurement of sulfate metabolites by LC-MS/MS. The concentrations of sulfate metabolites in healthy volunteers, including Caucasian and Asian volunteers, were measured.

The usefulness of sulfate metabolites to detect oral T misuse was evaluated after administration of a single oral dose to five Caucasian male volunteers. Using individual threshold limits epiandrosterone sulfate (epiA-S) improved the detection times (DTs) with respect to T/epitestosterone (E) ratio in all five volunteers. Androsterone (A), etiocholanolone (Etio) and two androstenediol sulfates also improved DTs for some volunteers. The most promising results were obtained using ratios between sulfates of epiA, A or androstenediol 1 and E, and also sulfates of epiA or androstenediol 1 and dehydroandrosterone (DHA). These ratios prolonged the DT of oral T administration, in some cases several days after administration, and therefore significantly improving the retrospectivity compared to sulfate concentrations or to the conventional T/E ratio.

Sulfate metabolites were also evaluated after a single intramuscular (IM) injection of T to six Caucasian and six Asian healthy male volunteers. Principal component analysis (PCA) was used to obtain the most useful markers for discrimination between pre- and post-administration samples. For Caucasian volunteers, a separation between pre- and post-administration samples was observed in PCA, whereas for Asian no separation was obtained. Seventeen ratios between sulfate metabolites were selected and further considered. DTs of each ratio were evaluated using individual thresholds for each volunteer, and the best results were obtained using ratios involving T and E sulfates in the denominator. The best marker was the ratio A-S/T-S which prolonged the DTs with respect to T/E ratio in all Caucasian volunteers and in two Asian volunteers. Other ratios between A-S or Etio-S and E-S, and, sulfates of Etio, DHA or epiA and T-S were also found adequate.

The data obtained in the project provide a comprehensive insight about the usefulness of endogenous sulfate metabolites as biomarkers for the detection of oral and IM T misuse. They can drastically increase the DTs with respect to the conventional T/E ratio, especially after oral T administration and, according to our results, its inclusion in the steroid profile is strongly recommended.

Publications:

- Esquivel A et al., Direct quantitation of endogenous steroid sulfates in human urine by liquid chromatography-electrospray tandem mass spectrometry. *Drug Test Anal.* 2018 Nov;10(11-12):1734-1743. doi: 10.1002/dta.2413.
- Esquivel A et al., Sulfate metabolites improve retrospectivity after oral testosterone administration. *Drug Test Anal.* 2019 Mar;11(3):392-402. doi: 10.1002/dta.2529.
- Esquivel A et al., Evaluation of sulfate metabolites as markers of intramuscular testosterone administration in Caucasian and Asian populations. *Drug Test Anal.* 2019 Apr 1. doi: 10.1002/dta.2598.