"Stability of new markers and steroidomic model in steroid profiling"

Project Overview

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Steroid profiling and analysis of the sterome provide valuable information on the homeostasis of the androgenic system in a doping control setting. New biomarkers based upon minor steroid metabolites showed to provide a substantial advantage to detect misuse with endogenous steroids. Within the framework of the biological passport for steroids, these metabolites have been included in an adaptive model as applied for the T/E ratio resulting in improved detection accuracy. Additionally, a lateral discrimination model was developed that could simplify the evaluation of a steroid profile greatly by condensing it to a single score. The input of this steroidomic model consists of traditional steroid metabolites complemented with new minor steroid metabolites found as good biomarkers. It was proved that the model could successfully differentiate with great sensitivity (more than twice better compared to the mere T/E) between normal and altered steroid profiles which originate from administration studies performed with various endogenous steroids.

However, the steroid profile is not only altered after the administration of endogenous steroids. Multiple confounding factors such as alcohol and medication also contribute to the variation in steroid profiles and might lead to misinterpretations of these new biomarkers and model. Therefore, it is necessary to study the new biomarkers and steroidomic model under circumstances where steroid profiles are not changed by intake of endogenous steroids. In this project, the effect of ethanol, corticosteroids, 5-reducatse inhibitors, hormonal contraceptives, therapeutic exogenous steroids and ACTH on an extended steroid profile will be investigated.

Results and Conclusions

The combination of extended steroid profiling using hydroxylated metabolites and longitudinal following from the biological passport has the potential to very sensitively detect alterations of the excreted androgens and metabolites. These more sensitive individual thresholds calculated by the Bayesian software can also be exceeded whenever confounding factors alter the steroid profile causing false negative atypical findings. Knowledge on these confounders is important to correctly interpret the steroid passport and inform its custodian in the best possible way.

EtG is the preferred **alcohol** markers to find alterations in primarily T/E, T/Andro, 5α/βAdiol and 7β-OH-DHEA/E. It is proposed to lower that EtG threshold to 20µg/ml for men and 10 µg/ml for women based on changes in sensitive steroid profile marker T/Andro.

Female's E and PD levels in the luteal phase are highly suppressed by **oral contraceptives**. The T/E ratio becomes more stable with use of oral contraceptives whereas Andro/Etio slightly increases. The ratios of DHEA (metabolites) over E increase mid-cycle while using OC whereas this they tend to decreased without contraceptives.

After **ACTH** use, adrenal androgens DHEA and Adion in females increased and E maximally increased to 10 times normal concentrations. The T/E ratio showed a significant reduction in women and a small reduction in men. Andro/Etio first slightly increased before showing a 50% reduction. Both parameters might be altered sufficiently in females to trigger atypical results with the adaptive model.

After use of **corticosteroids**, E concentrations were only suppressed after multiple high doses which also cause prolonged suppression of the endogenous corticosteroids. In steroid profiles, Andro values showed a decline affecting Andro/Etio similarly. DHEA and its metabolites presented relatively large decreasing trends to 60h after intake. With respect to the steroidomic model, no steroid profile changes were sufficient to alter abnormal steroid profile scores to exceed their threshold.

Mesterolone, as a model for **exogenous anabolic steroids** was used to investigate the general influence of a small amounts of exogenous steroids onto the steroid profile. For single small doses of non-endogenous steroids, it can be concluded that these do not trigger any alteration in the steroid profile, steroid passport or steroidomic model.

5α-reductase inhibitors like finasteride has a big impact on the steroid profile. Although the T/E ratio is unchanged, the effect on steroid profiles occurs downstream the metabolic pathway where 5α/β-Adiol and Andro/Etio show larger suppressions to 5-20% of their original values. Changing Andro concentrations also result in altered degradation parameters with 5α-Aadion/Andro almost reaching the WADA threshold for invalid steroid profiles.