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RECENT ADVANCES IN DOPING ANALYSIS (3)

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Evaluation of endogenous steroid profiles in urine (2) effects of ethanol intake reinvestigated

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Abstract

The effect of heavy ethanol intake (combined with standardized food intake) on the endogenous steroid profile was investigated in both males and females. All subjects showed a significant increase in T/E ratio after the intake of 1 gram ethanol per kg body weight. Besides on the T/E ratio, an effect of ethanol on concentrations of other endogenous steroids in urine were observed, but these were not consistent in all subjects.

Introduction

A female swimmer, declared positive on testosterone administration claimed the increased T/E to be due to ethanol intake at the evening before the urine sample was taken. She twice performed studies to imitate this event, of which the samples were sent to the laboratory. The first time only four samples were taken, the second time ten. Both times the T/E increased, exceeding the IOC limit of 6.

The effect of ethanol on the T/E ratio has been investigated earlier by Falk *et al.* in 1988 [1]. They found that intake of less than 1.0 g/kg bodyweight has small or negligable effect on the T/E ratio. However, intake of 2.0 g/kg body weight increased the T/E ratio with 30 - 90 %. Their investigation was limited to only 4 male subjects and urine samples were taken at t=0, t=6, t=14 and t=22 h.

Since we did not witness the imitation studies of this female swimmer, we were not able to conclude whether her T/E ratio was raised by ethanol or testosterone. But it stimulated us to reinvestigate the effects of ethanol on endogenous steroids in general and on the T/E ratio in particular.

Experimental design

Twelve subjects (age category: 20 - 35 years) participated in the study, six females (weight 60.6 ± 7.6 kg) and six males (weight 81.3 ± 6.0 kg). A reference day preceded three experimental days. Respective blood, urine and saliva samples were taken following the time schedule as illustrated in Table 1. On day 2, the first experimental day, food intake was standardized for all subjects: At 13.00 h (about 7 hours before ethanol intake) all subjects received the same standard hot meal and at 18.00 h (about 2 hours before ethanol intake) they had a non-fatty bread meal, after which food consumption was no longer allowed until the next morning (about 8 hours after finishing drinking). The ethanol was administered as red wine (Beaujolais 1990, 13%) over 3 hours, one-sixth portion of a dose of 1 g/kg bodyweight per half hour.

Table 1: Time schedule of sampling

20.00 h	20.00 h	20.00 h	20.00 h	7.00 h
day 1	day 2	day 3	day 4	
reference day	experimental days			
12.00 u blood	12.00 blood	12.00 blood		
urine sampling at timed intervals				
	saliva during experiment			

20.15 - 23.15: ethanol intake
1/6 portion per half hour

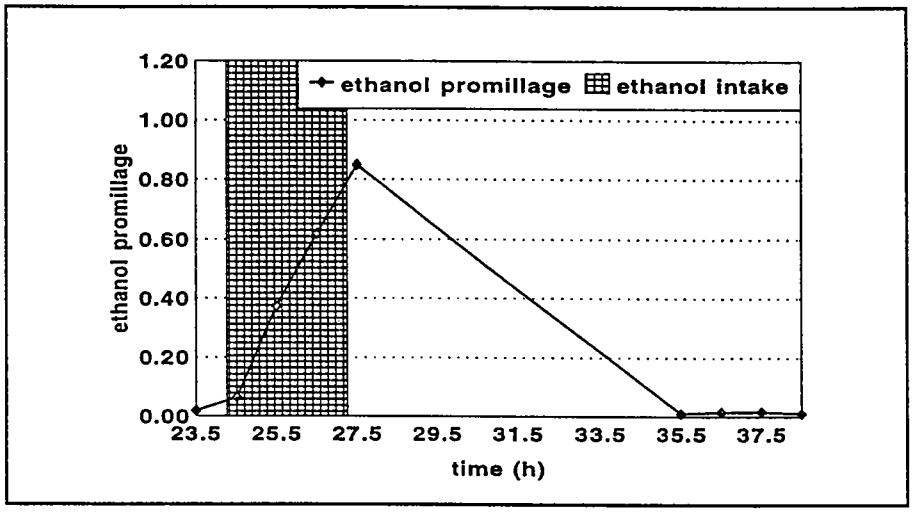
The respective saliva samples were analyzed for ethanol by GC/FID. The blood samples were analyzed by using immunoassay procedures for androstenedione (ADION), dehydroepiandrosterone-sulphate (DHEAS), testosterone (TESTO), dihydrotestosterone (DHT), sex hormone binding globuline (SHBG), luteinizing hormone and follicle stimulating hormone (FSH), respectively. The urine samples were analyzed by GC/MSD for testosterone (T), epitestosterone (E), androsterone (AO), etiocholanolone (EO), dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), 11β-hydroxy-androsterone (11β-OH-AO), 11β-hydroxy-etiocholanolone (11β-OH-EO), 5β-androstane-3α,17β-dione (5β-dione), 5α-androstane-3α,17β-diol (5α,3α-diol), 5α-androstane-3β,17β-diol (5α,3β-diol) and 5β-androstane-3α,17β-diol

(5β,3α-diol), respectively. All steroids were determined as their glucuronide-conjugates.

Results saliva samples

The intake of ethanol could obviously be followed by determining the ethanol promillage in the respective saliva samples, as illustrated in Figure 1.

Figure 1: The ethanol promillage in saliva samples of one subject



Results urine samples

Examples of the changes in testosterone and epitestosterone excretions and in the T/E ratio of one subject are given in Figure 2 and Figure 3, respectively.

Figure 2: Changes in the urinary excretions of testosterone and epitestosterone in one of the subjects.

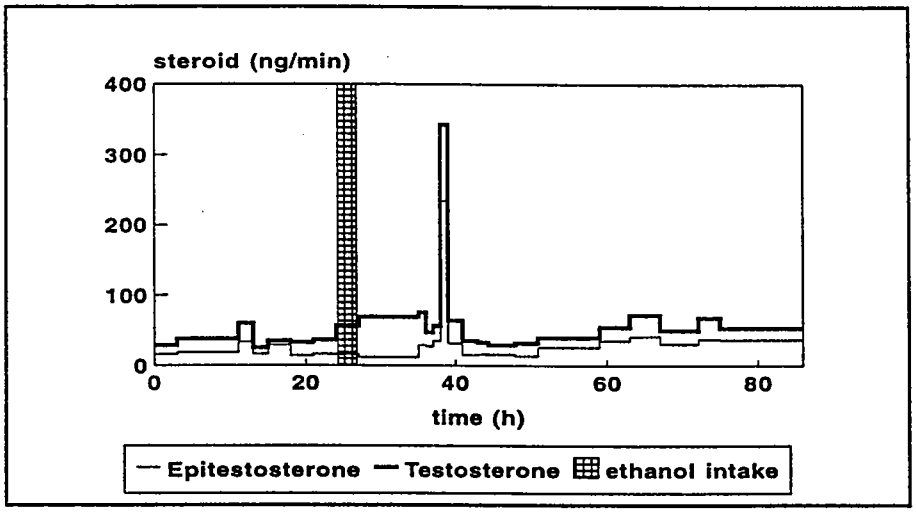
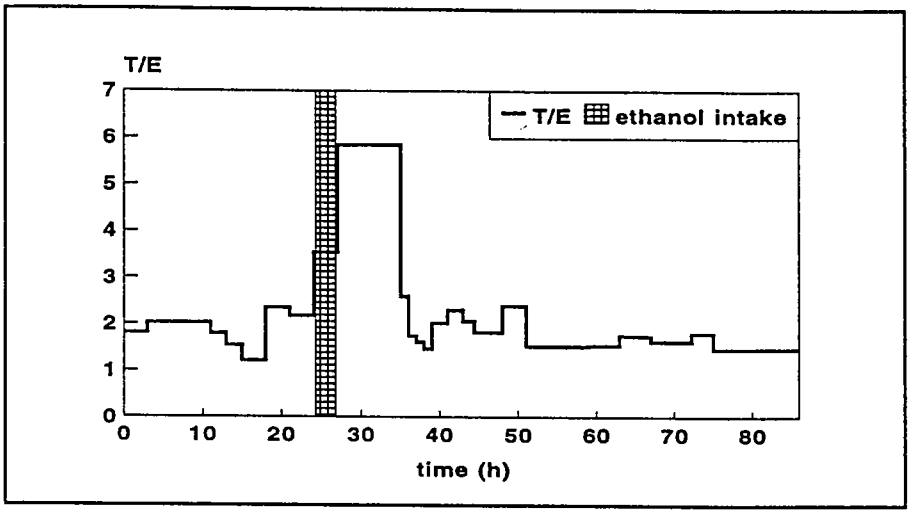


Figure 3: Changes in the T/E ratio in urine in one of the subjects.



The change in T/E ratio for all individual subjects is given Table 2.

Table 2: Changes in T/E ratio per subject after ethanol intake

subject	mean T/E ± S.D. without ethanol	highest T/E after ethanol intake	significant effect ?
F1 (T)	1.2 ± 0.51	3.1	YES
F2 (K)	1.3 ± 0.28	3.7	YES
F3 (A)	1.6 ± 0.20	4.5	YES
F4 (Mr)	0.3 ± 0.13	3.6	YES
F5 (Ms)	1.1 ± 0.11	2.0	YES
F6 (I)	1.0 ± 0.07	no data available	
M1 (D)	0.4 ± 0.03	1.1	YES
M2 (Er)	1.1 ± 0.11	1.6	YES
M3 (R)	1.6 ± 0.76	5.9	YES
M4 (Ed)	0.6 ± 0.04	1.0	YES
M5 (M)	1.0 ± 0.15	1.6	YES
M6 (K)	0.05 ± 0.01	0.09	YES

F1 - F6 = females
M1 - M6 = males

Although for all subjects the T/E ratio in urine increased significantly, it was not always caused by the same change in testosterone and/or epitestosterone excretions, which is shown in Table 3.

Table 3: Causes of increased T/E after ethanol intake per subject

subject	increase T/E	increase T	decrease E	decrease T	increase E
F1 (T)	YES *	YES *			YES NS
F2 (K)	YES *		YES *	YES NS	
F3 (A)	YES *	YES *			YES NS
F4 (Mr)	YES *	YES *			YES NS
F5 (Ms)	YES *	YES *			YES *
F6 (I)	no data available				
M1 (D)	YES *	YES *	YES NS		
M2 (Er)	YES *	YES *			YES NS
M3 (R)	YES *	YES NS	YES NS		
M4 (Ed)	YES *	YES NS	YES NS		
M5 (M)	YES *	YES NS	YES NS		
M6 (K)	YES *	YES *			YES NS

* = significant
NS = not significant
F1 - F6 = females
M1 - M6 = males

The effects of ethanol intake on the other endogenous steroids androsterone (AO), etiocholanolone (EO), dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), 11β-hydroxy-androsterone (11β-OH-AO), 11β-hydroxy-etiocholanolone (11β-OH-EO), 5β-androstane-3α,17β-dione (5β-dione), 5α-androstane-3α,17β-diol (5α,3α-diol), 5α-androstane-3β,17β-diol (5α,3β-diol) and 5β-androstane-3α,17β-diol (5β,3α-diol) varied per subject, which is shown in Table 4. Consequently, the effects of ethanol intake on some steroid ratios were not consistent, as demonstrated in Table 5.

Table 4: Effects of ethanol intake on some endogenous steroids for all subjects

Subjects	AO	EO	DHEA	DHT	11B-OH-AO	11B-OH-EO	5B-ADIONE	5a,3a DIOL	5a,3B-DIOL	5B,3a-DIOL
F1 (T)	-	-	-	-	-	-	x	-	-	-
F2 (K)	-	↓	↓	-	x	-	↑	↑	x	x
F3 (A)	↑	↑	NA	↑	NA	NA	↑	↑	x	↑
F4 (Mr)	-	-	-	↑	↑	-	-	↑	-	-
F5 (Ms)	-	↑	↑	-	↑	-	-	-	x	-
F6 (I)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
M1 (D)	↑	-	-	-	x	-	↑	↑	-	-
M2 (Er)	↓	↓	↓	-	-	↓	-	↑	x	↑
M3 (R)	-	-	↑	-	-	-	↑	-	x	x
M4 (Ed)	-	-	-	-	-	-	-	-	x	x
M5 (M)	-	-	-	↑	-	-	↑	-	x	x
M6 (K)	↑	-	-	-	↑	-	-	↑	↑	↑

Table 5: Effects of ethanol intake on some endogenous steroid ratios for all subjects

Subjects	AO/T	AO/E	EO/T	EO/E
F1 (T)	↓	↓	↓	↓
F2 (K)	↓	↓	↓	↓
F3 (A)	-	-	-	-
F4 (Mr)	-	↓	-	-
F5 (Ms)	-	-	-	-
F6 (I)	NA	NA	NA	NA
M1 (D)	↓	-	↓	-
M2 (Er)	↓	↓	↓	↓
M3 (R)	↓	↓	↓	↓
M4 (Ed)	↓	↓	↓	↓
M5 (M)	-	-	-	-
M6 (K)	-	-	↓	-

F1 - F6 = females
M1 - M6 = males
\- = no effect
↑ = increase
↓ = decrease
x = below detection limit
NA = not available

Results plasma samples

The plasma concentrations of all used parameters are indicated in Table 6 and Table 7, for the females and males respectively.

Table 6: Plasma concentrations in females

day 1: reference day
day 2: 13 hours after finishing ethanol intake
day 3: 37 hours after finishing ethanol intake

compound	DAY 1 mean ± S.D.(n=6)	DAY 2 mean ± S.D.(n=6)	DAY 3 mean ± S.D.(n=5)
ADION (nmol/l)	3.6 ± 1.5	3.3 ± 1.2	3.3 ± 1.3
DHEAS (µmol/l)	4.4 ± 1.7	4.6 ± 1.9	4.6 ± 1.6
LH (iu/l)	2.9 ± 4.6	2.7 ± 3.1	4.1 ± 3.8
FSH (iu/l)	5.7 ± 4.8	4.6 ± 4.0	5.1 ± 3.6
TESTO (nmol/l)	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3
DHT (nmol/l)	0.50 ± 0.21	0.51 ± 0.26	0.44 ± 0.18
SHBG (nmol/l)	138 ± 74		

Table 7: Plasma concentrations in males

day 1: reference day
day 2: 13 hours after finishing ethanol intake
day 3: 37 hours after finishing ethanol intake

compound	DAY 1 mean ± S.D.(n=6)	DAY 2 mean ± S.D.(n=6)	DAY 3 mean ± S.D.(n=6)
ADION (nmol/l)	3.5 ± 0.5	3.0 ± 0.6	2.9 ± 0.9
DHEAS (µmol/l)	8.7 ± 2.5	10.2 ± 2.4	8.6 ± 2.6
LH (iu/l)	3.9 ± 1.9	3.3 ± 1.4	5.4 ± 1.5
FSH (iu/l)	4.7 ± 1.8	4.8 ± 1.9	5.3 ± 1.6
TESTO (nmol/l)	20 ± 5	16 ± 5	18 ± 4
DHT (nmol/l)	2.3 ± 1.1	1.7 ± 0.6	2.0 ± 0.8
SHBG (nmol/l)	27 ± 10		

Discussion and conclusion

A female swimmer, declared to be positive on testosterone administration claimed that the increased T/E ratio in her urine was due tot ethanol intake. This problem rised to a re-investigation of the effects of ethanol on the endogenous steroid profile. The swimmer imitated twice the situation in which she was caught and both times the T/E increased, exceeding the IOC limit of 6. These findings were not supported by literature data, as Falk *et al.* described only a slight increase in the T/E ratio of four men after consumption of 2.0 g ethanol per kg body weight [1].

In this study, the effect of ethanol was studied in male and female subjects. It is known that the T/E ratio of women shows more variability than that of men [2]. Although Falk *et al.* describe that the amount of 1 gram ethanol per kg body weight has a negligable effect on the T/E ratio, we chose to study the effects at this amount of ethanol intake, as it is a more realistic situation than the intake of 2 gram ethanol per kg bodyweight. Additional to the effects of ethanol on testosterone, epitestosterone and the T/E ratio, we studied the total endogenous steroid profile in urine. In order to obtain a more controlled study, knowing the effects of food intake on the absorption of ethanol, the food intake of the subjects was standardized from 8 hours before till 8 hours after ethanol administration. Respective saliva samples were taken to get information about the blood ethanol concentration of the subjects and subsequent blood samples were taken to locate typical blood parameters which could be responsible for a change in urinary parameters.

For all subjects a significant increase in the T/E ratio was found. The average increase was higher in females (441% ± 426) than in males (216% ± 88). For the males, the increase in T/E was always caused by an increase in testosterone excretion, combined with either a decrease or a slight increase in epitestosterone excretion. For four females an increase in testosterone excretion was related with a decrease in epitestosterone levels, but in one female both testosterone and epitestosterone excretions decreased. Although all subjects showed an increased T/E ratio after the ethanol intake, their effect was not always related to the same change of the respective steroids concentrations. The observed effects on other urinary steroids or steroid ratios were not consistent.

No significant effects were found in the data obtained from the analysis of the respective blood samples. This is probably due to wrong time of sampling. Based on the results of Falk et al. the effect of ethanol was expected after 14 hours. The urine data in this study, however, showed a maximum increase in T/E between 15 minutes and 8 hours after ethanol intake. The

blood samples were taken 13 and 37 hours after ethanol administration, which appeared to be too late.

Saliva has shown to be an adequate specimen to follow the ethanol consumption. Also, differences in ethanol absorption were recognized as some subjects still had some ethanol in their saliva 8 hours after finishing ethanol intake, whereas most of them did not. These subjects were not used to consume large quantities of ethanol.

Although all cited effects are described to ethanol, a critical note must be made. Compared to literature data our results show a higher increase in T/E caused by ethanol (1.0 g/kg) intake with a maximum T/E 11 hours after first ethanol administration (or 8 hours after finishing). This may be explained by a more frequent sample collection and the standardized food intake.

In conclusion, ethanol can increase the T/E ratio. We therefore agree with Falk *et al.* that this phenomenon must be considered in cases where doping with testosterone is suspected. In our opinion, ethanol effects are more likely to be seen in 'out-of-competition' samples, as we still don't belief in the consumption of large quantities of ethanol before competition. Also, one should realize that effects were only noticed within 8 hours after finishing ethanol intake. Further research is required to determine the exact time of the most pronounced effect within this 8 hour interval. Additionally, it would be interesting to know if we can discriminate between ethanol intake and testosterone administration and therefore we recommend to check for ethanol in the required urine samples.

References

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