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Finasteride - a substance for manipulation in dope control ?

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Abstract

To study the influence of finasteride on the urinary steroidprofile and on the metabolism of anabolic androgenic steroids, several excretion studies with finasteride (Proscar ®, MSD Chibropharm GmbH, Germany) were performed:
An excretion study with 5 mg finasteride was performed with 1 volunteer. The urines were collected for 10 days and the profiles of endogenous urinary steroids were analysed by GC/MS. It could be shown, that finasteride led to obvious changes of several steroidprofile parameters. The excretion of 5-alpha-steroids like androsterone, 5α-androstane-3α, 17ß-diol, allo-tetrahydrocortisol, 11ß-OH-androsterone, and dihydrotestosterone decreased, whereas the excretion of the 5ß-steroids increased or didn't change. The results were obvious decreases of the ratios between epimeric 5α-and 5ß steroids like e.g. androsterone/ etiocholanolone, 5α-androstane-3α, 17ß-diol/5ß-androstane-3α, 17ß-diol, allo-tetrahydrocortisol/ tetrahydrocortisol etc. These changes could be detected for more than 8 days. The ratio testosterone/ epitestosterone was within the normal variation expected.
Further excretion studies with 5 mg finasteride were performed with volunteers, who received nandrolone and dihydrotestosterone. It could be shown that finasteride intake resulted in a decreased urinary excretion of norandrosterone, the main metabolite of nandrolone and a decrease of some of the steroid ratios, which are the main parameters for the detection of dihydrotestosterone. These results show, that finasteride is as potential masking agent. The significance of all these results for dope control is discussed.

Introduction

Finasteride is an inhibitor of 5-alpha reductase, the enzyme responsible for conversion of testosterone to dihydrotestosterone (fig. 1). It is administered orally in a dose of 5 mg daily for

the treatment of benign prostatic hypertrophy. Since a short time period it is also admitted in several countries (Germany, Switzerland, Australia, Newsealand and Sweden) for the treatment of men with hair loss (androgenetic alopecia) and it seems to become a socalled „life style drug". The recommended dose for the treatment of hair loss is 1 mg/day.
To get knowledge about the influence of this substance on the steroidprofile and on the metabolism of anabolic androgenic steroids, several excretion studies with finasteride were performed.

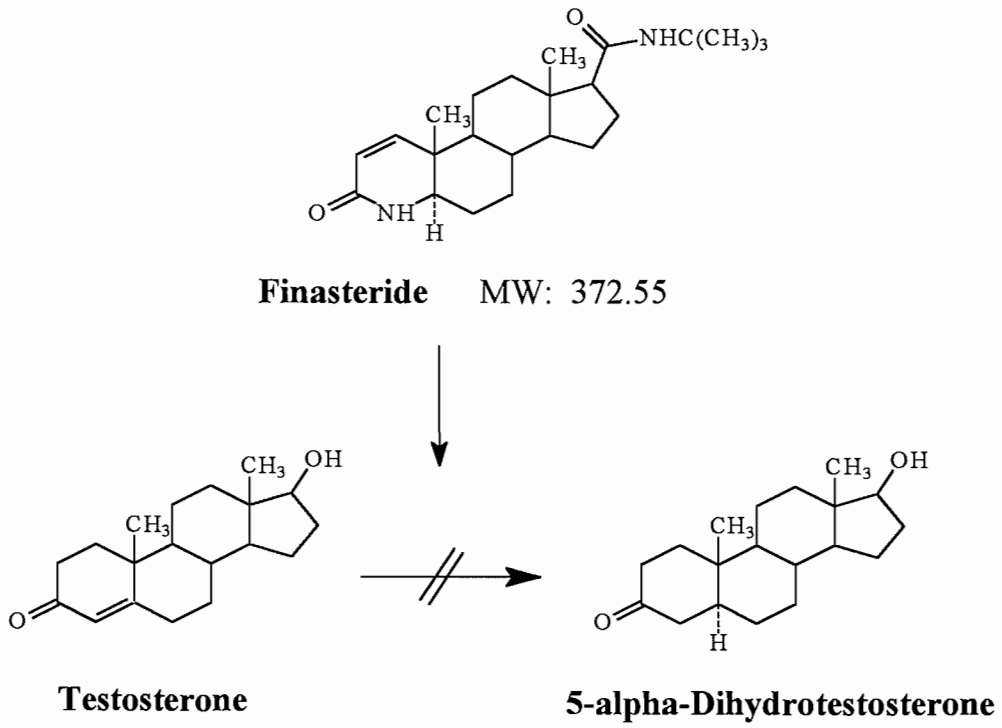


Fig. 1: Structure and effect of the 5α-reductase inhibitor finasteride.

Experimental

Excretion studies
All excretion studies were performed by the same male volunteer (43 years, 171 cm, 71 kg) by oral administration of 5 mg finasteride (1 tablet of Proscar ®, MSD Chibropharm GmbH, Germany). Between the excretion studies were pauses of several weeks.

1. Administration of 5 mg finasteride. Urines were collected before and up to 10 days after the administration.

2. Administration of
   a) 20 µg of nandrolone orally (20 µl of a methanolic solution of nandrolone (Serva) 1mg/ml was injected to a piece of bread and ingested)
   b) 5 mg finasteride. 6 hours after finasteride 20 µg of nandrolone orally.
   Urines were collected before and up to 10 hours after administration of the drugs.

3. Administration of
   a) 25 mg 5-alpha dihydrotestosterone sublingually (Anabolex ®, Samil, Rome,Italy).
   b) 25 mg 5-alpha dihydrotestosterone sublingually and simultaneous 5 mg finasteride.
   Urines were collected before and up to 48 hours after administration of the drugs

The urines were prepared according to the screening procedure of anabolic steroids (1). The parameters for the GC/MS analysis of the steroidprofile are described elsewhere by Donike et al. (2).

Detection of finasteride metabolite
2 ml of an urine collected 6 h after administration of 5 mg finasteride were prepared according to the screening procedure for anabolic steroids (1) without adding the internal standards. The dry extract was dissolved in methanol. The derivatisation was performed by flash alkylation with trimethyl-anilynium-hydroxide (TMAH). The GC/MS-parameters were as described by Donike et. al. (2). The temperature program was 180° C/ 10° C per min/ 320 °C/ 3 min.

Results and Discussion

Excretion studies

The excretion study 1 with 5 mg finasteride showed, that finasteride led to obvious changes of several steroidprofile parameters. The excretion of 5-alpha-steroids like androsterone (AND), 5α-androstane-3α, 17ß-diol (Adiol), allo-tetrahydrocortisol (ATHC), 11ß-OH-androsterone (11OHA), and dihydrotestosterone (DHT) decreased, whereas the excretion of the 5ß-steroids increased or did not change. This is shown in figure 2 for androsterone (AND) and etiocholanolone (ETIO). The results were obvious decreases of the ratios between epimeric 5α-and 5ß steroids like e.g. androsterone/ etiocholanolone (AND/ETIO), 5α-androstane-3α, 17ß-diol/5ß-androstane-3α, 17ß-diol (Adiol/Bdiol), allo-tetrahydrocortisol/ tetrahydrocortisol (ATHC/THC) etc. (see fig. 3 and tab. 1). The lowest levels for AND/ETIO and Adiol/Bdiol were reached between 12 and 24 hours after the application, whereas the ratio ATHC/THC showed the lowest level after 38 hours (tab. 1) These changes could be detected for more than 8 days. The ratio testosterone/ epitestosterone was within the normal variation (tab 1).

Tab. 1.: Ratios of androsterone/ etiocholanolone (AND/ETIO), 5α-androstane-3α, 17ß-diol/5ß-androstane-3α, 17ß-diol (Adiol/Bdiol), allo-tetrahydrocortisol/ tetrahydrocortisol (ATHC/THC), 11-hydroxy-androsterone/11-hydroxy-etiocholanolone (11OHA/11OHE) and testosterone/epitestosterone (TEST/EPIT) after the administration of 5 mg finasteride

time after application [h]	AND/ETIO	Adiol/Bdiol	ATHC/THC	11OHA/OHE	TEST/EPIT
-3.5	1.73	0.54	0.952	1.86	1.56
0.0	1.91	0.58	0.881	7.21	1.09
2.0	1.08	0.44	0.973	6.82	1.01
4.0	0.26	0.35	1.020	3.47	0.69
6.0	0.16	0.24	0.358	1.19	1.11
9.0	0.15	0.21	0.505	0.73	0.98
12.0	0.11	0.15	0.198	0.39	1.08
14.0	0.14	0.13	0.149	0.31	1.76
16.0	0.15	0.10	0.113	0.34	1.51
22.0	0.15	0.09	0.055	0.36	1.68
24.0	0.13	0.08	0.014	0.55	1.52
26.0	0.15	0.09	0.010	0.81	1.25
30.0	0.13	0.10	0.006	0.81	1.06
38.0	0.11	0.07	0.004	0.48	1.64
47.0	0.11	0.06	0.005	0.32	1.59
48.0	0.15	0.06	0.007	0.72	1.45
85.3 - 94.8	0.33	0.13	0.037	1.54	1.84
112.1 - 119.3	0.52	0.22	0.104	2.66	1.71
134.6 - 141.8	0.69	0.25	0.154	3.44	1.32
182.0 - 189.0	0.77	0.23	0.195	1.30	1.42
206.0 - 214.0	0.99	0.28	0.254	1.63	1.31
231.8 - 237.8	1.03	0.22	0.422	1.58	1.37

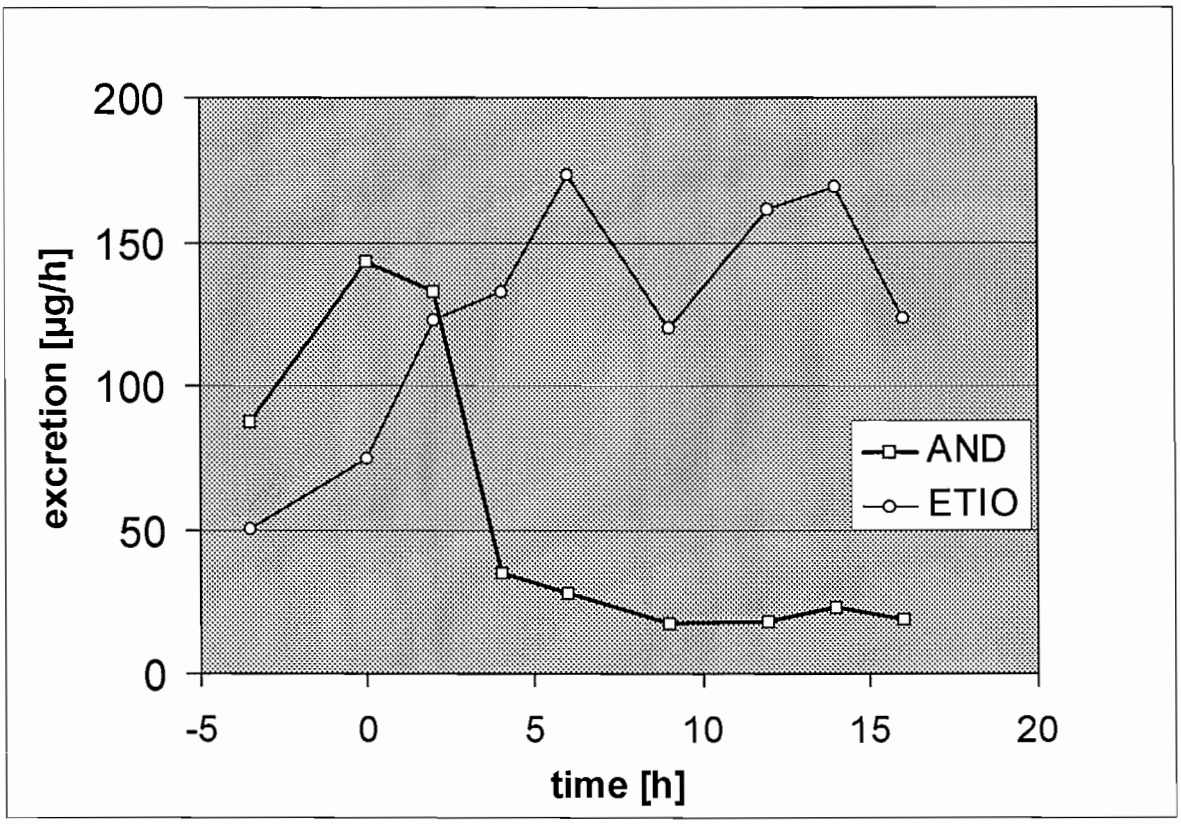


Fig. 2: Change of excretion rates of androsterone (AND) and etiocholanolone (ETIO) after the administration of 5 mg finasteride.

The change of the ratios between epimeric steroids after the application of finasteride is of importance in dope control, because these ratios, especially AND/ETIO and Adiol/Bdiol, are the most stable steroidprofile parameters (3, 4). These parameters are used for the individualisation of samples and to establish subject based reference ranges e.g. in longitudinal and endocrinological studies, which is not longer possible when finasteride is administered.

In the second set of excretion studies, 20 µg of nandrolone were administered without and with finasteride. After the application of finasteride it could be detected an obvious decrease of the excretion of the 5α-steroid norandrosterone, the main metabolite of nandrolone (fig. 4), and an increase of the excretion of the 5ß-metabolite noretiocholanolone. The maximum excretion rates for noretiocholanolone increased from 17 to 30 ng/min. Therfore the ratios norandrosterone/noretiocholanolone changed from about 3.5-4.5 without finasteride to 0.4-0.7 with finasteride. The suppression of the excretion norandrosterone is of great importance for dope control, because this metabolite is mainly used in the screening procedures to detect the

misuse of nandrolone, norandrostendione and norandrostendiol. With finasteride it may be possible to prevent a detection of such a misuse. Therefore this substance may an efficient masking agent.

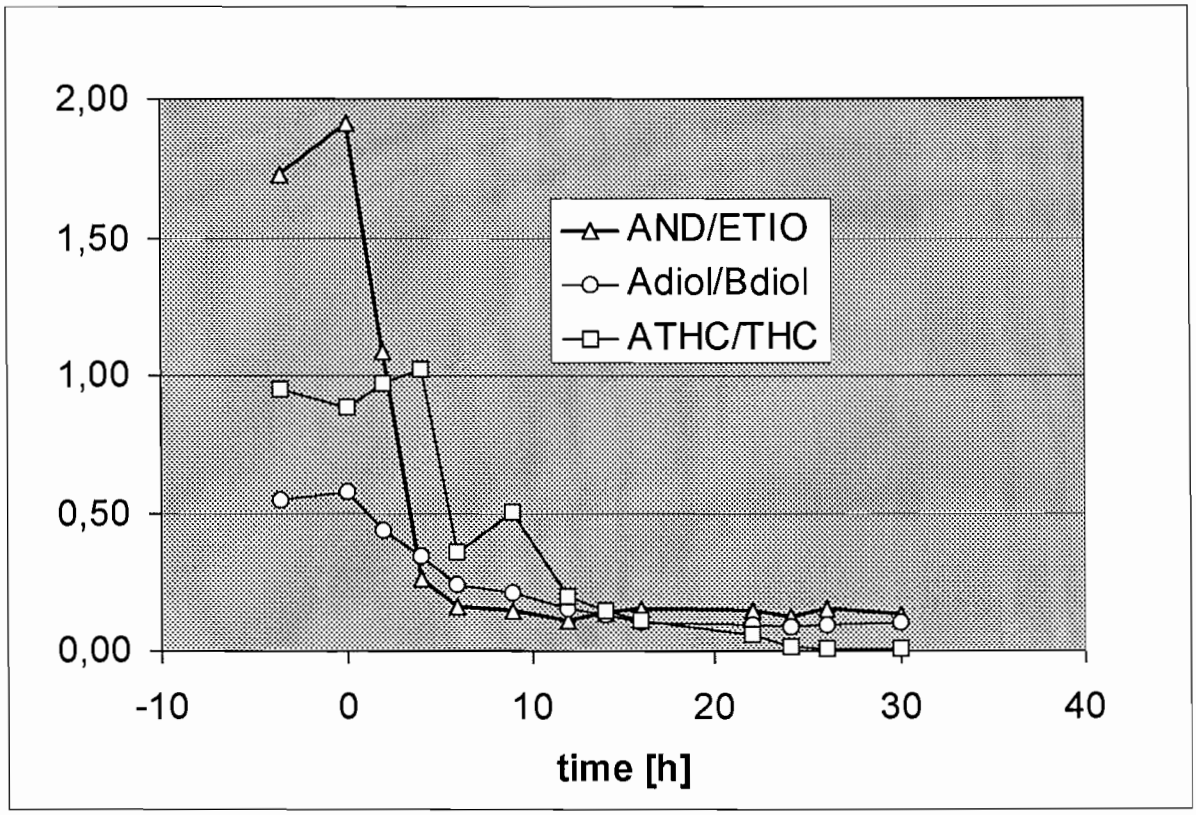


Fig. 3.: Ratios of androsterone/ etiocholanolone (AND/ETIO), 5α-androstane-3α, 17ß-diol/5ß-androstane-3α, 17ß-diol (Adiol/Bdiol) and allo-tetrahydrocortisol/ tetrahydrocortisol (ATHC/THC) after the administration of 5 mg finasteride

In the third set of excretion studies, 25 mg of 5-alpha dihydrotestosterone were administered sublingually without and with 5 mg finasteride. It was shown that finasteride can shorten the time period during which parameters for the detection of a misuse of DHT are above the population based – or individual reference limits and give a positive result (tab. 2, fig. 5). Based on individual reference limits, without finasteride, the DHT application could be detected with the parameter Adiol/Bdiol for 60 hours, whereas with finasteride the application could be detected only for 35 hours (tab. 2).

These results show, that finasteride may lead to an obvious change of the endogenous steroidprofile parameters which may cause serious problems for the interpretation of steroidprofiles e.g in longitudinal studies. Furthermore finasteride can be misused in dope

control for manipulation as a masking agent, i.e. to prevent the detection of 5-alpha metabolites of anabolic steroids or to manipulate characteristic steroidprofile ratios, which lead to positive results.

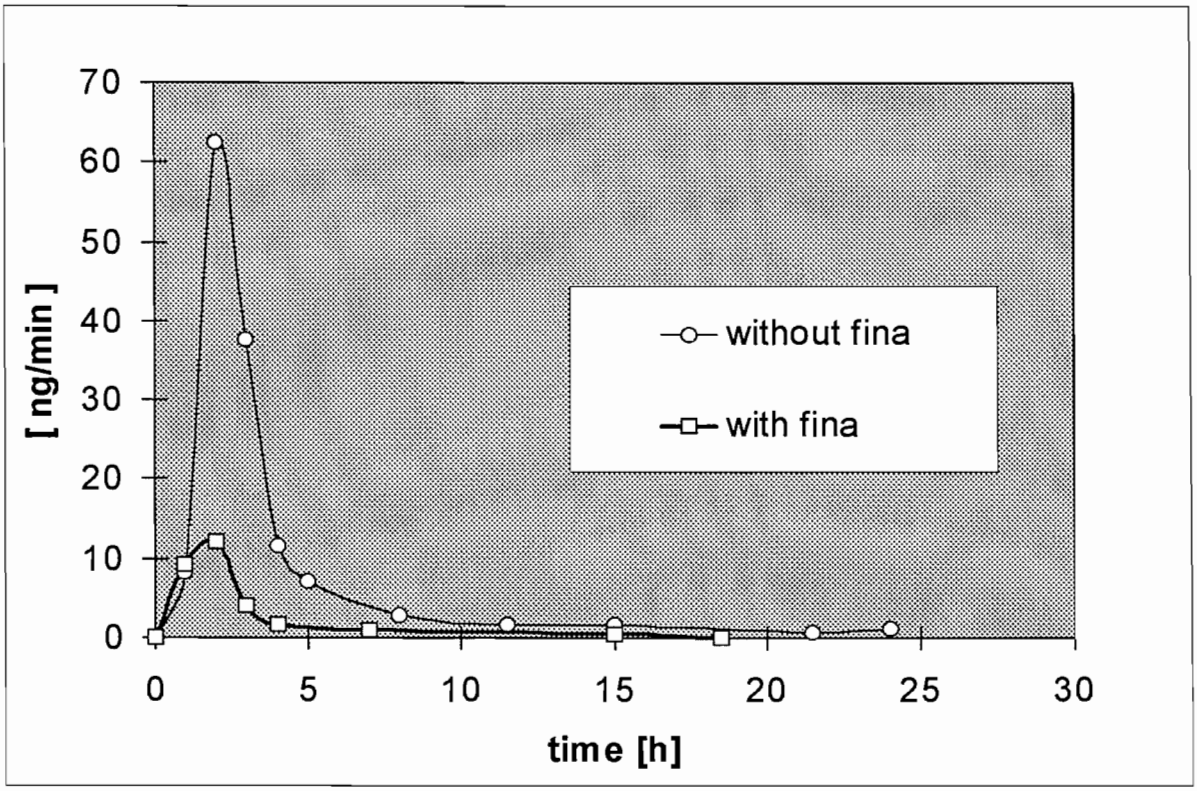


Fig. 4: Influence of finasteride on the excretion of norandrosterone after the administration of nandrolone (20 µg, orally)

Tab. 2 : Influence of finasteride on the characteristic parameters for the detection of dihydrotestosterone (DHT) misuse. Presented are the time periods, during which steroidprofile parameters were above the upper limits of the individual reference ranges of the volunteer and gave a positive result for misuse of DHT (administration of 25 mg DHT sublingually).

Parameter		without Fina	with Fina
cDHT [ng/ml] [1]	> 35	20 h	24 h
AND/ETIO	> 1.8	36 h	6 h
Adiol/Bdiol	> 0.4	60 h	35 h
DHT/ETIO [2]	> 10.7	32 h	24 h
DHT/EPIT	> 0.54	28 h	27 h

[1] concentration corrected for specific gravity of 1.020 g/cm^3
[2] DHT/ETIO value multiplied by 1000

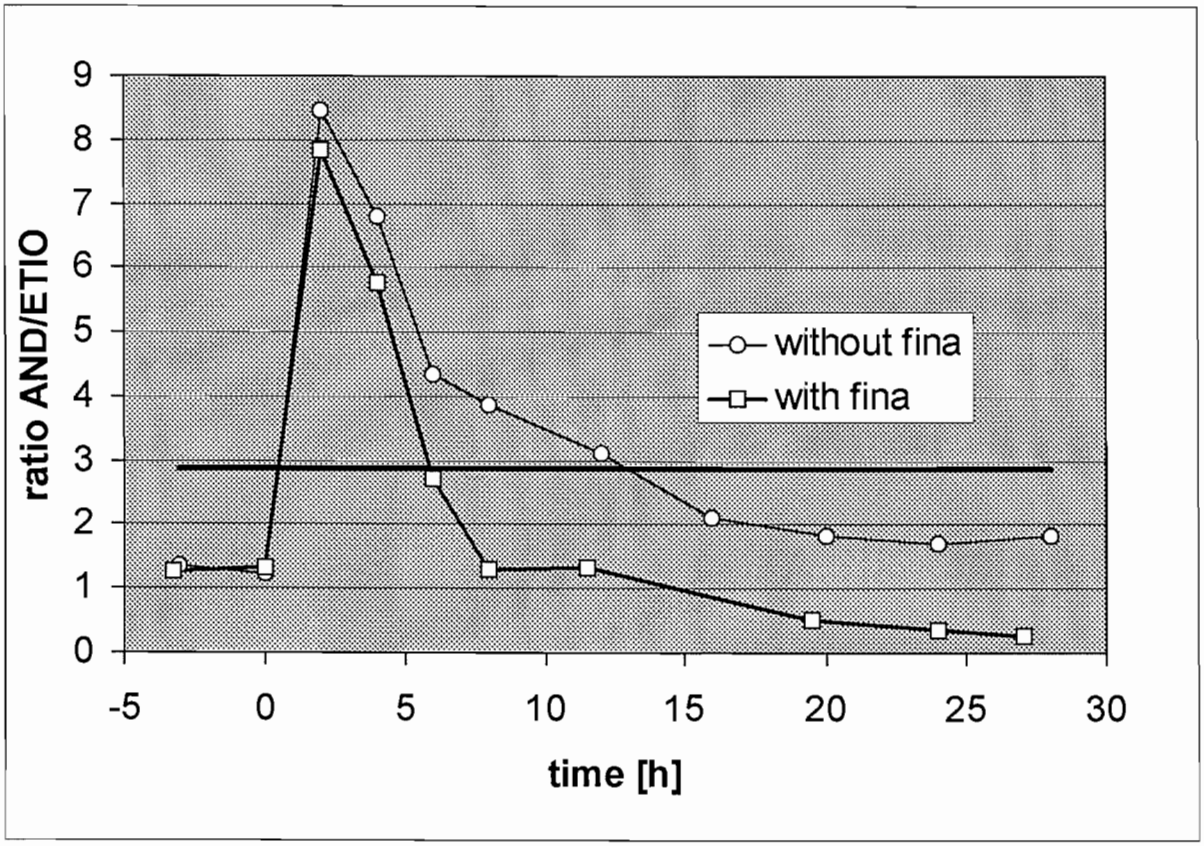
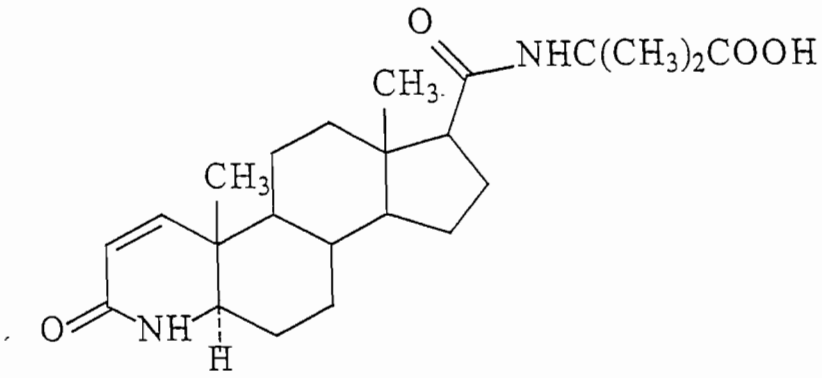


Fig. 5 : Administration of 25 mg dihydrotestosterone (DHT). Influence of finasteride on the time period, during which ratio androsterone/etiocholanolone (AND/ETIO) was above the upper limit of the population based reference range for male athletes (2) and gave a positive result for misuse of DHT. (— upper reference limit for ratio AND/ETIO)

Detection of finasteride

The main urinary metabolite of finasteride, the carboxy-finasteride is shown in fig 6. It is found in the free fraction (5). A full scan spectrum of this metabolite, from an urine collected 6 h after administration of 5 mg finasteride after flash alkylation with TMAH is shown in fig. 7. Further studies have to be performed to develop a method, which allows the analysis of the finasteride metabolite within an existing screening procedures in dope control.



$C_{23}H_{34}N_2O_4$

MW: 402.53

Fig. 6: The main metabolite of finasteride (5).

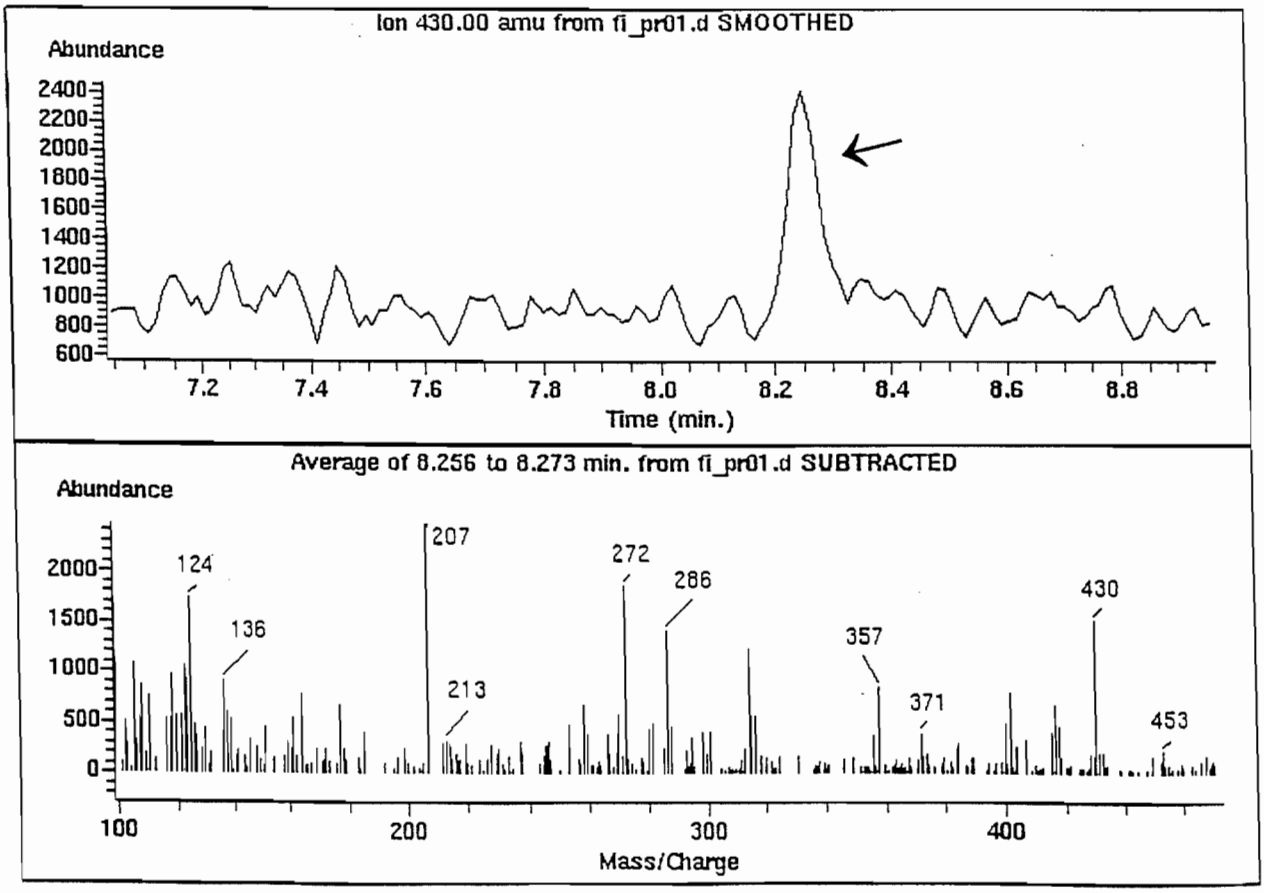


Fig. 7: Chromatogram of m/z 430 with the bis-methyl-derivative of the finasteride metabolite (Carboxy-finasteride) after flash alkylation with TMAH and its full scan spectrum.

References

1. Geyer, H., Schänzer, W., Mareck-Engelke, U., Nolteernsting, E., Opfermann, G.: Screening procedure for anabolic steroids-The control of the hydrolysis with deuterated androsterone glucuronide and studies with direct hydrolysis. In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (5). Sport und Buch Strauß, Köln (1998) 99-102

2. Donike, M., Ueki, M., Kuroda, Y., Geyer, H., Nolteernsing, E., Rauth, S., Schänzer, W., Schindler, U., Völker, E., Fujisaki, M.: Detection of dihydro-testosterone (DHT) doping - Alterations in the steroid profile and reference ranges for DHT and its 5α-metabolites. J. Sports.Med.Phys.Fitness, 35 (1995) 235-50.

3. Mareck-Engelke, U., Geyer, H., Donike, M.: Stability of steroid profiles. In: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (eds.) Proceedings of the 10th Cologne Workshop on Dope Analysis 7th to 12th June 1992, Sport und Buch Strauß, Köln (1993) 87-90.

4. Mareck-Engelke, U., Geyer, H., Donike, M: Stability of steroidprofiles (3): Ratios and excretion rates of endogenous steroids in male urines collected over 24 hours. In: M. Donike, H.Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (2). Sport und Buch Strauß, Köln (1995) 121-134

5. Carlin, J.R., Höglund, P., Erikkson, L.-O., Christafolo, P., Gregoire, S.L. et al.: Disposition and pharmakokinetics of [^{14}C] finasteride after oral administration in humans. Drug Metabolism and Disposition 20, (1992) 148-155