

Mareck U, Guddat S, Geyer H, Thevis M, Schänzer W

Urinary ethyl glucuronide in samples with elevated T/E ratios - evaluation of gender, sport discipline, type of control and concentration

Institute of Biochemistry, German Sport University, Cologne, Germany

Abstract

The elevation of testosterone/epitestosterone (T/E) ratios in urine following ethanol consumption is well known. A suitable indicator for ethanol application is the detection of ethyl glucuronide (ETG) in urine.

An evaluation of the frequency of increased T/E ratios in connection with ETG findings in different genders, different sports and different types of control (in competition, out of competition) was performed. Additionally the ETG concentration in the respective urine specimens was estimated.

713 doping control urine samples, which showed a T/E > 4 and which were tested negative by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) were analyzed for the presence of ETG with an LC-MS/MS method.

Out of the 713 doping control samples showing a T/E > 4 and negative GC/C/IRMS results, 105 samples (15%) contained ETG in a concentration greater than 0.5 µg/mL. Out of the 105 ETG containing samples 82 samples (78%) were collected out of competition (OOC), 86 samples (82%) originated from male athletes and 47 samples (45%) from endurance sports disciplines.

The evaluation of longitudinal studies in connection with these 105 ETG containing samples showed that the postulated threshold concentrations of ETG for the induction of the T/E increasing effects (48 µg/mL in male, respectively 15.5 µg/mL in female) are inappropriate and should be reconsidered.

Introduction

The potential influence of alcohol consumption on the urinary steroid profile is sufficiently known. An appropriate indicator for an ethanol application is the detection of ethyl glucuronide (ETG)[1]. Gender specific urinary ethyl glucuronide concentrations of 48 µg/mL (male) and 15.5 µg/mL (female) are proposed thresholds for a potential ethanol-induced suppression of steroids biotransformation [2]. Doping control urine samples showing elevated T/E ratios and ETG were evaluated regarding gender, sport discipline, type of control (in competition, out of competition) and ETG concentration [3].

Experimental

Between 2010 and 2012, steroid profiles of 44,687 doping control urine samples were determined by means of common procedures [4-6]. A total of 713 specimens (1.6%) showed a T/E > 4 and were tested negative by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS). The samples were analyzed for the presence of ETG by means of liquid chromatography-tandem mass spectrometry (LC-MS/MS)[3].

Results and Discussion

Out of the 713 specimens showing a T/E > 4 and negative GC/C/IRMS results, 105 samples (15%) contained ETG in a concentration greater than 0.5 µg/mL. Out of the 105 ETG containing samples 82 samples (78%) were collected out of competition (OOC), 86 samples (82%) originated from male athletes and 47 samples (45%) from endurance sports disciplines (Tab 1), mainly cycling (Tab 2).

	Incident	(%)
OOC	82	78
♂	86	82
Endurance sports disciplines	47	45

Table 1: Summary of main findings out of 713 specimens showing 105 (15%) ETG > 0.5 µg/mL (T/E > 4; IRMS neg)

Population	Sport discipline
Endurance	Cycling (29), canoe (8), rowing (5), skiing (1), skating (3), swimming (1)
Strength, martial arts	Weightlifting (4), tug of war (1), boxing (2), wrestling (1), karate (1), ju jitsu (1)
Team sports	Soccer (7), basketball (3), icehockey (6), volleyball (2), rugby (1), waterpolo (1), handball (1)
Misc	Athletics (13), equestrian (2), curling (1), gymnastic (1), sailing (1), dance sports (1), fencing (1), darts (4), bobsleigh (1), motor cycling (1)

Table 2: Summary of populations and corresponding sport disciplines

The evaluation of 34 longitudinal studies in connection with the 105 ETG containing samples showed 12 cases with triggering samples ($T/E > 4$) yielding ETG concentrations less than the postulated threshold values, as exemplified in Fig 1+2.

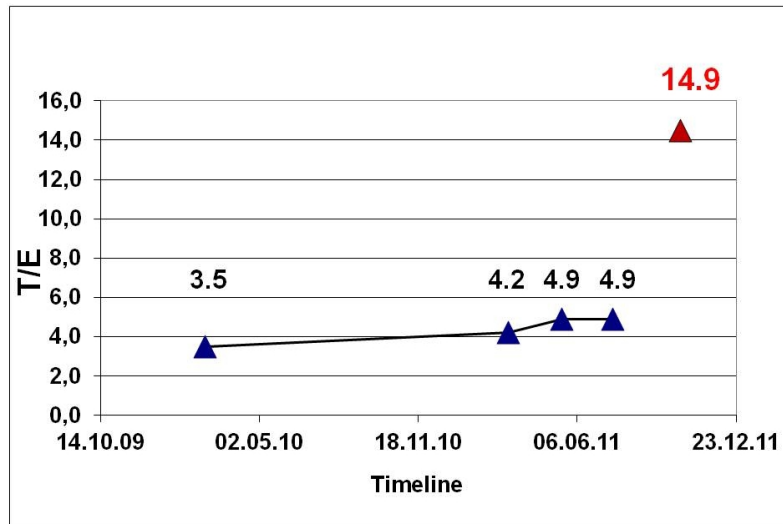


Figure 1: T/E longitudinal study (canoe / male) T/E 14.9: IRMS negative, ETG 41 µg/mL

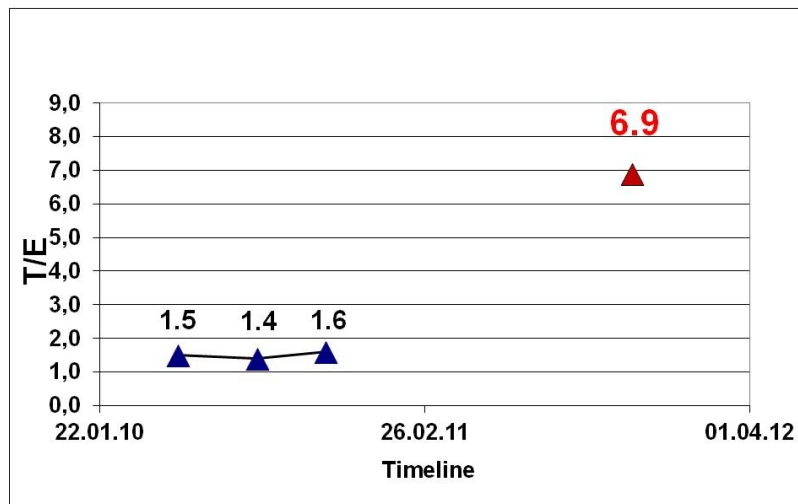


Figure 2: T/E longitudinal study (skating / female) T/E 6.9: IRMS negative, ETG 7 µg/mL

Conclusions

As 15% of the elevated T/E ratios are based on ethanol consumption, the determination of ETG in connection with steroid profiles is highly recommended.

The postulated threshold concentrations of ETG for the induction of the T/E increasing effects (48 µg/mL in male, respectively 15.5 µg/mL in female) are arguable too high and reconsideration is suggested.

References

- [1] Grosse J, Anielski P, Sachs H, Thieme D (2009) Ethylglucuronide as a potential marker for alcohol-induced elevation of urinary testosterone/epitestosterone ratios. *Drug Test. Analysis* **1**, 526-530.
- [2] Thieme D, Grosse J, Keller L, Graw M (2011) Urinary concentrations of ethyl glucuronide and ethyl sulfate as thresholds to determine potential ethanol-induced alteration of steroid profiles. *Drug Test. Analysis* **3**, 851-856.
- [3] Görgens C, Guddat S, Thomas A, Geyer H, Schänzer W (2011) Identification and quantitative determination of long-term alcohol markers ethylglucuronide and ethylsulfate in human urine by LC-MS/MS in doping control analysis. In: Schänzer W, Geyer H, Gotzmann A, Mareck U (eds.) *Recent Advances in Doping Analysis (19)*, Köln, pp. 309-312.
- [4] 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: *Recent Advances in Doping Analysis (12)*, Köln, pp. 65-68.
- [5] Geyer H, Schänzer W, Mareck-Engelke U, Nolteernsting E, Opfermann G (1997) Screening Procedure for Anabolic Steroids - The Control of the Hydrolysis with Deuterated Androsterone Glucuronide and Studies with Direct Hydrolysis. In: *Recent Advances in Doping Analysis (5)*, Köln, pp. 99-101.
- [6] Mareck U, Geyer H, Fußhöller G, Schwenke A, Haenelt N, Piper T, Thevis M, Schänzer W (2010) Reporting and managing elevated testosterone/epitestosterone ratios - Novel aspects after five years' experience. *Drug Test. Analysis* **2**, 637-642.

Acknowledgements

The authors wish to thank the Federal Ministry of the Interior of the Federal Republic of Germany and the Manfred Donike Institute (MDI) for support.