ZHANG Lisi, ZHANG Yinong, XIE Minhao[1], LIU Xin, WANG Jingzhu, YAN Yi[1], WANG Xintang[1], LI Yixuan[1]， WU Moutian*

Excretion Study with Different Preparations of 1-Testosterone**

China Doping Control Center, Beijing, China

[1]Sports Science College, Beijing Sport University, Beijing, China

INTRODUCTION

1-Testosterone, 17β-hydroxy-androst-1-en-3-one, is banned by The International Standard Prohibited List[1]. It has the identical molecular weight and molecular formula as testosterone. The only difference in their chemical structures between testosterone and 1-testosterone is the position of the double bond (Fig. 1). It was reported that 1-testosterone is a very potent

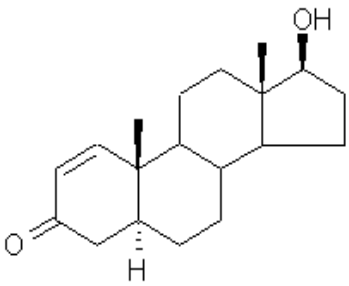

Fig. 1 Chemical structure of 1-testosterone

adrogen which binds selectively to the androgen receptor and transactivates androgen receptor dependent reporter genes. It has the same potency to stimulate the growth of the prostate, the seminal vesicles and the androgen sensitive levator ani muscle as testosterone[2].
1-Testosterone is now widely offered as a sport nutritional supplement. This paper presents the results of excretion study with different preparations of 1-testosterone.

SUBJECTS AND METHODS

Reagents

All chemical reagents used for analytical purpose were of Analytical Grade.

Instrumentation and Working Condition

GC/MS: Agilent 6890A/HP5973. The column used was a HP-1 17 m (0.2 mm i.d., 0.11

Before starting this study ethical committee application, study protocol had been prepared and approved by the local ethic committee in Beijing.

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μm film-thickness) with a head pressure of 91 kPa. The oven temperature program was: initial temperature: 180°C (0 min), +3.3°C /min → 231 °C, +30°C /min → 310°C (2 min).

The injector and the transfer line were held at 280°C. The split ratio was 10:1. Electron ionization with 70 eV was used. Scans were acquired from 50 to 500 amu with 0.25 sec/scan.

Excretion Study

The three nutritional supplements with 1-testosterone used in the study were: 5α-androst-1-ene-3-one-beta-undecanoate (UND) from Chemi-Sport Laboratories，USA, 1-2 softgels/time, 2-3 times daily, 50mg/softgel; 17β-hydroxyandrost-1-ene-3-one Tetrahydro -pyranyl ether (THP) from Molecular Nutrition, USA，1-2 caps./time, 2-3 times daily with meals, 25mg/cap; and 17β-hydroxy-5a-androst-1-ene-3-one Cypionate ether (CYP) with oral dispenser consume, from Vital Pharmaceuticals, Inc. USA, 1 to 3 cc's per day, 50mg/cc.

These three preparations were administered to 10 volunteers (5 males and 5 females) with the minimum dosage as manufactures' recommendations. Urine samples obtained from all

Tab. 1 Information about the volunteers

ID	Gender	Age	Weight (kg)	Dosage (mg)		
				UND	THP	CYP
01	Female	24	47	50	25	50
02	Female	29	62	50	25	50
03	Female	27	55	50	25	50
04	Male	37	80	50	25	50
05	Male	26	75	50	25	50
06	Male	22	72	50	25	50
07	Male	23	77.5	50	25	50
08	Female	30	55	50	25	50
09	Male	35	80	50	25	50
10	Female	27	47	50	25	50
	Mean±SD	28±4.9	65±13.4	50±0	25±0	50±0

volunteers were tested before administration to confirm the normal endocrine steroids profile.

A period of one month for clearance to normal steroids profile had been taken between the administrations with different preparations. All urine samples were collected for the then only in the morning for the further two days.

The following possible metabolites (Fig. 2) proposed by our previous study were monitored with routine procedure IV for total fraction. Among them Met.2 and Met.3 had been proved by synthesized standard, but Met.1 had not yet [3]

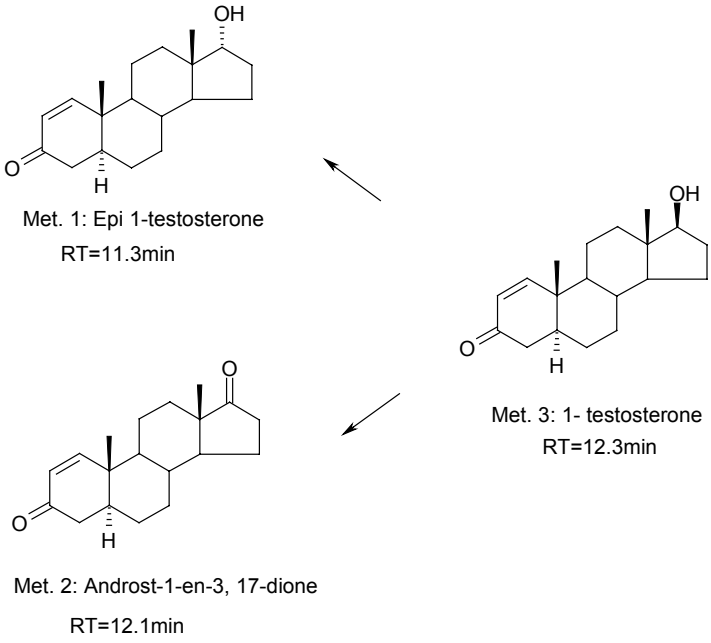
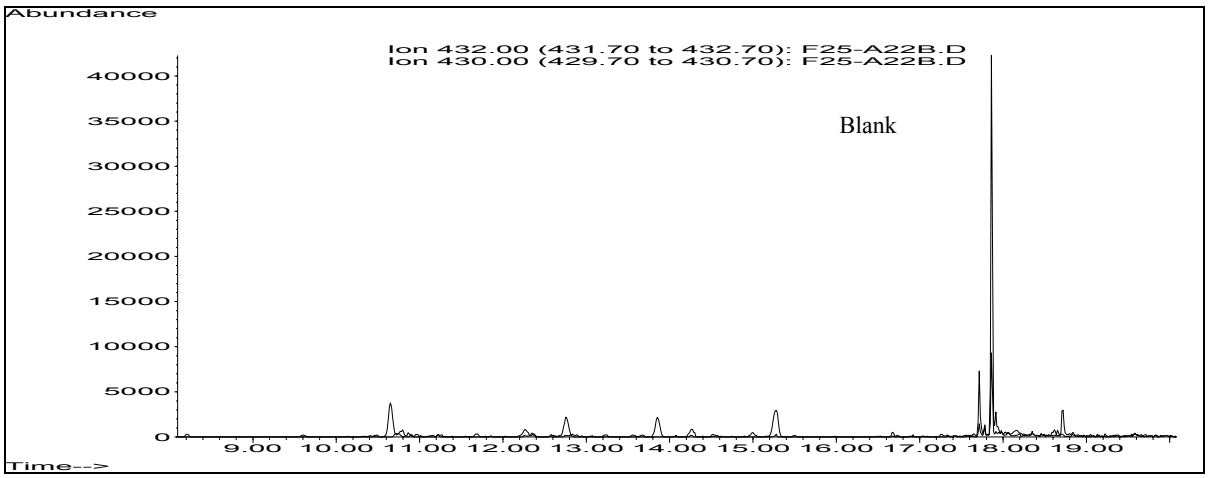


Fig. 2 Metabolites of 1-testosterone in human urine

RESULTS AND DISCUSSION

Results



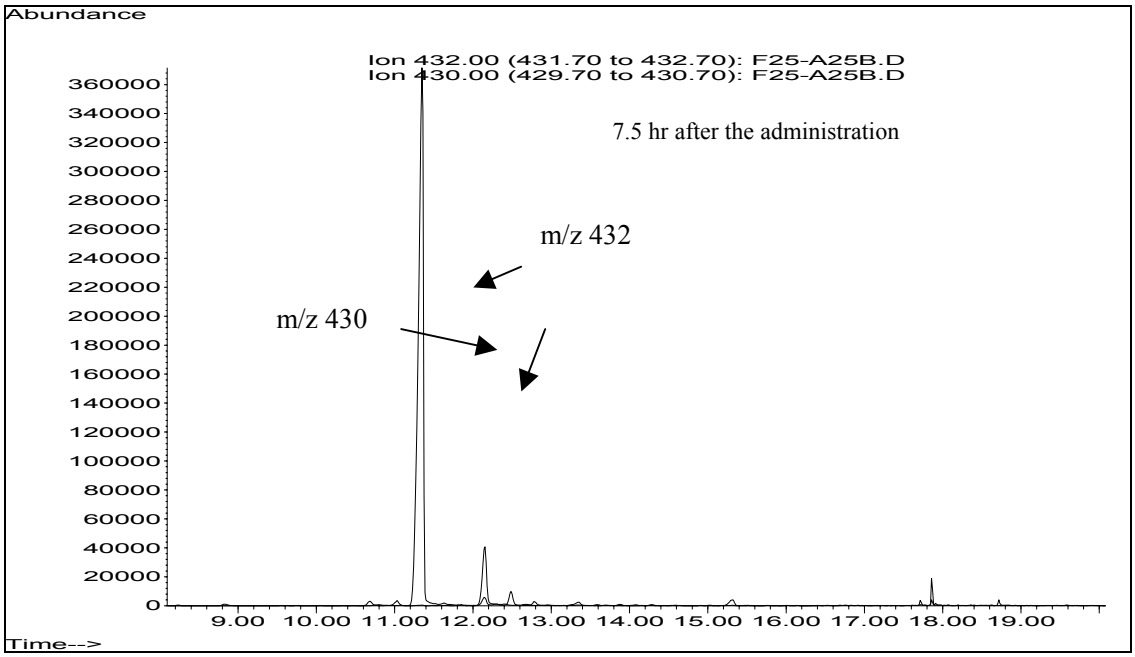


Fig. 3 EIC of urine from the volunteer No.1 (conjugated fraction)

Fig. 3 shows the extracted ion chromatogram of blank urine and the urine collected 7.5 hours after administration of 1-testosterone from the first volunteer. Fig.4, 5 and 6 shows the mass spectrum of the three metabolites, which were identified as 1-testosterone, androst-1-en-3, 17-dione and epi-1-testosterone compared with the reference literature[3].

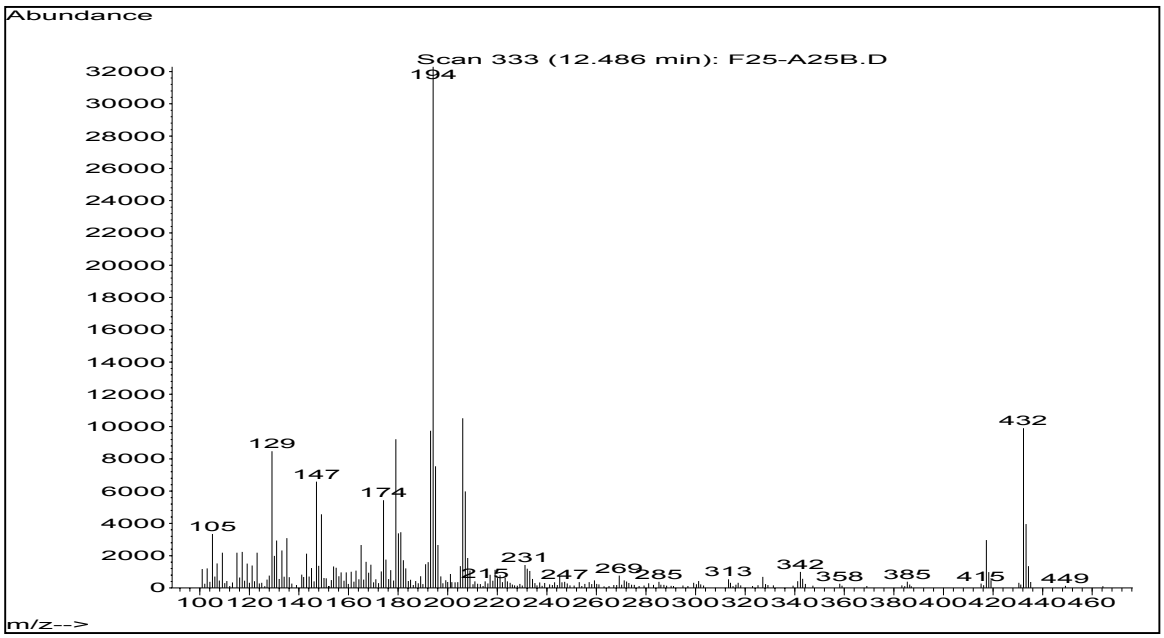


Fig. 4 Mass spectra of bis TMS-1-testosterone from urine

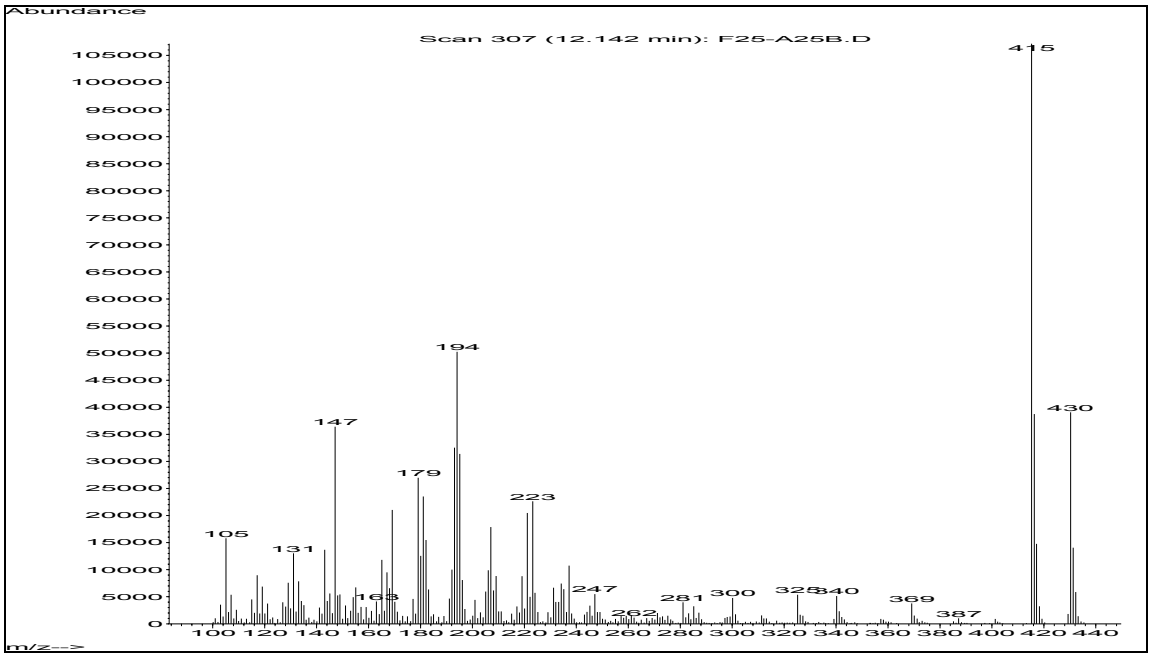


Fig. 5 Mass spectra of bis TMS-androst-1-en-3, 17-dione from urine

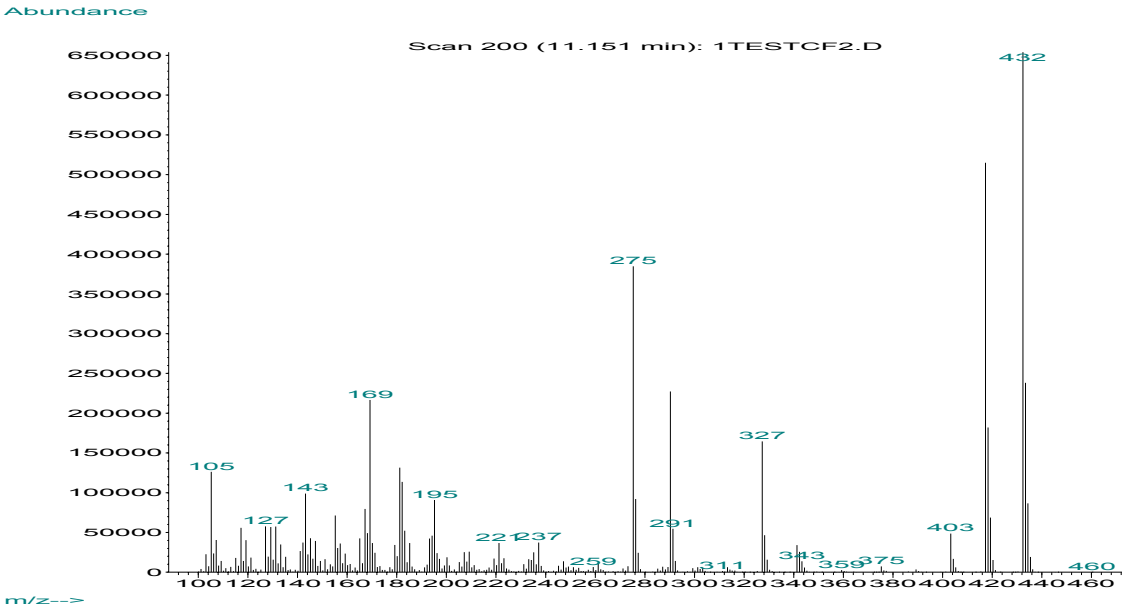


Fig. 6 Mass spectra of bis TMS-epi-1-testosterone from urine

The concentration of metabolites achieved maximum in 10 hours, and were detected at least 90 hours after the single dose of the three preparations, and regarding to the scatter graph (Fig. 7.), sexual difference did not affect on the metabolite profiles.

Met.1 (Epi-1-Testosterone) was the major metabolite for all volunteers administered with three different types of 1-testosteorne. For 9 out of 10 volunteers, Met.2 (Androst-1-en-3, 17-Dione) was the second major metabolite, but for one volunteer, Met.3 (1-Testosterone)

was excreted more than Met.2. And for one out of the 9 volunteers, the excretion amount of Met.2 and Met.3 were almost on the same level, which revealed significant individual difference (Tab.2).

Tab. 2 Area-Under-Curve Normalizing of the metabolites time curve

Volunteer 1#, 2#, 3#, 5#, 6#, 8#, 9#, 10#	**UND**		**THP**		**CYP**	
	Area	**Normalize**	**Area**	**Normalize**	**Area**	**Normalize**
MET1	20878.96	78.64%	6817.08	75.49%	46660.71	74.47%
MET2	4386.22	18.37%	1723.71	18.37%	14918.23	21.87%
MET3	761.57	3.00%	940.50	6.14%	5183.60	3.66%

Volunteer 4#	**UND**		**THP**		**CYP**	
	Area	**Normalize**	**Area**	**Normalize**	**Area**	**Normalize**
MET1	18802.70	61.01%	9942.18	53.50%	26555.57	44.06%
MET2	6610.10	21.45%	4748.31	25.55%	13764.09	22.84%
MET3	5405.35	17.54%	3891.83	20.94%	19945.01	33.10%

Volunteer 7#	**UND**		**THP**		**CYP**	
	Area	**Normalize**	**Area**	**Normalize**	**Area**	**Normalize**
MET1	16984.28	68.77%	3653.24	73.46%	46660.71	64.63%
MET2	3470.54	14.05%	610.07	12.27%	14918.23	15.96%
MET3	4243.33	17.18%	710.13	14.28%	5183.60	19.41%

Regarding the total excreted amount of Met 1, 1-testosterone cypionate ether with oral dispenser consume showed significant different to the other two preparations with oral administration. But no significant difference between two orally administered preparations was observed.

For endogenous steroid profile, only ID No. 2, one female volunteer showed a significant elevation of T/E ratio after administration with UND preparation but no similar trends were found after the administration with the other two preparations. Two of 5 female volunteers, ID No. 1 and 3, revealed a slightly abnormal change in T/E ratio. And T/E ratios of the remaining two female volunteers (ID No. 8 and 10) were kept stable after the administration of three 1-T preparations. T/E ratio of the all the male volunteers were quite stable. It is reasonable, because when the double bond of Δ1 is reduced, 1-T may be converted into 5α-dihydro-testosterone (DHT) without obvious effect on the T/E ratio. But the possible

reason for the large variation of T/E ratio with female volunteers is out of our knowledge we have so far.

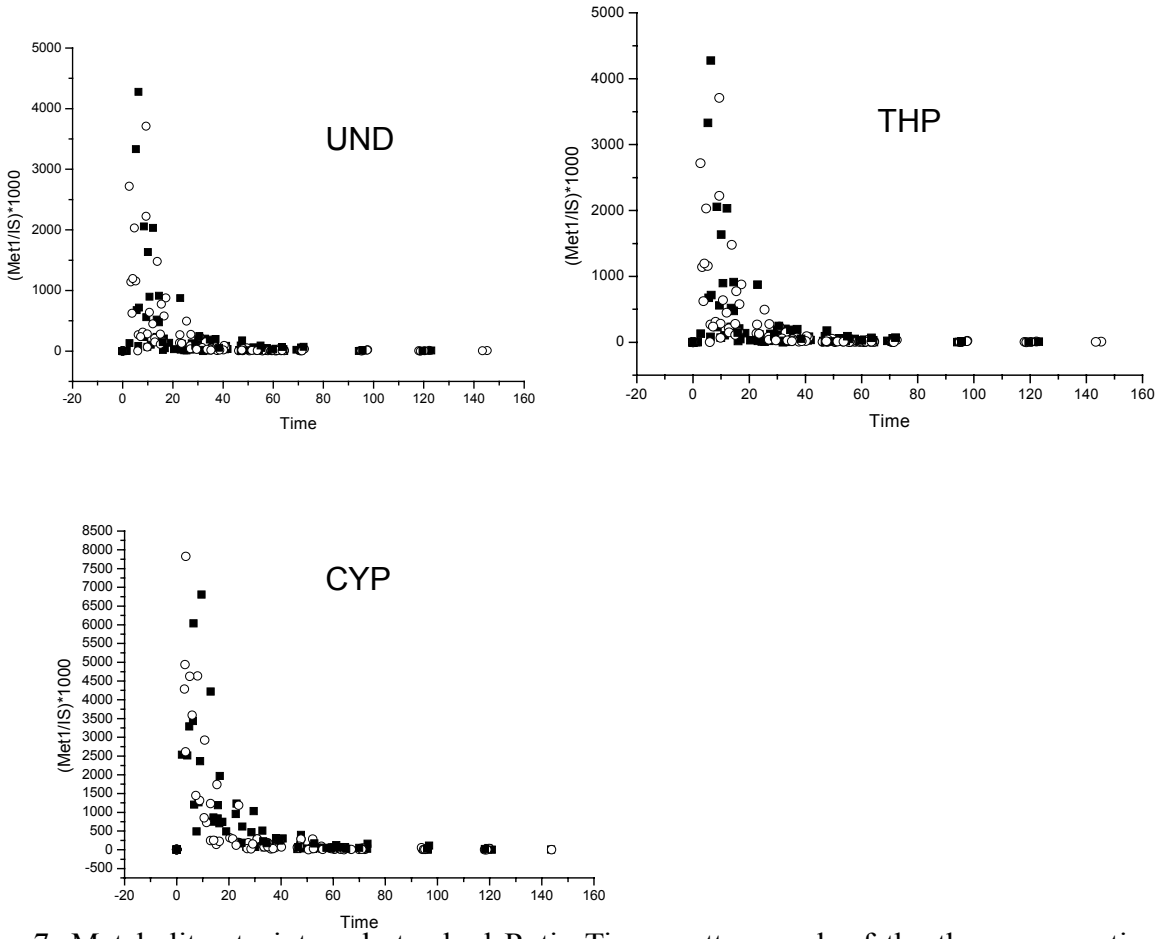


Fig. 7: Metabolites to internal standard Ratio-Time scatter graph of the three preparations. The solid spots represent data from female patients, and the circles represent data from male patients.

Tab. 2: T/E ratio data after administration.

ID	Gender	Normal Range	Max. UND	Max. THP	Max. CYP
1	Female	0.09±0.10	**1.14**	0.17	0.46
2	Female	0.08±0.10	**5.63**	0.10	0.21
3	Female	0.10±0.05	**1.66**	0.15	1.01
4	Male	1.10±0.30	1.33	1.42	2.19
5	Male	0.09±0.04	0.12	0.13	0.16

6	Male	0.19±0.03	0.22	0.21	0.54
7	Male	1.60±0.50	2.10	1.60	2.46
8	Female	0.03±0.01	0.18	0.05	0.09
9	Male	0.09±0.02	0.11	0.11	0.16
10	Female	0.40±0.20	0.62	0.22	0.42

Both the ratio for androsterone to etiocholanolone and 5α-AD to 5β- AD were greatly increased after administration (Tab.3 and Tab.4.), exceeding the diagnostic criteria for an adverse finding with DHT[4 - 6]. Especially, the volunteers administered CYP revealed an extremely abnormal change in An/Etio ratio, which was much more obvious than the other two preparations.

Tab. 3: An/Etio ratio data after administration.

ID	Gender	Normal Range	Max. UND	Max. THP	Max. CYP
1	Female	2.1±0.2	3.74	4.59	17.46
2	Female	1.1±0.1	1.74	1.79	3.07
3	Female	1.1±0.2	3.52	2.27	12.73
4	Male	1.0±0.2	3.61	2.69	7.19
5	Male	0.7±0.1	1.55	1.46	3.59
6	Male	1.3±0.1	2.68	1.56	6.14
7	Male	2.1±0.4	7.34	4.12	15.38
8	Female	1.7±0.2	8.33	2.61	28.42
9	Male	1.2±0.3	3.36	2.35	4.11
10	Female	1.2±0.2	3.11	2.48	17.30

Tab. 4: 5α-AD/5β- AD ratio data after administration

ID	Gender	Normal Range	Max. UND	Max. THP	Max. CYP
1	Female	1.5±0.2	3.86	8.89	8.32
2	Female	1.0±0.2	7.15	2.27	2.37
3	Female	0.7±0.2	2.02	4.29	6.15
4	Male	0.6±0.2	1.56	2.36	2.22
5	Male	0.6±0.2	1.21	2.46	1.92
6	Male	1.2±0.1	2.61	1.54	3.69
7	Male	0.6±0.2	1.79	2.17	3.84
8	Female	1.2±0.3	9.41	4.2	21.37
9	Male	1.2±0.4	2.26	2.46	2.05
10	Female	1.0±0.2	4.02	3.06	5.42

SUMMARY

The excretion study after administration of three kinds of 1-T preparations performed in ten volunteers showed the following points:

1) The presence of metabolites resulting from exogenous testosterone formulations including 1-testosterone, androst-1- ene-3,17-dione and epi-1-testosterone may be used as evidence for doping with 1-testosterone. The abnormal steroid profile (e.g. T/epiT ratio, 5α-/5β-androstanediol ratio, androsterone/etiocholanolone ratio.) can be proof of extra evidence of doping with 1-testosterone.
2) CYP with oral dispenser consume showed a more significant difference than the other two preparations with oral administration both in endogenous and exogenous steroid profiles. CYP affected strongly on endogenous profiles.
3) Larger variation of endogenous steroid profiles was observed with female volunteers than that with male volunteers.After a single dose, some metabolites of 1-testosterone can be detected at least for about 90 hours.

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