U. Mareck, H. Geyer, U. Flenker, T. Piper, M. Thevis, W. Schänzer

Population based reference ranges and additional screening marker for the improvement of the detection of dehydroepiandrosterone misuse

Institute of Biochemistry, German Sport University, Cologne, Germany

Extended Abstract

According to World Anti-Doping Agency (WADA) rules (WADA Technical Document – TD2004EAAS) urine samples containing dehydroepiandrosterone (DHEA) concentrations greater than 100 ng/mL shall be submitted to isotope ratio mass spectrometry (IRMS) analysis, whereas the DHEA concentrations have to be adjusted for a specific gravity value of 1.020.

In 2006, 11012 doping control urine samples from national and international federations were analyzed in the Cologne doping control laboratory, one hundred (0.9%) of them yielding concentrations of DHEA greater than 100 ng/mL*. Sixty eight percent of the specimens showed specific gravity values higher than 1.020, 52% originated from soccer players, 95% were taken in competition, 85% were male urines, 99% of the IRMS results did not indicate an application of testosterone (T) or related prohormones. Only one urine sample was reported as adverse analytical finding (AAF) having 319 ng/mL of DHEA (screening result), more than 10,000 ng/mL of androsterone and depleted carbon isotope ratio values for the testosterone metabolites androsterone (A) and etiocholanolone (E).
For the statistical evaluation the entire set of data generated in 2006 was used. The logarithmic distribution of the DHEA concentration indicated urine specimens containing DHEA values higher than 100 ng/mL as a normal part of this (uncontaminated) population. Significantly different DHEA concentrations were evaluated between specimens taken in- and out-of-competition, whereas females showed smaller DHEA values than males for both types of control. Also, a strong influence of the DHEA excretion on different sport disciplines was detected. The highest DHEA values were detected for game sports (soccer, basketball, handball, icehockey), followed by boxing and wrestling. The suggested Gaussian upper 99% reference limits for urinary DHEA calculated for the different sport disciplines showed in all cases higher values than the current cut-off-concentration of 100 ng/mL.

* For the quantitative determination of DHEA a one point calibration at 100 ng/mL employing [2,2,4,4-$^{2}H_4$]-etiocholanolone as internal standard was used.

In 2007, out of 13107 analyzed urine samples 24 specimens (0.2%) were submitted to IRMS, which contained more than 200 ng/mL DHEA. Comparable characteristics for those specimens to the suspicious DHEA population of 2006 were detected: 58% had specific gravity values higher than 1.020, 54% originated from soccer players, 96% were taken in competition, 75% were male urines, nearly all showing inconspicuous IRMS results. One urine sample was reported as AAF containing 377 ng/mL of DHEA, 7065 ng/mL of A and depleted carbon isotope ratio values for A and E.

Since the beginning of 2007, the DHEA metabolite 3α,5-cyclo-5α-androstan-6ß-ol-17-one (3α,5-cyclo) was implemented into the steroid profiling as an additional gas chromatography-mass spectrometry screening marker for DHEA abuse. Forty-two urine specimens showed concentrations of 3α,5-cyclo higher than the suggested threshold of 140 ng/mL, 2 urine samples yielded additionally DHEA concentrations higher than 200 ng/mL, one of them showing positive IRMS results as mentioned before.
The concentrations of 3α,5-cyclo were determined with 1200 ng/mL for the AAF detected in 2006, respectively 918 ng/mL for the AAF found in 2007.

In January 2008, DHEA misuse was detected in a urine specimen showing an elevated testosterone/epitestosterone ratio (~ 6.8). The steroid profile evaluation provided 192 ng/mL of DHEA, 99 ng/mL of 3α,5-cyclo and 7928 ng/mL of A, thus not indicating the urine suspicious for DHEA misuse. In general, after DHEA administration, depleted carbon isotope values of the target analytes are longer detectable than elevated concentrations of DHEA. However, in this “real” doping control sample the simultaneously application of different substances may be taken into account. Accordingly, the advantage in steroid profiling by monitoring multiple metabolites of the androgen pathway may cover the detection of the application of different prohibited (endogenous) steroids.

For an improved efficiency in detection of DHEA abuse an alteration of the threshold value for DHEA to 200 ng/mL and the addition of 3α,5-cyclo (proposed threshold value: 200 ng/mL) as secondary useful GC-MS screening marker is suggested. In any case, for the steroid profile evaluation the experienced scientist should include DHEA population based reference ranges.

For further details please refer to

Mareck *et al.* (2007) Detection of dehydroepiandrosterone misuse by means of gas chromatography-combustion-isotope ratio mass spectrometry. *Eur. J. Mass Spectrom.* **13**, 419-426.