

## PROJECT REVIEW

### “ $^{13}\text{C}/^{12}\text{C}$ -Analysis of Urinary Epitestosterone”

**W. Schänzer, T. Piper** (German Sport University, Cologne, Germany)

Epitestosterone is not an anabolic steroid but belongs to the WADA prohibited list of substances because of its potential to be used as a masking agent to lower the urinary testosterone to epitestosterone ratio. If this ratio exceeds 4, further investigations on the athletes' urine are followed up. So athletes try to lower their elevated T/E ratio after administration of testosterone or testosterone prohormones by the simultaneous intake of epitestosterone. Ready-for-use preparations are known from the former East German sports doping and are today back in the focus of interest due to the BALCO case. The preparation known as "The Cream" distributed by BALCO came out to be a composition of epitestosterone and testosterone. In order to detect such cases of doping, the WADA threshold for epitestosterone of 200ng/ml in urine is not sufficient. A more promising approach would be to distinguish between endogenous and exogenous steroid.

The method of choice in this situation is the measurement of the ratio of the two stable carbon isotopes  $^{12}\text{C}$  and  $^{13}\text{C}$  by gas chromatography / combustion / isotope ratio mass spectrometry (GC/C/IRMS). The method is employed routinely to discriminate endogenous from exogenous testosterone. It requires minimum amounts of carbon that are corresponding to about 10 to 100ng per compound. Unfortunately till now no method has been developed to clean up epitestosterone sufficient for GC/C/IRMS.

It is possible to measure the  $^{13}\text{C}/^{12}\text{C}$ -ratio of trace amounts of anabolic steroids, if a high grade of purification with a concomitant high recovery is achieved. On the one hand the aim of this project will be the development of a suitable method belonging to these requirements; on the other hand we need to set up a reference population to examine the natural occurring variance in  $^{13}\text{C}/^{12}\text{C}$ -ratios of epitestosterone to avoid false positive findings.

## **Determination of $^{13}\text{C}/^{12}\text{C}$ Ratios of Urinary EpiT and its Main Metabolites 5 $\alpha$ - and 5 $\beta$ -androsterone-3 $\alpha$ ,17 $\alpha$ -diol**

### **Results and Conclusions**

The presented method enables to detect the misuse of EpiT for a prolonged time and therefore complements previous approaches in both GC/C/IRMS and steroid profiling by means of GC/MS. The combination of both tools seems to be the most reliable way to detect a doping offence. As biological parameters always show a large variability, physiologically elevated ratios will sometimes be found. A consecutive investigation of these samples by GC/C/IRMS enables to elucidate whether a naturally elevation or a doping offence causes the suspicious finding.

As soon as more samples will have been investigated, the possible influence of gender on the urinary amounts of 17 $\alpha$ -OH-steroids might be elucidated. Further investigations on the related metabolism might then be helpful, too.

For doping control purposes, an excretion study with simultaneous administration of T and EpiT would be of interest to reveal the opportunities of the novel method to detect a doping offence in cases the T/EpiT ratio will not be elevated.