H. Geyer, W. Schänzer, U. Schindler and M. Donike:
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, 17β-diol (5αA3αD), 5α-androstane-3β, 17β-diol (5αA3βD) and epiandrosterone (EPIAND). The ratios of these steroids to etiocholanolone (ETIO), 5β-androstane-3α, 17β-diol (5βA3αD) 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/5αA3βD 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 candidates 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 steroid profile.
It is expected that the application of DHT will also lead to characteristic alterations in the steroid profile. 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,17β-diol (5αA3αD), 5α-androstane-3β,17β-diol (5αA3βD) 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 administration, it is recommended to administer DHT sublingually.

Fig. 1: Biosynthesis and metabolism of DHT: DHT (1), AND (2), 5αA3αD (3) 5αA3βD (4), 5α-androstane-3,17-dione (5)
Experimental

Steroids and Chemicals

Dihydrotestosterone, androsterone, etiocholanolone, 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, testosterone, epitestosterone, 11β-hydroxy-androsterone, 11β-hydroxy-etiocholanolone, pregnadiol, tetrahydro-cortisol, epiandrosterone, and methyltestosterone were purchased from SIGMA, D-82039 Deisenhofen, Germany. β-Glucuronidase from Escherichia coli K12 was obtained from Boehringer, D-68298 Mannheim, Germany. MSTFA, [16,16,17-3H3]-testosterone and [2,2,4,4-2H4]-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 wakup, then urine collection every 4 hours from 10:00 h to 22:00 h, another cumulative urine from 22:00 h to wakup, 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 administered 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 β-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 alkalisised 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/NH4I/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.
Quantification 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, 17β-diol, bis-TMS (5αA3αD), 5β-androstane-3α, 17β-diol, bis-TMS (5βA3αD) and m/z 421 for 5α-androstane-3β, 17β-diol, bis-TMS (5αA3βD), 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 [2,2,4,4-2H₄]-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>
<thead>
<tr>
<th>Table 1: Endogenous steroids: concentrations of the working solution and resulting concentrations per mL of urine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>reference substances</td>
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<tr>
<td></td>
</tr>
<tr>
<td>androsterone</td>
</tr>
<tr>
<td>etiocholanolone</td>
</tr>
<tr>
<td>testosterone</td>
</tr>
<tr>
<td>epitestosterone</td>
</tr>
<tr>
<td>5α-androstane-3α, 17β-diol</td>
</tr>
<tr>
<td>5β-androstane-3α, 17β-diol</td>
</tr>
<tr>
<td>5α-androstane-3β, 17β-diol</td>
</tr>
<tr>
<td>epiandrosterone</td>
</tr>
<tr>
<td>dihydrotestosterone</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Internal standard: concentrations of the working solution and resulting concentrations per mL of urine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference substances</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>17α-methyltestosterone</td>
</tr>
<tr>
<td>[2,2,4,4-2H₄]-etiocholanolone</td>
</tr>
<tr>
<td>[16,16,17-2H₃]-testosterone</td>
</tr>
<tr>
<td>[16,16,17-2H₃]-epitestosterone</td>
</tr>
<tr>
<td>[2,2,4,4-2H₄]-11β-hydroxyandrosterone</td>
</tr>
</tbody>
</table>
Results and Discussion

Derivatisation and GC-MS-analysis

The derivatisation with MSTFA/NH₄I 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)

<table>
<thead>
<tr>
<th>Substanz</th>
<th>RT</th>
<th>Index</th>
<th>m/e</th>
</tr>
</thead>
<tbody>
<tr>
<td>androsterone</td>
<td>10.36</td>
<td>2506</td>
<td>434</td>
</tr>
<tr>
<td>d₅-etiocholanolone</td>
<td>10.46</td>
<td>2511</td>
<td>438</td>
</tr>
<tr>
<td>etiocholanolone</td>
<td>10.54</td>
<td>2515</td>
<td>434</td>
</tr>
<tr>
<td>5α-androstane-3α,17β-diol</td>
<td>10.68</td>
<td>2522</td>
<td>241</td>
</tr>
<tr>
<td>5β-androstane-3α,17β-diol</td>
<td>10.82</td>
<td>2529</td>
<td>241</td>
</tr>
<tr>
<td>epiandrosterone</td>
<td>11.78</td>
<td>2578</td>
<td>434</td>
</tr>
<tr>
<td>5α-androstane-3β,17β-diol</td>
<td>12.15</td>
<td>2597</td>
<td>421</td>
</tr>
<tr>
<td>d₅-epitestosterone</td>
<td>12.15</td>
<td>2597</td>
<td>435</td>
</tr>
<tr>
<td>epitestosterone</td>
<td>12.19</td>
<td>2599</td>
<td>432</td>
</tr>
<tr>
<td>dihydrotestosterone</td>
<td>12.41</td>
<td>2610</td>
<td>434</td>
</tr>
<tr>
<td>d₅-testosterone</td>
<td>12.99</td>
<td>2640</td>
<td>435</td>
</tr>
<tr>
<td>testosterone</td>
<td>13.03</td>
<td>2642</td>
<td>432</td>
</tr>
<tr>
<td>methyltestosterone</td>
<td>14.82</td>
<td>2733</td>
<td>446</td>
</tr>
</tbody>
</table>

After derivatisation with MSTFA/NH₄I, 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 isomers 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-etiocholanolone. 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)
Fig. 2: DHT, bis-TMS (I) and 11-keto-etiocholanolone, bis-TMS (II) after different incubation times with MSTFA/NH₄I.

a) 15 min at 60° C
b) 30 min at 60° C
Changes of the steroid profile after sublingual administration of DHT

After the sublingual administration of 25 mg DHT obvious alterations of steroid profile parameters can be observed. In Figure 3 and 4 are presented as examples the screening printouts of the steroid profiles of V1 before and after the application of DHT. As expected, the concentrations of DHT and its 5α metabolites AND, 5αA3αD, 5αA3βD and EPI AND 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 5β-metabolites (ETIO, 5βA3αD and 5βA3βD) 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/cm³ with the following formula [12,13]:

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

- $Conc_{corr}$: corrected concentration
- $Conc_m$: 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 5αA3βD 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 5β-epimers, the ratios of DHT and its metabolites with the 5β-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/5βA3αD 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: Steroid profile of V1 before the application of DHT (all derivatives are per-trimethylsilylated)

1. AND and ETIO
2. 5αA3αD and 5βA3αD
3. DHT
4. 5αA3βD
5. EPIAND
Fig. 4: Steroid profile of V1 4 hours after the sublingual application of 25 mg DHT (all derivatives are per-trimethylsilylated)

1 AND and ETIO
2 5αA3αD and 5βA3αD
3 DHT
4 5αA3βD
5 EPIAND
|    | 22     | 143    | 391    | 258    | 72     | 118    | 1368   | 3730   | 6      | 20     | 48 - 60 hr | 36 - 48 hr | 24 - 36 hr | 20 - 24 hr | 16 - 20 hr | 12 - 16 hr | 8 - 12 hr | 4 - 6 hr | 2 - 4 hr | 0 - 2 hr | 0 - 24 hr |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|------------|-------------|-------------|-------------|-------------|-------------|-----------|----------|---------|---------|---------|----------|
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 22 | 4.1    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 16 | 2.2    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 20 | 1.7    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 24 | 1.4    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 28 | 1.0    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 32 | 0.8    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 36 | 0.6    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 40 | 0.4    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 44 | 0.2    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
| 48 | 0.1    |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |
|    |        |        |        |        |        |        |        |        |        |        |            |             |             |             |             |             |           |          |         |         |          |          |

Table 4: Mean and standard deviation of the serum concentrations after sublingual administration of 25 mg of DHT (mean ± SD) (n=4 volunteers).
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.

<table>
<thead>
<tr>
<th>Substance</th>
<th>-24 - 0 h [µg] mean ± stdev</th>
<th>0 - 24 h [µg] mean ± stdev</th>
<th>24 - 48 h [µg] mean ± stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHT</td>
<td>18.0 ± 5.7</td>
<td>759.8 ± 235.5</td>
<td>38.2 ± 11.5</td>
</tr>
<tr>
<td>AND</td>
<td>3795.5 ± 990.6</td>
<td>10576.6 ± 2494.0</td>
<td>4387.6 ± 1793.3</td>
</tr>
<tr>
<td>5αA3αD</td>
<td>101.6 ± 27.8</td>
<td>1653.5 ± 829.9</td>
<td>201.1 ± 79.9</td>
</tr>
<tr>
<td>5αA3βD</td>
<td>16.6 ± 3.9</td>
<td>128.6 ± 52.6</td>
<td>46.1 ± 30.0</td>
</tr>
<tr>
<td>EPIAND</td>
<td>12.1 ± 3.1</td>
<td>49.1 ± 26T.8</td>
<td>40.5 ± 35.6</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>5αA3αD/5βA3αD</th>
<th>AND/ETIO</th>
<th>DHT/ETIO*</th>
<th>DHT/EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±stdev</td>
<td>mean±stdev</td>
<td>mean±stdev</td>
<td>mean±stdev</td>
</tr>
<tr>
<td>-24-0</td>
<td>0.29 ± 0.14</td>
<td>1.18±0.24</td>
<td>4.87±2.67</td>
<td>0.36±0.06</td>
</tr>
<tr>
<td>0-2</td>
<td>3.16 ± 2.34</td>
<td>4.61±2.66</td>
<td>444.62±554.30</td>
<td>22.25±17.90</td>
</tr>
<tr>
<td>2-4</td>
<td>4.78±1.62</td>
<td>7.71±3.29</td>
<td>637.94±299.36</td>
<td>45.51±29.38</td>
</tr>
<tr>
<td>4-6</td>
<td>3.41±1.63</td>
<td>5.21±0.88</td>
<td>372.58±171.74</td>
<td>36.38±28.68</td>
</tr>
<tr>
<td>6-8</td>
<td>3.66±1.81</td>
<td>3.94±1.09</td>
<td>310.39±138.13</td>
<td>30.59±20.62</td>
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<tr>
<td>8-12</td>
<td>3.09±2.02</td>
<td>2.93±1.06</td>
<td>125.64±68.38</td>
<td>12.50±6.53</td>
</tr>
<tr>
<td>12-16</td>
<td>1.72±1.04</td>
<td>1.90±0.41</td>
<td>50.21±20.93</td>
<td>3.70±1.64</td>
</tr>
<tr>
<td>16-20</td>
<td>1.20±0.68</td>
<td>1.58±0.40</td>
<td>24.25±14.72</td>
<td>1.63±0.78</td>
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<tr>
<td>20-24</td>
<td>0.86±0.49</td>
<td>1.46±0.29</td>
<td>17.60±7.84</td>
<td>1.67±0.85</td>
</tr>
<tr>
<td>24-36</td>
<td>0.70±0.08</td>
<td>1.45±0.02</td>
<td>14.11±2.79</td>
<td>1.38±0.13</td>
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<tr>
<td>36-48</td>
<td>0.43±0.09</td>
<td>1.24±0.03</td>
<td>9.65±1.69</td>
<td>0.90±0.06</td>
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<tr>
<td>48-60</td>
<td>0.37±0.04</td>
<td>1.30±0.09</td>
<td>7.71±1.04</td>
<td>0.76±0.05</td>
</tr>
</tbody>
</table>

* DHT/ETIO value multiplied with 1000
Reference ranges for the judgment 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 parameters are necessary.

In this study subject based reference ranges for each volunteer were calculated from the steroid profile 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 steroid profile [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 steroid profile parameters 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/5βA3αD 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.

<table>
<thead>
<tr>
<th>parameter</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
</tr>
</thead>
<tbody>
<tr>
<td>c DHT corr.**</td>
<td>20.0</td>
<td>32.8</td>
<td>60.3</td>
<td>60.0</td>
</tr>
<tr>
<td>AND/ETIO</td>
<td>20.0</td>
<td>20.5</td>
<td>16.8</td>
<td>28.0</td>
</tr>
<tr>
<td>5αA3αD/5βA3αD</td>
<td>60.0</td>
<td>44.5</td>
<td>56.3</td>
<td>45.0</td>
</tr>
<tr>
<td>DHT/ETIO*</td>
<td>52.0</td>
<td>36.5</td>
<td>60.3</td>
<td>28.0</td>
</tr>
<tr>
<td>DHT/5βA3αD</td>
<td>20.0</td>
<td>36.5</td>
<td>60.3</td>
<td>28.0</td>
</tr>
<tr>
<td>DHT/EPI</td>
<td>52.0</td>
<td>28.5</td>
<td>60.3</td>
<td>60.0</td>
</tr>
<tr>
<td>5αA3βD/5βA3αD</td>
<td>36.0</td>
<td>36.5</td>
<td>60.3</td>
<td>&gt;60.0</td>
</tr>
</tbody>
</table>

* 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].

<table>
<thead>
<tr>
<th>parameter</th>
<th>upper limit of reference range</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
</tr>
</thead>
<tbody>
<tr>
<td>c DHT corr.**</td>
<td>20.55</td>
<td>n.d.</td>
<td>32.8</td>
<td>52.3</td>
<td>60.0</td>
</tr>
<tr>
<td>AND/ETIO</td>
<td>2.86</td>
<td>12.0</td>
<td>12.8</td>
<td>6.3</td>
<td>12.0</td>
</tr>
<tr>
<td>5αA3αD/5βA3αD</td>
<td>1.53</td>
<td>20.0</td>
<td>20.5</td>
<td>8.3</td>
<td>8.0</td>
</tr>
<tr>
<td>DHT/ETIO*</td>
<td>8.22</td>
<td>n.d.</td>
<td>28.5</td>
<td>36.3</td>
<td>45.0</td>
</tr>
<tr>
<td>DHT/EPI</td>
<td>0.73</td>
<td>28.0</td>
<td>28.5</td>
<td>&gt;60</td>
<td>60.0</td>
</tr>
</tbody>
</table>

* 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/5βA3αD of volunteer 2 after application of 25 mg DHT with subject- and population based upper reference limits of this ratio

REFERENCES


