Project Summary

“The detection and confirmation of androstenediol abuse in athletes”
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It is known that anabolic steroids are very effective in promoting muscle growth. The detection of synthetic anabolic steroids is now very effectively carried out by WADA laboratories but the effective detection of the abuse of naturally occurring anabolic steroids is still a major challenge. Doping control laboratories need criteria that allow endogenous steroids to be distinguished from their synthetic analogues in urine. For some endogenous steroids such as testosterone the criteria have been established and measurements of the T/E ratio coupled with isotope ratio measurements can effectively detect such doping. There are however several other endogenous androgenic steroids which can potentially increase active androgen levels and boost performance. 4-androstenediol and 5-androstenediol are two such prohormones which are banned but for which there is incomplete understanding of their metabolism. Recent evidence indicates that at least in some individuals the administration of 4-androstenediol does not lead to a significant change in the T/E ratio. Thus there is a need for improved detection capabilities which can not only detect the abuse of endogenous androgens but also specify which steroid has been administered. It is proposed to use the same methodology which has been developed in this laboratory for the detection of dehydoepiandrosterone (DHEA) abuse. Specific markers arising from 4-androstenediol and 5-androstenediol abuse will be looked for in both the glucuronide and sulfate steroid fractions. Once identified these compounds will be used both for GC-MS screening and for GC-C-IRMS confirmation procedures.

Results and Conclusions

"The detection of androstenediol abuse in athletes"

Compound specific detection (CSD) of endogenous steroid abuse using complementary Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) techniques is an important requirement for effective doping control in sport. This project was undertaken to determine if 4-androstenediol (4-ADIOL; androst-4-ene-3β,17β-diol) and 5-androstenediol (5-ADIOL; androst-5-ene-3β,17β-diol) administration could be selectively identified in urine. Single and multiple oral doses of 4- and 5-ADIOL were performed on two volunteers with human ethics approval and informed consent to monitor changes in urinary endogenous steroid excretion and 13C content.

The results showed significant increases in the excretion of androsterone glucuronide and etiocholanolone glucuronide from 4- and 5-ADIOL administration. This behaviour, which is similar to that observed for dehydroepiandrosterone and androstenedione in previous studies, is an effective screening indicator of endogenous steroid administration. 4-ADIOL was found to produce the diagnostic urinary dehydrogenation products: androst-2,4-diene-17-one and androst-3,5-diene-17-one. These can be used as screening markers in the GC-MS steroid profile to identify suspicious samples that require confirmation by carbon isotope ratio (δ13C) analysis using GC-C-IRMS. Differences in post-administration detection times based on Δδ13C values, relative to δ13C measurement of an endogenous reference compound, were found between glucuronide and sulfoconjugated steroids that were dependent on the individual's metabolism. Preferential glucuronide excretors provided a detection period of up to 72 hours, while sulfoconjugate excretors may provide up to 68 hours.

The administration of 5-ADIOL did not result in the urinary excretion of the diagnostic diene marker compounds, thereby enabling a distinction to be made between the 4- and 5-ADIOLs. Additionally, CSD between 4- and 5-ADIOL may be performed using δ13C analysis of the etiocholanolone sulfoconjugate relative to the sulfoconjugates of androsterone, epiandrosterone and dehydroepiandrosterone. The detection of 5-ADIOL doping based on Δδ13C values, derived from sulfoconjugates greater than 4.0 ‰ is capable of achieving a 60 hour post-administration time.