PROJECT REVIEW

"Potential of recently reported testosterone metabolites as markers for testosterone misuse in challenging cases"

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Testosterone misuse is the most detected doping offence in screening analysis. Nowadays, the ratio between testosterone and epitestosterone (T/E) is measured in the screening method. The exogenous nature of testosterone in those samples showing a T/E higher than 4 is confirmed by GC-C-IRMS. Although this approach gives satisfactory results in most of the testosterone misuse cases, there are some situations in which the value of 4 is not reached even after acknowledged testosterone administration. Those problematic situations include (i) detection of testosterone misuse in population with low basal T/E values, (ii) long term detection of testosterone misuse after oral intake and (iii) detection of testosterone misuse after topical administration.

Recently, four new testosterone metabolites have been reported in our laboratory after treatment of the sample with basic media. The detection of these metabolites in urine samples collected from a volunteer after oral testosterone administration showed that they substantially improved the detection of testosterone misuse in that individual case. Therefore, the addition of these metabolites into screening methods seems to be a promising complement to the commonly used T/E ratio.

Therefore, this project aims to check the usefulness of the quantitative detection of these metabolites for the detection of testosterone misuse in the challenging cases mentioned above: (i) detection of testosterone misuse in population with low basal T/E values, (ii) long term detection of testosterone misuse after oral intake and (iii) detection of testosterone misuse after topical administration. For this purpose, a quantitative method will be developed and validated. Afterwards, the method will be applied to samples collected after different testosterone applications.

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Result and Conclusion

Recently, some testosterone metabolites appearing in urine after alkaline treatment have been reported in our laboratory. In this project, the applicability of these metabolites for doping control purposes has been evaluated.

In the first step of the project a quantitative method for the detection of alkaline released metabolites in human urine was developed and validated. Analytical figures (linearity, precision, accuracy and limits of quantification) of the validated method were appropriate for the quantification of both basal levels present in urine of non-treated subjects and concentration levels present after testosterone administration. Besides the satisfactory figures, the method involves an easy and rapid sample treatment which favors its application to the large number of samples required for the development of the project.

In a second step, the validated method was applied to 173 urine samples and reference population concentrations were calculated. Using the software refval a concentration threshold was established for every metabolite and for the ratios calculated between them. The applicability of these thresholds was evaluated. Similarly to T/E, these population based thresholds are useful when large doses of endogenous steroids are administered like in the oral administration of testosterone. However, in small doses, these thresholds are normally not exceeded and, therefore, individual threshold values like the ones used in the Athlete Biological Passport seem to be the option of choice in order to apply these metabolites in the fight against doping.

In the last step, the applicability of these metabolites for the detection of testosterone misuse has been checked. After oral administration, the quantification of these metabolites and several ratios between them allowed for the increase on the retrospectivity. The best marker seems to be 1,4-androstadien-3,17-dione (boldione). The quantification of this marker after alkaline treatment of the urine increased between 3 and 6 times the retrospectivity of the detection of oral administration of testosterone. Ratios involving boldione after alkaline treatment of the sample have been found to be also useful for the detection of administration of single topic dose of testosterone. Although ratios involving 15-AD seem to be useful for this purpose, the absence of the reference material avoid for the quantification of this metabolite.

Although the preliminary results shown in this project seem to indicate that the inclusion of testosterone metabolites released after alkaline treatment in the Athlete Biological Passport can be useful for the detection of endogenous steroid consumption, several parameters have to be evaluated before. Among them, it is remarkable the evaluation of the potential of these metabolites as markers for testosterone administration in those subjects with low basal T/E values.

Publications/presentations related to the project
Publications:

1.- A. Fabregat, O. J. Pozo, J. Marcos, J. Segura, R. Ventura. Quantification of testosterone and metabolites released after alkaline treatment in human urine. Drug Test. Anal. 2 (2010) 630-636

2.- A. Fabregat, O. J. Pozo, J. Marcos, J. Segura, R. Ventura. Alternative markers for the long-term detection of oral testosterone misuse. Steroids 76 (2011) 1367-1376

3.- A. Fabregat, O. J. Pozo, P. Van Renterghem, P. Van Eenoo, J. Marcos, J. Segura, R. Ventura. Detection of dihydrotestosterone gel, oral dehydroepiandrosterone and testosterone gel misuse through the quantification of testosterone metabolites released alter Basic treatment. Drug Test. Anal. 3 (2011) 828-835

Presentations:

1.- A. Fabregat, O.J. Pozo, J. Marcos, J. Segura, R. Ventura, "Nuevas herramientas analíticas en el control antidopaje: utilidad de diferentes modos de barrido en la detección y caracterización de marcadores alternativos de la administración exógena de testosterona". Oral comunication presented in the V meeting of the spanish society of mass spectrometry. Málaga (Spain) 2011.

2.- A. Fabregat, O. J. Pozo, J. Marcos, P. Renterghem, P. Eenoo, J. Segura, R.Ventura, "Application of recently reported testosterone metabolites for the detection of endogenous steroids misuse. Oral communication presented in the 29th Cologne Workshop in Dope analysis. Cologne (Germany) 2011.