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Short communication

Increased urinary testosterone/epitestosterone ratios found in Swedish athletes in connection with a national control program Evaluation of 28 cases

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Abstract

In connection with a national anti-doping control program, including analysis of 8946 urine samples, 28 athletes were found to have delivered samples free from xenobiotic anabolic steroids but with an increased testosterone/epitestosterone (T/E) ratio (>6). Unannounced testing of the above athletes produced 2–4 additional urine samples during the next 2–3 months. A low degree of variation of the T/E ratio, with a C.V. below 30% was found in 17 of the subjects whereas 10 had a C.V. varying from 31% to 43%. One subject with a high urinary T/E ratio (10.5) had a C.V. of this ratio of 126% and also an extremely high ratio between testosterone and LH in urine. It has been reported that non-users of testosterone have T/E ratios fluctuating around a mean with a C.V. that will not exceed 30%. We found that administration of testosterone to seven healthy volunteers resulted in urinary T/E ratios that varied with a C.V. ranging from 67% to 130% during the following 4 weeks. It is concluded that among the above 28 cases, only one can be regarded as a clear case of testosterone doping. Although the vast majority of Swedish athletes have urinary T/E ratios below six, there is a subfraction with a constant higher ratio, possibly due to genetic factors.

Keywords: Testosterone; Epitestosterone; Steroids

1. Introduction

In 1982, the Medical Commission of the International Olympic Committee (IOC) decided that a testosterone (T)/epitestosterone (E) ratio in urine above six has to be considered as a positive doping test [1]. It was later shown, however, that there are subjects with elevated T/E ratios due to low urinary excretion of E. It was therefore recommended to conduct additional unannounced testing of athletes who have a T/E ratio between six and ten before considering legal action [2]. On the other hand, it is known that there are athletes who are using exogenous testosterone, yet their T/E ratios never exceed six.

In Sweden, since 1992, athletes found to have produced a urine sample devoid of xenobiotic anabolic steroids but with a T/E ratio above six have to deliver three additional urine samples within the next 2–3 months. The results of all tests are reported to the Swedish Doping Commission and an evaluation is made by the analytical, medical and legal experts in the commission. Among the 8946 tests performed, 28 samples were obtained that resulted in repeated testing and evaluation by the Commission. The results of these repeated tests are reported here.

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2. Experimental

The T/E ratio was determined with a method for screening of anabolic steroids by GC–MS [3]. A 2-ml volume of urine was extracted by using C_{18} Sep-Pak cartridge, deconjugated with β-glucoronidase from *E. coli* ((Boehringer Mannheim, Germany), extracted with butylmethyl ether and derivatised as trimethylsilyl derivatives. Since 1994 a modified method was used. This modification was proposed by Manfred Donike in 1994 and involves an extraction of free steroids before the enzymatic hydrolysis of the conjugated steroids and also an extraction with *n*-pentane instead of diethyl ether. These steps reduce interfering compounds, allowing a correct estimation of T and E. Deuterated T and E are used as internal standards. These standards are also used for correction of the T/E ratio (Fig. 1).

LH was determined in urine by immunoassay, using the DELFIA system. This method has recently been controlled and accepted in a program organised by the European Council. In this program several samples were sent out from the Drug Control Centre in London to seven IOC-accredited laboratories, including the Huddinge Laboratory.

For comparison, seven healthy volunteers received a single injection of 200 mg of testosterone enanthate [4]. Samples were collected over 30 days on 10 different occasions.

3. Results and discussion

The results are presented in Table 1. The mean T/E ratio in the population studied was found to be 7.0 and the mean coefficient of variation (C.V.) 42%. The T/E ratio means ranged from 4.2 to 12.4 whereas the C.V. ranged from 5 to 126%.

In carefully performed longitudinal studies, Hatton et al. [5] and Donike et al. [6] concluded that the T/E ratios normally fluctuate around the mean "plus or minus 30%" and "with a C.V. which will not exceed 30%". As shown in Table 1, 17 of the 28 subjects studied here had T/E ratios that varied with a C.V. less than 30%. It is noteworthy that one of these subjects had a T/E ratio above 10.0.

Ten of the subjects studied had T/E ratios that varied with a C.V. between 31 and 43% during the time interval studied (Table 1). One subject (subject 9) had a T/E ratio that varied extensively from 20 to 1 during the study. This corresponds to a C.V. of 126%.

Table 2 shows the T/E ratios in the testosterone enanthate administration study: their C.V. values varied from 67% to 130%.

From the above information it may be concluded that the 17 subjects with T/E ratios varying with a C.V. less than 30% are not likely to be testosterone users. The subject with the highly variable T/E ratio (subject 9 in Table 1) has most probably administered testosterone. The ten cases with T/E ratios varying with a C.V. between 31% and 43% may be regarded to be in a grey area. It should be pointed out, however, that the subjects studied by Donike with T/E ratios fluctuating around a mean with a C.V. less than 30% all had relatively low T/E ratios. The imprecision is likely to increase with decreasing levels of E and thus higher variations can be expected when measuring high T/E ratios. In view of this, it seems likely that C.V. values between 31% and 43% are possible also in non-testosterone users if the T/E ratios are high due to genetic reasons.

The ratio between T and LH may give valuable information in unclear cases of testosterone doping [7]. Administration of testosterone increases the T/LH ratio dramatically and it has been suggested that this ratio exceeds 340 in cases of testosterone doping [7].

As expected the subject believed to be a testosterone user (subject 9 in Table 1) had a very high T/LH ratio, clearly exceeding 340. One of the subjects with a constantly high T/E ratio, believed to be a non-testosterone user (subject 14) also had a high T/LH ratio close to 340. This high T/LH ratio was however remarkably constant with a very low C.V. All the other cases had T/LH ratios below 340.

4. Conclusion

To summarise, only one of the 28 subjects investigated here is likely to be a testosterone-user. At the present state of knowledge neither the T/E ratios nor the T/LH ratios would allow us to take legal action against any of the subjects in the grey area. If more thorough investigations had been possible, including

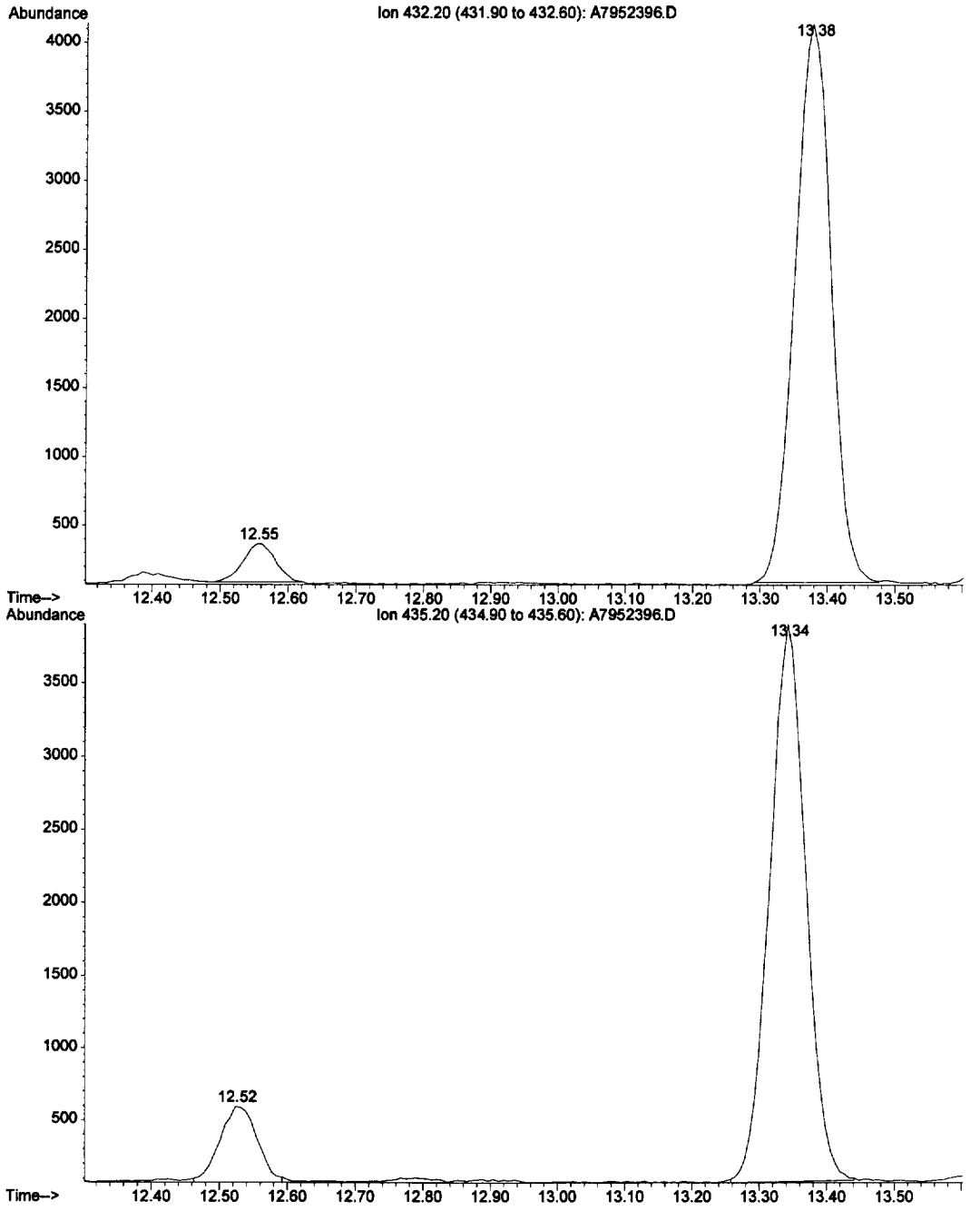

Fig. 1. Chromatogram (upper) from a urine sample with elevated T/E ratio (11.9) showing ion 432 of the TMS-derivative of epitestosterone (t_R = 12.55) and testosterone (t_R = 13.38). Chromatogram (lower) showing ion 435 of the TMS-derivative of D3-epitestosterone (t_R = 12.52) and D3-testosterone (t_R = 13.34).

Table 1
Biochemical parameters measured in the 28 subjects

Subject	Number of samples	T/E			T/LH		
		Mean	S.D.	C.V.(%)	Mean (nmol/U)	S.D. (nmol/U)	C.V.(%)
1	4	12.4	4.4	36	53	42	79
2	3	9.0	0.4	5	139	179	128
3	4	8.8	0.8	9	65	25	39
4	4	6.3	1.0	17	96	36	38
5	4	10.0	1.4	14	92	16	18
6	4	5.5	0.9	16	41	12	29
7	3	7.5	0.7	9	59	24	41
8	5	8.2	0.8	10	67	38	57
9	3	8.3	10.5	126	493	847	172
10	4	7.0	1.7	24	70	25	36
11	4	5.7	2.2	38	48	28	58
12	4	9.8	1.2	12	53	35	66
13	4	8.6	2.3	26	37	6	16
14	5	5.6	1.3	28	333	32	10
15	4	4.8	1.6	33	117	97	83
16	4	6.1	1.3	21	73	48	66
17	4	6.0	1.4	24	55	24	43
18	4	4.5	1.8	41	54	37	68
19	4	5.7	2.2	38	48	28	58
20	3	7.7	2.4	31	58	42	72
21	5	4.7	1.9	39	155	100	64
22	3	5.5	2.0	37	91	55	61
23	4	4.2	1.6	38	60	43	71
24	4	6.6	1.5	22	61	29	48
25	4	6.5	1.4	21	81	79	97
26	4	4.9	2.1	43	69	41	60
27	4	10.3	2.5	24	116	46	40
28	3	9.9	1.8	18	18	14	81

the ketoconazol test [8] and measuring the ratio between T and 17-hydroxyprogesterone in serum [6], the situation might have been different.

Table 2
T/E in urine from 7 volunteers after administration of testosterone enanthate

Subject	Number of samples	T/E		C.V. (%)
		(Mean	±S.D.)	
1	10	3.1	2.1	67
2	10	2.2	2.1	94
3	10	32.2	38.0	118
4	10	0.7	0.9	130
5	10	26.3	29.9	114
6	10	6.1	6.1	101
7	10	7.8	8.6	109

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