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Effect of metandienone abuse on the urinary steroid profile in body builders

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1. Abstract

Urinary steroid profiles have been determined in recreational body builders, self-administering anabolic steroids, who were subjected to a routine health assessment at the Institute's of Sport outpatient clinic. The subjects were classified according to their current pattern of anabolic intake: "on-cycle" (3 - 6 weeks) or "off-cycle" (a 3 - 4 week intermission in taking drugs). According to data obtained at interviews, the subjects used testosterone, metandienone, 19-nortestosterone and metenolone. The subjects used steroids at dosages varied from 3.3 up to 30-fold greater than the therapeutic ones. Steroid profiles were determined according to the protocol proposed by Donike with deuterised internal standards mixture, on a HP5970B GC-MS device equipped with 12,5 m HP-1 column. Luteinizing hormone (LH) levels were determined by using Serono Serozyme 1 assay kits. High concentrations of testosterone and low concentrations of epitestosterone, as well as elevated T/LH ratios, were detected in all subjects taking high doses of testosterone and metandienone. On the other hand , the subjects taking only high doses of metandienone exhibited very low levels of both testosterone and epitestosterone, with the T/E ratio being significantly higher than 6. The concentrations of androsterone and etiocholanolone were below normal levels. The results presented showed that high doses of metandienone could elevate the T/E ratio due probably to an unbalanced suppression of synthesis of epitestosterone and testosterone.

2. Introduction

Determination of the T/Et ratio has been one of the most important elements of antidoping analysis since 1982, when it was introduced as an indicator of synthetic testosterone abuse. Its value exceeding 6.0 has been accepted as a proof of ingestion of a testosterone-containing drugs. In some cases, however, this has been disputed due to sometimes naturally elevated T/Et ratio, which calls for running additional tests. It is obvious that an elevated T/Et ratio results not only from an increased concentration of testosterone but also from a low concentration of epitestosterone, caused by a congenital defect of its synthesis or elimination with urine [2].

The synthesis and metabolism of epistestosterone have not been yet fully investigated, although it is known that it takes place in both testicles and adrenal cortex [2] and the conversion of exogenous testosterone into epitestosterone being negligible [2].

Studies conducted by Donike and coworkes have shown that a long-term abuse of anabolic androgenic steroids (AAS) disturbs the steroid profile which can be expressed primarily as a decrease of concentration of steroid metabolites in urine. The authors suggested that the concentrations of testosterone metabolites in the urine of normal subjects should not be lower than 766.4 ng/ml for androsterone (A), 700.9 ng/ml for etiocholanolone (E), 8.2 ng/ml for epitestosterone (Et) and 0.45 for the A/E ratio [2,3].

De laTorre and others reported that a prolonged stanazolol abuse led to a significantly decreased excretion of androsterone and etiocholanolone in urine as much as to a 8 - 10-fold decrease of urinary testosterone and even greater (20 - 30-fold) decrease of epitestosterone. This could induced an elevation of T/Et ratio in the subjects. On the other hand, no such effect has been observed after metenolone used[1].

Our previous results indicated that elevated T/Et ratios could be caused, in same cases, not from an increased excretion of testosterone but rather a low concentration of epistestosterone. Table 1 presents the results of analyses of samples B in 8 athletes, who showed the T/Et ratio above 6 in samples A. In 6 of the eight subjects the concentrations of epitestosterone was found to be below 8.2 ng/ml.

The aim of the study was, therefore to investigate possible changes in urinary steroid profiles in subjects taking AAS, and metandienone in particular, for a long time. Further, we intended to examine whether metandienone can lead to considerable abnormalities of urinary steroid profiles reflected by an elevated T/Et ratio (above 6).

2.0. Material and Methods

2.1. Urine samples were obtained anonymously from 10 bodybuilders routinely examined medically at the Sports Medicine Outpatient Clinic of the Institute of Sport. All of them were long-term anabolic steroid abusers (up to 12 years). The subjects were assigned to two groups according to their actual status of anabolic intake: "on cycle" (3-6 weeks) - 4 persons (age: 24 ±4,8, body mass 87,8 ±12,6, height 175,6 ±4,9, time of abuse 2,5 ±1,2 years) and "off cycle" (3-4 weeks of break in taking drugs - 6 persons (age 30,4 ±7,5, body mass 89,6 ±1,9, height 172,8 ±6,5, time of abuse 4 ±4,3 years). According to data obtained at interviews, the subjects used testosterone, metandienone, 19-nortestosterone and metenolone. These steroids were used at dosages 3.3 to 30-fold greater than the therapeutic ones.

2.2. Reference steroids

Testosterone (T) and epitestosterone (Et) were purchased from Steraloids (Wilton, N.H., USA), methyltestosterone from Fluka, the mixture of deuterised (D3) etiocholanolone, testosterone, epitestosterone and 11β-hydroxyandrosterone was donated by Prof. M. Donike, Cologne.

2.3. Materials

Methanol A.R. (POCh, Poland); diethylether A.R. (Lab-Scan, Ireland), redistilled before use over calcium hydride (Merck); MSTFA (Macherey Nagel, Germany); β-glucuronidase ex e. coli from Boehringer, Mannheim, (Germany); TMS-imidazole (Pierce, USA); TMS-J and dithioerithritol (Aldrich, USA); XAD-2 resin (Serva, Germany); EIA kits (SEROZYME) for LH determination were supplied by Serono (U.K.).

2.4. Sample preparation

Urine samples were processed according to routine procedure used in the laboratory for the analysis of conjugated and free fractions of steroids (Donike et al.. [2]). Urine samples were absorbed with XAD-2 resin, washed with methanol, water and eluted with methanol. The dried residue was redissolved in phosphate buffer (pH 7), followed by hydrolysis with β-glucuronidase (E. coli) at 50°C for 1 hour. After adjustment of pH to 9, free steroids were extracted with 5 ml of diethyl ether. The solvent was removed under the stream of nitrogen and the residue dissolved in silylation mixture MSTFA/TMSJ following heating for 15 min at 60°C.

2.5. GC/MSD analysis

The analysis was performed with 5970B MSD device coupled with II HP 5890GC and 7673 autosampler. HP-1.12 m, 0,2 mm i.d., 033 μm film thickness column was used for separation. The injector operated in split mode 1:10. The oven temperature program was as follows: initial temperature 180°C maintained for 0.1 min. then increased at a rate of 2°C/min up to 230°C, then at a rate of 15°C/min up to 280°C and maintained for 5 min. Mass spectra were obtained in SIM mode.

2.6. LH determination

Urinary LH was determined with SEROZYME assay kits (Serono, U.K.) Assay calibration was performed according to manufacturer's instruction; three quality control samples were included in each run.

3. Results and Discussion

Basic components of urinary steroid profiles (testosterone, epitestosterone, androsterone, etiocholanolone, T/Et and A/E ratios and LH) of 4 subjects taking different AAS drugs for a period from 3 to 5 weeks are presented in Table 2. All subjects took metandienone in doses varied between 385 and 980 mg. In the group of subjects a very low concentrations of epitestosterone were found (0.06 - 1.4 ng/ml) which resulted in elevated T/Et values. In one case a high testosterone concentration (219.5 ng/ml) was detected. That particular subject admitted the use of Omnadren 250 (2750 mg during four weeks) besides metandienone and metenolone. In that case, the T/Et and T/LH ratios was markedly elevated (157 and 732, respectively). The concentrations of androsterone and etiocholanolone were within normal ranges. In subject No. 3, testosterone concentrations was normal (51 ng/ml) but

epitestosterone was very low (0.06 ng/ml) which resulted in a unbelievable high T/Et = 883 (due to only mathematical calculation). In three other cases, when testosterone was not administered, very low concentrations androsterone and etiocholanolone were detected.. It is worth mentioning that A/E ratio was in all cases within normal values (over 0.45).

The components of steroid profiles in the same subjects after an intermission of 3 to 10 weeks in taking AAS are presented in Table 2a. It should be emphasised that after 10 weeks of abstaining from taking AAS, both epitestosterone and T/Et returned to normal values and only in subject No. 3 the epitestosterone concentration exceeded 8.2 ng/ml. A break in taking metandienone, lasting as long as 8.5 weeks, did not normalise the steroid profile. In all these cases urine analyses for metandienone metabolites were negative.

Table 3 illustrates the effect of metandienone on the concentration of epitestosterone and T/Et ratio in three subjects while taking the drug after 3 - 4 weeks of intermission. In two subjects, who took AAS for 3 and 4 years, in cycles of 2 and 5 weeks, respectively, the epitestosterone concentrations were remarkably decreased and the T/Et ratio increased to 91 and 12.5. Androsterone and etiocholanolone levels were very low. After an intermission (3 and 4 weeks, respectively), a slow recovery regarding the steroid profile components was observed. In the third subject (a first time user), metandienone resulted in a 3-fold decrease in the testosterone concentration and a 8-fold decease in epitestosterone. The unbalanced changes in concentration of testosterone and epitestosterone caused a 3-fold increased T/Et ratio. In this subject a fast recovery to normal values testosterone and epitestosterone was observed.

Table 4 presents the steroid profiles of subjects being "off cycle". In this group, only two out of 6 subjects showed concentrations of epitestosterone greater than 8.2. Even a two year break in taking drugs, following 12 years of continuous use of metandienone and other SAA (case No. 6), could not bring the testosterone concentration (3.7 ng/ml) back to normal. The epitestosterone level, however was found to be normal after the cessation of the drugs used.

The results of the investigations suggest that the decreased level of epitestosterone and corresponding high value of T/Et ratio, could probably be induced by the intake of metandienone. This finding is in agreement with data previously published by Donike et al. [2] who reported that metandienone intake could suppress the epitestosterone synthesis as well as the testosterone and its main metabolites. It should be stressed however, that in same cases the suppression of epitestosterone is not equal to suppression of testosterone, what in result can cause an elevation of the T/Et ratio.

Normalisation of the steroid profile after the cessation of metandienone intake is rather slow process and not going parallelly with testosterone and epitestosterone. Epitestosterone usually, normalises slower than testosterone and its metabolites (especially, in chronic abusers). These processes lasted much longer than disappearance of metandienone metabolites in urine.

The results of this study also suggests that for a proper assessment of an elevated urinary T/Et ratio, quantitative data of testosterone, epitestosterone, as well as of androsterone, etiocholanolone and LH are indispensable. Low concentrates of testosterone and even lower concentrations of epitestosterone, as well as of androsterone and etiocholanolone may constitute indices of a prolonged used of metandienone. In contrast to subjects taking synthetic testosterone the use of metandienone could decreased LH levels in urine but the T/LH ratio would remain low. In any case, proper conclusion should be support by further long-lasting investigations on individual basis.

References

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Table 1. Results of analyses of samples B of athletes in whose samples A the T/Et values exceeded 6.0

Subject No.	Age	T/Et	[T]	[Et]	[A]	[E]	A/E
1.	20	7.8	138.3	**19.4**	996	169	0.6
2.	23	9.4	108.0	**12.8**	1733	2769	0.6
3.	21	8.2	63.6	**7.8**	3011	2463	1.2
4.	20	9.3	47.4	**5.0**	1445	1477	1.0
5.	18	6.5	24.6	**3.8**	646	619	1.1
6.	19	11.8	41.6	**3.6**	2593	2998	0.9
7.	19	9.5	21.9	**2.3**	1562	1299	1.2
8.	18	10.4	16.4	**1.6**	405	351	1.1

Legend: **[T]** - Testosterone; **[Et]** - Epitestosterone; **[A]** - Androsterone; **[E]** - Etiocholanolone. All concentrations in ng/ml (means of three analyses each)

Table 2. Urinary steroid profiles in body builders

- subjects „on cycle"

Subject No.	Weeks on cycle	Drugs	Dose (mg)	[T] ng/ml	[Et] ng/ml	T/Et	[A] ng/ml	[E] ng/ml	A/E	LH mU/ml	T/LH
2	4	**metandienone** testosterone metenolone	**980** 2750 500	219.5	**1.4**	157	3862	3225	1.2	0.3	731.6
3	4	**metandienone** nandrolone methenolone	**630** 400 1100	51.0	**0.06**	883	457	213	2.2	0.2	255.0
9	3	**metandienone** metenolone nandrolone	**630** 1100 400	6.6	**0.2**	33	240	64	3.75	0.8	8.2
4	5	**metandienone**	**385**	3.7	**0.3**	12	234	347	0.67	0.2	18.5

Table 2a. Urinary steroid profiles in same body builders

- persons „off cycle"

Subject No	Weeks off taking drugs	[T] ng/ml	[Et] ng/ml	T/Et	[A] ng/ml	[E] ng/ml	A/E
2.	3	14.6	**0.6**	24.3	646	962	0.7
3.	10	72.6	**15.4**	4.3	2346	1983	1.3
9.	10	12.2	**3.4**	3.6	598	407	1.5
4.	8.5	1.7	**0.2**	8.5	115	120	1.0

Table 3. Influence of metandienone intake on urinary concentrations of epitestosterone and other androgenes

Subject No	Abuse time (in years)	Metandienone intake		[T] ng/ml	[Et] ng/ml	T/Et	[A] ng/ml	[E] ng/ml	A/E
		weeks	dose-mg						
1.	3	2 on*	315	36.7	**0.4**	91.7	683	827	0.8
1.a		4 off		25.8	**4.7**	5.8	735	2080	0.35
4.	4	5 on	385	3.7	**0.3**	12.3	234	347	0.67
4.a		3 off		1.7	**0.2**	8.5	115	120	1.0
7.	first cycle	1 on	90	36.8	**38.1**	0.9	1686	2011	0.83
7.a		6 on	840	11.5	**4.3**	2.6	726	730	1.0
7.b		4 off		20.3	**22.1**	0..9	1244	1271	1.0

* also metenolone - 100 mg

Table 4. Urinary steroid profiles of body builders

- persons „off cycle"

Subject No.	Time off drugs in weeks	Drugs taken - mg/cycle		[T] ng/ml	[Et] ng/ml	T/Et	[A] ng/ml	[E] ng/ml	A/E	LH mIU/ml	T/LH
1.	4	**metandienone** metenolone testosterone	600 240 500	25.8	**4.7**	5.8	735	2080	0.3	1.5	17.2
8.	24	**metandienone**	840	30.6	**12.8**	2.6	1431	1771	0.8	3.2	9.6
10.	3	**metandienone** methenolone testosterone	3000 1260 1260	14.3	**0.8**	17.9	510	889	0.5	0.2	71.5
11.	12	**metandienone** testosterone nandrolone	300 1820 2750	19.2	**6.8**	2.7	1854	1726	1.1	2.0	9.6
12.	3	**metandienone** nandrolone	840 900	17.3	**7.8**	2.3	635	1666	0.4	4.4	5.1
6.	104	**metandienone** metenolone testosterone stanozolol clostebole	504 2520 2400 2000 1200	3.7	**13.6**	0.3	699	899	0.7	3.4	1.1