"*Useful information about recently reported testosterone metabolites related with doping control analysis*"

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Project Summary

Testosterone misuse is the most detected doping offence in screening analysis by means of the ratio between testosterone and epitestosterone (T/E).

Recently, four testosterone metabolites have been reported in our laboratory after alkaline treatment of the sample. In a previous WADA project (reference 10A16OP), we have shown that the detection of these metabolites and the ratio between them is useful for the detection of testosterone misuse after oral and topic administration. Therefore, the addition of these metabolites into screening methods seems to be a promising complement to T/E.

Besides the cases already tested, the measurement of T/E has also additional limitations like (i) its use in samples with low basal T/E or (ii) it can be affected by external factors like alcohol intake. Most of these external factors are related with the glucuronidation process. Since the basic released metabolites are not glucuronidated, they can be less affected by these factors providing a complementary tool for testosterone misuse detection.

The knowledge about the phase II metabolic pathway which is associated with the presence of these metabolites can provide useful information for the antidoping control field. Among other adbantages, knowing the phase II metabolite would theoretically allow for including these metabolites in already existing screening procedures.

Therefore, the goal of this follow-up project is to evaluate of the usefulness of the quantitative detection of the metabolites released after basic treatment for the detection of testosterone misuse. In order to achieve this main goal, the project will be divided in three specific objectives: (i) detection of testosterone misuse in population with low basal T/E values, (ii) study of the effect of several factors which can vary the steroidal profile in the values of these metabolites and, (iii) elucidation of the phase II metabolic pathway related with the occurrence of these metabolites.

Results and Conclusions

Recently, four new testosterone metabolites (Δ1-AED, Δ6-AED, Δ6-T and Δ15-AD) have been reported in our laboratory after alkaline treatment of the

urine. These metabolites and the ratio between them were found to be useful for the detection of oral testosterone misuse. Therefore, the addition of these metabolites into screening methods seemed to be a promising complement to the steroid profile.

The goal of this follow-up project was to evaluate the usefulness of the detection of these metabolites for doping control. For this purpose, the project was divided in three specific objectives: (i) elucidation of the metabolic pathway responsible of the occurrence of these metabolites, (ii) study of the effect of several factors which can vary the steroidal profile in the values of these metabolites and (iii) detection of testosterone misuse by these metabolites.

We demonstrated that the studied metabolites are produced from the degradation of cysteine conjugates. The formation of these metabolites implies an unreported metabolic biotransformation: 6,7-dehydrogenation as phase I metabolism followed by conjugation with glutathione and subsequent transformation to cysteine conjugates. The postulated pathway was supported by studies with human hepatocyte cells systems. Analogously to testosterone, this pathway might also be present in other steroids, opening the possibility of targeting additional biomarkers. This fact was confirmed by the detection of 24 boldione metabolites (11 conjugates with cysteine and 13 conjugated with N-acetylcysteine).

The influence of several factors in the excretion of the studied metabolites was evaluated. Degradation, freeze/thaw cycles and infradian variability studies showed moderate variations (below 40%) for these metabolites. UGT2B17 polymorphism does not influence the excretion of cysteinyl compounds whereas the intake of exogenous substances (alcohol or 5α-reductase inhibitors) dramatically affects their excretion. During pregnancy only the excretion profile of Δ1-AED increased. Overall, the presented data describes the stability of the urinary cysteinyl steroids under the influence of many factors, proving their potential as suitable parameters to be included in the steroid profile.

The usefulness of cysteinyl markers for the detection of T im and T gel misuse was studied. Among cysteinyl metabolites Δ1-AED/Δ15-AD was shown to be the best marker after T im allowing the detection in all studied cases. The use of this marker also allowed the detection of T gel misuse in almost all volunteers with T/E around 1. Worse results were observed for the detection of T gel misuse in volunteers with T/E below 0.2. Anyway, the use of cysteinyl markers did not increase the detection capabilities of the current steroid profile questioning their usefulness for doping control analysis.