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Intramuscular administration of 5α-dihydrotestosterone heptanoate: changes in urinary hormone profile

Sarah B. Coutts,[1] Andrew T. Kicman,[1*] Derek T. Hurst,[2] and David A. Cowan[1]

A recommended confirmatory procedure for detecting 5α-dihydrotestosterone (DHT) doping in male athletes proposed the use of the urinary concentration ratio of DHT to epitestosterone (EpiT) as the primary marker and those of 5α-androstane-3α,17β-diol (5α-Adiol) to EpiT, luteinizing hormone (LH), and 5β-androstane-3α,17β-diol (5β-Adiol) as secondary markers. Here we investigate the effects on these markers of intramuscular administration of DHT heptanoate (250 mg) to six healthy men. Within 24 h of administration all four markers greatly exceeded the published discrimination limits, remaining above these limits for 10–14 days. All ratios returned to basal values by day 28. In contrast to results after percutaneous administration, 5β-Adiol excretion decreased, probably as a consequence of greater suppression of testicular steroidogenesis. Results were largely in agreement with those obtained after percutaneous administration, although probably augmented by the larger dose and the different route of delivery.

Anabolic-androgenic steroids are frequently found to be misused in the field of sports. Data collected from laboratories accredited by the International Olympic Committee (IOC)[3] indicate that cheaters appear to favor administration of testosterone (T), which is commonly detected by abnormal urinary concentration ratios of T to epitestosterone (EpiT). Ueki et al. *[1]* claim that in an attempt to beat this test, some cheaters are switching to dihydrotestosterone (DHT; the active 5α-reduced metabolite of T), which is known not to perturb the T/EpiT ratio *[2]*. Recently, we proposed a confirmatory procedure for detecting 5α-DHT in male athletes *[3]*. The urinary concentration ratio of DHT/EpiT was chosen to be the primary marker for detection of DHT doping; 5α-androstane-3α,17β-diol (5α-Adiol; the main metabolite of DHT)/EpiT, 5α-Adiol/luteinizing hormone (LH), and 5α-Adiol/5β-androstane-3α,17β-diol (5β-Adiol; a metabolite of T) were chosen as secondary markers.

Because we developed a method capable of detecting percutaneous DHT administration *[3]*, we wanted to investigate the robustness of the test by studying how well these markers were suited to the detection of DHT doping when other routes of administration were used. Oral administration may be convenient for the cheater, but for steroids that are produced endogenously, it is not the ideal route of delivery because of extensive first-pass metabolism. Nevertheless, DHT may be taken orally by some sports competitors, and some work has been done in this area *[4, 5]*. First-pass metabolism can be circumvented by administering formulations designed for sublingual absorption, but nonetheless some of the dose may be swallowed. Investigations into the changes in the urinary steroid profile after sublingual application to four male volunteers *[6, 7]* concluded that DHT/E, 5α-Adiol/5β-Adiol, and androsterone/etiocholanolone (A/E) were suitable parameters of detection.

Intramuscular administration and, in particular, injection of esterified compounds prolong the activity of steroids because of a depot effect. Investigations of the possible clinical use of crystalline DHT *[8]* and DHT heptanoate *[9]* found that intramuscular injection gave a prompt and sustained increase in DHT, and concluded that such preparations could provide an effective and convenient method of replacement therapy. Such a future clinical use would increase the risk of underground availability of licensed DHT compounds. Even without such a supply, esters of DHT would be relatively simple

[1] Drug Control Centre, King's College London, Manresa Rd., London SW3 6LX, UK.
[2] School of Life Sciences, Kingston University, Kingston upon Thames, Surrey KT1 2EE, UK.
* Author for correspondence. Fax 44-171-351-2591; e-mail a.kicman@kcl.ac.uk.

[3] Nonstandard abbreviations: IOC, International Olympic Committee; DHT, 5α-dihydrotestosterone; EpiT, epitestosterone (17α-hydroxyandrost-4-ene-3-one); 5α-Adiol, 5α-androstane-3α,17β-diol; 5β-Adiol, 5β-androstane-3α,17β-diol; LH, luteinizing hormone; T, testosterone; A, androsterone; E, etiocholanolone; D_3, trideuterated; HPT, hypothalamic–pituitary–testicular; G, glucuronide.

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to synthesize by the underground chemist and could easily be formulated for intramuscular delivery.

In a previous pilot study *[2]* to detect DHT abuse in the field of sports, two male volunteers were injected intramuscularly with DHT heptanoate, and the subsequent perturbations in the urinary hormone ratios were evaluated with the use of peak height abundances, as determined by GC-MS. Our primary objective in this study was to quantify by GC-MS the changes in urinary steroid concentrations and hormone concentration ratios after intramuscular administration of DHT heptanoate to six men. We formulated a dose of 250 mg, equivalent in mass to licensed formulations of T heptanoate, e.g., Primoteston®. For synthesis of the heptanoate ester, 5α-DHT was reacted with heptanoyl chloride rather than heptanoic anhydride to eliminate the possibility of heptanoic acid being generated in the reaction. For quantification of urinary hormones a mixture of internal standards was used, as described previously *[3]*, although a trideuterated analog of 5α-DHT (D_3-DHT), synthesized by hydrogenation of D_3-T as described herein, was also included.

Materials and Methods

MATERIALS

Materials were obtained as described previously *[3]*. Vacutainer Tubes® were supplied by Becton Dickinson and D_3-EpiT by Ultrafine Chemicals. All other calibrators, reagents, and solvents were supplied by Sigma-Aldrich Chemical Co.

SYNTHESIS OF DHT HEPTANOATE

Synthesis of DHT heptanoate was done by acylation of the 17β-hydroxyl group of DHT. DHT (25 g) was dissolved in dichloromethane (250 mL). Heptanoyl chloride (16 mL) was added, together with 4-(*N*,*N*-dimethylamino)pyridine (3.75 g) as a catalyst. The flask was stoppered with an anhydrous calcium chloride tube, the entire apparatus was protected from the light, and the reaction mixture was stirred magnetically at regular intervals. After 1 week the reaction mixture was washed with sodium hydroxide (2 × 75 mL, 1 mol/L), hydrochloric acid (2 × 75 mL, 1 mol/L), and then deionized water until an aqueous layer of neutral pH was obtained. Evaporation of the organic layer resulted in a yellow, waxy solid that was further dried in a desiccator for several days. The product was recrystallized with the use of acetone/water and characterized by electron impact full-scan MS with an ion-trap detector (ITD 800; Finnigan MAT) coupled to a gas chromatograph (Model 5890A; Hewlett-Packard) fitted with a HP-1 methyl silicone capillary column *[3]*. Purity of the compound was also assessed by nuclear (^{1}H) magnetic resonance spectroscopy (AMX 400 NMR spectrometer; Bruker Spectrospin). The DHT heptanoate was prepared for injection (250 mg in 1 mL of arachis oil and benzoyl alcohol solution) at St. Thomas' Hospital, London.

SYNTHESIS OF [16,16,17-$^2H_3$]5α-DHT

[16,16,17-$^2H_3$]T (20 mg) was dissolved in tetrahydrofuran (10 mL), a solvent reported to favor the production of the 5α- over 5β-isomer during hydrogenation reactions involving 3-oxo-4-ene compounds and without any of the starting material remaining *[10]*. A catalyst (palladium on activated charcoal, 20 mg) was added, and the mixture was hydrogenated for 2 h while being stirred. The reaction products, the 5α- and 5β-isomers of [16,16,17-$^2H_3$]DHT, were separated by means of their differing solubilities in acetonitrile/H_2O. The product containing both isomers was dissolved in the minimum volume (450 μL) of acetonitrile, but addition of an approximately equal volume of water, to our surprise, caused precipitation of a portion of the 5α-isomer. This portion was isolated by removal of the supernatant after centrifugation. The chemical and isotopic purity of this [16,16,17-$^2H_3$]5α-DHT was assessed by full-scan MS. With the use of a 25-m HP-1 methyl silicone column (Hewlett-Packard) and operating conditions described elsewhere *[3]*, the retention time/methylene units of the bis-trimethylsilyl derivatives of D_3-5α- and D_3-5β-DHT (*m/z* 437) were 22.2 min/26.21 methylene units and 17.0 min/24.56 methylene units, respectively.

ADMINISTRATION AND SAMPLE COLLECTION

5α-DHT heptanoate (250 mg) was administered intramuscularly to six healthy male volunteers (ages 23 to 28 years). Ethical permission and informed consent were obtained in accordance with our institution. Each subject gave a brief medical history and stated that they were not taking any medication likely to interfere in the study nor were they competing athletes at county level or above. Urine samples (24-h pooled) were collected on days −2 to 5 and on days 7, 10, 14, 21, and 28, except on the day of administration, when the collections were divided into two 12-h periods. Total volumes were recorded, and the samples were divided into appropriate aliquots. Urine samples for steroid analysis were stored at −20 °C, and for LH analysis samples were frozen rapidly in liquid nitrogen and then stored at −70 °C.

ANALYSIS OF URINE

Urinary steroid concentrations and the A/E peak-height ratio were determined by selected ion monitoring GC-MS as described elsewhere *[3]*. The trideuterated internal standard mixture consisted of D_3-DHT in addition to D_3-T, D_3-EpiT, and D_3-5α-Adiol, in final concentrations of 50, 50, 50, and 100 μg/L, respectively.

Urinary LH concentration was determined with the Serono immunoenzymetric assay, both by direct measurement and after ultrafiltration, according to the method described previously *[3* and references therein*]*.

Results

Synthesis of DHT heptanoate resulted in 91.6% recovery (31.8 g) of crude product. Recrystallization in acetone/

water gave a white product that was considered pure after subsequent analysis by both full-scan MS and nuclear magnetic resonance. Hydrogenation of D_3-T gave a 50.4% yield (10.1 mg) of the combined 5α- and 5β-isomers of D_3-DHT; separation of the two isomers produced 4.19 mg of D_3-5α-DHT.

Validation of the steroid assay has been described elsewhere *[3]*. The four quality controls analyzed in each assay (n = 6 assays) showed a similar between-assay imprecision and gave urinary concentrations of steroid analytes within 2 SD of the mean values reported previously.

In our previous paper *[3]*, the unpaired Student's *t*-test rather than the paired *t*-test was used in determining the statistical significance between samples collected before and after DHT administration. We used the unpaired test because in the context of sports the detection of doping with endogenous steroids by comparing changes in an individual's urinary hormone profile over time (longitudinal profiling) requires collection of multiple samples and is therefore relatively expensive and time-consuming. Although a similar statistical evaluation of intramuscular administration data would be preferable, the wide range of basal values observed in this study together with the considerable variation in individual responses to intramuscular injection gave a data set that was not thought to form part of a normal distribution. For this reason, and also because of the limited number of observations, nonparametric statistics were applied, and the significance of changes in the urinary hormone profile was assessed by a one-sided Mann–Whitney test. The threshold for significance was chosen at $P \leq 0.05$. Mean excretion rates after DHT heptanoate administration are shown in Fig. 1, together with a profile of the maximum and minimum daily excretions to give an indication of the spread of the data. After injection, the mean 24-h excretion rates of DHT and 5α-Adiol increased significantly compared with the basal mean [day −1 and day −2 shown not to differ significantly (two-sided Mann–Whitney test)], maximizing within the first 24 h of sampling with the rates ~8 times basal. Excretion rates remained significantly augmented (one-sided Mann–Whitney test; basal < administration) until day 10 and still did not return to basal concentrations until day 28. The wide separation of the maximum and minimum excretion profiles indicates the large degree of variability in individual responses, particularly in the period immediately after administration. Despite this variability, the excretion profiles of all individuals were found to follow a similar trend.

Accompanying the increase in DHT and 5α-Adiol was a decrease in excretion rates of T, EpiT, 5β-Adiol, and LH (Fig. 1). T, EpiT, and LH all followed a similar pattern of decline, with maximum suppression for all three analytes occurring by days 4 or 5 and approximating 20% of the basal concentrations. Even with the wide range of basal values shown in the graphs, excretion rates of all three analytes were significantly suppressed (one-sided Mann–Whitney; basal > administration) between days 1 and 7, with T showing additional significance on day 10. With 5β-Adiol, the fall in excretion rate was not found to be significant because of the wide range of basal values. However a decrease from basal was observed in all six volunteers, and if excretion rates were first calculated as a percentage of the values obtained on day −1, then administration values were found to be significantly lower (one-sided Mann–Whitney; basal > administration) than basal (day −2) between days 1 and 14.

From our previous study, the hormone ratio DHT/EpiT was proposed as the primary marker, with 5α-Adiol/EpiT, 5α-Adiol/LH, and 5α-Adiol/5β-Adiol all as secondary markers with discrimination limits of 2, 11.6, 112.4, and 4.3, respectively. Values exceeding these limits were shown to be indicative of doping with DHT *[3]*. The responses to intramuscular administration can be seen in Fig. 2, in which ratios of samples from each individual are displayed at seven selected times: two at basal, one each at the period where the applicable discrimination limit was first exceeded, at the maximum, at the times where the ratio decreased to just above and below the limit, and finally at 28 days after administration. For any individual, when a ratio in all the samples collected did not exceed the corresponding limit, then selected points were plotted to give an illustration of the changes in ratio over time.

After administration, ratios of all volunteers increased rapidly, in most cases exceeding the given discrimination limits within the first 24 h after injection and remaining above these limits until about day 10. All ratios had returned to basal by day 28. Although all individuals responded in a similar way, there was considerable variation in the degree of response. One volunteer in particular had relatively low basal values and although he did respond to administration, his ratios exceeded only the discrimination limit for 5α-Adiol/5β-Adiol. Nevertheless, statistical evaluation of the data (one-sided Mann–Whitney; basal < administration) showed all ratios for the group to be significantly augmented from day 0 to day 7.

The histograms in Fig. 2 show how many of the samples exceeded the discrimination limits on each day. With the exception of the ratio of 5α-Adiol/5β-Adiol, five of the six volunteers gave samples for which the ratios exceeded the discrimination limits. The one exception was the samples collected from the individual with low basal concentration ratios, which resulted, in part, from the urine having a larger average basal EpiT excretion than the other volunteers (218 μg/24 h compared with a range for the rest of the group of 21–116 μg/24 h). With 5α-Adiol/5β-Adiol, two out of the six volunteers gave samples whose ratios did not exceed the discrimination limit; samples from these volunteers were characterized by having relatively large average basal excretions of 5β-Adiol compared with the rest of the group (630 and 534 μg/24 h compared with a range for the rest of the group of 55–160 μg/24 h).

Changes in the T/EpiT ratio after administration (Fig.

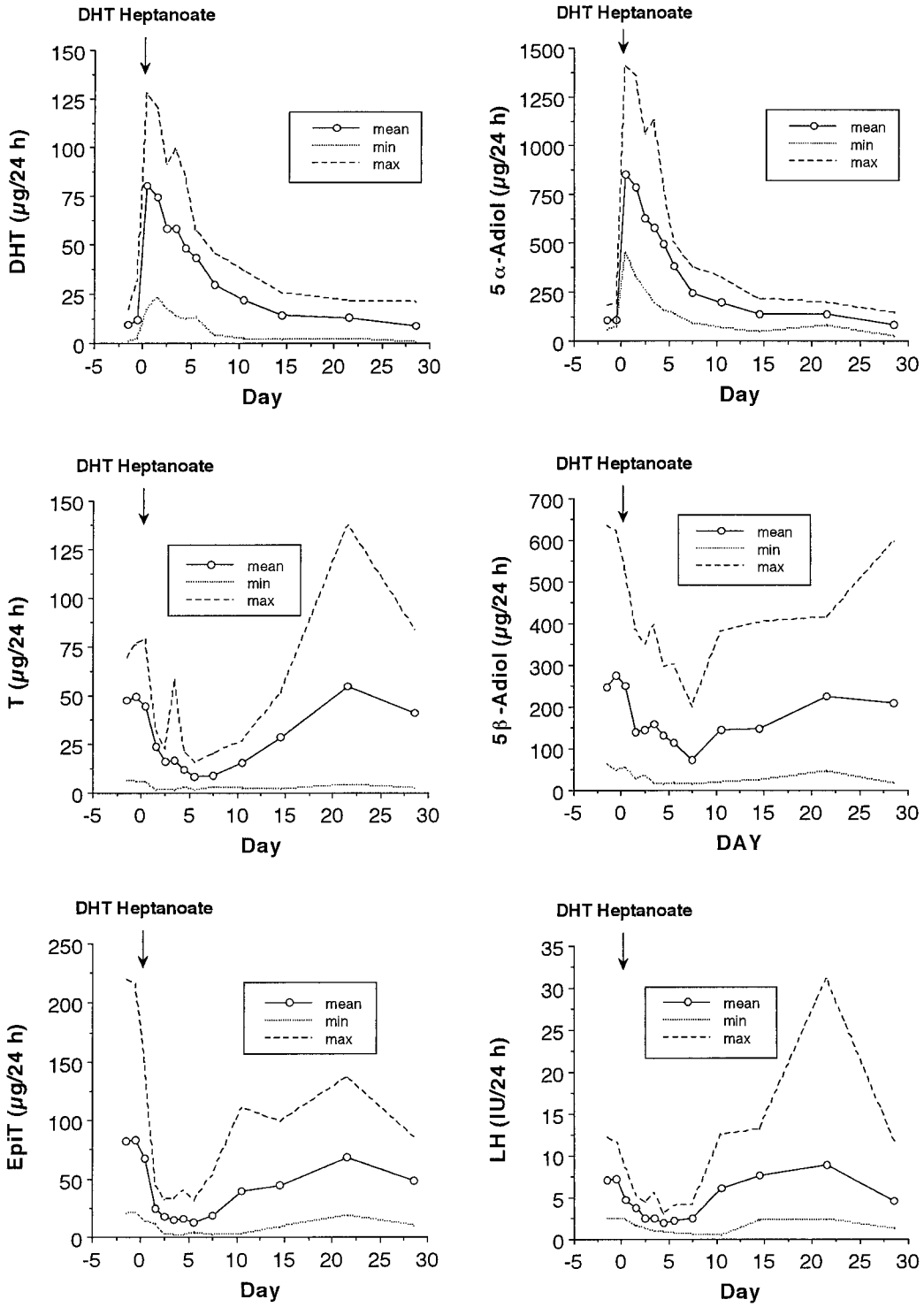

Fig. 1. Effect of intramuscular administration of DHT heptanoate (250 mg) on the urinary excretion rates of DHT, 5α- and 5β-Adiol, T, EpiT, and LH in six healthy men (profiles of mean, maximum, and minimum excretion rates).

3) were not significant (one-sided Mann–Whitney; basal < administration) as a group. Nonetheless, in five of the six individuals, the T/EpiT ratio in all samples collected during the first 3 days after administration was greater than the respective basal ratio.

The peak-height ratio of A/E was also determined on selected days (Fig. 4), this being a marker chosen by some IOC-accredited laboratories. The A/E ratio was found to be significantly increased (one-sided Mann–Whitney; basal < administration) between days 1 and 5, increasing

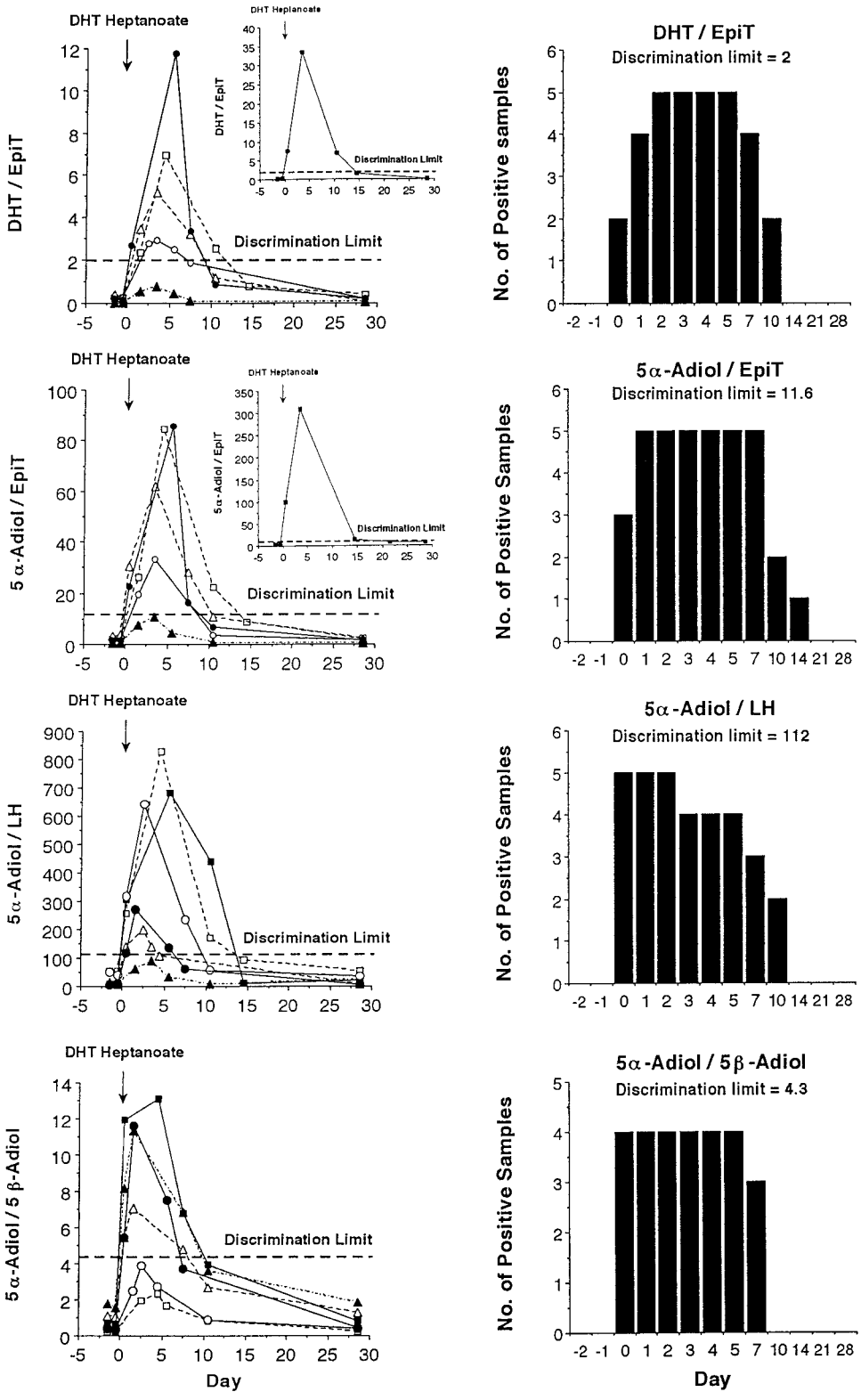

Fig. 2. Effect of intramuscular administration of DHT heptanoate (250 mg) on the urinary concentration ratios DHT/EpiT, 5α-Adiol/EpiT, 5α-Adiol/LH, and 5α-Adiol/5β-Adiol on selected days (see text) in six healthy men.

Graphs of DHT/EpiT and 5α-Adiol/EpiT for one individual are shown as *insets* because of the particularly large responses in these ratios.

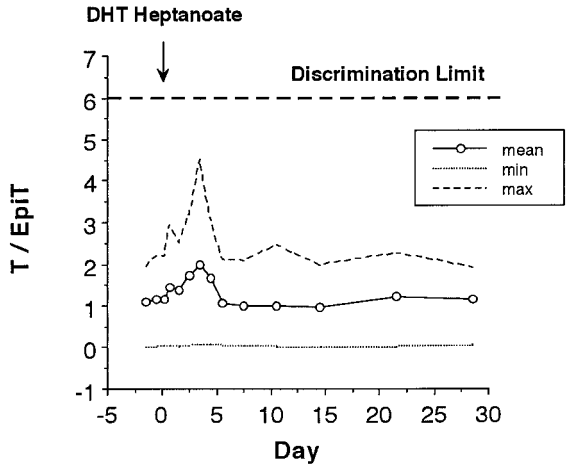

Fig. 3. Effect of intramuscular administration of DHT heptanoate (250 mg) on the urinary concentration ratio of T/EpiT in six healthy men (profile of mean, maximum, and minimum ratio).

~3-fold in the first 24–48 h, and then slowly returning to basal.

Discussion

We have shown previously *[3]* that DHT administered percutaneously (125 mg, twice daily) for 3 days caused substantial increases in the excretion rate of DHT and its 5α-reduced metabolite 5α-Adiol, while decreasing the excretion rates of the hormones T, EpiT, and LH. Similar findings occurred with intramuscular administration, although in general the responses were more marked, a fact we attribute to the larger dose and different route of administration. With an intramuscular route of delivery, all the drug injected is bioavailable, whereas with percutaneous administration, only ~10% of the dose is able to penetrate the skin. Excretion rates of DHT and 5α-Adiol rose ~8-fold, maximum excretion being attained within the first 24 h after administration and then slowly declining. T, EpiT, and LH also showed a more marked response with excretion rates decreasing to ~20–25%. This pattern of suppression was consistent with the administered DHT causing a negative feedback effect on the hypothalamic–pituitary–testicular (HPT) axis.

The hormone concentration ratios DHT/EpiT, 5α-Adiol/EpiT, 5α-Adiol/LH, and 5α-Adiol/5β-Adiol all increased after percutaneous administration, and with the exception of 5α-Adiol/5β-Adiol, mean increases for the group exceeded the discrimination limits. With intramuscular administration the increases in concentration ratios were more marked, the means for the group clearly exceeding the discrimination limits of all four markers. Consistent with a depot preparation, these effects were sustained, and by day 10 the ratios still exceeded the respective limits in all cases except 5α-Adiol/5β-Adiol. A return to basal values did not occur until about day 28.

On an individual basis, five of the six volunteers administered intramuscular DHT had concentration ratios that for several days exceeded the discrimination limits for DHT/EpiT and 5α-Adiol to EpiT and LH. This compares with respective numbers of 6, 7, and 5 volunteers out of the 10 from the percutaneous study, whose ratios exceeded the discrimination limits on day 3 of administration, the day in which ratios for the group were most augmented. Therefore, under our proposed confirmatory procedure, five of the six volunteers intramuscularly administered DHT heptanoate would be considered positive. That one volunteer would have escaped detection, despite the dose administered and its direct route of delivery, might suggest that our discrimination limits are too large. This favors investigating the development of a discrimination function incorporating several hormone concentration ratios.

The implementation of longitudinal profiling in all IOC laboratories would also be particularly useful for individuals whose concentration ratios did not exceed the discrimination limits but were nevertheless significantly increased above basal values. By comparison of the urinary

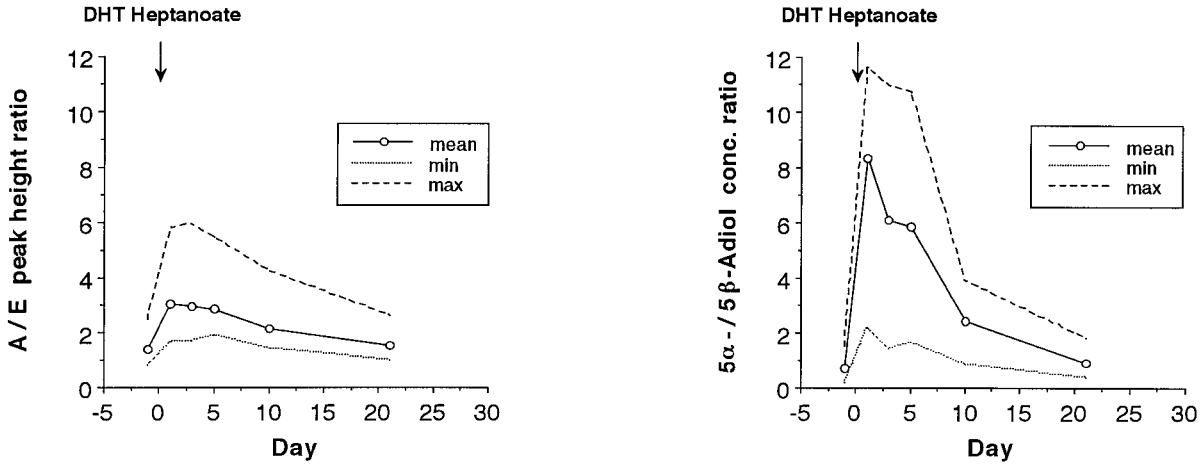

Fig. 4. Comparison of the effect of intramuscular administration of DHT heptanoate (250 mg) on the urinary peak-height ratio of A/E with the concentration ratio of 5α-Adiol/5β-Adiol in six healthy men (profiles of mean, maximum, and minimum ratios).

hormone profiles of individuals over a period of time, a more sensitive test based on differences in ratio could be established, and thus detection of doping would depend on the significance of changes in concentration ratios as opposed to the ratios themselves exceeding discrimination limits.

A decrease in the excretion rate of 5β-Adiol was observed in all six individuals, although the overall change was not found to be significant because of the considerable range of basal values observed. However, when individual changes in excretion rate were analyzed as a percentage of the basal value for that volunteer, decreases in the excretion rate of 5β-Adiol were found to be significant. This result contrasts with the findings of the percutaneous study, where there appeared to be no change in the individual rates of 5β-Adiol excretion despite suppression of the HPT axis. 5β-Adiol glucuronide (G) is believed to have more than one origin *[3]*. After DHT administration, 5β-Adiol from an extrasplanchnic source would be expected to fall, suppression of the HPT axis resulting in a decrease in availability of one of its major precursors, T. 5β-Adiol G arising from hepatic metabolism of adrenal precursors would however be expected to remain unaffected. Such a situation would result in an overall decrease in 5β-Adiol G excretion rates, and this appears to be the case with intramuscular administration. With percutaneous administration there was also suppression of the HPT axis, and although this was not so marked, we expected to see some change in 5β-Adiol G excretion. That the excretion rates remained unchanged is difficult to explain, but it is possible that the adrenal contribution to the pool of urinary 5β-Adiol G is perhaps the more important, the decrease from an extrasplanchnic source having a lesser influence on the overall urinary excretion rate.

The decreases in the excretion rate of 5β-Adiol G after intramuscular administration, together with the larger increases in the excretion rate of 5α-Adiol G, are responsible for the greater response in the ratio of 5α-Adiol/5β-Adiol compared with that observed with percutaneous administration. Samples from 4 of the 6 volunteers had ratios that exceeded the discrimination limit for 6 days after injection, compared with only 2 of the 10 from the percutaneous study that were greater than the limit on the day in which this ratio was most augmented.

The peak-height ratio of A/E was not shown to be a sensitive marker of percutaneous DHT administration because steroids of adrenal origin contribute largely to the formation of G conjugates of A and E. The A/E ratio, however, is reported to be a good marker of oral administration *[4, 5]*, and we suggested previously that large doses and administration by other routes may also have a greater effect on this ratio. After sublingual DHT administration (25 mg) *[6]*, an ~7-fold increase in the concentration ratio of A/E was reported, although this ratio was the least sensitive of the ratios studied and remained above basal for the shortest time interval after administration. Consideration should also be given to the possibility that some of the sublingual formulation may have been swallowed, resulting in first-pass metabolism of DHT to AG.

Intramuscular injection caused an ~3-fold increase that was found to be significant. However, compared with the concentration ratio of 5α-Adiol/5β-Adiol (Fig. 4), the relative increases in the peak-height ratio of A/E compared with basal are smaller. 5α-Adiol/5β-Adiol is our least sensitive marker in terms of exceeding the respective discrimination limit, but in terms of perturbation, it does nevertheless increase immediately after administration in all individuals. Hence we favor the ratio of 5α-Adiol/5β-Adiol as a marker for longitudinal profiling, although natural intraindividual variation would have to be fully explored before we would recommend this ratio for such profiling. The general stability of steroid profiles for doping control purposes is currently being explored (e.g., *[11]*).

The concentration ratio of T/EpiT was expected to remain unchanged after DHT treatment because of suppression of the HPT axis, resulting in an equal reduction in the secretion of T and EpiT from the testis. The excretion rates of T did not, in fact, decrease as rapidly as those of EpiT, thus giving rise to a small increase in T/EpiT shortly after administration. Such changes were not significant as a group because of the spread of data and the limited number of individuals, but samples collected from five of the six individuals showed ratios that had approximately doubled over basal by 3 days after administration. This result could possibly be explained by the greater binding affinity of DHT compared with T with plasma sex hormone-binding globulin. DHT generated from the administration of the heptanoate ester will displace T from sex-hormone-binding globulin, whereas EpiT, which is not bound by this protein, remains unaffected. Work by Pugeat et al. *[12]*, while showing no change in the binding affinity of T after chronic percutaneous DHT treatment, did find an ~3-fold increase in the percentage of unbound T after acute percutaneous DHT administration (three doses of 250 mg). Given a similar situation after intramuscular injection, the increased amount of free T produced by displacement could partially compensate for the decrease in secretion of T from the testis.

In summary, the proposed confirmatory procedure for detecting DHT doping in male athletes with the use of the concentration ratios DHT/E, 5α-Adiol/E, 5α-Adiol/LH, and 5α-Adiol/5β-Adiol was applied to samples from volunteers administered intramuscular DHT heptanoate. The discrimination limits of all four markers were exceeded, the larger dose and the different route of administration augmenting the responses seen previously with percutaneous administration. The markers were able to detect a normal replacement dose of DHT heptanoate, a dose that would be thought modest in terms of doping in

sport, up to 10 days after administration. This provides confidence, therefore, that doping with DHT esters by sports competitors, either obtaining such preparations from illegal synthesis or underground availability of future licensed preparations, can be detected, and thus further demonstrates the suitability of these hormone concentration ratios as a method of confirming DHT abuse.

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References

1. Ueki M, Fujisaki M, Ikekita A, Hiruma T, Okano M. Some followed up cases of doping with naturally occurring steroids—testosterone and dihydrotestosterone. In: Donike M, Geyer H, Gotzman A, Mareck-Engelke U, eds. Recent advances in doping analysis (3): proceedings of the 13th Cologne workshop on dope analysis, 12–17 March 1995. Köln: Sport und Buch Strauß, 1996;231–46.
2. Southan GJ, Brooks RV, Cowan DA, Kicman AT, Unnadkat N, Walker CJ. Possible indices for the detection of the administration of dihydrotestosterone to athletes. J Steroid Biochem Mol Biol 1992;42:87–94.
3. Kicman AT, Coutts SB, Walker CJ, Cowan DA. Proposed confirmatory procedure for detecting 5α-dihydrotestosterone doping in male athletes. Clin Chem 1995;41:1617–27.
4. Cartoni GP, Giarrusso A, Ciardi M, Rosati F. Capillary gas chromatography and mass spectrometry detection of anabolic steroids II. J High Resolut Chromatogr Chromatogr Commun 1985;8:539–43.
5. Schänzer W, Horning S, Donike M. Synthesis and metabolism of [16,16,17-$^{2}H_3$]-5α-dihydrotestosterone. In: Donike M, Geyer H, Gotzman A, Mareck-Engelke U, eds. Recent advances in doping analysis (3): proceedings of the 13th Cologne workshop on dope analysis, 12–17 March 1995. Köln: Sport und Buch Strauß, 1996:201–13.
6. Geyer H, Schänzer W, Schindler U, Donike M. Changes of the urinary steroid profile after sublingual application of dihydrotestosterone (DHT). In: Donike M, Geyer H, Gotzman A, Mareck-Engelke U, eds. Recent advances in doping analysis (3): proceedings of the 13th Cologne workshop on dope analysis, 12–17 March 1995. Köln: Sport und Buch Strauß, 1996;215–29.
7. Donike M, Ueki M, Kuroda Y, Geyer H, Nolteernsting E, Rauth S, et al. Detection of dihydrotestosterone (DHT) doping: alterations in the steroid profile and reference ranges for DHT and its 5α-metabolites. J Sports Med Phys Fitness 1995;35:235–50.
8. Diaz-Sanchez V, Larrea F, Ulloa-Aguirre A, Garza-Flores J, Richards E, Veayra F. Absorption of dihydrotestosterone (DHT) after its intramuscular administration. Fertil Steril 1989;51:493–7.
9. Keenan BS, Eberle AJ, Sparrow JT, Greger NG, Panko WB. Dihydrotestosterone heptanoate: synthesis, pharmacokinetics, and effects on hypothalamic-pituitary-testicular function. J Clin Endocrinol Metab 1987;64:557–62.
10. Combe MG, Henbest HB, Jackson WR. Aspects of stereochemistry, Part XXI. Hydrogenation of 3-oxo-4-ene steroids over a palladium-calcium carbonate catalyst. J Chem Soc 1967;C:2467–9.
11. Mareck-Engelke U, Geyer H, Donike M. Stability of steroid profiles: the circadian rhythm of urinary ratios and excretion rates of endogenous steroids in male. In: Donike M, Geyer H, Gotzman A, Mareck-Engelke U, eds. Recent advances in doping analysis (2): proceedings of the 12th Cologne workshop on dope analysis, 10–15 April 1994. Köln: Sport und Buch Strauß, 1995;121–33.
12. Pugeat M, Lejeune H, Dechaud H, Emptoz-Bonneton A, Fleury MC, Charrie A, et al. Effects of drug administration on gonadotrophins, sex steroid hormones and binding proteins in humans. Horm Res 1987;28:261–73.