

Piper T<sup>1</sup>, Degenhardt K<sup>2</sup>, Federherr E<sup>3</sup>, Thomas A<sup>1</sup>, Saugy M<sup>2</sup>, Thevis M<sup>1</sup>

## **Effect of changes in the deuterium content of drinking water on the hydrogen isotope ratio of urinary steroids in the context of sports drug testing**

Center for Preventive Doping Research / Institute of Biochemistry, German Sport University Cologne, Cologne, Germany<sup>1</sup>; Swiss Laboratory for Doping Analysis, University Center of Legal Medicine, Geneva and Lausanne, Centre Hospitalier Universitaire Vaudois and University Lausanne, Epalinges, Switzerland<sup>2</sup>; Elementar Analysensysteme GmbH, Hanau, Germany<sup>3</sup>

### **Abstract**

The hydrogen isotope ratio (HIR) of body water and, therefore, of all endogenously synthesized compounds in humans, is mainly affected by the HIR of ingested drinking water. As a consequence, the entire organism and all of its synthesized substrates will reflect alterations in the isotope ratio of drinking water, which depends on the duration of exposure. To investigate the effect of this change on endogenous urinary steroids relevant to doping control analysis, the hydrogen isotope composition of potable water was enriched for 250 ‰ and maintained at this level for two weeks for two individuals. The steroids under investigation were 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol, 5 $\alpha$ -androst-16-en-3 $\alpha$ -ol, 3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one (ANDRO), 3 $\alpha$ -hydroxy-5 $\beta$ -androstane-17-one (ETIO), 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol, and 5 $\beta$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (excreted as glucuronides) and ETIO, ANDRO and 3 $\beta$ -hydroxyandrost-5-en-17-one (excreted as sulfates). The HIR of body water was estimated by determination of the HIR of total native urine, to trace the induced changes. The hydrogen in steroids is partly derived from the total amount of body water and cholesterol-enrichment could be calculated by use of these data. Although the sum of changes in the isotopic composition of body water was 150 ‰, shifts of approximately 30 ‰ were observed for urinary steroids. Parallel enrichment in their HIR was observed for most of the steroids, and none of the differences between the HIR of individual steroids was elevated beyond recently established thresholds. This finding is important to sports drug testing because it supports the intended use of this novel and complementary methodology even in cases where athletes have ingested water of different HIR, a plausible and, presumably, inevitable scenario while traveling.

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