“GC/C/IRMS analysis of testosterone and nandrolone metabolites after the administration of testosterone enanthate and nandrolone decanoate in healthy volunteers”

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Project overview

Anabolic androgenic steroids (AAS) behave differently in the human body. The human organism deals with these compounds differently in respect of uptake, distribution into different organs, metabolism and excretion. We recently demonstrated that ¾ of Oriental people have a severely compromised capacity to excrete testosterone in the urine compared to only 10 % in people from the west. This is a confounder in the doping test program and therefore it is urgent to find new biomarker and approaches.

In the way towards personalised test programmes, Bayesian inference techniques are known to suit particularly well. Another approach is to verify the origin of testosterone by using IRMS.

To further improve the new individualised steroid profile passport, we will conduct humanstudies with nandrolone and different doses of testosterone in order to assess the sensitivity and specificity of IRMS and to learn more how administrated AAS are metabolized

Results and Conclusions

The urinary testosterone glucuronide to epitestosterone glucuronide (T/E) ratio is a biomarker included in Steroid Module of the Athlete Biological Passport (ABP) which has improved doping tests for steroids. However, the ratio is greatly affected by a genetic deletion polymorphism in the UGT2B17 enzyme which is the major catalyst of testosterone metabolism. Suspect urine doping tests are further analyzed with gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS) to determine the origin of the androgen.

We investigated the sensitivity of the steroidal module and the IRMS analysis in subjects administered with three doses of testosterone enanthate (500, 250, and 125 mg), in relation to the UGT2B17 polymorphism. All subjects carrying the UGT2B17 enzyme reached the traditionally used threshold, a T/E ratio of 4, after all three administered doses, whereas none of the subjects devoid of this enzyme reached a T/E of 4. However, using the ABP and IRMS analysis, all three doses generated a positive result with a high degree of sensitivity. Our results demonstrate that administration of one single dose, as small as 125 mg testosterone enanthate, could be detected with the ABP in combination with IRMS. Since IRMS is sensitive to testosterone doping independent of UGT2B17 genotype, also very small changes in the steroidal passport should be investigated with IRMS.

In our study of the excretion profile of nandrolone and the 19-norandrosterone (19-NA) and 19-noretiocholanolone (19-NE) metabolites after one single i.m. dose of 150 mg nandrolone decanoate (ND) to healthy volunteers we were able to demonstrate that 19-NA is detectable for 9 months in about half of the individuals. This is the first study where GC-C-IRMS analysis has been performed after an i.m administration of ND in a controlled study group. The absolute δ19-NA (‰) was in the same range as observed previously, with values between -29.1 -and -34.7 in 19-NA positive samples confirming the presence of 19-NA of exogenous origin. The use of the well characterized androsterone (A) as an endogenous reference compound or any other compound not modified by exogenous administration is also possible. Here we show that 11-oxoandrostenedione and pregnanediol may also serve as endogenous reference compounds to detect exogenous origin of 19-NA 3-9 months after one single dose of 150 mg ND. Interesting to note, exogenous 19-NA was traced in GC/C/IRMS in a sample where 19-NA was below the decision limit.

In summary, GC/C/IRMS analysis confirmed the presence of exogenous derived steroids in both our studies, regardless of UGT2B17 genotype, dose, and time, further supporting the strength of using this methodology.