

PROJECT REVIEW

“Improved methodology for detecting and confirming the abuse of glucocorticosteroids.”

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The aims of this project are to further develop and validate cost effective methods for the analysis of corticosteroids which will be adequate to meet the current and future requirements of the World Anti-Doping Agency (WADA). WADA has mandated that corticosteroids be part of the analytical suite of substances routinely tested by all laboratories during competition testing. Basic methodology was developed using the parent corticosteroids to allow detection of this class of substances. There is however an urgent need to further develop such methods to allow a better understanding of their metabolism and detection, which will allow inclusion of major metabolites easily into the screening procedures. We have already developed methodology using LC/MS/MS which is capable of detecting the fourteen synthetic corticosteroids currently specified by WADA. We will now fully investigate the metabolites of synthetic corticosteroids with a view to including them in the screening method and thus enhance the laboratories' capability to detect corticosteroid abuse. Until this has been done there will be neither an understanding of the biological modification and excretion of these substances nor an understanding of the difference between allowed routes of administration and the procedures that are banned.

Once an expanded method has been developed and validated it is proposed to use the method to measure the natural corticosteroids and their metabolites and precursors in urine samples from elite athletes. Administration studies on these compounds will further allow selection of parameters. The aim is to determine normal levels and attempt to find means such as ratios of corticosteroid levels to precursor or related compounds which are indicative of corticosteroid abuse.

The use of CIRMS to detect the exogenous intake of natural corticosteroids has been proposed. The final stage of this project will be to develop and apply such methodology to investigate its potential for confirming the abuse of natural corticosteroids.

Improved methodology for the detection and confirmation of abuse in sport

Results and Conclusions

LC-MS/MS Screening

An accurate and robust stable isotope dilution LC-MS/MS assay for the corticosteroid screening ratios F/THS and THF/THS was developed and utilized for the analysis of both reference population and administration study urine samples. Thresholds or “cut-offs” as proposed from statistical analysis of the profiling data was used to examine administration study results, with “detection periods” of up to 18 hours observed. In summary then, these preliminary results compare favourably to the previous results obtained by AFLD and show validity for the THF/THS ratio as a marker of endogenous corticosteroid abuse (Meklat, Tabet et al. 2009). However, it is noted that significant intra-individual differences were observed so further investigation into this phenomenon will be undertaken. Additionally, the concept of individual reference ranges needs to be investigated, as this may further improve the sensitivity of the THF/THS ratio to detect suspicious urine samples (Kicman and Cowan 2009). For individual reference ranges to be of assistance in identifying suspicious urine values, it must be shown that the interindividual variation for the THF/THS ratio is quite large relative to the intra-individual variation. That is, the THF/THS ratio for any individual must be relatively stable over time. Further statistical analysis of the reference population (i.e. n = 44, duplicate samples) and administration study data will be undertaken to test this concept.

GC-C-IRMS Confirmation

The “corticosteroid specific” GC-C-IRMS method developed by AFLD was implemented at ASDTL (Buisson et al. 2009). Validation procedures highlighted the overall extraction recovery differences for some analytes, however during subsequent GC-C-IRMS analysis no fractionation was observed. Preliminary analysis of two cortisone acetate administration studies have confirmed previous findings from AFLD. Future ongoing NMI funded work includes the analysis of a reference population from Australian and New Zealand and more administration subjects.