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Changes of the Urinary Steroidprofile after Sublingual Application of Dihydrotestosterone (DHT).

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Abstract

DHT was administered sublingually in doses of 25 mg to four male volunteers. From 24 hours before the application till 90 hours after the application urine samples were collected. The glucuronides of DHT and its metabolites were analysed as per-trimethylsilylated derivatives with a gas chromatographic/mass spectrometric method. The administration of DHT led to increases of the excretion of DHT and its 5α-metabolites androsterone (AND), 5α-androstane-3α,17ß-diol (5αA3αD), 5α-androstane-3ß,17ß-diol (5αA3ßD) and epiandrosterone (EPIAND). The ratios of these steroids to etiocholanolone (ETIO), 5ß-androstane-3α,17ß-diol (5ßA3αD) and epitestosterone (EPI) also increased. For doping control purposes the concentration of DHT (corrected by the specific gravity) and the ratios DHT/ETIO, DHT/EPI, 5αA3αD/5αA3ßD and AND/ETIO are suitable parameters for detection of DHT. Prerequesit is the knowledge of subject based or population based reference ranges.

Introduction

The use of anabolic androgenic steroids (AAS) by athletes is banned by the International Olympic Committee (IOC) and National and International sport Federations. This ban is controlled worldwide by the analysis of urine samples of athletes by a gas chromatographic/ mass spectrometric (GC/MS) method introduced by Donike [1]. The identification is based on comparison of the EI-mass spectra and GC retention times of the isolated and steroids [2] and/or their metabolites with those of authentic reference substances [3].

To defeat doping controls, athletes have changed from the application of synthetic AAS to the application of endogenous androgens. From the biochemical/physiological point of view possible candidats of endogenous androgens for misuse in sports are testosterone, androstendione, epitestosterone and DHT.
For the detection of testosterone doping Donike and coworkers [4,5,6] developed a method based upon GC/MS measurements of changes of the urinary steroidprofile.

It is expected that the application of DHT will also lead to characteristic alterations in the steroidprofile. As DHT is characterized by a hydrogen irreversibly fixed in the 5α-position, the detection of exogenous DHT can be based on increased urinary excretion of DHT and/or its main 5α-metabolites, androsterone (AND), 5α-androstane-3α,17ß-diol (5αA3αD), 5α-androstane-3ß,17ß-diol (5αA3ßD) and epiandrosterone (EPIAND), as discussed by several working groups [7,8,9]. The metabolism of DHT is presented in figure 1.

Nowadays, DHT preparations for medical use have been withdrawn from the international market, except a DHT containing lotion for transdermal application (Andractim, Piette, Belgique and Besins-Iscovesco, France). But their exist rumours that oral and injectable preparations are still available on the black market. The easiest and most probable route of administration for athletes is the oral route. To circumvent the first pass deactivation in the liver after oral adminisration, it is recommended to administer DHT sublingually.

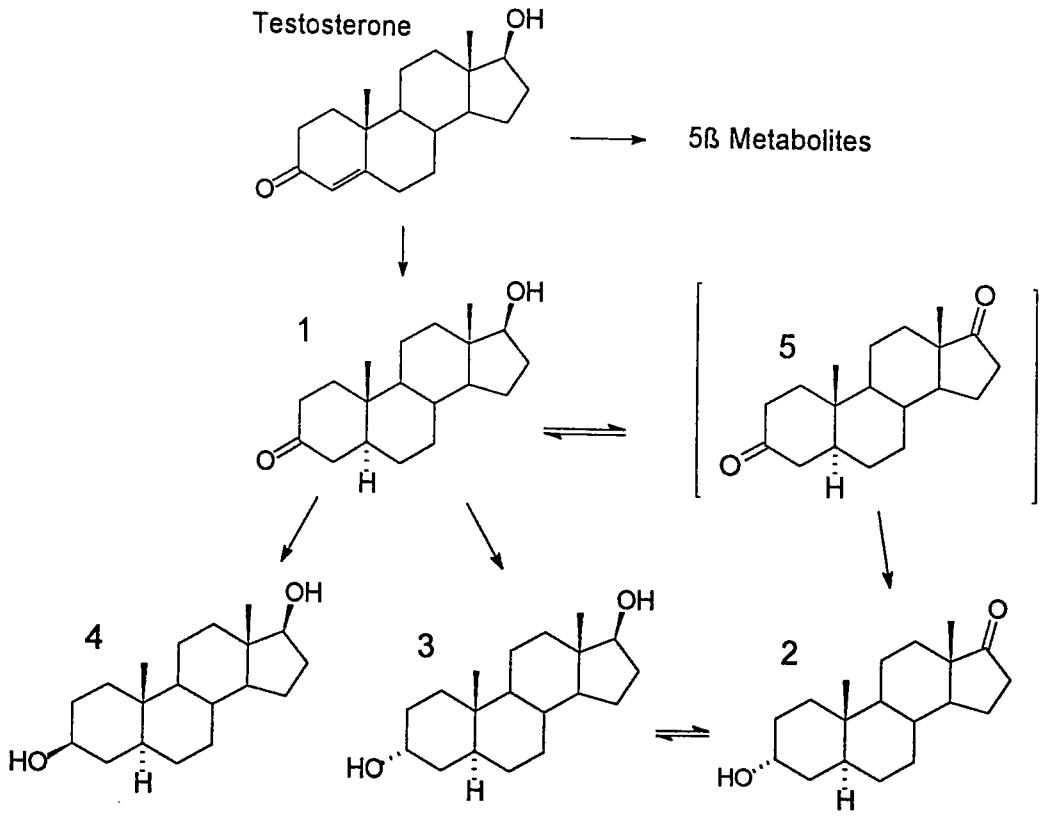


Fig. 1: Biosynthesis and metabolism of DHT: DHT (1), AND (2), 5αA3αD (3) 5αA3ßD (4), 5α-androstane-3,17-dione (5)

Experimental

Steroids and Chemicals

Dihydrotestosterone, androsterone, etiocholanolone, 5α-androstane-3α,17ß-diol, 5ß-androstane-3α,17ß-diol, 5α-androstane-3ß,17ß-diol, testosterone, epitestosterone, 11ß-hydroxy-androsterone, 11ß-hydroxy-etiocholanolone, pregnandiol, tetrahydro-cortisol, epiandrosterone, and methyltestosterone were purchased from SIGMA, D-82039 Deisenhofen, Germany. ß-Glucuronidase from Escherichia coli K12 was obtained from Boehringer, D-68298 Mannheim, Germany. MSTFA, [16,16,17-$^2H_3$]-testosterone and [2,2,4,4-$^2H_4$]-etiocholanolone were synthesized in the Cologne laboratory [10,11]. All other reagents and solvents were of analytical grade and glass distilled before used.

Excretion study

DHT (25 mg tablets, Anabolex, Samils.r.l.,Rome, no longer marketed) was administered to four male volunteers (V1-V4) sublingually: V1, (75 kg, 43 years), V2 (65 kg, 38 years), V3 (65 kg, 43 years), V4 (88 kg, 33 years) having given their informed consent.
Urine was collected for 24 hours before DHT application to determine the basal values and during 90 hours after application of DHT.
In the basal period, sample collection included a cumulative urine collection from 22:00 h to wakeup, then urine collection every 4 hours from 10:00 h to 22:00 h, another cumulative urine from 22:00 h to wakeup, and the urine collection at 10 h of the experiment's day. Directly after delivering the 10:00 h urine, 25 mg of DHT were administerd sublingually. Within the next 12 hours, urine was collected every 2 hours and then, for the next 24 hours, every 4 hours. For another 2 days samples were collected as described for the basal 36 hours period. The exact time of urine delivery was noted and the volume of each portion measured.

Isolation of steroids

2 ml of urine were adsorbed on Amberlite XAD-2 polystyrene resin. The XAD-2 column (pasteur pipette, with a glass pearl put into the restriction to regulate the effluent and to carry the XAD-2 bed, height: ca. 20 mm) was washed with 2 ml of twice distilled water, and conjugated and unconjugated steroids were eluted with 2 ml methanol. To the methanolic eluate 20 µl of the internal standard solution were added. The methanolic eluate was evaporated to dryness, and the residue was enzymatically hydrolysed with 50 µl ß-glucuronidase from Escherichia coli K12 (Boehringer, 6800 Mannheim, Germany) in 1 ml 0.2 M phosphate buffer, pH 7.0 for 1 hour at 50°C. After hydrolysis, the buffer solution was alkalised with 250 µl 5% potassium carbonate solution, and the steroids were extracted with 5 ml tert.butylmethyl ether. After centrifugation, the organic layer was transferred into a glass tube and evaporated to dryness under vacuum.

Derivatization for GC/MS

The dry residue was dissolved in 100 µl of MSTFA/NH_4I/ethanethiol,TMS (1000:2:6 v/w/v) and heated for 15 min at 60°C.
3 µl were injected directly into the injection port.

GC/MS quantitation

Quantitation of excreted steroids were performed with a GC/MS system [GC/MSD Hewlett-Packard (GC 5890/MS 5971A)], with the electron impact set at 70 eV, column: Hewlett-Packard, HP1, fused silica capillary column cross-linked methyl silicone (OV 1), 17 m, ID 0.20 mm, film thickness 0.11 µm. The carrier gas was helium (1 ml/min, split 1:10), and the temperature program was as follows: initial temperature 180°C, program rate 3°C/min to 229°C, 40°C/min up to final temperature 310°C.
Selected ion monitoring (SIM) was used with the following ions: m/z 434 for DHT,bis-TMS, androsterone,bis-TMS and etiocholanolone,bis-TMS, m/z 241 for 5α-androstane-3α,17ß-diol,bis-TMS (5αA3αD), 5ß-androstane-3α,17ß-diol,bis-TMS (5ßA3αD) and m/z 421 for 5α -androstane-3ß,17ß-diol,bis-TMS (5αA3ßD), m/z 432 for testosterone,bis-TMS and epitestosterone,bis-TMS.
Urine concentrations were calculated by the peak areas of the detected signals relative to the internal standard [2,2,4,4-$^{2}H_4$]-etiocholanolone m/z 438.
For calibration of the GC/MS instrument, a mixture of the reference substances (Table 1) and internal standards (Table 2) with the indicated concentrations per ml of urine was derivatized. To the urine XAD-2 extract a mixture of internal standards was added with the concentrations indicated in table 2.

Table 1: Endogenous steroids: concentrations of the working solution and resulting concentrations per ml of urine.

reference substances	working solution [µg/ml]	urine [ng/ml]
androsterone	200	2000
etiocholanolone	200	2000
testosterone	4	40
epitestosterone	4	40
5α-androstane-3α,17ß-diol	8	80
5ß-androstane-3α,17ß-diol	18	180
5α-androstane-3ß,17ß-diol	10	100
epiandrosterone	10	100
dihydrotestosterone	10	100

Table 2: Internal standard: concentrations of the working solution and resulting concentrations per ml of urine.

Reference substances	working solution [µg/ml]	urine [ng/ml]
17α-methyltestosterone	50	500
[2,2,4,4-$^{2}H_4$]-etiocholanolone	50	500
[16,16,17-$^{2}H_3$)-testosterone	9	90
[16,16,17-$^{2}H_3$]-epitestosterone	1.5	15
[2,2,4,4-$^{2}H_4$]-11ß-hydroxy-androsterone	24	240

Results and Discussion

Derivatisation and GC-MS-analysis

The derivatisation with MSTFA/NH_4I leads to the per-trimethylsilyl derivatives of the steroids of interest. The retention times and retention indices are summerized in Table 3. In addition the ions used for the acquisition in the routine screening procedure are listed.

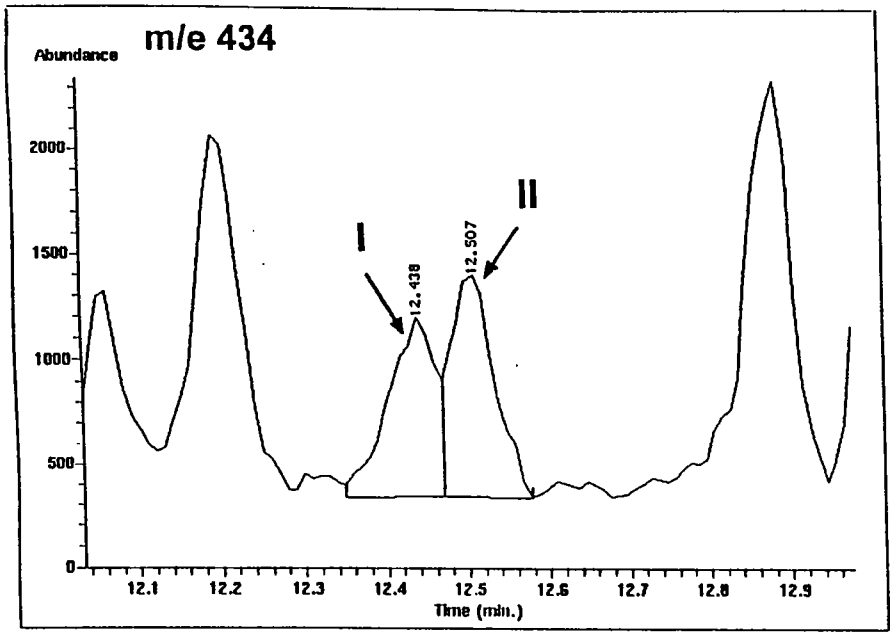
Table 3: Retention times, retention indices and ion traces used in the general procedure for dihydrotestosterone, its metabolites, the respective internal standards, and the relevant endogenous steroids (as per-trimethylsilyl derivatives)

Substanz	RT	Index	m/e
androsterone	10.36	2506	434
d_4-etiocholanolone	10.46	2511	438
etiocholanolone	10.54	2515	434
5α-androstane-3α,17ß-diol	10.68	2522	241
5ß-androstane-3α,17ß-diol	10.82	2529	241
epiandrosterone	11.78	2578	434
5α-androstane-3ß,17ß-diol	12.15	2597	421
d_3-epitestosterone	12.15	2597	435
epitestosterone	12.19	2599	432
dihydrotestosterone	12.41	2610	434
d_3-testosterone	12.99	2640	435
testosterone	13.03	2642	432
methyltestosterone	14.82	2733	446

After derivatisation with MSTFA/NH_4I, DHT is enolized to two bis-TMS isomers: TMS-3-enol,17-TMS ether (5-6%) and TMS-2-enol,17-TMS ether (94-95%). The minor isomer is eluted 0.2 min before the main isomer and baseline separated. The relation of both isomeres was constant and reproducible. The main isomer was quantified.

The bis-TMS derivative of DHT is coeluted with the bis-TMS derivative of 11-keto-eticholanolone. The fragment m/z 434 of this substance may lead to wrong evaluations of the DHT signal (Fig. 2 a). After a longer reaction time with the derivatisation reagent the bis-TMS derivative of 11-keto-etiocholanolone is completely converted to the tris-TMS derivative, which is well separated from DHT (Fig. 2b)

a)



b)

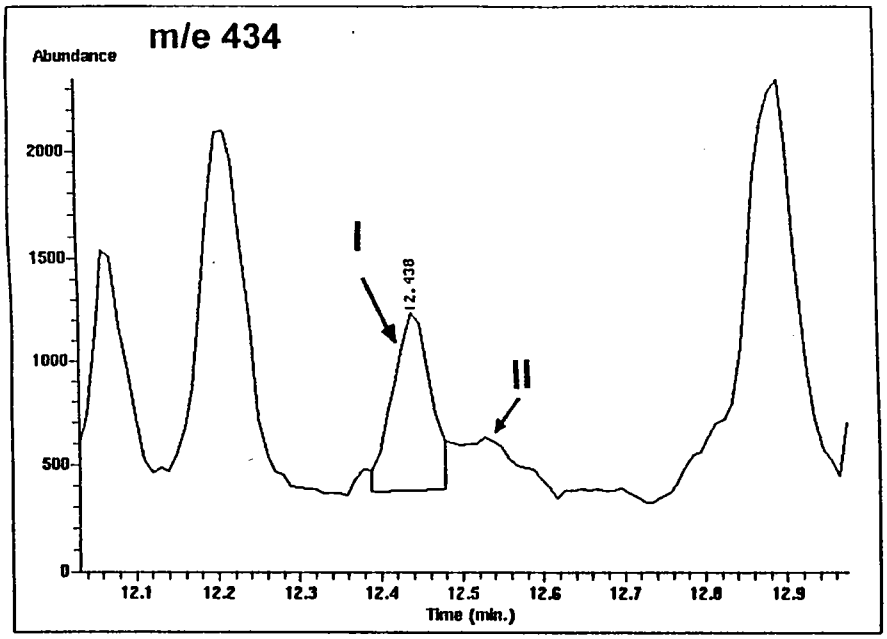


Fig. 2: DHT, bis-TMS (I) and 11-keto-etiocholanolone, bis-TMS (II) after different incubation times with MSTFA/NH_4I.
a) 15 min at 60° C
b) 30 min at 60° C

Changes of the steroidprofile after sublingual administration of DHT

After the sublingual administration of 25 mg DHT obvious alterations of steroidprofilparameters can be observed. In Figure 3 and 4 are presented as examples the screening printouts of the steroidprofiles of V1 before and after the application of DHT. As expected, the concentrations of DHT and its 5α metabolites AND, 5αA3αD, 5αA3ßD and EPIAND increase (Tab. 4). The maxima of the concentrations and excretion rates of all steroids are reached within the 2 - 4 hours interval after the DHT application. The concentrations and excretion rates of the 5ß-metabolites (ETIO, 5ßA3αD and 5ßA3ßD) and EPI are not influenced.
To have the possibility to compare concentrations of different urine samples in a similar way as excretion rates, it is necessary to correct them. Routinely we correct urinary steroid concentrations to a specific gravity of 1.020 g/cm^3 with the following formula [12,13]:

$$Conc_{corr} = \frac{1.020 - 0.998}{s.g. - 0.998} \bullet Conc_m$$

$Conz_{corr}$: corrected concentration
$Conz_m$: measured concentration
s.g.: specific gravity
0.998 : water value
1.020 : standard value of s.g.

Compared to the basal values, the average increase of the amount of excreted DHT is much greater than those of the other DHT metabolites (Tab. 5). In the first 24 hours after the DHT application the average increase of the excreted amount of DHT is about 40-fold. The excretion of 5αA3αD and 5αA3ßD increase by a factor of about 16 respectively 7. The increase of the AND excretion by a factor of about 3 is less than the increase of the 5α-diols because a substantial amount of AND is endogenously produced.

As the administration of DHT only increases the excretion of 5α-steroids but not the excretion of the corresponding 5ß-epimeres, the ratios of DHT and its metabolites with the 5ß-epimeric steroids and EPI increase as presented in Table 6.
The most obvious changes show the ratios of DHT/ETIO and DHT/EPI with about 130-fold increases. The ratios 5αA3αD/5ßA3αD and AND/ETIO is increased by a factor of about 16 respectively 7. As for the concentrations and excretion of DHT and its metabolites also the ratios show maximum values in the 2-4 hours interval.

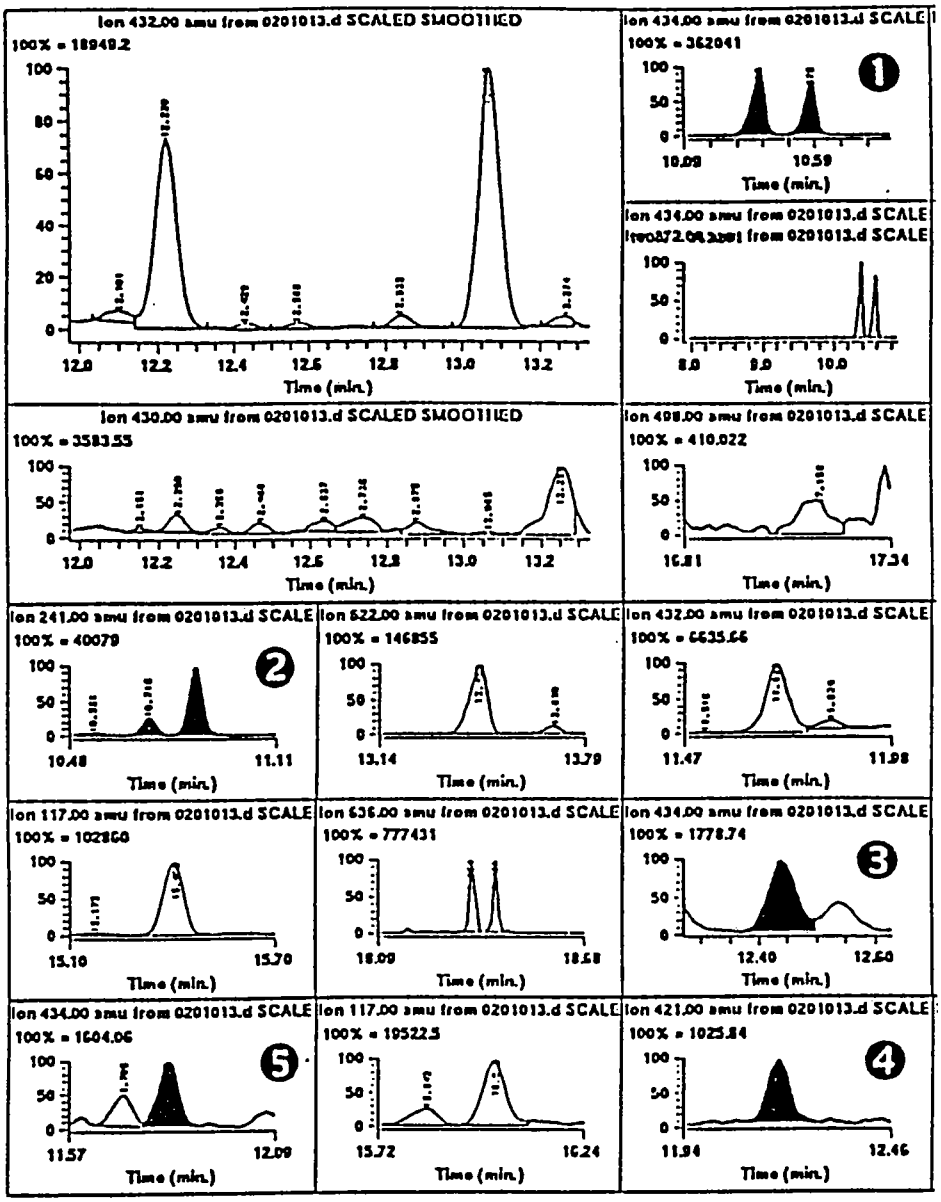


Fig. 3: Steroidprofile of V1 before the application of DHT
(all derivatives are per-trimethylsilylated)

❶ AND and ETIO
❷ 5αA3αD and 5ßA3αD
❸ DHT
❹ 5αA3ßD
❺ EPIAND

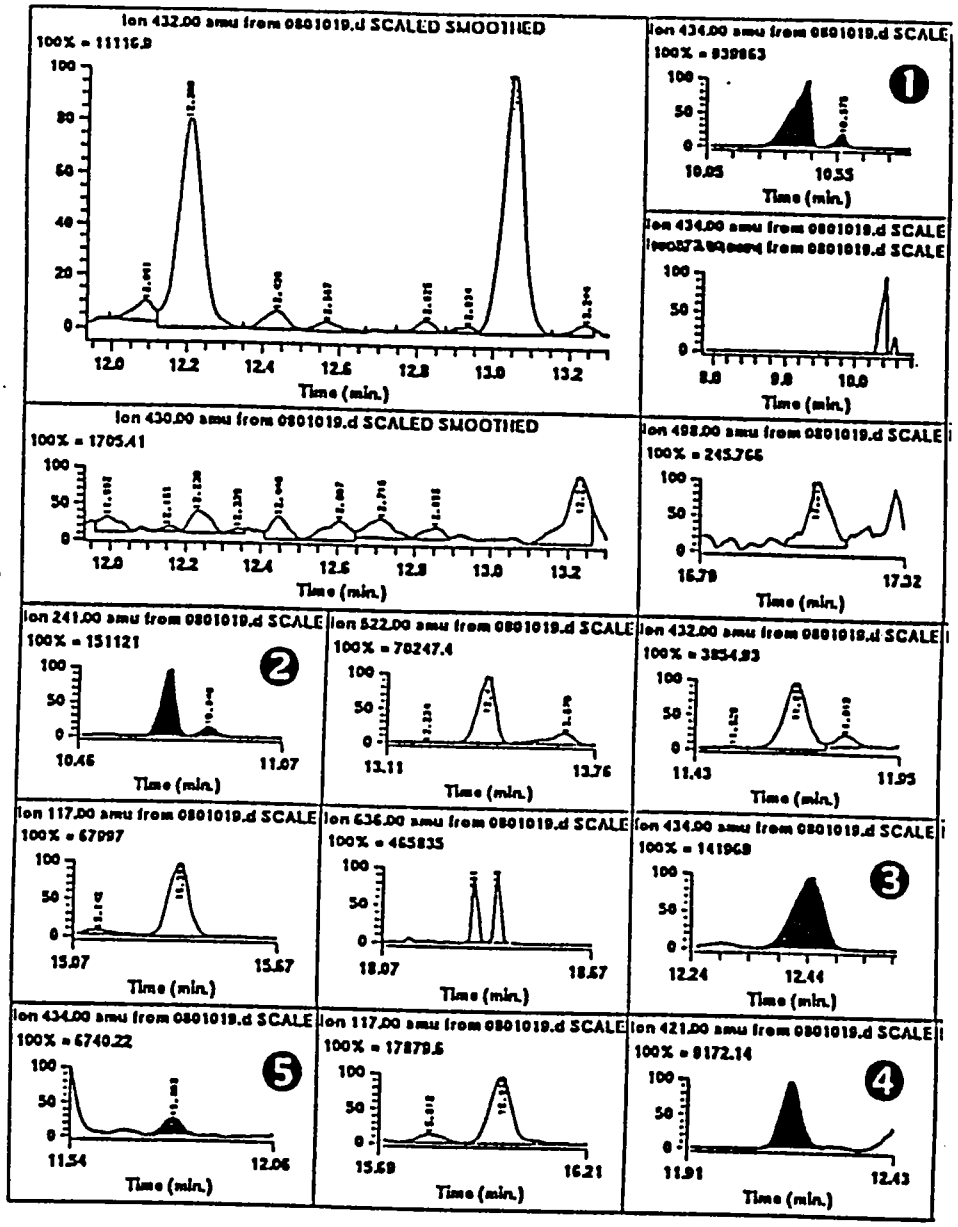


Fig. 4: Steroidprofile of V1 4 hours after the sublingual application of 25 mg DHT (all derivatives are per-trimethylsilylated)

❶ AND and ETIO

❷ 5αA3αD and 5ßA3αD

❸ DHT

❹ 5αA3ßD

❺ EPIAND

Table 4: Mean and standard deviation of the steroid concentrations after sublingual administration of 25 mg of DHT (mean of 4 volunteers).

Steroid	DHT [ng/ml]		AND [ng/ml]		5αA3αD [ng/ml]		5αA3ßD [ng/ml]		ETIO [ng/ml]		5ßA3αD [ng/ml]		EPI [ng/ml]	
	mean	stdv	mean	stdv	mean	stdv	mean	stdv	mean	stdv	mean	stdv	mean	stdv
-24-0 h	13	5	3639	1045	95	33	15	4	3062	949	375	194	37	15
0 - 2 h	758	415	10930	5509	783	350	120	59	2784	1446	302	133	40	19
2 - 4 h	1977	598	25037	7447	1766	559	255	36	3468	1184	383	97	84	60
4 - 6 h	1142	782	14758	5602	1265	687	132	67	2907	1102	594	699	38	30
6 -8 h	1063	590	13218	5675	1245	532	160	85	3442	1491	438	398	37	14
8 - 12 h	288	223	6168	5064	762	524	90	53	2065	1205	249	135	22	16
12 - 16 h	101	82	3501	1868	564	596	77	65	1792	725	315	178	28	17
16 - 20 h	64	38	4012	847	383	236	75	59	2763	1199	417	317	41	20
20 - 24 h	37	10	3484	1832	205	92	54	40	2421	1119	286	182	24	7
24 - 36 h	26	8	3790	2024	173	99	35	23	2619	1256	327	169	32	20
36 - 48 h	21	7	3636	1049	144	46	28	17	3075	1209	373	280	37	16
48 - 60 h	20	6	3730	1363	118	42	22	7	2844	528	330	141	35	22

Table 5: Amounts of excreted steroids before (-24 - 0 h) and after (0 - 24 h, 24 - 48 h) the application of 25 mg DHT. Presented are the means and standard deviations of the four volunteers.

Substance	-24 - 0 h [µg] mean ± stdev	0 - 24 h [µg] mean ± stdev	24 - 48 h [µg] mean ± stdev
DHT	18.0 ± 5.7	759.8 ± 235.5	38.2 ± 11.5
AND	3795.5 ± 990.6	10576.6 ± 2494.0	4387.6 ± 1793.3
5αA3αD	101.6 ± 27.8	1653.5 ± 829.9	201.1 ± 79.9
5αA3ßD	16.6 ± 3.9	128.6 ± 52.6	46.1 ± 30.0
EPIAND	12.1 ± 3.1	49.1 ± 26T.8	40.5 ± 35.6

Table 6: Steroid ratios relevant for the detection of DHT application. Mean and standard deviation of the 4 volunteers of the DHT excretion study, 25 mg DHT sublingually.

	5αA3αD/5ßA3αD mean±stdev	**AND/ETIO** mean±stdev	**DHT/ETIO*** mean±stdev	**DHT/EPI** mean±stdev
-24-0	0.29 ± 0.14	1.18±0.24	4.87±2.67	0.36±0.06
0-2	3.16 ± 2.34	4.61±2.66	444.62±554.30	22.25±17.90
2-4	4.78 ±1.62	7.71±3.29	637.94±299.36	45.51±29.38
4-6	3.41 ±1.63	5.21±0.88	372.58±171.74	36.38±28.68
6-8	3.66 ±1.81	3.94±1.09	310.39±138.13	30.59±20.62
8-12	3.09 ± 2.02	2.93±1.06	125.64±68.38	12.50±6.53
12-16	1.72 ±1.04	1.90±0.41	50.21±20.93	3.70±1.64
16-20	1.20 ± 0.68	1.58±0.40	24.25±14.72	1.63±0.78
20-24	0.86±0.49	1.46±0.29	17.60±7.84	1.67±0.85
24-36	0.70±0.08	1.45±0.02	14.11±2.79	1.38±0.13
36-48	0.43±0.09	1.24±0.03	9.65±1.69	0.90±0.06
48-60	0.37±0.04	1.30±0.09	7.71±1.04	0.76±0.05

* DHT/ETIO value multiplied with 1000

Reference ranges for the judgement of a DHT application

To decide if DHT related parameters are influenced by the application of exogenous DHT, subject based and/or population based reference ranges of the DHT related parmeters are necessary.

In this study subject based reference ranges for each volunteer were calculated from the steroidprofile values of the 24 hours pretest collection period. The upper limit of the individual reference range of an parameter was defined as mean + 3 x standard deviation [14].
In Table 7 are presented the time periods in which ratios lie above the upper limits of the subject based reference ranges. The ratio AND/ETIO shows the shortest increase after DHT application. Nevertheless this ratio is very important for the detection of DHT doping because it is the most stable parameter of the steroidprofile [15,16]

Another basis for the judgement of a DHT application are population based reference ranges. Such reference ranges for DHT related parameters were calculated by Donike et. al. [17]. In Table 8 are presented the time intervals in which steroidprofileparameters are above the upper limits of population based reference ranges after the administration of DHT. For individuals with borderline values of some parameters (see V1 in Tab.8), subject based reference ranges are more suitable for the decision making process.
Additionally subject based reference ranges will allow a more precise prediction of an DHT application than population based reference ranges, if the upper limits of the subject based reference ranges are lower than the upper limits of the population based reference ranges. This is shown in Figure 5 for the ratio 5αA3αD/5ßA3αD of volunteer 2. The increase of the ratio after DHT application is longer detectable with the use of the upper subject based reference limit.

For routine doping control subject-based reference ranges are not available, but they may be established if DHT doping is suggested by collecting retrospective data of previous tests and prospective data by unannounced out of competition controls.

Table 7: Time interval in hours after the administration of 25 mg DHT in which steroid ratios of the four volunteers (V1-V4) are above the upper limits of the subject based reference ranges. The upper limits of the subject based reference ranges were calculated from the means of the pretest values (-24 - 0 h) + 3 x standard deviation.

parameter	V1	V2	V3	V4
c DHT corr.**	20.0	32.8	60.3	60.0
AND/ETIO	20.0	20.5	16.8	28.0
5αA3αD/5ßA3αD	60.0	44.5	56.3	45.0
DHT/ETIO*	52.0	36.5	60.3	28.0
DHT/5ßA3αD	20.0	36.5	60.3	28.0
DHT/EPI	52.0	28.5	60.3	60.0
5αA3ßD/5ßA3αD	36.0	36.5	60.3	> 60.0

* DHT/ETIO value multiplied with 1000

** concentration of DHT [ng/ml] corrected by specific gravity (see formula above)

Table 8: Time interval in hours after the administration of 25 mg DHT in which steroid ratios of the four volunteers (V1-V4) are above the upper limits of population based reference ranges. The reference ranges are calculated from 4631 dope control samples of male athletes [17].

parameter	upper limit of reference range	V1	V2	V3	V4
c DHT corr.**	20.55	n.d.	32.8	52.3	60.0
AND/ETIO	2.86	12.0	12.8	6.3	12.0
5αA3αD/5ßA3αD	1.53	20.0	20.5	8.3	8.0
DHT/ETIO*	8.22	n.d.	28.5	36.3	45.0
DHT/EPI	0.73	28.0	28.5	>60	60.0

* DHT/ETIO value multiplied with 1000

** concentration of DHT [ng/ml] corrected by specific gravity (see formula above)

n.d. cannot be determined because pretest values are already above reference limit

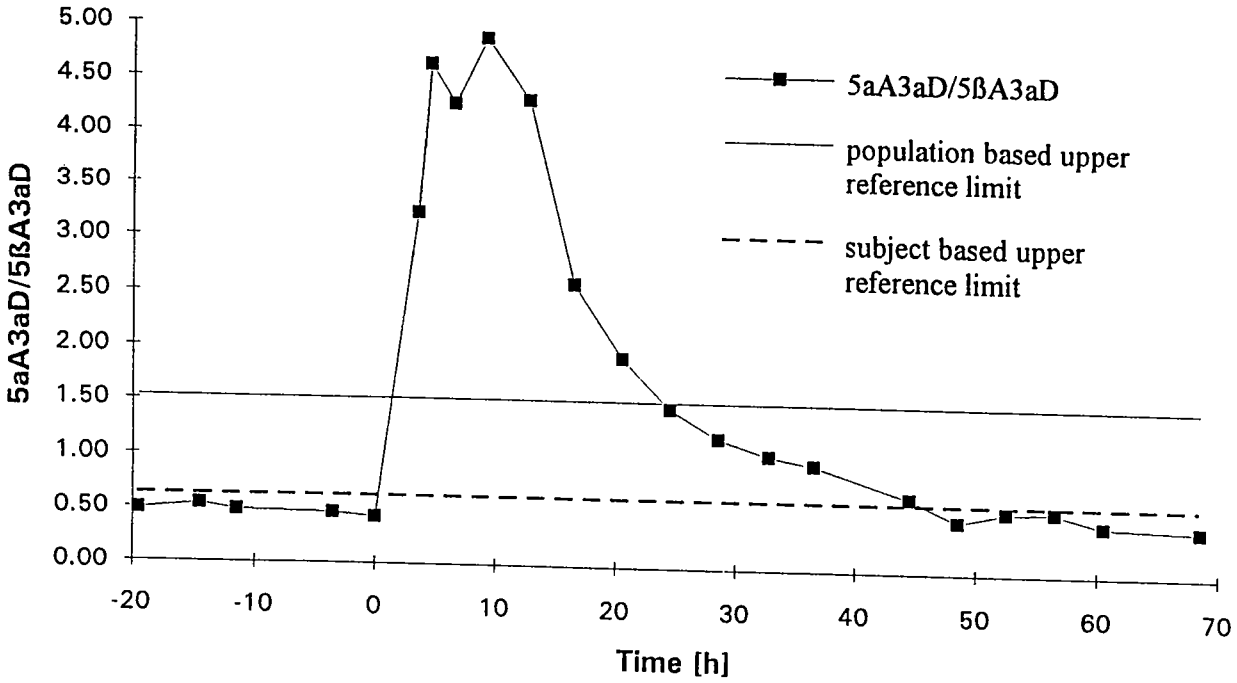


Fig. 5: Ratio of 5αA3αD/5ßA3αD of volunteer 2 after application of 25 mg DHT with subject- and population based upper reference limits of this ratio

REFERENCES

1. DONIKE, M.; ZIMMERMANN, J.; BÄRWALD, K.-R.; SCHÄNZER, W.; CHRIST, V.; KLOSTERMANN, K.; OPFERMANN, G.: Routinebestimmung von Anabolika in Harn. *Deutsche Zeitschrift für Sportmedizin* **35** (1984) 14-24.
2. DONIKE, M.; ZIMMERMANN, J.: Zur Darstellung von Trimethylsilyl-, Triethylsilyl- und tert.-Butyldimethylsilyl-enoläthern von Ketosteroiden für gas-chromatographische und massenspektrometrische Untersuchungen. *J. Chromatogr.* **202** (1980) 483-486.
3. SCHÄNZER, W.; DONIKE, M.: Metabolism of anabolic steroids in man : synthesis and use of reference substances for identification of anabolic steroid metabolites. *Analytika Chimica Acta* **275** (1993) 23-48.
4. DONIKE, M.; BÄRWALD, K.-R.; K.-R.; KLOSTERMANN, K.; SCHÄNZER UND, W.; ZIMMERMANN, J.: Nachweis von exogenem Testosteron. *in: Sport: Leistung und Gesundheit. Edt.: H.Heck, W.Hollmann, H.Liesen, R.Rost, Deutscher Ärzte Verlag Köln* (1983) 293.

5. DONIKE, M.; ADAMIETZ, B.; OPFERMANN, G.; SCHÄNZER, W.; ZIMMERMANN, J.; MANDEL, F.: Die Normbereiche für Testosteron- und Epitestosteron-Urinspiegel sowie des Testosteron-/Epitestosteron-Quotienten. *in: Training und Sport zur Prävention und Rehabilitation in der technisierten Umwelt. I.-W.Franz, H.Mellerowicz, W.Noack (Eds.), Springer Verlag, Berlin, Heidelberg* (1985) 503-507.
6.. ZIMMERMANN, J.: *Untersuchungen zum Nachweis von exogenen Gaben von Testosteron* Hartung-Gorre Verlag Konstanz, Dissertation Deutsche Sporthochschule Köln, 1986
7. MOGHISSI, E.; ABLAN, F.; HORTON, R.: Origin of plasma androstanediol glucuronide in men. *J. Clin. Endocrinol. Metab.* **59** (1984) 417-421.
8. VIERHAPPER, H.: Formation of androstanediol from 13C-labeled testosterone in humans. *Steroids* **55** (1990) 177-180.
9. SOUTHAN, G.J.; BROOKS, R.V.; COWAN, D.A.; KICMAN, A.T.; UNNADKAT, N.; WALKER, C.J.: Possible indices for the detection of the administration of dihydrotestosterone to athletes. *J. Steroid Biochem. Molec. Biol.* **42** (1992) 87-94.
10. DONIKE, M.: N-Methyl-N-trimethylsilyl-trifluoracetamid, ein neues Silylierungsmittel aus der Reihe der silylierten Amide. *J. Chromatogr.* **42** (1969) 103-104.
11. SCHÄNZER, W.; DONIKE, M.: Synthesis of deuterated steroids for GC/MS quantification of endogneous steroids. *in: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke (Eds.) Recent advances in doping analysis (2).. Sport und Buch Strauß, Köln* (1995) 93-112.
12. DONIKE, M.; RAUTH, S.; SAMPLE, B.: Excretion of ephedrine and endogenous steroids under conditions of controlled water intake and of water diuresis. *in: Donike, M., H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (Eds.) 10th Cologne Workshop on Dope Analysis, 7th to 12 th June 1992 - Proceedings. Sport und Buch Strauß, Köln* (1993) 163-176.
13. RAUTH, S.: Referenzbereiche von urinären Steroidkonzentrationen und Steroidquotienten - Ein Beitrag zur Interpretation des Steroidprofils in der Routinedopinganalytik. *Dissertation, Deutsche Sporthochschule Köln* 1994.
14. DONIKE, M.; MARECK-ENGELKE, U.; RAUTH, S.: Statistical evaluation of longitudinal studies, part 2: The usefulness of subject based reference ranges. *in: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke (Eds.). Recent advances in doping analysis (2) Sport und Buch Strauß , Köln* (1995) 157-165.
15. MARECK-ENGELKE U., GEYER H. AND DONIKE M.: Stability of steroid profiles. *in: M. Donike, , H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (Eds.) 10th Cologne Workshop on Dope Analysis, 7th to 12 th June 1992 - Proceedings. Sport und Buch Strauß, Köln* (1993) 87-89.
16. MARECK-ENGELKE U., GEYER H. AND DONIKE M.: Stability of steroid profiles (3): The Circadian Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Male. *in: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke (Eds.) Recent advances in doping analysis (2) Sport und Buch Strauß, Köln* (1995) 121-133.
17. DONIKE, M., UEKI, M., KURODA, Y., GEYER, H., NOLTEERNSING, E., RAUTH, S., SCHÄNZER, W., SCHINDLER, U., VÖLKER, E., FUJISAKI, M.: Detection of dihydrotestosterone (DHT) doping - Alterations in the steroid profile and reference ranges for DHT and its 5α-metabolites. *J. Sports.Med.Phys.Fitness, in press.*