PROJECT REVIEW

"Alternative Steroid Profile"

F.T. Delbeke, P. Van Eenoo (Ghent University, DoCoLab, Ghent, Belgium), **W. Schänzer** (German Sport University, Cologne, Germany)

Naturally occurring steroids (e.g. testosterone, 4-androstenedione, dehydroepiandrosterone, etc.) are misused in sports. These steroids are predominantly metabolised to testosterone, epitestosterone, androsterone, etiocholanolone, dehydroepiandrosterone, androstenedione, dihydrotestosterone and 3,5-androstanediols.

Hence, screening for the misuse of naturally occurring anabolic steroids has been performed traditionally by monitoring the concentrations and ratios of several endogenous steroids (e.g. the testosterone to epitestosterone ratio).

Although intake of naturally occurring steroids results in an increase of these steroids, it should be noted that these steroids are endogenous and naturally present in urine samples. Hence, the mere presence of such a substance can not constitute a doping offence. Only when a threshold concentration/ratio is exceeded misuse of an endogenous steroid is established.

One of the problems when setting up threshold levels is that these are based on population statistics and there is a huge inter-individual variation in urinary concentrations of the monitored steroids.

A marked intra-individual increase in the concentration of an endogenous steroid (after misuse) might therefore remain unnoticed in doping control laboratories. Indeed, doping control laboratories do not have any information on the identity of an athlete and her/his individual reference range for the monitored steroids.

Although the major metabolic pathway of endogenous steroids leads to the formation of the mentioned steroids, a few minor metabolic pathways result in the formation of oxygenated/hydroxylated metabolites. Intake of a naturally occurring steroid results in very low urinary concentrations of these metabolites, and preliminar research has shown that individual detection times can be similar to those obtained using the "traditional steroid profiling".

However, very few data on the natural urinary concentrations of these steroids are available. This research project would aim at the identification of suitable metabolites for the detection of misuse of endogenous steroids, the determination of population based reference ranges and comparison of detection times of misuse between the newly developed and traditional screening method and GC/C/IRMS.

Moreover, recently several of the mentioned oxygenated/hydroxylated metabolites steroids have become available on the prohormone market (e.g. 7-keto-DHEA, 6-oxo, formestane, etc.).

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Results and Conclusions

Using a comprehensive GC/MS method the usefulness of naturally occurring minor steroids metabolites was investigated for the detection of misuse with small doses of various formulations of endogenous steroids (oral T undecanoate, T gel, DHT gel, oral DHEA) in sports. Of 24 endogenous steroids, precursors and steroid metabolites the reference ranges were established and applied upon excretion urines. It was concluded that decision limits based upon population statistics were inadequate to detect the misuse of small amounts of steroids and steroid gels. To detect oral administration of T, the best markers were T, T/E, DHT, 4-OH-Adion en 6α-OH-Adion for maximally 24h. Oral use of DHEA was best detectable by screening for DHEA, 7β-OH-DHEA and 16α-OH-DHEA. These specific biomarkers maximally exceeded their population based reference limit for 24h whereas a non-specific steroid ratio like 5βαβ-Adiol/5ααβ-Adiol could indicate DHEA misuse until maximally 60h after intake.

Minor steroid metabolite ratios were investigated in a longitudinal way and implemented as potential biomarkers within the context of the adaptive Bayesian model as used in the Biological Passport. Using this individual approach, detection accuracy could be further improved. Following steroid ratios were found to provide valuable additional information in addition to the T/E ratio and proposed as additional biomarkers: 6α-OH-Adion/16α-OH-DHEA, 4-OH-Adion/16α-OH-Adion, 7α-OH-T/7β-OH-DHEA and DHT/5βαβ-Adiol were identified as very promising biomarkers to detect a single oral T administration for at least 30h post-administration. For detection of DHT administration, best markers are DHT/E, DHT/5βαβ-Adiol and 5ααβ-Adiol/5βαβ-Adiol with maximal detection times up to 78h. The best biomarkers to detect DHEA are 16α-OH-DHEA/E, 7β-OH-DHEA/E, DHEA/E and 5βαβ-Adiol/5ααβ-Adiol with maximal detection times up to 60h.

According to the traditional WADA (TD2004) criteria for screening, 11% of excretion urines until 7 days after administration were identified with atypical steroid profiles of which 95% was confirmed by IRMS. Screening with the Alternative Steroid Profiling strategy led to an additional 14% more atypical steroid profiles of which 72% could be confirmed by isotopic analysis applying compound specific the $\Delta\delta^{13}C$ criteria for IRMS.

This study proves the usefulness of minor steroid metabolites in steroid profiling as well as the relevance of direct individual monitoring of steroid profiles of athletes in the biological passport concept. The detection sensitivity of steroid profile screening can be substantially enhanced leading undoubtedly to more confirmable positive urine samples for misuse with endogenous steroids.