

H. Geyer, U. Mareck, N. Haenelt, W. Schänzer

Atypical steroid profiles in connection with ethanol findings in urine

Institute of Biochemistry, Center for Preventive Doping Research, German Sport University, Cologne, Germany

Introduction

In 2008, the Cologne laboratory interpreted seven longitudinal studies (5 male, 2 female athletes) in which the following observations were made: In all of these studies, one testosterone/epitestosterone (T/E) value exceeded the range of T/E ratios normally observed in these athletes (see Tab1 and Fig. 1). Also the testosterone (T) concentration exceeded the normally determined levels in samples obtained from these athletes, and concentrations above 300 ng/mL were observed (see Tab 1). Additionally, extremely low ratios of androsterone/testosterone (AND/T) were found, which were far below the athletes' normal ranges (see Fig. 1). Although all these changes indicated an application of testosterone or testosterone prohormones, the results of the Carbon-Isotope-Ratio determination (CIR) gave no indication of an application of testosterone or related prohormones

Analysis for ethanol

50 µl of each suspicious urine sample were analysed by gas- chromatography (with headspace injection) for ethanol in the Institute of Forensic Medicine of the University of Cologne.

Steroid profile analysis and Carbon Isotope Ratio analysis (CIR)

For the steroid profile analyses and CIR analyses previously described methods were used (1-3).

Results and Discussion

The analysis of the suspicious samples demonstrated the presence of ethanol in all samples, indicating an application of ethanol before the out-of competition sample collection (see Tab. 1).

The observations of altered steroid profiles are in accordance with results of earlier studies, investigating the influence of ethanol on the steroid profile (4-6), but the changes of the steroid profiles observed in these seven cases were much more obvious than in the controlled studies. The mechanism behind these changes is not fully understood, but it is most probably connected with a competitive inhibition of enzymes involved in the phase I metabolism of testosterone. The competitive inhibition of e.g. 17 β -hydroxysteroid-dehydrogenases may lead to the observed high urinary testosterone and low androsterone and etiocholanolone concentrations.

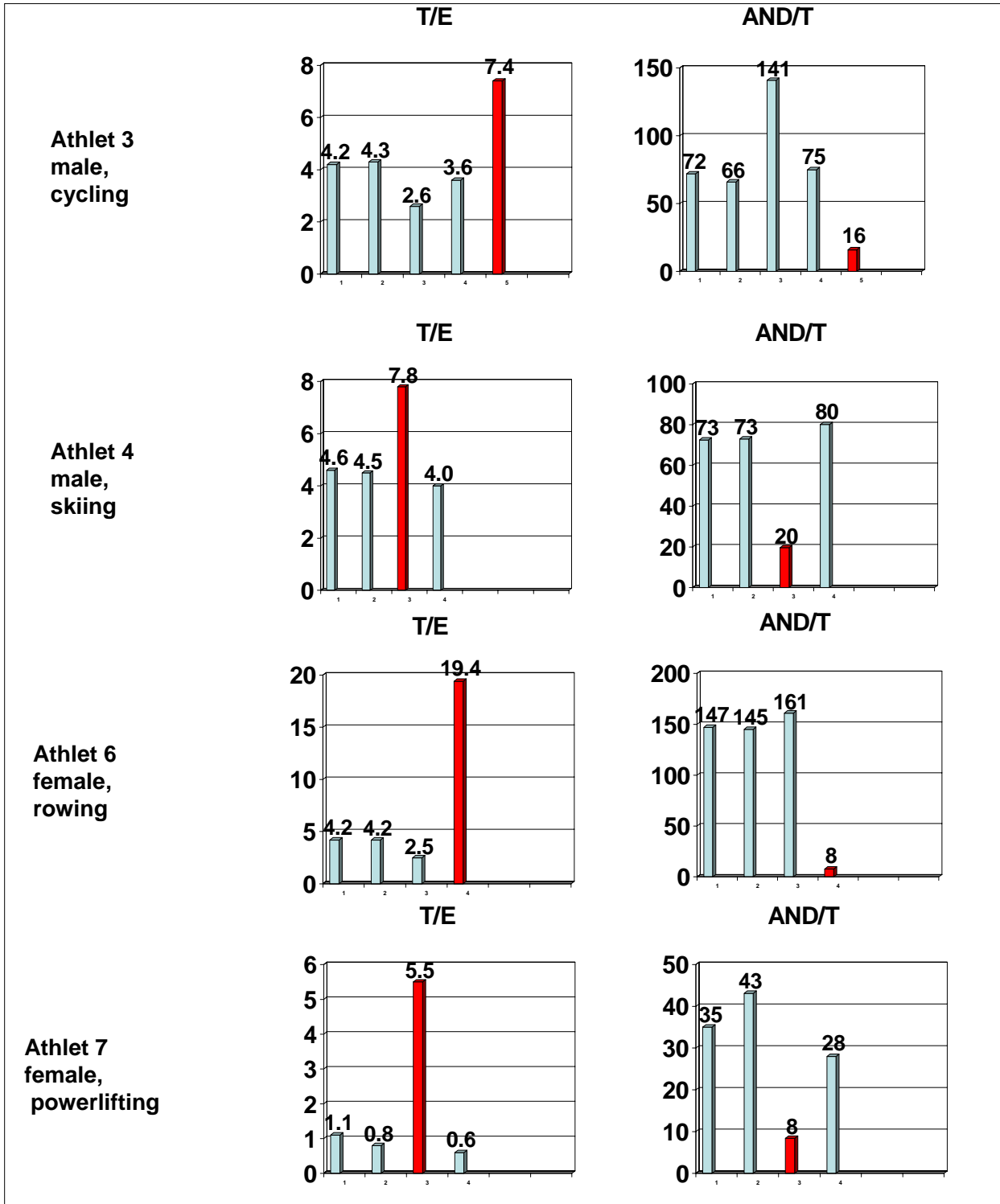
Conclusion

From the results it can be concluded that for some cases (abnormal T/E, abnormal AND/T and negative CIR) the analysis of urinary ethanol concentrations or other ethanol parameters (e.g. urinary ethylglucuronide) may be useful to prevent a misinterpretation of steroid profiles.

References

1. Geyer H., Schänzer W, Mareck-Engelke U, Nolteernsting E, Opfermann G. (1998) Screening procedure for anabolic steroids-The control of the hydrolysis with deuterated androsterone glucuronide and studies with direct hydrolysis. In: Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds.) Recent advances in doping analysis (5). Sport und Buch Strauß, Köln, 99-102
2. Mareck U, Thevis M, Guddat S, Gotzmann A, Bredehöft M, Geyer H, Schänzer W. (2004) Comprehensive sample preparation for anabolic steroids, glucocorticosteroids, beta-receptor blocking agents, selected anabolic androgenic steroids and buprenorphine in human urine. In: Schänzer W, Geyer H, Gotzmann A, Mareck U (eds.) Recent advances in doping analysis (12). Sport und Buch Strauß, 65-68
3. Piper T, Mareck U, Geyer H, Flenker U, Thevis M, Platen P, Schänzer W. (2008) Determination of ¹³C/¹²C ratios of endogenous urinary steroids: method validation, reference population and application to doping control purposes. Rapid Commun. Mass Spectrom. 22, 2161-2175
4. Falk O, Palonek E, Björkhem I (1988) Effect on the ratio between testosterone and epitestosterone in urine. Clin. Chem. 7 (1988) 1462-1464
5. Mareck-Engelke U, Geyer H, Schindler U, Flenker U, Iffland R, Donike M (1996) Influence of Ethanol on Steroid Profile Parameters. In: Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (Eds.) Recent advances in doping analysis (3) Sport und Buch Strauß, Köln, 143-156
6. Seppenwoolde-Waasdorp TJ, de Boer D, Van Engelen HM, Vrijdag AD, Maes RA (1996) Evaluation of endogenous steroid profiles in urine (2). Effects of ethanol intake reinvestigated. in: In: Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (Eds.) Recent advances in doping analysis (3) Sport und Buch Strauß, Köln, 157-165

Fig. 1: Urinary testosterone/ epitestosterone (T/E) and androsterone/testosterone (AND/T) ratios obtained from longitudinal studies of four athletes. The black bars represent values of samples, where additionally ethanol was detected. The detailed values of the samples represented by the black bars are presented in table 1



Tab 1: Steroid profiles of urine samples of seven athletes with atypical T/E ratios. All samples show negative CIR data and contain ethanol. (s.g. specific gravity, AND androsterone, ETIO etiocholanolone, E epitestosterone, T testosterone, ADIOL 5 α -androstane-3 α ,17 β -diol, BDIOL 5 β -androstane-3 α ,17 β -diol, CIR Carbon Isotope Ratio

Athlet	Sports	sex	s.g.	AND	ETIO	E	T	ADIOL	BDIOL	T/E *	AND/ETIO	AND/T	AND/E	ADIOL/ BDIOL	CIR data	ethanol
				[ng/ml]	[ng/ml]	[ng/ml]	[ng/ml]	[ng/ml]	[ng/ml]							[g/L]
1	boxing	male	1.019	1783	2895	25.1	167.7	120.3	578.5	6.6	0.6	10.6	71.0	0.2	negative	0.33
2		male		894	875	27.0	252.0			8.2	1.0	3.5	33.1		negative	0.30
3	cycling	male	1.029	1520	1488	10.9	95.2	75.5	411.9	7.4	1.0	16.0	139.4	0.2	negative	0.22
4	skiing	male	1.031	7776	4043	50.2	397.5	565.4	1239.8	7.8	1.9	19.6	154.9	0.5	negative	0.25
5	handball	male	1.020	672	808	12.6	87.6	88.9	335.1	6.9	0.8	7.7	53.3	0.3	negative	0.71
6	rowing	female	1.024	367	502	2.2	46.8	47.0	220.9	19.4	0.7	7.8	166.8	0.2	negative	0.80
7	powerlifting	female	1.011	254	610	5.5	30.2	16.6	145.4	5.5	0.4	8.4	46.2	0.1	negative	2.42