RECENT ADVANCES
IN DOPING ANALYSIS
(6)

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Nagano Strategy against Natural Hormone Doping - Testosterone, DHT, Androstenedione and DHEA –

Nagano strategy against natural hormone doping
- Testosterone, DHT, Androstenedione and DHEA -

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Doping control laboratory for the XVIII Winter Olympic Games, Nagano 1998 was temporarily established by Tokyo laboratory in co-operation with NAOC. Three series of steroid analysis procedures were applied during the games.

1. Screening of combined fraction steroids (Free+Glucuronide+Sulfate) by MSD.
2. High sensitive screening by means of HR-SIM with MS resolution 10,000.
3. Further analysis of natural steroids by GC/combustion/high precision carbon isotope ratio mass spectrometer (GC/C/IRMS).

MSD and HR-MS screening were applied on all the samples. Some samples that meet any of the following conditions were subjected to the further analysis by CIRMS.

- Urinary T/ET ratio > 3.0
- DHEA/THF > 1.0 and DHEA/THE > 1.0
- (DHEA ratio to Tetrahydrocortisol, Tetrahydrocortisone)
- Elevated free androstenedione excretion at significant level
- (Low androsterone/androstenedione < 25 may be indicative to doping)
- DHT suspects (when compared to our published criteria)

Above criteria was applied not for sanction but for the selection of samples for the further analyses.

The steroids were fractionated into free, gluco- and sulfo-conjugated steroids by triethylamino hydroxypropyl Sephadex LH-20 at the first step in the analysis. Hydroxy steroids in the glucuronide fraction were analyzed as their acetate according to the similar procedure that was developed by Shakleton et.al.. DHEA-sulfate and epiandrosterone-sulfate were analyzed after hydrolysis with sulfatase from Ampurallia and following acetylation with acetic anhydride (delta value of reagent = -45.8).
Carbon isotope ratio of DHEA-sulfate, 5β- and 5α-androstan-3α,17β-diol-glucuronides, pregnanediol-glucuronide was evaluated. Following observation are considered to be due to natural hormone doping. 

a. Significant decrease in $^{13}$C-content of the molecule of the target steroids.
b. Acute intra-individual variation of the delta values of the target steroids.
c. Significant difference of $^{13}$C-content among precursor(s) and the metabolite(s).

Naturally elevated urinary DHEA is frequently found especially among Mongoloids, thus DHEA doping could not be detected by the concentration alone. DHEA/THF and DHEA/THE were found to be the useful sign to indicate DHEA doping. CIRMS allowed the clear discrimination between exogenous and endogenous steroids.

Conclusion:
Decrease in the $^{13}$C-contents of steroids is indicative to doping with naturally occurring steroids. However, normal delta-value not always means negative since the existence of synthetic steroids with animal origin is not well known.

Remarks:
The details of this presentation will be published in

Toxin Reviews for the Journal of Toxicology - Special issues on doping -