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RECENT ADVANCES IN DOPING ANALYSIS (3)

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Some Followed up Cases of Doping with Naturally Occurring Steroids
- Testosterone and Dihydrotestosterone –
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Some followed up cases of doping with naturally occurring steroids
– *Testosterone and Dihydrotestosterone* –

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Summary

This paper describes the results of followed up study on six doping cases with endogenouly occurring androgen. Testosterone (T) is one of the major androgenic anabolic steroid abused by sports men. As Testosterone doping became detectable, 5 α-Dihydrotestosterone (DHT) has been systematically abused by athletes or prescribed by their medical staff as a substitute of Testosterone. For most laboratories, the detection of DHT doping was difficult since no authorized criteria to indicate DHT doping was available.

Our procedure for the detection of DHT doping consists of a) statistics of steroid profile database, b) personal test history and c) pharmacokinetics of steroids. An administration of DHT results in a large increase of urinary excretion of Androsterone and 5α-Androstan-3α,17β-diol (5α,3α-A_2). Elevated excretion in absolute amount of DHT, 5α,3α-A_2, and relative amount of DHT or it's 5α-metabolites to the corresponding 5β-endogenous steroids were indicative to DHT administration. No significant intraindividual variation was observed in these parameters while the athletes participated to the high altitude training. Steroid profiles of some followed up athletes indicated the alteration due to administration of T and/or DHT. A gray zone athlete with normal DHT value has also been followed by further unannounced testing since the other parameters were still indicative to DHT administration, but the all parameters fell within the normal range at the later test. Difference in the personal steroid profile in this case exceeded the range of day-to-day variation.

Our individual based testing procedure was successfully applied on the several recent international sports events, and our strategy uncovered the problems of Dihydrotestosterone doping in top sports.

Introduction

Current doping definition of IOC medical commission involves some endogenously occurring hormones[1)], and its abuse is an escalating problem in sports. The major problem was stem from availability of criteria and the procedure to be used for the

differentiation of exogenous hormone from endogenous one. Criterion to indicate Testosterone doping is based on statistics and expressed as cut-off value for Testosterone to Epitestosterone ratio (T/ET). It is mandatory to follow the athlete with T/ET>6 by further investigation before declaring positive. Comparison of steroid profiles between races shows the differences in T/ET ratio. Mean T/ET ratio from Caucasian races is roughly 1 and those of Mongolian races is 0.5[2]. Few cases with elevated T/ET were recently considered to be due to a normal physiologic condition[3]. DHT is a major active metabolite of Testosterone and its abuse is also regarded as doping. However, DHT doping has never been successfully detected according to the laboratory statistics reviewed by the medical commission of IOC. Possible parameters to indicate DHT administration were proposed based on the application study to volunteers by Southan et. al.[4], but the followed up data on real DHT doping cases have never been reported in the literature yet. In this paper, applicability, stability of recommended DHT parameters, and the results of investigation on followed up DHT cases are discussed.

Materials and Protocol

Reference urine samples were collected in Dou-Ba city at Qing Hai prefecture in Federal Republic of China on August through October 1993. Totally ten Japanese and Chinese walking athletes, five females and males each, were participated to high altitude training. It was a part of the research project on trainability at high altitude granted by the Japanese Ministry of Education, Science, Sports and Culture. The day-to-day variations of steroid profile have been monitored for up to 51 days. Altitudes from the sea level were ranged between 13m (Tokyo) and 3,000m (Lake Qing Hai).

The real urine samples were collected from athletes at several occasions, namely, at out-of-competition testing between competitions (OOCT), at unannounced testing conducted in athlete village at several days before the major international games and during the Asian Games in Hiroshima 1994. All samples except OOCT samples sent by the international sports federation (IF) were collected in Japan.

Determination of steroid glucuronide was performed by known routine screening procedure that approved by the medical commission of IOC[3]. Improved procedures using n-Pentane as an extraction solvent was utilized at confirmation stage since target steroids in some cases were co-eluted with polar interfering substances such as Trimethoprim.

Two cases with T/ET around 10 were found by unannounced testing and the T/ET values were informed to the responsible IF without declaring positive. These cases were followed by further testing during the games, and information to follow the

personal steroid profiles were released to the laboratory as the code number. Then the investigation of previous test results of the athlete was recommended by the laboratory to the responsible IF and the Sports Medicine Committee of the Olympic Council of Asia (SMC-OCA) since intraindividual variation of the steroid profiles in two cases suggested the alteration due to steroid doping.

All the urine samples taken during the Asian Games '95 were analyzed for steroid profiles, and the statistical evaluation was made in Tokyo laboratory. Calculation and the verification of reference interval of DHT parameters were made by Cologne staff using IFCC standard method.

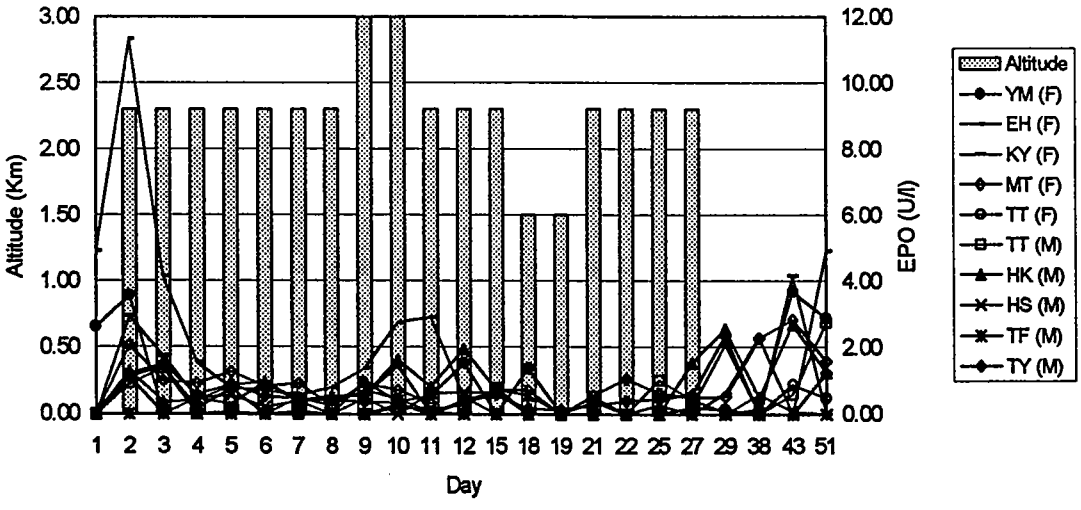
Stability of steroid profiles in comparison with urinary Erythropoietine

It is well known that the production of Erythropoietine (EPO) is stimulated when a normal adult is exposed to the lower oxygen pressure in the air, and such stress may affect on an excretion of the other hormones. It is expected that EPO is the most sensitive stress marker for high altitude training. Urinary EPO was therefore selected as the reference hormone at high altitude training. Steroid profiles of the same urine samples as EPO were measured.

Trends in urinary Erythropoietine

Figure-1 shows the trends in urinary excretion of EPO measured during the joint training. The results clearly showing both inter- and intraindividual variations. EPO level of athletes came from flat ground to participate to the training was highly depends on the altitude. Such shift was notable especially in light weight Japanese female athletes with low Hematocrit value.

Figure-1 The relationships between urinary EPO and altitude.



Stimulation of urinary EPO excretion was minor in nine Chinese athletes who were

the permanent residence in Qing Hai city, but an other Chinese female came from flat ground also showed the elevated urinary EPO as observed in Japanese.

It was obvious that the acute change of urinary EPO concentration is affected by the oxygen pressure, and the trends can be used as a sign of adaptability of athletes to the environment at the mountain.

Stability of steroid profiles

Steroid profiles of the same athlete were measured according to the screening procedure-4. Figure-2-a and -2-b represent the stability of urinary DHT concentration. The change in urinary excretion of DHT was minor both in two crowds. The coefficients of variation (CV) for Japanese and Chinese were 23-71% and 17-101% respectively.

Fig.2-a Relationships Between Altitude and DHT Excretion of Walking Athlete (Japanese Subject)

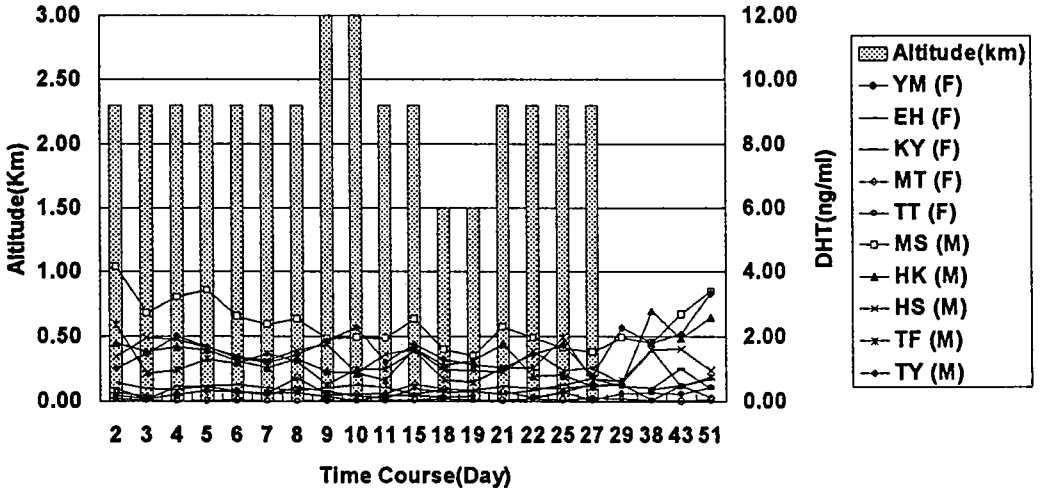
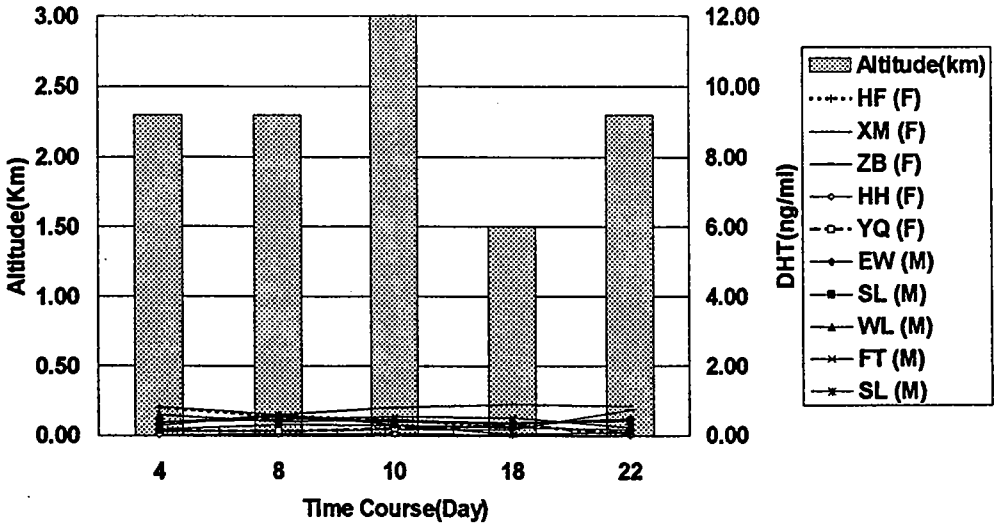


Fig.2-b Relationships Between Altitude and DHT Excretion of Walking Athlete (Chinese Subject)



The difference in CV of urinary DHT between two crowds was provably caused by analytical error because DHT concentration was near around the limit of quantification especially in Chinese. The other parameters, namely the ratios of Androsterone(AND)/Etiocholanolone (ETIO), 5α,3α-A2/5β,3α-A2, and absolute amount of 5α,3α-A2, T, ET etc. were also checked but no significant change was observed. (Figure-3a, -3-b, -4-a, -4-b)

Fig.-3-a Stability of Androsterone/Etiocholanorone Ratio
(Japanese Walking Athlete)

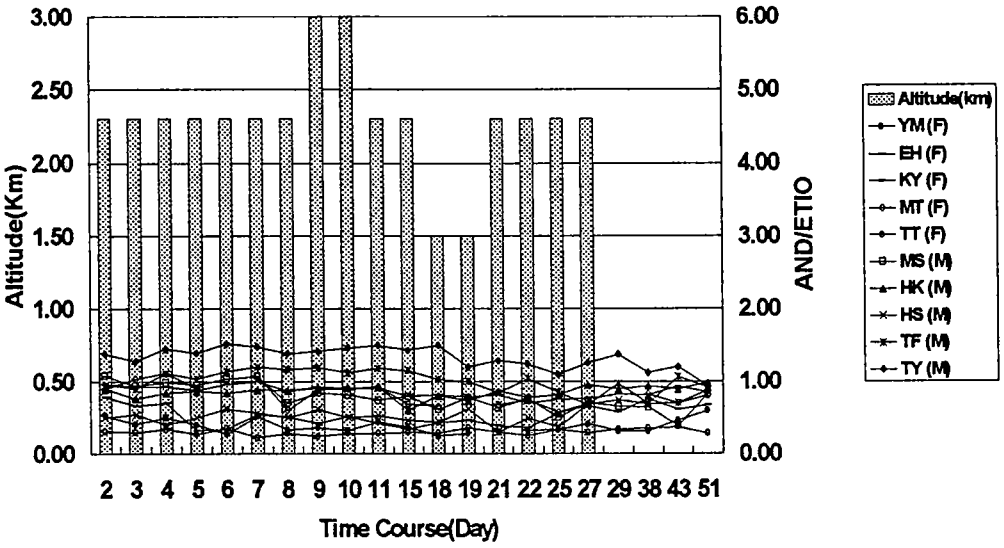
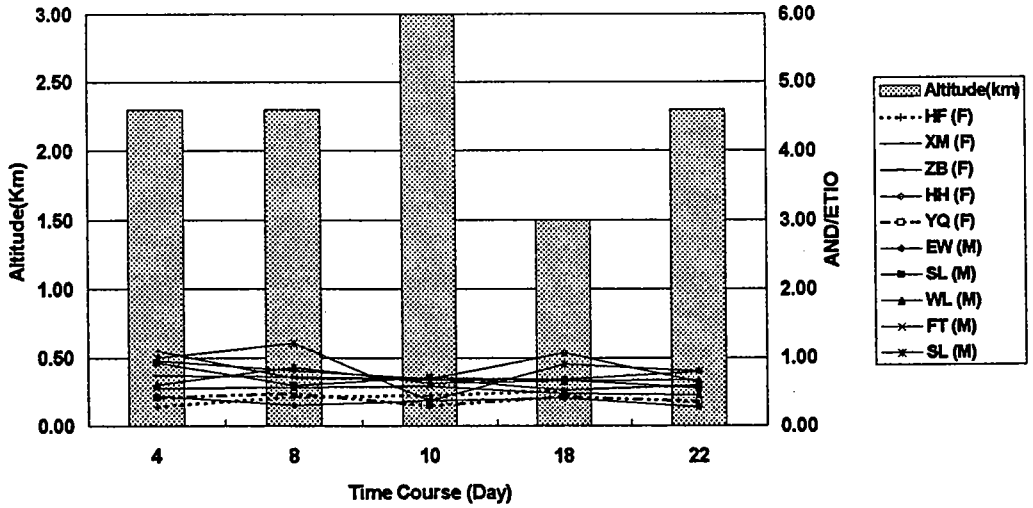


Fig.3-b Stabiliy of Androsterone/Etiocholanorone Ratio
(Chinese Walking Athlete)



Mean value, standard deviation and coefficient of variation (CV) of each steroid were summarized in Table-1-a and Table-1-b. The most stable parameter was AND/ETIO ratio as reported by Mareck-Engelke et.al.[5], and the ratio of 5α,3α-

$A_2/5\beta,3\alpha-A_2$ was also stable. In most specimen, CV of the personal steroid profile was not grater than 100%.

Intraindividual variations of urinary steroids were not small, however, our investigation confirmed the stability of personal steroid profiles.

Fig.4-a The Trends of 5α/5β-Androstane-3α,17β-diol Ratio
(Japanese Walking Athlete)

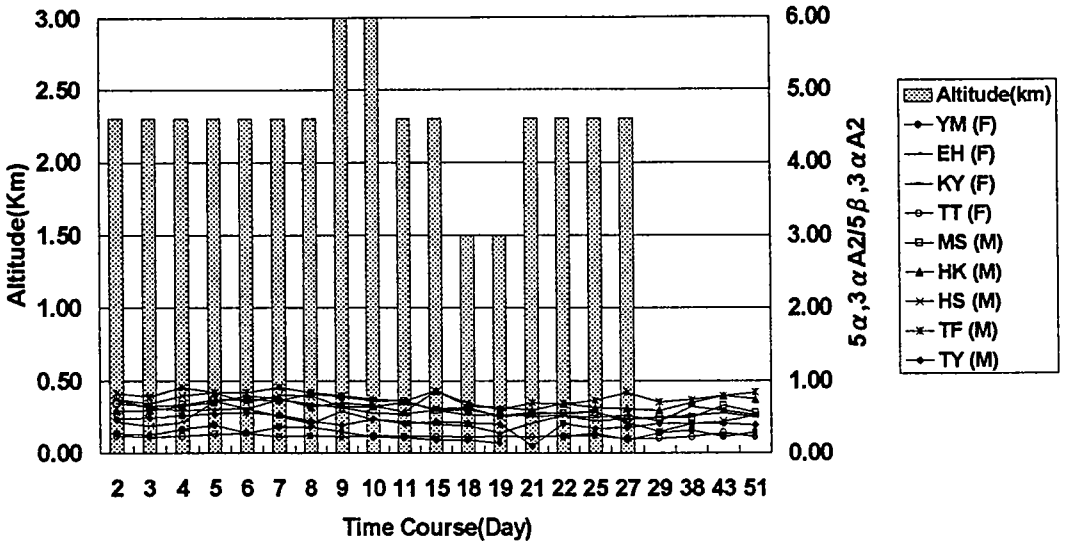
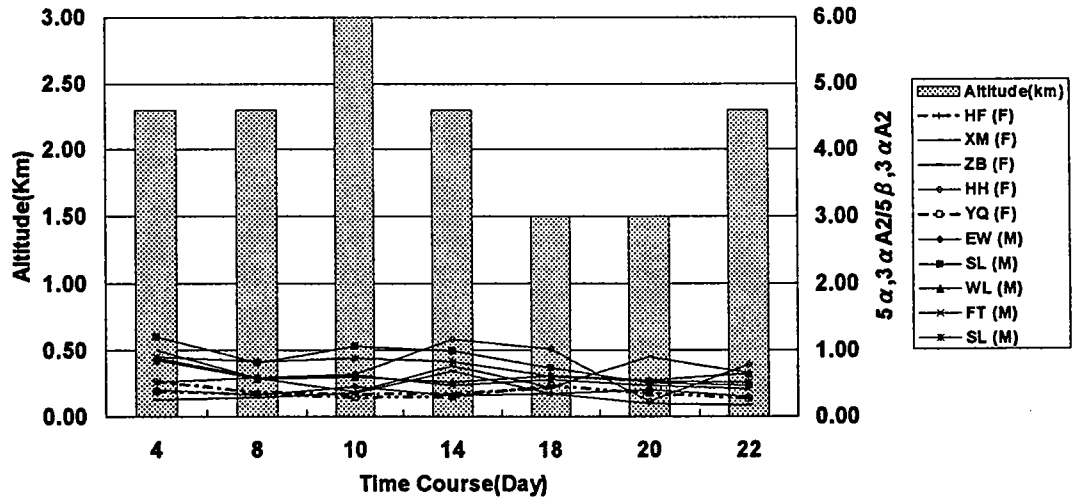


Fig.-4-b Stability of 5α/5β-Androstane-3α,17β-diol Ratio
(Chinese Walking Athlete)



Case study and Discussion

Two similar suspicious cases of doping with native androgen was found from unannounced testing. Reconstructed SIM chromatograms from a suspicious female athlete are shown in the figure-5. The SIM chromatogram in upper was obtained from the urine of unannounced testing that was conducted by IF at 5 days before

competition. The steroid profiles showed the elevated T/ET ratio of 11.4. Consequently, an additional urine sample was collected at competition and the SIM chromatogram was shown at lower in Figure-5.

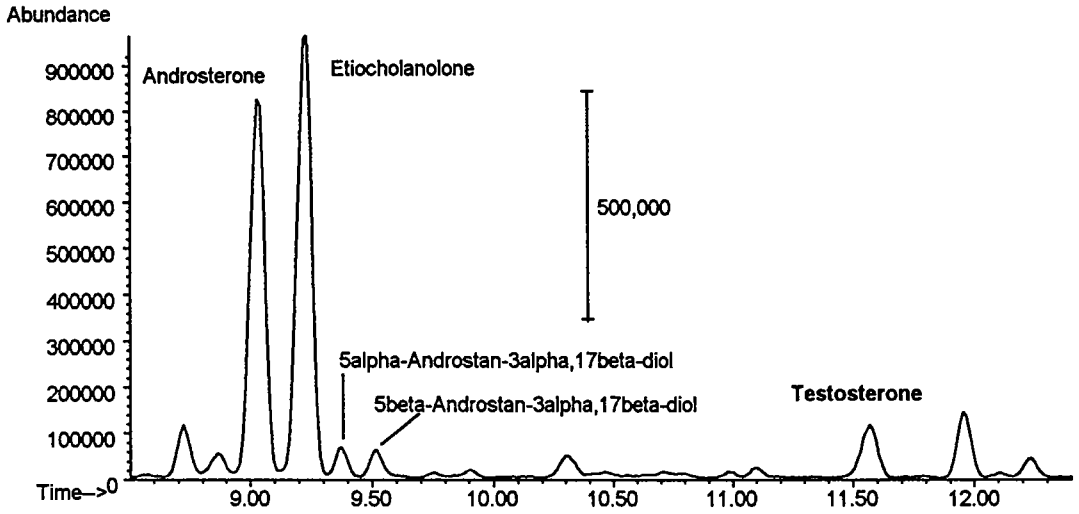


Fig.5-a The Urinary Steroid Profiles of Female Athlete
(5 Days Before Competition)

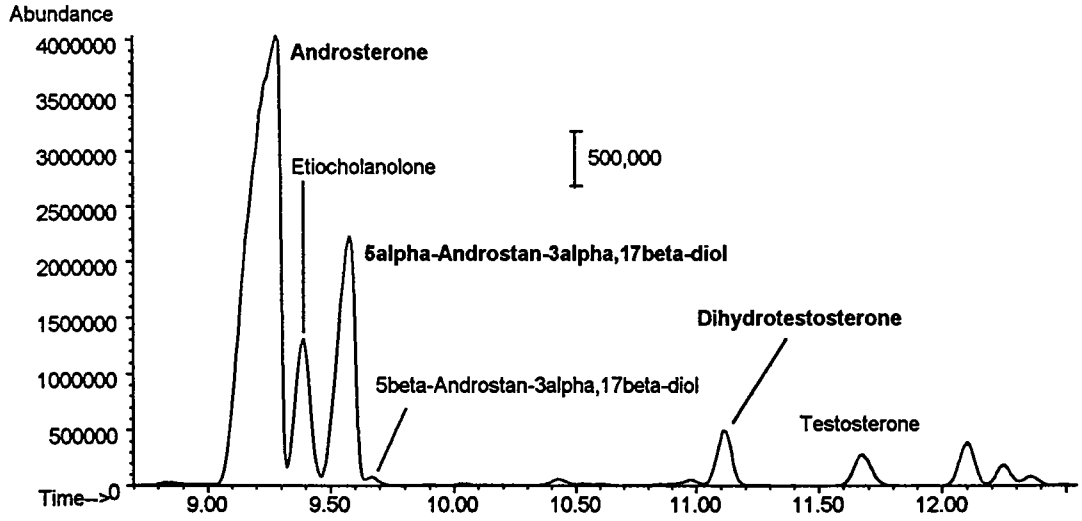


Fig.5-b The Urinary Steroid Profiles of Female Athlete
(at Competition)

Figure-5 The alteration of steroid profiles due to Dihydrotestosterone administration.

The profile indicated that firstly the athlete has administered T and then the substitution from T to DHT was done prior to the competition.

Major deference between two urine samples from a same athlete was the excretion in much grater absolute amount of DHT, $5\alpha,3\alpha$-A_2 and AND. Such trends are

normally indicative to Testosterone administration because these steroids are the metabolite of Testosterone via DHT pathway.(Figure-6) Questions to be answered were the suppression of T/ET ratio, and no increase in Testosterone and it's 5β-metabolites. As shown in table-2, the T/ET ratio fell rapidly to 50% over 5 days.
Increase in relative amount of 5α-steroids to the relating 5β-steroids, namely the ratios of AND/ETIO, 5α,3α-A_2/5β-androstan-3α,17β-diol (5β,3α-A_2), DHT/ETIO and DHT/ET of the urine from competition, were highly significant when compared to those ratios of previous urine.

Table-2 Personal steroid profile history of a female athlete.

Date	T	T/ET	AND	AND/ETIO	DHT	5α,3αA_2	5α/5β*
Sep30-94	14.1	11.4	753	0.9	4.4	50.0	1.0
Oct05-94	14.4	5.7	4,782	5.7	212.0	1,274.0	56.6
Ratio %	102%	50%	635%	620%	4,775%	2,548%	5,443%

*) 5α,3αA_2 to 5β,3αA_2 ratio (Unit:ng/ml or ng/ng)

Amount of urinary steroids listed in Table-2 except T was 6 to 48 times higher than the previous value, and the differences were exceeded the range of day-to-day variation. The data could therefore be interpreted that the athlete have administered T in September then it was substituted to DHT. Such case might not be detected if the test was performed only in one occasion by measuring T/ET ratio as a sole criterion. The alteration of steroid profiles in this case were in good agreement with the metabolic pathway of DHT. Major part of the steroids shown in the figure-6 is detected as the glucuronide except Epiandrosterone.
Southan et. al. reported the increasing of urinary excretion rates of DHT, 5α,3α-A_2 and 5α,3β-A_2 after DHT administration when compared to those of T, ET, 5β,3α-A_2, and Luteinizing Hormone[4]. Our hypothesis on the followed up case was also in good agreement with the results of deuterated DHT application study performed by Donike et.al.[6]. Application of 16,16,17-Trideutero-DHT to a volunteer resulted in the urinary excretion of the absolute amount of 16,16-Dideutero-AND, 16,16,17-Trideutero-DHT and 16,16,17-Trideutero-5α,3α-A_2, and most part of the metabolites were excreted as glucuronide.
Urinary concentration of metabolites of T, and the ratio of each 5α-metabolite to the corresponding 5β-metabolite are, therefore, selected for the calcuration of reference intervals of possible DHT parameters since 5α-reduction from T to DHT was almost negligible.

Reference Intervals

The reference intervals for the possible DHT parameters were calculated based on the analytical results of real urine samples that were taken during the Asian Games in

Hiroshima 1994 and unannounced testing prior to the games. Investigation and comparison of the data from the Asian Games in Hiroshima 1994 with those from the former Asian Games in Beijing 1990 was made by Donike et.al. in order to eliminate the possibility of difference in steroid profiles caused by the nationality. From the re-investigation of the analytical results of the urine of Asian Games in Beijing, no inter-country variation among far east Asian countries was found[6]. The urine samples of athletes from the country concerned with DHT doping were not used for the calculation because all suspicious cases of DHT were found from only one country at the Asian Games in Hiroshima. The calculation and the verification of reference intervals were done also in Cologne laboratory using IFCC recommended method[7]. DHT concentration was corrected by the specific gravity since the urine density of athlete from aquatic sports is frequently diluted.

Most significant parameters were DHT corrected by specific gravity, $5\alpha,3\alpha$-A_2/$5\beta,3\alpha$-A_2 and AND/ETIO, so the interpretation of steroid profiles for decision making was done according to the comparison of these three parameters with the 97.5% upper reference limit as the cut-off value. (Table-3)

Investigation of previous test results and follow up study by further testing

Personal test history was recorded in the steroid database of Tokyo laboratory in three females and two males with DHT doping. In these cases, athletes were tested more than once.(Table-4) In one female, an OOCT result that conducted three months before the Asian Games, one unannounced test result and three competition test results were available. The earliest test results of this athlete showed no abnormal result. In all cases, the intraindividual variation of DHT parameters exceeded the range of daily rhythm and upper limit of reference interval. Elevated ratio of $5\alpha,3\alpha$-A_2/$5\beta,3\alpha$-A_2 was most evident and was not caused by low $5\beta,3\alpha$-A_2 concentration but caused by high $5\alpha,3\alpha$-A_2. An acute change in concentration suggests the short half-life of DHT so that the urinary excretion in absolute and relative amount of $5\alpha,3\alpha$-A_2 seemed to be more sensitive parameter for the first screening of DHT doping.

One other female was not declared as positive but followed by further testing since not all the parameters exceeded the reference range. Later test of this athlete was conducted at long after the games.(Table-5) The followed up results represented no significant difference in the urinary excretion of ETIO, ET and $5\beta,3\alpha$-A_2. On the other hand the elevated ratios of $5\alpha,3\alpha$-A_2/$5\beta,3\alpha$-A_2, AND/ETIO, and higher concentration of DHT in the previous urine were highly significant when compared with the latest test results. Alteration of the steroid profiles that found in the previous urine of this athlete was therefore equivalent to those observed after DHT application.

Conclusion

Parameters to indicate Dihydrotestosterone doping were selected and the stability of these parameters were confirmed. Our strategy to detect DHT doping has been applied on the recent international sports event and the results uncovered the problem of doping with naturally occurring steroid. An individual based steroid profiling allowed the more sensitive detection of steroid doping. Thus, the false positive due to the statistical error can be avoided.

Acknowledgment

Author thanks to Professor Manfred Donike with great sorrow for his untimely passing. Our success in this work has been honored by his great help.

This paper is dedicated to Mr. Shinji Ueki. His fight against prostatic cancer has been of great hint to create the strategy against DHT doping.

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Table-1-a The Day-to-day Variations of Urinary Steroids in Japanese Walking Athletes (n=21) (ng/ml)

		T	ET	AND	ETIO	11OHA	11OHE	DHT	DHT corr.	5α, 3αA2	5β, 3αA2	5α/β, 3αA2	T/ET	AND/ ETIO	mDHT/ ETIO	DHT/ ET
TY (M)	Average	47.9	30.8	851	655	416	47	1.6	1.5	15.5	35.9	0.4	1.6	1.3	2.53	0.05
	SDn-1	12.7	9.2	249	248	281	45	0.6	0.5	6.5	15.0	0.1	0.2	0.2	0.48	0.01
	C.V.(%)	27%	30%	29%	38%	67%	96%	35%	32%	42%	42%	22%	14%	12%	19%	16%
HS (M)	Average	7.7	35.8	1,176	2,111	245	147	1.3	1.0	26.0	42.4	0.6	0.2	0.6	0.62	0.04
	SDn-1	2.2	7.8	371	395	273	160	0.4	0.4	9.7	13.1	0.1	0.0	0.1	0.11	0.01
	C.V.(%)	29%	22%	32%	19%	112%	109%	29%	35%	37%	31%	24%	14%	24%	18%	21%
MS (M)	Average	6.2	28.6	2,007	2,599	223	91	2.4	1.8	51.3	81.8	0.6	0.2	0.8	0.95	0.09
	SDn-1	1.2	7.0	655	799	132	39	0.7	0.7	12.0	19.7	0.1	0.0	0.2	0.30	0.02
	C.V.(%)	20%	25%	33%	31%	59%	43%	30%	37%	23%	24%	17%	13%	24%	32%	29%
HK (M)	Average	4.0	49.0	1,477	1,726	226	175	1.3	1.1	37.3	55.7	0.7	0.1	0.9	0.76	0.03
	SDn-1	1.5	14.0	548	555	163	94	0.6	0.5	12.2	19.4	0.1	0.0	0.1	0.20	0.01
	C.V.(%)	38%	29%	37%	32%	72%	54%	46%	44%	33%	35%	12%	17%	10%	26%	27%
MF (M)	Average	3.5	18.6	1,464	1,454	204	40	1.1	1.1	21.0	26.6	0.8	0.2	1.0	0.74	0.06
	SDn-1	1.2	5.9	471	436	71	15	0.5	0.3	8.5	9.4	0.1	0.0	0.2	0.20	0.02
	C.V.(%)	35%	32%	32%	30%	35%	36%	45%	26%	40%	35%	10%	13%	15%	27%	33%
YM (F)	Average	3.3	6.2	401	1,011	66	30	0.2	0.2	4.5	16.9	0.3	0.6	0.4	0.20	0.04
	SDn-1	1.3	3.4	184	243	51	31	0.1	0.1	5.1	18.1	0.1	0.3	0.1	0.08	0.02
	C.V.(%)	39%	54%	46%	24%	77%	102%	48%	40%	116%	107%	26%	43%	25%	43%	55%
EH (F)	Average	5.2	12.8	472	895	78	21	0.4	0.3	5.6	11.9	0.5	0.4	0.5	0.51	0.04
	SDn-1	1.3	4.3	219	240	52	8	0.1	0.1	1.8	3.7	0.1	0.1	0.1	0.13	0.01
	C.V.(%)	24%	34%	46%	27%	66%	41%	23%	28%	32%	31%	21%	32%	26%	25%	31%
TY (F)	Average	0.5	4.3	509	1,676	109	78	0.0	0.0	4.1	17.6	0.2	0.1	0.3	0.03	0.01
	SDn-1	0.2	2.4	142	332	75	47	0.0	0.0	1.2	4.8	0.0	0.0	0.0	0.02	0.01
	C.V.(%)	42%	54%	28%	20%	69%	60%	71%	68%	29%	27%	8%	27%	14%	61%	60%
MT (F)	Average	0.7	9.5	1,419	1,806	136	115	0.3	0.4	41.2	25.5	1.6	0.1	0.8	0.18	0.04
	SDn-1	0.2	3.6	288	507	36	51	0.2	0.3	26.4	8.9	0.8	0.0	0.2	0.09	0.02
	C.V.(%)	24%	38%	20%	28%	26%	44%	51%	65%	64%	35%	48%	39%	20%	49%	53%
KY (F)	Average	0.7	10.9	2,270	2,663	361	73	0.4	0.3	18.3	32.7	0.6	0.1	0.9	0.14	0.04
	SDn-1	0.2	4.9	490	635	205	29	0.2	0.2	4.4	9.3	0.1	0.0	0.1	0.06	0.02
	C.V.(%)	33%	46%	22%	24%	57%	40%	54%	53%	24%	28%	14%	47%	9%	45%	57%

Table-1-b The Day-to-day Variations of Urinary Steroids in Chinese Walking Athletes (n=7) (ng/ml)

		T	ET	AND	ETIO	11OHA	11OHE	DHT	DHT corr.	5α, 3αA2	5β, 3αA2	5α/β, 3αA2	T/ET	AND/ ETIO	mDHT/ ETIO	DHT/ ET
EW (M)	Average	3.3	20.8	757	2,059	39	139	0.2	0.2	9.6	26.3	0.4	0.2	0.4	0.11	0.01
	SDn-1	0.9	5.2	144	285	14	58	0.1	0.0	2.1	6.1	0.1	0.0	0.1	0.03	0.00
	C.V.(%)	26%	25%	19%	14%	35%	42%	26%	21%	22%	23%	16%	16%	16%	25%	33%
SL (M)	Average	3.3	15.4	686	934	19	8	0.4	0.3	16.5	24.3	0.8	0.2	0.7	0.42	0.02
	SDn-1	1.0	3.2	221	176	10	9	0.1	0.1	5.9	17.3	0.3	0.0	0.2	0.22	0.01
	C.V.(%)	29%	21%	32%	19%	51%	113%	38%	40%	36%	71%	33%	17%	21%	51%	43%
WL (M)	Average	2.8	9.7	1,134	1,662	93	31	0.5	0.4	10.6	17.0	0.6	0.3	0.7	0.29	0.05
	SDn-1	0.8	3.9	249	394	37	20	0.1	0.1	4.6	6.5	0.1	0.1	0.1	0.04	0.01
	C.V.(%)	28%	41%	22%	24%	40%	64%	17%	16%	44%	38%	17%	17%	13%	15%	28%
FT (M)	Average	2.3	19.9	812	1,472	69	204	0.5	0.4	10.3	19.9	0.5	0.1	0.5	0.32	0.02
	SDn-1	1.0	7.0	295	463	100	169	0.1	0.1	4.5	9.8	0.1	0.0	0.1	0.04	0.00
	C.V.(%)	44%	35%	36%	31%	144%	83%	30%	26%	44%	49%	14%	10%	10%	12%	10%
SL (M)	Average	1.7	14.6	642	706	95	41	0.3	0.3	10.0	13.3	0.7	0.1	0.9	0.34	0.02
	SDn-1	0.6	6.4	327	256	63	50	0.2	0.3	7.0	7.8	0.2	0.0	0.3	0.23	0.01
	C.V.(%)	34%	44%	51%	36%	66%	122%	78%	75%	69%	59%	23%	23%	30%	68%	62%
HF (F)	Average	5.4	5.1	447	1,053	45	41	0.5	0.4	3.7	9.4	0.4	1.0	0.5	0.38	0.09
	SDn-1	3.8	3.5	192	506	25	27	0.4	0.3	2.1	5.6	0.1	0.5	0.1	0.21	0.05
	C.V.(%)	70%	67%	43%	48%	55%	66%	79%	63%	56%	59%	20%	50%	23%	55%	59%
XM (F)	Average	4.4	6.1	1,143	1,418	156	78	0.7	0.5	11.8	18.7	0.7	0.8	0.8	0.51	0.15
	SDn-1	2.2	4.2	211	284	74	66	0.2	0.1	2.4	5.3	0.2	0.3	0.2	0.17	0.07
	C.V.(%)	52%	69%	18%	20%	47%	84%	28%	27%	20%	28%	34%	31%	19%	33%	48%
ZB (F)	Average	1.2	1.4	387	600	54	22	0.2	0.3	2.0	6.7	0.3	0.9	0.7	0.48	0.27
	SDn-1	0.6	0.8	158	246	36	15	0.2	0.4	0.9	2.5	0.2	0.2	0.1	0.65	0.43
	C.V.(%)	50%	61%	41%	41%	67%	70%	101%	139%	44%	37%	53%	23%	17%	137%	159%
HH (F)	Average	0.3	5.7	405	478	78	45	0.0	0.0	2.1	2.7	0.8	0.1	0.9	0.09	0.01
	SDn-1	0.1	2.8	193	267	54	66	0.0	0.0	1.3	1.7	0.3	0.0	0.2	0.05	0.01
	C.V.(%)	45%	49%	48%	56%	69%	147%	55%	53%	61%	64%	40%	58%	24%	56%	85%
YQ (F)	Average	0.3	4.0	370	950	52	90	0.2	0.2	1.7	5.0	0.3	0.1	0.4	0.19	0.06
	SDn-1	0.1	2.7	83	220	14	71	0.1	0.1	0.5	1.4	0.1	0.1	0.1	0.08	0.04
	C.V.(%)	55%	68%	23%	23%	26%	79%	41%	36%	32%	29%	19%	91%	15%	44%	72%

Table-3 The reference intervals for DHT parameters

(Data from the Asian Games in Hiroshima '94)

(mDHT=DHT X 1,000)

		DHT	DHT correc.	5aA2/ 5bA2	AND/ETIO	mDHT /ETIO	DHT/ ET
Reference Intervals for Male							
High	97.50%	27.74	26.03	2.58	2.85	28.62	1.63
	90.00%	29.29	28.78	3.70	3.41	32.96	2.45
	Confidence	22.40	21.06	2.43	2.53	23.74	1.27
Low	2.50%	0.45	0.45	0.22	0.67	0.85	0.05
	90.00%	0.61	0.70	0.26	0.70	1.05	0.06
	Confidence	0.30	0.20	0.16	0.54	0.48	0.03
Data number		342	342	344	344	343	342
Reference Intervals for Female							
High	97.50%	13.90	12.13	1.88	2.20	10.76	2.72
	90.00%	18.94	17.03	2.92	2.58	15.31	5.40
	Confidence	11.13	9.63	1.73	2.02	9.16	1.92
Low	2.50%	0.20	0.37	0.21	0.41	0.47	0.08
	90.00%	0.50	0.56	0.24	0.48	0.77	0.10
	Confidence	0.04	0.04	0.06	0.41	0.12	0.05
Data number		155	155	155	156	156	156

DHT corr.=(1.020-0.998)/(Density-0.998)*DHT (DHT corrected by density)

5aA2/5bA2=ratio of 5 α -Androstan-3 α ,17 β -diol / 5 β -Androstan-3 α ,17 β -diol

Table 4 Followed Up Cases of Doping with Dihydrotestosterone

(mDHT=DHT X 1,000)

Date	Sex	pH	Density	T	ET	AND	ETIO	11OHA	11OHE	DHT	DHT corr.	5α,3β -A_2	5α/5β, 3β -A_2	T/ET	AND/ ETIO	mDHT /ETIO	DHT/ ET
Sep-30	F	6.1	1.012	14.1	1.2	753	815	104	8	4.6	7.2	50.0	1.0	11.4	0.9	5.58	3.70
Oct-05		6.8	1.010	14.4	2.5	4,782	839	253	63	212.0	388.7	1,274.0	56.6	5.7	5.7	252.83	83.27
Jun-27	F	6.5	1.016	1.6	1.8	494	822	89	115	2.7	3.3		0.3	0.9	0.6	3.29	1.52
Sep-30		5.8	1.008	1.1	1.2	1,765	888	93	102	35.2	77.4	238.8	10.6	0.9	2.0	39.63	29.12
Oct-04		5.8	1.012	3.2	2.3	2,707	1,359	214	220	57.0	89.5	414.9	12.7	1.4	2.0	41.93	24.77
Oct-07		6.5	1.006	1.1	1.0	1,464	648	111	54	17.4	47.9	191.7	17.7	1.1	2.3	26.91	17.51
Oct-08		6.1	1.016	3.4	3.8	3,012	1,568	321	429	49.7	60.7	439.8	10.2	0.9	1.9	31.69	13.23
Sep-30	F	6.1	1.016	0.5	2.1	1,307	1,180	96	106	2.6	3.1	66.6	12.8	0.2	1.1	2.18	1.21
Oct-05		6.8	1.014	1.2	4.2	2,794	1,110	202	212	20.9	28.7	390.2	62.4	0.3	2.5	18.80	4.94
Oct-07		6.5	1.012	0.7	2.1	1,878	749	156	30	10.0	15.7	228.4	70.5	0.3	2.5	13.32	4.76
Sep-30	M	5.1	1.002	1.3	0.1	217	172	216	42	0.4	2.5	2.5	0.4	9.2	1.3	2.61	3.10
Oct-07		5.8	1.012	9.6	2.4	3,976	720	272	174	54.7	86.0	689.0	70.5	3.9	5.5	75.98	22.44
Oct-03	M	6.1	1.016	6.2	4.8	4,116	1,155	300	76	68.4	83.6	730.7	49.5	1.3	3.6	59.26	14.22
Oct-04		5.0	1.022	5.9	2.7	3,797	1,074	316	184	82.8	75.9	614.8	56.1	2.2	3.5	77.09	30.82
Oct-08		5.5	1.022	5.0	2.8	4,510	1,071	370	228	104.2	95.5	680.6	41.9	1.8	4.2	97.29	36.79

DHT corr.=(1.020-0.998)/(Density-0.998)*DHT

(Unit:ng/ml)

5α/5β,3β -A_2=5α-Androstan-3α,17β -diol to 5β-Androstan-3α,17β -diol ratio

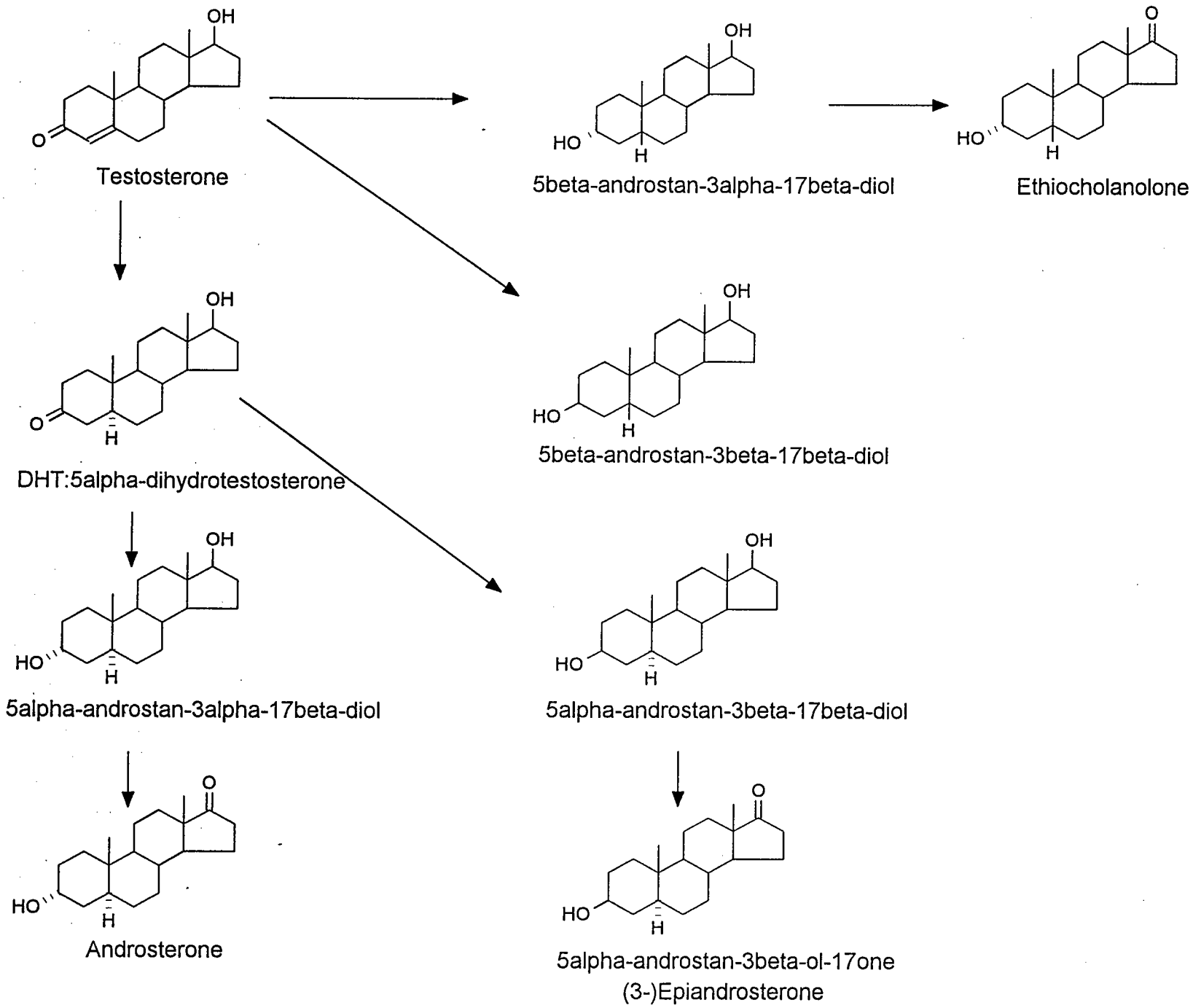

Figure-6 Metabolism of 5alpha-Dihydrotestosterone (DHT)

Table-5 Followed Up results of Gray Zone Female Athlete

(mDHT=DHT X 1,000)

Occasion	pH	S.G.	T	ET	AND	ETIO	11OHA	11OHE	DHT	DHT corr.	5α,3α-A_2	5β,3α-A_2	5α/5β 3α-A_2	T/ET	AND/ ETIO	mDHT / ETIO	DHT/ ET
1-1	6.1	1.016	0.7	5.5	2,881	1,124	242	99	4.1	5.1	183.2	5.9	42.0	0.1	2.6	3.68	0.75
1-2	5.5	1.024	0.5	5.5	2,826	1,380	220	173	3.2	2.7	156.2	9.4	22.5	0.1	2.0	2.34	0.59
1-3	5.3	1.025	0.5	6.4	3,119	1,538	508	249	1.8	1.4	97.6	9.2	14.3	0.1	2.0	1.15	0.28
2-1	5.5	1.019	0.1	2.0	1,108	1,098	194	294	0.1	0.1	5.7	8.0	1.1	0.1	1.0	0.05	0.03
2-2	5.5	1.032	0.2	4.0	1,561	1,533	330	307	0.1	0.0	9.1	13.7	1.0	0.0	1.0	0.05	0.02

The urine samples were collected at two series of the competition respectively.
The second series of samples were collected at 10 months after the first competition.