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Review

Factors influencing the steroid profile in doping control analysis

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Steroid profiling is one of the most versatile and informative screening tools for the detection of steroid abuse in sports drug testing. Concentrations and ratios of various endogenously produced steroidal hormones, their precursors and metabolites including testosterone (*T*), epitestosterone (E), dihydrotestosterone (DHT), androsterone (And), etiocholanolone (Etio), dehydroepiandrosterone (DHEA), 5α-androstane-3α,17β-diol (Adiol), and 5β-androstane-3α,17β-diol (Bdiol) as well as androstenedione, 6α-OH-androstenedione, 5β-androstane-3α,17α-diol (17-epi-Bdiol), 5α-androstane-3α,17α-diol (17-epi-Adiol), 3α,5-cyclo-5α-androstan-6ß-ol-17-one (3α,5-cyclo), 5α-androstanedione (Adion), and 5β-androstanedione (Bdion) add up to a steroid profile that is highly sensitive to applications of endogenous as well as synthetic anabolic steroids, masking agents, and bacterial activity. Hence, the knowledge of factors that do influence the steroid profile pattern is a central aspect, and pharmaceutical (application of endogenous steroids and various pharmaceutical preparations), technical (hydrolysis, derivatization, matrix), and biological (bacterial activities, enzyme side activities) issues are reviewed. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: sport; doping; urinary steroid profile; GC-MS; anabolic steroids

INTRODUCTION

Steroid profiling in urine is a commonly employed method of clinical endocrinology, which is frequently used, for instance, for newborn screenings to detect enzyme deficiencies.[1,2] The methodology was adapted and introduced in doping controls in 1983 by Donike *et al*.[3,4] to allow for the determination of *testosterone* (T) misuse in sports, and later for the identification of the administration of related compounds. 'The urinary steroid profile'[5] is composed of concentrations and ratios of various endogenously produced steroidal hormones, their precursors, and metabolites including T, epitestosterone (E), dihydrotestosterone (DHT), androsterone (And), etiocholanolone (Etio), dehydroepiandrosterone (DHEA), 5α-androstane-3α,17β-diol (Adiol), and 5β-androstane-3α,17β-diol (Bdiol). The interrelation of these substances is illustrated in Fig. 1,[6,7] whereas the exact pathway leading to the formation of E has not yet been elucidated.[8] The alteration of one or more of the concentrations of these parameters interferes with the naturally well-balanced system and raises suspicion in routine doping controls either by increased or decreased concentrations and ratios. In order to improve the comprehensiveness and significance of this approach, an enclosure of additional parameters was recommended,[3,4,9–14] which facilitates and supports the detection of surreptitious administrations of compounds such as T, E, or DHEA administration as well as bacterial activity. Therefore, androstenedione (precursor of T) and 6α-OH-androstenedione (metabolite of androstenedione), 5β-androstane-3α,17α-diol (17-epi-Bdiol) and 5α-androstane-3α,17α-diol (17-epi-Adiol), 3α,5-cyclo-5α-androstan-6ß-ol-17-one (3α,5-cyclo, metabolite of DHEA), or 5α-androstanedione (Adion) and 5β-androstanedione (Bdion) were utilized, respectively. Moreover, steroids with an origin independent of the androgen metabolism are monitored, serving as so-called endogenous reference compounds (ERC) for gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) in confirmatory analyses,[15] which include, e.g. pregnanediol (PD), 11β-hydroxy-androsterone (11-OH-And), and 11β-hydroxy-etiocholanolone (11-OH-Etio). Additionally, the artificial formation of 19-norsteroids[16] and the endogenous production of boldenone (B) and B metabolites,[17,18] which were observed in a few cases, are considered. A summary of steroids, their retention times, and characteristic fragment ions commonly monitored for steroid profiling are presented in Table 1 using GC-MS conditions as published elsewhere.[10,19]

Steroid profiles, as determined routinely in doping control, provide essential information for several purposes as they are recorded, stored, and attributed to individuals

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Table 1. Retention times (RT) and ion traces (*m/z*) of endogenous steroids which are commonly monitored for steroid profiling (as TMS derivatives)

Substance	Abbreviation	RT	*m/z*
3α,5-cyclo-5α-androstan-6β-ol-17-one, bis-TMS	3α,5-cyclo	7.58	432
5β-androstane-3α,17α-diol, bis-TMS	17-epi-Bdiol	8.64	241
5α-estran-3α-ol-17-one, bis-TMS	Norandrosterone	8.94	405, 420, 315
5α-androstane-3α,17α-diol, bis-TMS	17-epi-Adiol	9.12	241
5β-androstanedione, bis-TMS	Bdion	9.19	432, 417, 275, 290
5β-androst-1-en-17β-ol-3-one, bis-TMS	B-M1	9.19	194, 432, 417
5β-estran-3α-ol-17-one, bis-TMS	Noretiocholanolone	9.88	405, 420, 315
Androsterone, bis-TMS	And	10.43	434
Etiocholanolone, bis-TMS	Etio	10.61	434
5α-androstane-3α,17β-diol, bis-TMS	Adiol	10.78	241
5β-androstane-3α,17β-diol, bis-TMS	Bdiol	10.91	241
Dehydroepiandrosterone, bis-TMS	DHEA	11.76	432
Epiandrosterone, bis-TMS	epiAnd	11.85	434
5α-androstanedione, bis-TMS	Adion	12.11	432, 417, 275, 290
5α-androstane-3β,17β-diol, bis-TMS	Trans-Adiol	12.26	421
Epitestosterone, bis-TMS	E	12.28	432
Dihydrotestosterone, bis-TMS	DHT	12.51	434
Boldenone, bis-TMS	B	12.85	206, 430, 415
Testosterone, bis-TMS	T	13.12	432
11β-hydroxy-androsterone, tris-TMS	11-OH-And	13.43	522
11β-hydroxy-etiocholanolone, tris-TMS	11-OH-Etio	13.67	522
Methyltestosterone, bis-TMS (ISTD)	MT	14.92	446
5β-pregnane-3α,20α-diol, bis-TMS	Pregnanediol (PD)	15.43	117
5β-pregnane-3β,17α,20β-triol, tris-TMS	Pregnanetriol (PT)	15.60	435, 345, 255

incrementally with each collected sports drug testing sample. Hence, it is of utmost importance that laboratory standards are comparable within the group of World Anti-Doping Agency (WADA)-accredited drug testing sites to enable correlation of data obtained from one athlete on different occasions. This is guaranteed by comprehensive validations and proficiency tests, and the use of isotope-dilution mass spectrometry (IDMS) employing certified reference material allows the precise and accurate evaluation of steroid profile data. Several studies demonstrated only small intraindividual variations of steroid profile parameters, especially within the ratios utilized for doping control purposes such as T/E, And/Etio, And/T, and Adiol/Bdiol.[20] These ratios were not influenced by exercise or severe physical endurance performance,[21,22] by menstrual cycle,[23,24] by circadian[23,25] or annual rhythms.[24] Hence, steroid profiles are valuable, for instance, for the management of elevated T/E ratios according to WADA guidelines.[26,27] Longitudinal and retrospective evaluation of doping control samples in terms of steroid profiles enable the detection of abnormal alterations that trigger subsequent analyses such as confirmatory GC/C/IRMS measurements. Moreover, considerable long-term stability of intraindividual steroid profiles support the detection of manipulation (e.g. urine substitution) in doping control urine sample collection and provide one of the most important parameters for specimen individualization in sports drug testing. A recent example showed that screening a database containing 14 224 urinary steroid profiles of athletes for specific values of four characteristic steroid ratios (T/E, And/Etio, And/T, and Adiol/Bdiol) yielded three samples that were all derived from one athlete.[28] However, limitations of the prospects of steroid profiling for identification purposes were also reported recently. Robinson *et al.*[29] described a case of seven doping control urine samples collected during a cycling stage race with moderately elevated T/E ratios. Different pattern classification tools were tested to categorize the most similar steroid profiles, but none of the models enabled a clear classification of the different urine samples. Finally, genetic profiling demonstrated that only three of seven samples originated from the same cyclist.

Nevertheless, the great importance of urinary steroid profiles and their utility in doping controls are manifold, and the information obtained is vital to the successful screening for steroidal anabolic agents. Thus, knowledge of factors that influence the steroid profile pattern is of central importance, in addition to parameters such as specific gravity, pH-value, gender, sport discipline, and time of sampling; other factors such as pharmaceutical, technical, and biological issues need consideration when interpreting steroid profile patterns, which are outlined in the following text.

POPULATION BASED- *VERSUS* SUBJECT-BASED REFERENCE RANGES FOR THE DETECTION OF TESTOSTERONE (T) MISUSE

In 1982, the test adopted by the International Olympic Committee (IOC) for detection of T administration was based on the T/E ratio.[3] The T/E laboratory reporting threshold

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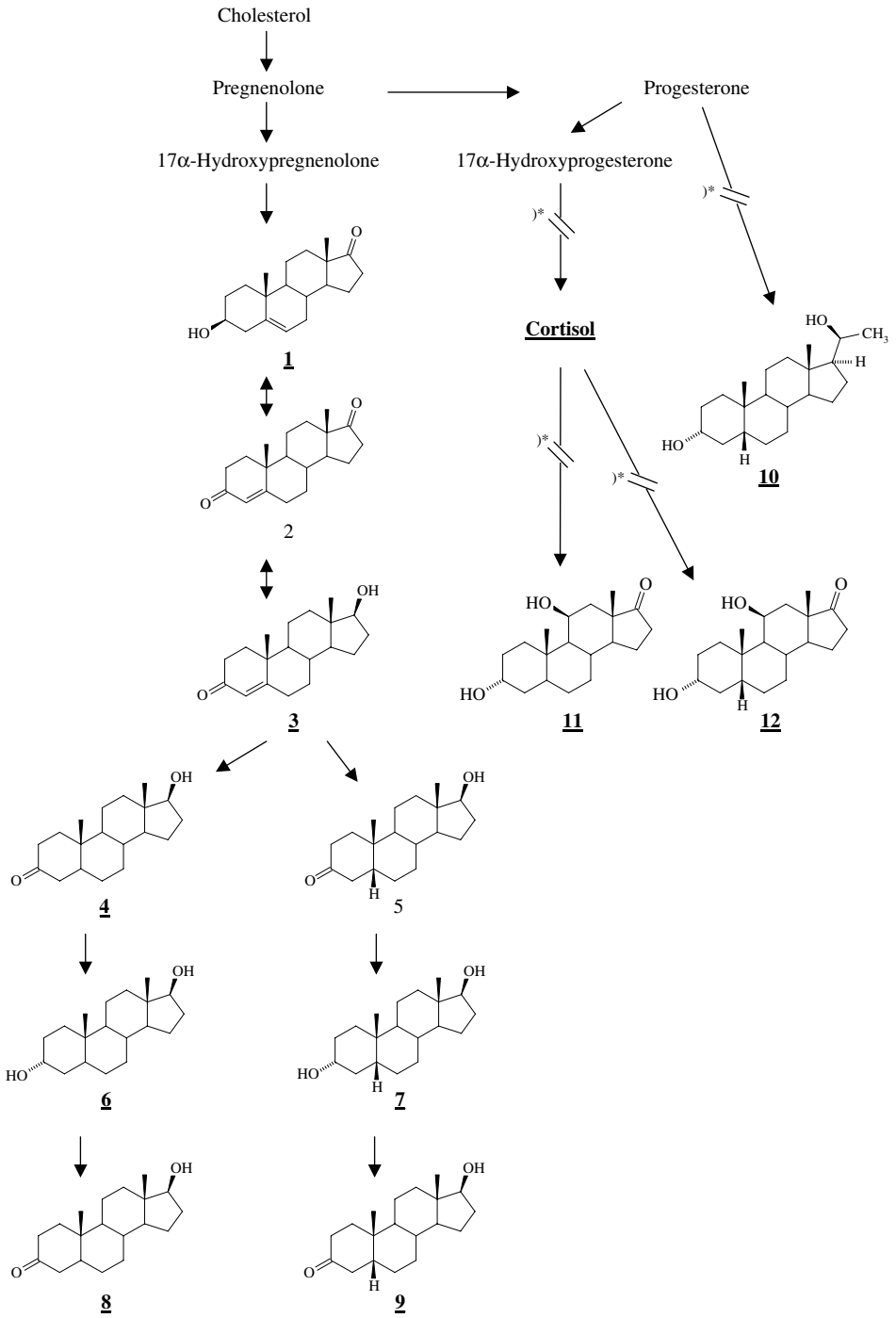

Figure 1. Biosynthesis of endogenous steroids: DHEA [**1**], 4-Androstene-3,17-dione [**2**], Testosterone [**3**], Dihydrotestosterone [**4**], 5β-Dihydrotestosterone [**5**], 5α-Androstane-3α,17β-diol (Adiol) [**6**], 5β-Androstane-3α,17β-diol (Bdiol) [**7**], Androsterone [**8**], Etiocholanolone [**9**], Pregnanediol [**10**], 11β-Hydroxyandrosterone [**11**], 11β-Hydroxyetiocholanolone [**12**].)* several steps included. Steroids analyzed for urinary steroid profiling are written in bold letters and underlined.

was derived empirically from an observed distribution of measurements in specimens collected from a large number of individuals and established at T/E = 6. With an adverse finding, it was mandatory to investigate the T/E results from previous and subsequent tests, i.e. assessing the T/E ratio intraindividually. The reason that elevated T/E ratios need a follow-up before they are declared as adverse finding is the occurrence of naturally elevated T/E ratios.[30] Since 2005, the WADA has changed the reporting threshold for T/E from 6 to 4 in order to improve the sensitivity for the detection of T misuse. The application of intraindividual T/E profiling was first discussed by Donike *et al*. in 1994.[31,32] He demonstrated that subject-based reference ranges react sensitively to variations and provide a better doping control approach than the ones utilizing population-based reference ranges. Hence, subject-based reference ranges of endogenous hormone concentrations or ratios such as T/E have been considered reliable tools to monitor various kinds of doping, employing endogenous steroids. Results of longitudinal urinary steroid profile studies[20,22–25] outlined the low variability within the

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biosynthesis of endogenous steroids and that the metabolic pathway is in agreement with the stationary, homeostatic model for calculating subject-based reference ranges.

In agreement with Donikes' publications[31,32] Sottas *et al.*[33] improved this method recently by proposing the Bayesian screening test[34] for the detection of abnormal values in longitudinal biomarkers. This test compares sequential measurements of a biomarker against previous readings performed on the same individual. The importance of such approaches was stressed by comprehensive studies concerning testosterone gel (T-gel) administrations[35] (*vide infra*). In a recent study 18 healthy male volunteers were treated for 6 weeks continuously and intermittently with T-gel. The discriminating power of individual reference ranges was significantly superior to conventional population-based reference ranges, especially for volunteers showing basal values for T/E less than 1. The mean of one participant's pretest T/E ratio was 0.44. During the period of T-gel application, all T/E values remained far below the cut-off limit of 4, but all values were above the upper limit of the individual reference range of 0.79.[35]

Even now, in general, only atypical findings are followed up. The importance of having access to steroid profile data of all athletes' samples in order to initiate further analytical tests (isotope ratio mass spectrometry = IRMS) or target doping controls may be a new possibility for steroid profiling in future doping control.

GENOTYPE-DEPENDENT VARIATION OF T/E

T/E ratios vary among athletes of different ethnic origin. Typically, Asian people have lower urinary T/E values (<0.5) than Caucasians (approximately 1.0) and, thus, androgen doping exerts weaker effects on the T excretion in the Asian population, increasing the risk of false-negative results.[35] Recent studies described the lack of the UGT2B17 gene in some individuals.[36] In 2006, a Swedish group investigated urine samples from 74 Korean and 122 Swedish men for T- and E-glucuronides.[37,38] The distribution of the natural logarithms of urinary T concentrations showed a distinct bimodal pattern in both groups, suggesting a monogenic inheritance. When the UGT2B17 genotypes were compared with urinary T levels, all the individuals homozygous for the UGT2B17 deletion genotype had no or negligible amounts of urinary T. This genotype was seven times more common in the Korean (66.7%) than in the Swedish population (9.3%).[37,38] These data are in accordance with evaluations of reference ranges for Asian and Caucasian male and female athletes calculated from databases of the Asian Games 1994, the previous Asian Games 1990, and the routine doping control samples of Caucasian athletes measured in Cologne 1994[39] as well as with the results of the population-based reference ranges of 5101 male and 1694 female athletes.[40]

PREGNANCY

Pregnancy causes characteristic changes in the female steroid profile. Resulting from a missing feedback mechanism, the excretion of PD is significantly increased. The population-based reference ranges of PD for females are between 80 and 3000 ng/ml.[40] In the early stages of pregnancy between 5000 and 10 000 ng/ml of PD were detected, increasing to more than 20 000 ng/ml shortly before delivery.[41] In addition, estrogenous substances are excreted in high amounts, with values increasing during the course of pregnancy and interfering with the internal standard used for the steroid profile evaluation. As a consequence, invalid results may be obtained.[41]

19-Norandrosterone is detected in the later course of pregnancy, probably formed as a side product[42] during the conversion of steroids by aromatization. Urinary concentrations of 19-norandrosterone during pregnancy were studied in five pregnant women, who collected morning urine samples once per week during the whole course of pregnancy. In addition, 50 spot urine specimens from different pregnant women were analyzed. The detection of 19-norandrosterone was possible from the 14th week of pregnancy and the concentrations in healthy pregnant women showed an increase during the course of pregnancy with mostly less than 5 ng/ml. Only 7% of all samples showed higher concentrations with a maximum value of 16 ng/ml.[43]

INFLUENCE OF PHARMACEUTICAL PREPARATIONS

Endogenous steroids

The administration of T or its precursors, androstenediol, androstenedione, DHEA or a T metabolite, DHT, or a masking agent such as E are proven to alter one or more of the parameters of the urinary steroid profile.[9,19,39,44–50] Elevated levels of urinary metabolites, which are part of the steroid profile are not consistent with normal endogenous production[23–25,31,32,51] and can result from the intake of these steroids. Consequently, the origin of many steroidal analytes has to be evaluated, preferably by means of GC/C/IRMS enabling the differentiation of endogenously produced and administered compounds.[12,52–62]

Testosterone (T)

After the oral application or injection of exogenous T, the most obvious changes in the steroid profile are the increase of the T concentration and the ratio T/E,[3,4] and the decrease of the And/T ratio. Dehennin and Matsumoto[44] reported increased urinary excretions of T metabolites, and decreased excretions of conjugates of E and its precursor androgen 5-androstene-3β,17α-diol after long-term administration of T enanthate to normal men. The suppression of urinary E was also described in investigations concerning male hormonal long-term contraception by administration of depot T.[63–65]

However, administration studies with stable-isotope-labeled T (1,2 dideutero-T) demonstrated the conversion of T to E to a minor extent,[4] indicating a possible metabolic pathway from T to E, which could result in a limited increase of T/E ratios upon T administration.[4]

According to WADA rules, urine samples showing T/E values equal or greater than 4, or concentrations of T (equivalent to the glucuronide) greater than 200 ng/ml (adjusted for a specific gravity value of 1.020) are suspicious for T administration.[26] More criteria for the evaluation

of urinary steroid profiles, based upon population-based reference ranges of 5101 male and 1694 female athletes, were calculated by Rauth.[40]

Testosterone gel (T gel)

In contrast to oral applications or injected depot formulations, the transdermal administration of T gel leads to an increase of serum T and a decrease of luteinizing hormone (LH). Major effects on the urinary steroid profile were alterations in the ratios Adiol/E, And/E, and T/E, which resulted from a decrease of E and an increase of T and Adiol concentrations. The results also indicated that transdermally administered T is converted to 5α-metabolites (And and Adiol) rather than to 5β-metabolites (Etio and Bdiol), probably due to a high 5α-reductase activity of the skin.[66] First studies on the traceability of T-gel misuse demonstrated that individual reference ranges of steroid profile parameters were more suitable than population-based reference ranges, and adequate target analytes for GC/C/IRMS measurements were T and Adiol.[35]

Dihydrotestosterone (DHT)

The application of exogenous DHT leads to an increased renal elimination of DHT, its 5α-metabolites Adiol, 5α-androstane-3β,17β-diol, And, and epiandrosterone (epiAnd),[39,67–69] while corresponding 5-β-steroids and E are not influenced. Therefore steroid profiles influenced by the application of DHT are characterized by increased ratios of DHT/Etio, DHT/E, Adiol/Bdiol, and And/Etio. The criteria for a detection of DHT doping are based on reference ranges of 4631 male and 1341 female athletes[39] (Table 2).

5α-androstanedione (Adion)

The administration of Adion, an intermediate product of the T metabolism, yielded changes in accordance to DHT applications (*vide supra*), but to a different extent; however, Adion was not excreted in administration studies.[9]

Androstenedione

4-Androstene-3,17-dione (androstenedione) is known to be a biosynthetic precursor of T (Fig. 1). Different groups investigated urinary steroid profiles after the administration of androstenedione.[4,14,47,70,71] Potential urinary markers of androstenedione applications include high levels of And, Etio, T, DHT, and abnormal alterations of T/E values. Uralets and Gilette[71] further reported the presence of a OH-androstenedione metabolite, and Lévesque and Ayotte[14] described 6α-hydroxyandrostenedione, 6β-hydroxyandrosterone, 6β-hydroxyetiocholanolone, and 6β-hydroxyepiandrosterone as additional characteristic metabolites following the application of androstenedione. Catlin *et al*.[48] demonstrated an increase of urinary excreted E and its two metabolites 17-epi-Bdiol and 17-epi-Adiol after androstenedione intake, while 5-androstene-3β,17α-diol (E-precursor) was decreased. Consequently, elevated ratios of 17-epi-Bdiol and 17-epi-Adiol/E-precursor and the presence of 6α-hydroxyandrostenedione have been suggested as androstenedione administration markers.

Table 2. Parameters of the steroid profile, indicating the application of exogenous DHT

Parameter	Men	Women
c DHT [ng/ml][a]	>21	>18
And/Etio	>2.9	>2.1
Adiol/Bdiol	>1.5	>1.3
DHT/Etio[b]	>8.2	>8.5
DHT/E	>0.73	>2.3
c Adiol [ng/ml][a]	>204	>89
c And [ng/ml][a]	>9103	>7562

[a] Concentration corrected to a specific gravity of 1.020 g/cm^3.
[b] DHT/Etio value multiplied with 1000.

Epitestosterone (E)

E is regarded as a masking agent in sports drug testing[72] as its administration results in a decreased T/E ratio, which represents one of the most important steroid profile parameters. Classified documents[73] saved after the collapse of the former German Democratic Republic (GDR) revealed that since 1983, the pharmaceutical company VEB Jenapharm had produced preparations of E propionate exclusively for the governmental doping program. The administration of E with T simultaneously or sequentially enables an athlete to manipulate the result for T administration if the test is based solely on determination of the T/E ratio. The WADA Technical Document for reporting and evaluation of endogenous steroids[26] has recommended further GC/C/IRMS analyses for urine specimens containing concentrations of E (equivalent to the glucuronide) greater than 200 ng/ml (adjusted for a specific gravity value of 1.020) to prove the exogenous or endogenous origin of the analyte. The use of exogenous E increases the excretion of E and the 17α-diols, 17-epi-Bdiol, and 17-epi-Adiol. Hence, the E concentration, the T/E ratio, and those obtained from the above-mentioned 17α-diols and steroids that are not influenced by the E-metabolism (e.g. 17β-diols) were considered suitable for the detection of an E application.[9]

Dehydroepiandrosterone (DHEA)

Few publications are available regarding alteration of urinary steroid profile parameters after administration of DHEA. In 1964, Brooks and Giuliani identified DHEA as a precursor of E by administration of [^{3}H] DHEA.[74] In 1997, Shackleton *et al*. characterized 5-androstene-3β,17β-diol, And, and Etio as metabolites of DHEA using IRMS analysis.[12] A year later, Dehennin *et al*.[46] reported DHEA glucuronide concentrations higher than 300 ng/ml in post-administration urine specimens, and Bowers *et al*. observed in a study involving nine women and seven men that urinary DHEA glucuronide concentrations did not exceed 90 ng/ml[45] under normal conditions. These observations were most likely the basis for fixing the DHEA threshold value at 100 ng/ml for doping control purposes.[26] Recent studies comprising more than 20 000 doping control samples collected in 2006 and 2007, however, demonstrated that DHEA values higher than 100 ng/ml represent a normal part of this (uncontaminated) population.[10,11] Significantly

different DHEA concentrations were evaluated between specimens taken in- and out-of-competition, whereas females showed smaller DHEA values than males for both types of control. Also, a strong influence of DHEA excretion on different sport disciplines was detected, with highest values for game sports, followed by boxing and wrestling, whereas the suggested Gaussian upper 99% reference limits for urinary DHEA calculated for the different sport disciplines showed in all cases, higher values than the current cut-off concentration of 100 ng/ml.[10,11] Since the beginning of 2007, the DHEA metabolite 3α,5-cyclo with a suggested threshold concentration of 140 ng/ml was implemented as an additional GC/MS screening marker for DHEA abuse.[75] For an improved efficiency in detection of DHEA abuse, an alteration of the threshold values for DHEA and 3α,5-cyclo to 200 ng/ml each and a matching of both parameters are suggested.[11]

Effects after long-term application of anabolic androgenic steroids

Long-term applications of anabolic androgenic steroids (AAS) have shown to reduce the endogenous production of androgenic steroids via negative feedback mechanisms leading to a decreased urinary excretion.[21,76] Moreover, a suppression of the 5α-reductase activity was reported causing ratios of And/Etio far lower than 1,[21,77] which are commonly found between 0.5 and 2.9 for male and between 0.4 and 2.2 for female athletes,[40] respectively. This special pattern remained also after cessation of the drug and was observed over prolonged time periods in the former years in sports with frequently detected abuse of AAS (e.g. weightlifting, powerlifting, bodybuilding).[21,76,77] Shortly after withdrawal of AAS, an increase of the T/E ratio was described resulting from a faster recovery and increase of the urinary excretion of T than of E.[21]

Designer steroids

Chemically modified steroids (designer steroids), including tetrahydrogestrinone and norbolethone have demonstrated to pose a threat to the integrity of the sport community. In 2002, the anabolic steroid norbolethone (13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-3-one) was detected in a doping control urine sample, although this compound has never been commercially available. This drug was used by a professional athlete to obtain an unfair advantage in competition.[78] In 2003 and 2005, the discovery of other steroids, tetrahydrogestrinone (THG, 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,9,11-trien-3-one) and 'madol' (17α-methyl-5α-androst-2-ene-17β-ol, also known as *desoxy-methyltestosterone* (DMT) substantiated the abuse of designer steroids in the world of sport.[79,80] Resulting from the close relationship to other AAS, long-term application of designer steroids also leads to a suppression of the excretion of urinary endogenous steroids, probably being a first indicator for the presence or recent misuse of a designer steroid.[78]

Glucocorticosteroids

Glucocorticosteroids are widely used in sports medicine for the treatment of conditions such as asthma and acute injuries. Their use is prohibited when administered orally, rectally, intravenously, or intramuscularly and requires a therapeutic use exemption (TUE) approval. Other routes of administration (intra-articular/periarticular/peritendinous/epidural/intradermal injections and inhalation) require an abbreviated TUE, except dermatological preparations which are not prohibited.[72] Long-term applications of synthetic glucocorticosteroids reduce the production of the endogenous steroids cortisol and cortisone via negative feedback mechanism leading to a decrease of their urinary excretion.[81] On the 'androgenic' steroid profile no effects have been observed yet.

With regard to athletes, the most interesting systemic effect of the glucocorticosteroids is energy production by stimulation of gluconeogenesis and mobilization of amino acids and fatty acids.[82] On the basis of confiscation reports and interviews,[83,84] corticosteroids belong to the group of commonly used doping substances in sports.

Probenecid

Probenecid is a competitive inhibitor of renal tubular transport mechanism and, because it inhibits the resorption of uric acid, one of its main therapeutic indications is the treatment of hyperuricemia. Probenecid may also interfere with renal elimination of certain compounds and, thus, reduce their concentrations in urine. Most of the AAS and their metabolites undergo phase-II metabolism, and probenecid appears to reduce the excretion of the associated glucuronide conjugates. In 1988, administration of probenecid was banned by the IOC Medical Commission because its use was considered as a 'pharmacological manipulation' that altered the integrity and validity of urine samples. Geyer *et al*.[85] showed the substantial reduction of the urinary excretion of both, the endogenous androgenic and the synthetic anabolic steroids.

Diuretics/water diuresis

In contrast to probenecid, diuretics increase the urine flow and lead to reduced steroid concentrations and high volumes of urine. This group of substances is regarded as masking agents according to WADA rules.[72] Moreover, drinking of large volumes of water also leads to low urinary steroid concentrations; however, the androgen ratios are not influenced by diuresis.[86]

Amineptine

Metabolites of amineptine (trade name: Survector), a tricyclic antidepressant drug, may lead to an inhibition of the hydrolysis of steroid glucuronides in common sample preparation procedures employing β-glucuronidase from *Escherichia coli (E.coli)*. The steroid profile patterns show suppressed concentrations of And and Etio, whereas T and E yield normal values. An uncharacterized metabolite of amineptine, which coelutes with And and Etio, generates abundant fragments at m/z 192 and 191; hence, the implementation of m/z 191 for screening purposes is recommended. Metabolites of amineptine are further detected in screening procedures dedicated in the determination of stimulants (commonly referred to as screening 1). Completion of the glucuronide hydrolysis is

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achieved by using ß-glucuronidase/arylsulfatase from *Helix pomatia*.[9]

Ethanol

The oral intake of ethanol in high amounts can increase the T/E ratio and decrease the And/T ratio by an elevated excretion of T-glucuronide and decreased elimination of And-glucuronide.[87,88] This effect was found more pronounced for female than for male volunteers, whereas the changes in the steroid profile ratios were always connected with the presence of ethanol in urine.[89] This important result has always to be taken into account if high T/E ratios are found in doping control urine samples, especially in 'out-of-competition' controls. To avoid false positive results, ethanol should be analyzed in the respective suspicious urine specimen, e.g. by headspace/GC.[89]

Finasteride/Dutasteride

5α-Reductase inhibitors such as finasteride and dutasteride are prohibited in sports according to the WADA[72] since 2005. This class of drugs is used therapeutically to treat benign prostatic hyperplasia as well as male baldness by decreasing 5α-reductase activity. Accordingly, metabolic pathways of endogenous as well as synthetic steroids are influenced, which complicates the evaluation of steroid profiles in sports drug testing. The evaluation of urinary steroid profiles demonstrated the intense effect of finasteride on numerous crucial analytical parameters, in particular the production of 5α-steroids such as And and Adiol, which was significantly reduced. For doping control analysis, the use of 5α-reductase inhibitors causes considerable problems because steroid profile parameters, which are commonly considered stable, are highly affected.[19] Owing to the fact that less than 1% of unchanged dutasteride is excreted into the urine, neither dutasteride nor its metabolites are detectable in urine, and other complementary biological samples are needed.[90]

Oral contraceptives

The application of oral contraceptives leads to an increase of the T/E ratio resulting from a suppression of the E excretion, whereas the ratios And/Etio, Adiol/Bdiol as well as the excretion rates of And and T are not influenced. Also PD is detected as a stable intraindividual parameter. Following cessation of administration, a decrease of the T/E ratio is observed resulting from an increase of the E excretion.[91]

Trimethoprim

Trimethoprim is an antibiotic/antiinfectious agent frequently combined with sulfamethoxazol when used as medication. Trimethoprim metabolites and sulfamethoxazol metabolites coelute and interfere with endogenous steroids, which can disturb chromatography and lead to wrong evaluations of steroid profile parameters. Amelioration of this effect is accomplished by extraction of the steroid fraction with *n*-pentane instead of *tert*-butyl methyl ether (TBME).[92]

Cyclodextrin

Owing to its ability to form inclusion compounds as a result of the cyclic structure with molecules of molecular masses up to approximately 300 Da, cyclodextrin is supposed to be suitable for manipulation of urine. Preliminary investigation on adulteration of urine with γ-cyclodextrin was carried out utilizing seven quality control urine samples fortified with cyclodextrin. Two of the analyzed samples showed decreased signal intensities for all steroids in the steroid profile.[93]

Human chorionic gonadotrophin (HCG)

HCG is a glycoprotein hormone misused by some male athletes to stimulate endogenous T production or to prevent testicular atrophy during prolonged administration of AAS. As the testes contribute to ~95% of the pool of urinary T and E-glucuronide in eugonadal men,[94,95] HCG stimulation leads to a simultaneous increase in the urinary excretion rate of both E and T resulting in only minor changes in the urinary T/E ratio.[96] de Boer *et al*. reported a suppression of the changes in the urinary T/E ratio by HCG and T co-administation in a case study.[97] However, no significant influence on steroid profile parameters by an administration of HCG was observed, whereas HCG itself is detected in urine by means of commercially available immunoassays.

Luteinizing hormone (LH)

LH, a peptide hormone, which is secreted in pulses by the pituitary gland, stimulates the Leydig cells to produce T in males. In women, LH plays an important role for the ovulation; during the female menstrual cycle a strong variability of LH concentrations in blood is detected.[98] Depending on missing data of urinary LH concentrations, a detection of LH misuse in doping control urine samples in sport has not been possible yet. Voss *et al*.[99] established urinary LH reference values for men and women, analyzing LH concentrations of 483 urine specimens collected from European male and female athletes as well as morning urine samples of one male and two female volunteers (with and without oral contraceptive) collected for a time period of 1 month. Steroid profiles obtained after the administration of a single dose of 75-IU LH (Luveris from Serono) showed only weak effects on the excretion of urinary T and E. Also, no detection of LH misuse by quantitation of urinary LH was possible in the respective urine specimens.

Ketoconazole

The so-called 'ketoconazole-test' was used for the detection of exogenous T application before the direct analysis by means of GC/C/IRMS became possible. The test is based on the suppressive effects of ketoconazole on steroidogenesis. The antifungal agent ketoconazole inhibits the enzyme cytochrome P450 17α-hydroxylase/17,20-lyase that converts intermediate products in the steroidogenic pathway to T; hence, the application of ketoconazole leads to an inhibition of the T biosynthesis and subsequent suppression of urinary steroid profile parameters.[100,101] Consequently, administration of ketoconazole to athletes having misused T will not influence the T/E ratio, as T does not originate from an endogenous production. In contrast, a naturally elevated T/E ratio decreases due to the inhibiting effect of ketoconazole, which is more evident on T than on E.[102]

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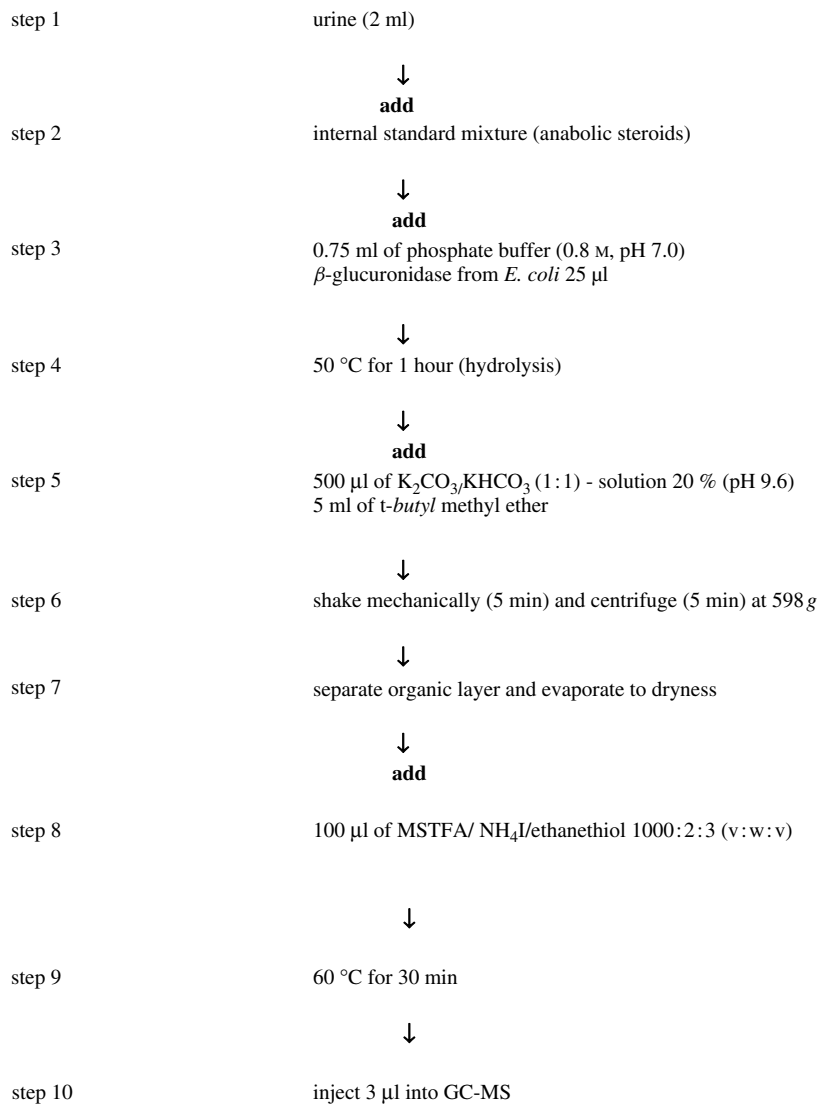

Figure 2. Flow scheme for the sample preparation for the screening of anabolic steroids in human urine.

Nutritional supplements (*Tribulus terrestris*, ZMA)

Nutritional supplements advertised as natural T boosters were tested by administration studies and analysis of the urinary steroid profiles of volunteers. Application of the *Tribulus terrestris* formulation Tribulin showed no significant changes in urinary LH and T profiles or T/E ratios.[103–105] Also, the high-dose zinc supplement ZMA had no significant effects regarding serum T levels and the metabolism of T in subjects consuming a zinc-sufficient diet.[106]

Table 3. Inhibition of hydrolysis in doping control urine samples analyzed in 2006 and 2007 in Cologne

	2006	2007
Samples analyzed	11 012	13 108
Hydrolysis inhibition	50 (0.45%)	49 (0.37%)
pH >8	33 (66%)	18 (37%)
Male	31 (62%)	30 (61%)
Female	17 (34%)	18 (37%)
In-competition	37 (74%)	40 (82%)
Out-of-competition	13 (26%)	9 (18%)

INFLUENCE OF TECHNICAL PARAMETERS

A common sample preparation flow scheme for the hydrolysis of steroid conjugates, the extraction of aglycons, and subsequent derivatization with GC/MS analysis is depicted in Fig. 2[107–111] that will provide an overview of the most important technical and (bio)chemical sample preparation parameters.

Hydrolysis of glucuronic acid conjugates

The time required to complete the hydrolysis of glucuronic acid conjugates using β-glucuronidase from *E.coli* varies between the different steroid glucuronides.[21] T- and E-glucuronide are cleaved completely within several minutes while And- and Etio-glucuronide require extended hydrolysis times. This is presumably depending on their higher urinary concentrations; however, the hydrolysis of Etio-glucuronide is completed faster than the cleavage of And-glucuronide. The incomplete generation of aglycons results in characteristic patterns of the steroid profile. The concentrations of And and Etio are reduced whereas the concentrations of T and E are situated within the respective

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population-based reference group. The corresponding ratios of And/T and And/E are considerably decreased, and the ratio And/Etio is usually far below 1. Such patterns are also observed after an application of amineptine that represents one reason for the inhibition of enzymatic hydrolysis efficiency (*vide supra*). In order to test for the completeness of hydrolysis, deuterated And glucuronide ([2,2,3,4,4,-$^2H_5$]-And glucuronide) is used as internal standard and allows, in combination with the unconjugated internal standard [2,2,4,4,-$^2H_4$]-Etio, an estimation of the hydrolytic activity of the β-glucuronidase by comparison of peak areas of both equimolarly added compounds. The control of the hydrolysis step enables to perform a direct cleavage of steroid conjugates in urine specimens without a preceding solid phase extraction.[111]

In Table 3, the evaluation of incidences of hydrolysis inhibition in doping control urine samples analyzed in 2006 and 2007 in Cologne is presented. Approximately 0.5% of all measured samples showed an incomplete hydrolysis. For these specimens, a second sample preparation was performed using solid phase extraction (SPE) as additional cleanup step leading in most cases to satisfying results. Only 5 out of 99 samples in 2 years were prepared a third time using β-glucuronidase/arylsulfatase from *H. pomatia* instead of β-glucuronidase from *E.coli* (Fig. 2, step 3). In both years the respective urine samples showed similar characteristics. Most of them were taken in competition, and approximately 25% of the respective specimens showed pH values higher than 8. According to common sample preparation protocols, the pH value is adjusted to 7 before addition of the enzyme (Fig. 2, step 3), which necessitated highly concentrated buffer reagents and, thus, high ion intensities in several samples of strongly elevated pH values. Consequently, inhibition of hydrolysis was observed that was corrected only by using sample preparation methods including SPE.

Coelution

A prerequisite for the identification and quantitation of anabolic and endogenous steroids by GC/MS is a clean chromatographic and mass spectrometric signal. However, coeluting substances lead to asymmetric chromatographic signals and impure mass spectra of the substances of interest. Possible methods for enhanced separation of coeluting substances are (1) the change of the gas-chromatographic temperature program, (2) the use of columns with different polarities, or (3) the use of other derivatives and/or the addition of purification steps to the sample preparation.

Extraction with *n*-pentane instead of TBME (Fig. 2, step 5) is a very simple but still efficient method to improve purification of most anabolic and endogenous steroids by removal of interfering substances. These include species that coelute with the substances of interest, e.g. 11-oxo-etiocholanolone coeluting with E, 11-oxo-epiandrosterone coeluting with T and 11β-hydroxy-etiocholanolone coeluting with metenolone, and trimethoprim metabolites and sulfamethoxazol metabolites coeluting with T and E. However, this cleanup step is not suitable for polar steroids such as stanozolol-metabolites, 6β-hydroxy-metandienone, fluoxymesterone, and corticosteroids but for confirmatory analyses of most other anabolic steroids and elevated T/E ratios.[92]

Derivatization

Various steroids relevant for an interpretation of the urinary steroid profile comprise at least one keto function. This functionality is commonly derivatized by means of enolization and trimethylsilylation (TMS) which is accomplished using *in-situ* formed trimethyl iodosilane.[3,112] An incomplete derivatization leads to altered steroid profile values, e.g. low And/T values, as ions derived from bis-TMS are accounted for in GC/MS measurements. The completeness of derivatization is controlled by monitoring the ion at m/z 272 representing the molecular ion after elimination of trimethylsilanol from the mono-TMS derivatives of And and Etio. If these two substances are detected in GC/MS analyses, the derivatization is incomplete, which is corrected by the addition of approximately 20 µl of a more concentrated derivatization reagent.[113]

Matrix issues

The calibration of GC/MS systems for the determination of endogenous steroids with reference standards has frequently caused problems arising from different reasons, which are mainly attributed to matrix effects. The major issues include signal enhancement for steroids measured from urine specimens and nonlinear calibration curves measured from pure reference material samples.[114,115] Such phenomena are suggested to result from a protective property of the biological matrix (e.g. urine) and are proposed to be associated with the injection port, the analytical column, the ion source, or the mass selective detector.

In 1995, Geyer *et al.*[116] tested various options and matrices while searching for a solution to overcome the matrix issue(s), e.g. the preparation of an artificial matrix that could imitate the features of a steroid-free urine. First, injections of calibration standards after analyses of urine specimens demonstrated that the protective nature of the matrix remains for a few injections ('memory effect') but does not provide a reasonable option. Second, the addition of a series of synthetic diisopropylamines [DIPAs, starting from diisopropyl-tetradecyl-propylamines (C-14) to diisopropyl-tricosyl-propylamines (C-23)] to calibration standards was tested.[117] The correctness of the calibration factors in calibration standards prepared with DIPAs was controlled by the comparison of the steroid ratios measured from the (deuterated) internal standards in the calibration samples and each urine specimen. Since 2006, synthetic urine has become available (e.g. LGC-Promochem, Wesel, Germany). This artificial urine has been employed by different WADA-accredited laboratories and represents a convenient way to imitate a biological matrix for calibration standards.

INFLUENCE OF MICROORGANISM

Bacterial activities

First indicators for bacterial activities in urine samples are elevated pH values. Frequently, the steroid profiles of such urine specimens are altered due to the formation

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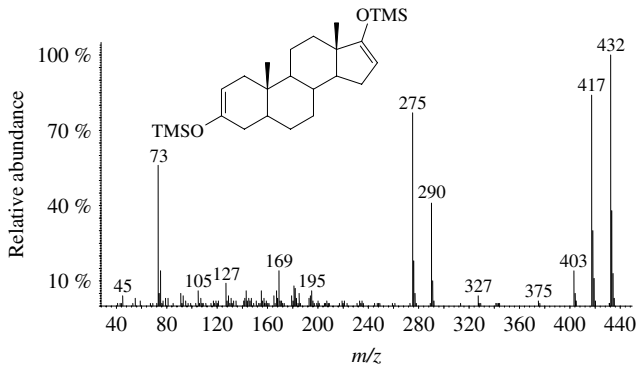

Figure 3. Mass spectrum of 5α-androstane-3,17-dione, bis-TMS 2,16-dienol.

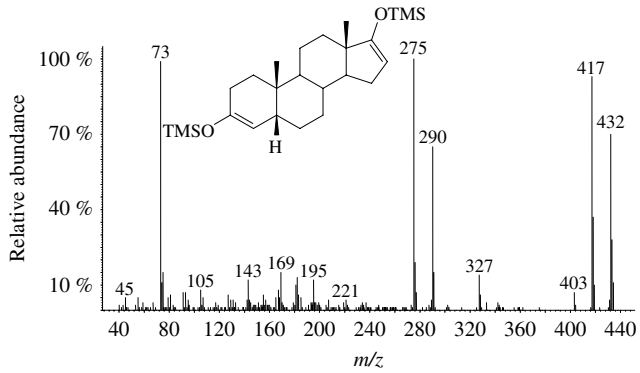

Figure 4. Mass spectrum of 5β-androstane-3,17-dione, bis-TMS 3,16-dienol.

Table 4. Bacterial activity in doping control urine samples analyzed in 2006 and 2007 in Cologne

	2006	2007
Samples analyzed	11 012	13 108
Bacterial activity	184 (1.7%)	289 (2.2%)
pH >8	63 (34%)	85 (29%)
Male	100 (54%)	162 (56%)
Female	78 (42%)	122 (42%)
In-competition	100 (54%)	181 (63%)
Out-of-competition	84 (46%)	108 (37%)

of 5α-androstane-3,17-dione (5α-androstanedione = Adion) (Fig. 3) and 5β-androstane-3,17-dione (5β-androstanedione = Bdion) (Fig. 4). The formation of these steroids is based on a bacterial deconjugation of And- and Etio-glucuronide followed by a bacterial 3-hydroxysteroid-dehydrogenase activity.[9,13] Because of the bacterial deconjugation, high amounts of steroids, normally excreted as conjugates (as And and Etio) are observed as aglycons. Another effect of bacterial activity on steroid profiles, which is more rarely observed, is the increase of the T concentration leading to elevated T/E ratios. The formation of T resulting from a bacterial hydrolysis of 5-androstene-3β,17β-diol sulfate followed by a 3β-hydroxy-Δ^5steroid-dehydrogenase and steroid-Δ-isomerase activity, was suggested as the bacterially derived T that was not conjugated. Hence, the artificial T is well separated from T-glucuronide during sample preparation

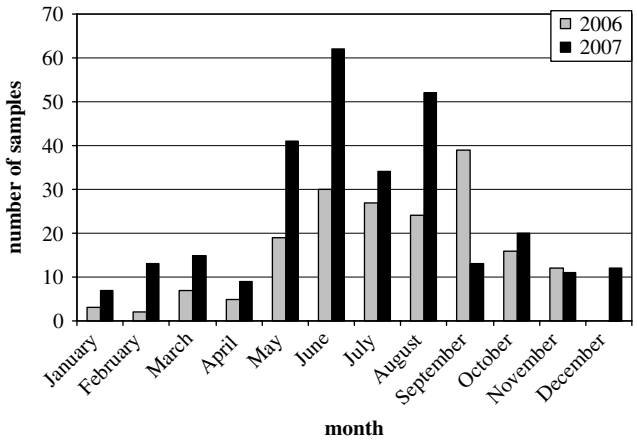

Figure 5. Distribution of urine samples demonstrating bacterial activity over the course of 2 years.

by liquid–liquid extraction before the hydrolysis (Fig. 2, step 1).[9,13]

These considerations have been included into the WADA technical document for 'Reporting and Evaluation of T/E ratios': specimens containing more than 5% of free T and/or E are not valid for reporting Adverse Analytical Findings of endogenous steroids.[26]

In Table 4 and Fig. 5, the evaluation of doping control urine samples showing formation of Adion and/or Bdion is illustrated. Approximately 2% of the specimens analyzed in

Table 5. Influence of the application of endogenous steroids on the steroid profile

Substance	Increase	Decrease
Testosterone	T, T/E	And/T
Testosterone gel	T, Adiol, T/E, Adiol/E, And/E	E
Dihydrotestosterone	DHT, Adiol, 5α-androstane-3β,17β-diol, And, epiAnd DHT/Etio, DHT/E, Adiol/Bdiol, And/Etio	
5α-Androstanedione	DHT, Adiol, 5α-androstane-3β,17β-diol, And, epiAnd DHT/Etio, DHT/E, Adiol/Bdiol, And/Etio	
4-Androstene-3,17-dione	And, Etio, T, DHT, E, 17-epi-Bdiol, 17-epi-Adiol, T/E	5-androstene-3β, 17α-diol
Epitestosterone	E, 17-epi-Bdiol, 17-epi-Adiol	T/E
Dehydroepiandrosterone	And, Etio, 5-androstene-3β,17β-diol, 3α,5-cyclo-DHEA	

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a period of 1 year showed bacterial activity, but only 30% of them had elevated pH values (>8). The consideration of gender and type of control (in- or out-of-competition) was not significant, but the distribution of urine samples demonstrating bacterial activity varied over the course of the year (Fig. 5). Apparently, inappropriate storage/transportation conditions at elevated temperatures initiate or stimulate the effect of bacterial contamination on urinary steroids.

Side activities of enzymes during sample preparation

The use of β-glucuronidase/arylsulfatase from *H. pomatia* for the enzymatic hydrolysis may lead to an increased ratio of T/E and formation of 4-androstenedione.[21,118] Reasons for these changes are side activities (3β-hydroxy-Δ^5steroid-dehydrogenase and steroid-Δ-isomerase) in the *H. pomatia* preparation, which convert 5-androstene-3β,17β-diol to T and DHEA to 4-androstenedione. Similar effects were observed using β-glucuronidase from *E. coli* but the side activities were attributed to bacteria originating from outdated phosphate buffers as demonstrated, for instance, with 5-androstene-3β,17β-diol, which was successfully converted to T under such conditions.[21]

Formation of 19-norsteroids from endogenous steroids

In 2005, Grosse *et al*.[16] reported the formation of 19-norsteroids by demethylation of endogenous steroids in routine doping control samples. Since 2006, the unequivocal determination of the origin of urinary norandrosterone traces is possible by means of GC/C/IRMS.[119]

Table 6. Influence of pharmaceutical preparations on the steroid profile

Substance	Effect
Anabolic androgenic steroids	Suppression of endogenous steroids, And/Etio <1, T/E increase after withdrawal
Designer steroids	Suppression of endogenous steroids
Glucocorticosteroids (synth)	Suppression of cortisol, cortisone
Probenecid	Suppression of endogenous steroids, supp synthetic anabolic steroids
Diuretics	Small steroid concentrations, no effect on T/E
Amineptine	Inhibition of hydrolysis, supp And + Etio
Ethanol	Increase of T, T/E and decrease of And, And/T
Finasteride/ Dutasteride	Decrease of And, Adiol, and And/Etio $\ll$1
Oral contraceptives	Suppression of E, increase of T/E
Trimethoprim	Coelution with T and E (m/z 432)
Cyclodextrin	Decrease of endogenous steroids
HCG	Weak effects on T + E (not detectable)
LH	Weak effects on T + E (not detectable)
Ketoconazole	Suppression of endogenous steroids
Tribulus terrestris, ZMA	No effect

Table 7. Influence of technical and microbiological parameters on the steroid profile

Parameter	Effect
Inhibition of hydrolysis	Supp And + Etio
Coelution	Asymmetric chromatographic signals
Incomplete derivatization	And- and Etio-mono-TMS detectable (m/z 272)
Matrix problems	Response for known steroids is higher in urinary matrix than in pure standard mixture; different area ratios of steroids in urine and pure standard calibration curves for steroids in pure standard solutions are not linear
Bacterial activity	Formation of Adion and Bdion, increase of T (unconjugated) and T/E
Side activities	Formation of 4-androstenedione, increase of T/E

Endogenous production of boldenone (B)

In 1994, Schänzer *et al*. detected B and its two main metabolites 17β-hydroxy-5β-androst-1-en-3-one and 3α-hydroxy-5β-androst-1-en-17-one in urine samples of two laboratory staff members not treated with B.[17] A hypothesis was presented that B production resulted from bacterial activity in the gut, which converted T or androst-4-ene-3,17-dione (AED) to androsta-1,4-diene-3,17-dione (ADD). Transport of this metabolite to the liver via enterohepatic circulation, could result in metabolic conversion to B and metabolites. van de Kerkhof and co-workers conducted an *in-vitro* experiment in 1999 in which feces of an athlete, suspect for a B-positive doping control sample, were incubated in non-specific media with T or AED as metabolic precursors.[18] Minor traces of B and its metabolite were detected, but the experimental design suffered from missing optimal incubation conditions and precursor concentrations. In 2007, experiments on production of 'endogenous boldenone' were performed by Leinonen *et al*.[120] Large intestinal models of humans were simulated to study different conditions for the formation of B and its metabolites. In authentic colon conditions, no formation of B was detected but in simulations with enhanced oxygen addition, the increased redox-potential led to the formation of large ADD quantities. Furthermore, fecal contaminations of urine samples and their storage at room temperature were found suitable to yield B and its metabolites under aerobic conditions. Thus, GC/C/IRMS analysis is the only reliable method to verify exogenous and endogenous B.

CONCLUSIONS

The urinary steroid profile consisting of concentrations and ratios of various endogenously produced steroidal hormones is a valuable tool in doping control analysis. The

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majority of parameters are not influenced by exercise, severe physical endurance performance, menstrual cycle, circadian rhythms, or annual rhythms. However, the application of pharmaceutical preparations such as T and T prohormones, AAS, probenecid, or finasteride (Tables 5 and 6) lead to strong variations of steroid profile ratios following partial suppression or enhancement of the endogenous steroids. The influence of technical parameters such as inhibition of hydrolysis, incomplete derivatization, or matrix problems as well as biological issues including bacterial activity that causes the formation of different steroids, e.g. 5α- and 5β-androstanedione, 19-norsteroids or B are shown in Table 7. All these facts necessitate consideration by an experienced scientist to reliably interpret steroid profile patterns.

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REFERENCES

1. Shackleton CHL. Profiling steroid hormones and urinary steroids. *Journal of Chromatography* 1986; **379**: 91.
2. Vestergaard P, Sayegh JF, Mowat JH, Hemmingsen L. Estimation after multicolumn liquid chromatography of common urinary neutral steroids with an application to the assay of plasma 17-oxosteroids. *Acta Endocrinologica* 1978; **88**: 88.
3. Donike M, Bärwald KR, Klostermann K, Schänzer W, Zimmermann J. Nachweis von exogenem testosterone (The detection of exogenous testosterone). In *Sport: Leistung und Gesundheit*, Heck H, Hollmann W, Liesen H, Rost R (eds). Deutscher Ärzte Verlag: Köln, 1983; 293.
4. Zimmermann J. *Untersuchungen zu Nachweis von exogenen Gaben von Testosteron*. Thesis, Deutsche Sporthochschule Köln, 1986.
5. Donike M. Steroid profiling in Cologne. In *10th Cologne Workshop on Dope Analysis*, 7th to 12th June 1992, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, Rauth S (eds). Sport und Buch Strauß: Köln, 1992; 47.
6. Träger L. *Steroidhormone, Biosynthese, Stoffwechsel, Wirkung*. Springer-Verlag: Berlin-Heidelberg-New York, 1977.
7. Shimada K, Mitamura K, Higashi T. Gas chromatography and high-performance liquid chromatography of natural steroids. *Journal of Chromatography A* 2001; **935**: 141.
8. Bellemare V, Faucher F, Breton R, Luu-The V. Characterization of 17α-hydroxysteroid dehydrogenase activity (17α-HSD) and its involvement in the biosynthesis of epitestosterone. *Biochemistry* 2005; **6**: 12.
9. Geyer H, Schänzer W, Mareck-Engelke U, Donike M. Factors influencing the steroid profile. In *Recent Advances in Doping Analysis (3)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1995; 95.
10. Mareck U, Geyer H, Flenker U, Piper T, Thevis M, Schänzer W. Detection of dehydroepiandrosterone misuse by means of gas chromatography-combustion-isotope ratio mass spectrometry. *European Journal of Mass Spectrometry* 2007; **13**: 419.
11. Mareck U, Geyer H, Flenker U, Piper T, Thevis M, Schänzer W. Population based reference ranges and additional screening marker for the improvement of the detection of dehydroepiandrosterone misuse. In *Recent Advances in Doping Analysis (16)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2008; (in press).
12. Shackleton CHL, Roitman E, Phillips A, Chang T. Androstanediol and 5-androstenediol profiling for detecting exogenously administered dihydrotestosterone, epitestosterone, and dehydroepiandrosterone: potential use in gas chromatography isotope ratio mass spectrometry. *Steroids* 1997; **62**: 665.
13. Ayotte C, Charlebois A, Lapointe S, Barriault D, Sylvestre M. Validity of urine samples: microbial degradation. In *Recent Advances in Doping Analysis (4)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1996; 127.
14. Lévesque JF, Ayotte C. Criteria for the detection of androstenedione oral administration. In *Recent Advances in Doping Analysis (7)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1999; 169.
15. Cawley AT, Flenker U. The application of isotope ratio mass spectrometry to doping control. *Journal of Mass Spectrometry* 2008; DOI: 10.1002/jms.1437.
16. Grosse J, Anielski P, Hemmersbach P, Lund H, Mueller RK, Rautenberg C, Thieme D. Formation of 19-norsteroids by in situ demethylation of endogenous steroids in stored urine samples. *Steroids* 2005; **70**: 499.
17. Schänzer W, Geyer H, Gotzmann A, Horning S, Mareck-Engelke U, Nitschke R, Nolteernsting E, Donike M. Endogenous production and excretion of boldenone (17beta-hydroxyandrosta-1,4-dien-3-one), an androgenic anabolic steroid. In *Recent Advances in Doping Analysis (2)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1994; 211.
18. van de Kerkhof DH, van der Voort PM, de Boer D, Maes RAA. Confirmation of endogenous boldenone production: a procedure for an *in vitro* experiment. In *Recent Advances in Doping Analysis (7)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1999; 161.
19. Thevis M, Geyer H, Mareck U, Flenker U, Schänzer W. Doping-control analysis of the 5α-reductase inhibitor finasteride: determination of its influence on urinary steroid profiles and detection of its major urinary metabolite. *Therapeutic Drug Monitoring* 2007; **29**: 236.
20. Mareck-Engelke U, Geyer H, Donike M. Stability of steroid profiles. In *10th Cologne Workshop on Dope Analysis*, 7th to 12th June 1992, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, Rauth S (eds). Sport und Buch Strauß: Köln, 1992; 87.
21. Geyer H. *Die Gas-chromatographisch/massenspektrometrische Bestimmung von Steroidprofilen im Urin von Athleten*. Thesis, Deutsche Sporthochschule Köln, 1986.
22. Nitschke R. *Steroidprofile und Veränderungen biochemischer Parameter bei Hochleistungsradrennfahreren während zwei Rundfahrten*. Thesis, Deutsche Sporthochschule Köln, 1996.
23. Mareck-Engelke U, Geyer H, Donike M. Stability of steroid profiles (4): the circadian rhythm of urinary ratios and excretion rates of endogenous steroids in female and its menstrual dependency. In *Recent Advances in Doping Analysis (2)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1994; 135.
24. Mareck-Engelke U, Flenker U, Donike M. Stability of steroid profiles (5): the annual rhythm of urinary ratios and excretion rates of endogenous steroids in female and its menstrual dependency. In *Recent Advances in Doping Analysis (3)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1995; 177.
25. Mareck-Engelke U, Geyer H, Donike M. Stability of steroid profiles (3): the circadian rhythm of urinary ratios and excretion rates of endogenous steroids in male. In *Recent Advances in Doping Analysis (2)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1994; 121.
26. World Anti-Doping Agency: WADA Technical Document – TD2004 EAAS. Reporting and evaluation guidance for testosterone, epitestosterone, T/E ratio and other endogenous steroids. Available: http://www.wada-ama.org/rtecontent/document/end_steroids_aug_04.pdf (accessed 01.04.2007).
27. World Anti-Doping Agency. Guideline: reporting and management of elevated T/E ratios. Available: http://www.

wada-ama.org/rtecontent/document/GuidelineReporting ManagementElevatedTERatios.pdf (accessed 07.04.2008).
28. Thevis M, Geyer H, Mareck U, Sigmund G, Henke J, Henke L, Schänzer W. Detection of manipulation in doping control urine sample collection: a multidisciplinary approach to determine identical urine samples. *Analytical and Bioanalytical Chemistry* 2007; **388**: 1539.
29. Robinson N, Castella V, Saudan C, Sottas PE, Schweizer C, Dimo-Simonin N, Mangin P, Saugy M. Elevated and similar urinary testosterone/epitestosterone ratio in all samples of a competition testing: suspicion of a manipulation. *Forensic Science International* 2006; **163**: 148.
30. Oftebro H. Evaluating an abnormal urinary steroid profile. *The Lancet* 1992; **339**: 941.
31. Donike M, Rauth S, Mareck-Engelke U, Geyer H, Nitschke R. Evaluation of longitudinal studies, the determination of subject based reference ranges of the testosterone/epitestosterone ratio. In *Recent Advances in Doping Analysis*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, Rauth S (eds). Sport und Buch Strauß: Köln, 1993; 33.
32. Donike M, Mareck-Engelke U, Rauth S. Statistical evaluation of longitudinal studies, part 2: the usefulness of subject based reference ranges. In *Recent Advances in Doping Analysis (2)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1994; 157.
33. Sottas PE, Saudan C, Schweizer C, Baume N, Mangin P, Saugy M. From population- to subject-based limits of T/E ratio to detect testosterone abuse in elite sports. *Forensic Science International* 2008; **174**: 166.
34. Sottas PE, Saudan C, Schweizer C, Kamber M, Saugy M. Bayesian detection of abnormal values in longitudinal biomarkers with an application to T/E ratio. *Biostatistics* 2007; **8**: 285.
35. Geyer H, Flenker U, Mareck U, Platen P, Piper T, Schmechel A, Schrader Y, Thevis M, Schänzer W. The detection of the misuse of testosterone gel. In *Recent Advances in Doping Analysis (15)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2007; 133.
36. Wilson W, de Villena FPM, Lyn-Cook BD, Chatterjee PK, Bell TA, Detwiler DA, Gilmore RC, Valladeras IC, Wright CC, Threadgill DW, Grant DJ. Charcterization of a common deletion polymorphism of the UGT2B17 gene linked to UGT2B15. *Genomics* 2004; **84**: 707.
37. Ekström L, Jakobsson J, Garle M, Rane A. Large differences in testosterone excretion in Korean and Swedish men are strongly associated with a UDP-glucuronosyl transferase 2B17 polymorphism. In *Recent Advances in Doping Analysis (14)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2006; 211.
38. Jakobsson J, Ekström L, Inotsume N, Garle M, Lorentzon M, Ohlsson C, Roh H-K, Carlström K, Rane A. Large differences in testosterone excretion in Korean and Swedish men are strongly associated with a UDP-glucuronosyl transferase 2B17 polymorphism. *Journal of Clinical Endocrinology and Metabolism* 2006; **91**: 687.
39. Donike M, Ueki M, Kuroda Y, Geyer H, Nolteernsting 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. *Journal of Sports Medicine and Physical Fitness* 1995; **35**: 235.
40. Rauth S. *Referenzbereiche von urinären Steroidkonzentrationen und Steroidquotienten. Ein Beitrag zur Interpretation des Steroidprofils in der Routinedopinganalytik*. Thesis, Deutsche Sporthochschule Köln, 1994.
41. Mareck-Engelke U, Geyer H, Schänzer W. The interpretation of female steroid profiles. In *Recent Advances in Doping Analysis (5)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1997; 51.
42. Lund H, Jathun S, Fedorcsak P, Storeng R, Torjesen P, Hemmersbach P. Synthesis of nandrolone in the human ovary. In *Recent Advances in Doping Analysis (10)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2002; 23.
43. Mareck-Engelke U, Schultze G, Geyer H, Schänzer W. The appearance of urinary 19-norandrosterone during pregnancy. *European Journal of Sport Science* 2002; **2**: 1.
44. Dehennin L, Matsumoto AM. Long-term administration of testosterone enanthate to normal men: alterations of the urinary profile of androgen metabolites potentially useful for detection of testosterone misuse in sport. *Journal of Biochemistry and Molecular Biology* 1993; **44**: 179.
45. Bowers LD. Oral dehydroepiandrosterone supplementation can increase the testosterone/epittestosterone ratio. *Clinical Chemistry* 1999; **45**: 295.
46. Dehennin L, Ferry M, Lafarge P, Pérès G, Lafarge JP. Oral administration of dehydroepiandrosterone to healthy men: alteration of the urinary androgen profile and consequences for the detection of abuse in sport by gas chromatography-mass spectrometry. *Steroids* 1998; **63**: 80.
47. Bassindale T, Cowan DA, Dale S, Hutt AJ, Leeds AR, Kicman AT. Disposition of androstenedione and testosterone following oral administration of androstenedione to healthy female volunteers; influence on the urinary T/E ratio. In *Recent Advances in Doping Analysis (10)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2002; 51.
48. Catlin DH, Leder BZ, Ahrens BD, Hatton CK, Finkelstein JS. Effects of androstenedione administration on epitestosterone metabolism in men. *Steroids* 2002; **67**: 559.
49. Leder BZ, Catlin DH, Longcope C, Ahrens B, Schoenfeld DA, Finkelstein JS. Metabolism of orally administered androstenedione in young men. *Journal of Clinical Endocrinology and Metabolism* 2001; **86**: 3654.
50. Lévesque JF, Ayotte C. The oral administration of DHEA: the efficiency of steroid profiling. In *Recent Advances in Doping Analysis (7)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1999; 213.
51. Mareck-Engelke U, Geyer H, Donike M. Stability of steroid profiles (2): excretion rates from morning urines. In *Recent Advances in Doping Analysis*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, Rauth S (eds). Sport und Buch Strauß: Köln, 1993; 85.
52. Becchi M, Aguilera R, Farizon Y, Flament MM, Casabianca H, James P. Gas chromatography/combustion/isotope-ratio mass spectrometry analysis of urinary steroids to detect misuse of testosterone in sport. *Rapid Communications in Mass Spectrometry* 1994; **8**: 304.
53. Horning S, Geyer H, Machnik M, Schänzer W, Hilkert A, Oeßelmann J. Detection of exogenous testosterone by $^{13}C/^{12}C$ analysis. In *Recent Advances in Doping Analysis (4)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1996; 275.
54. Shackleton CHL, Phillips A, Chang T, Li Y. Confirming testosterone administration by isotope ratio mass spectrometric analysis of urinary androstanediols. *Steroids* 1997; **62**: 379.
55. Flenker U, Horning S, Nolteernsting E, Geyer H, Schänzer W. Measurement of $^{12}C/^{13}C$-ratios to confirm misuse of endogenous steroids. In *Recent Advances in Doping Analysis (6)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1998; 243.
56. Aguilera R, Becchi M, Casabianca H, Hatton CK, Catlin DH, Starcevic B, Pope HG. Improved method of detection of testosterone abuse by gas chromatography/combustion/isotope ratio mass spectrometry analysis of urinary steroids. *Journal of Mass Spectrometry* 1996; **31**: 169.
57. Aguilera R, Catlin DH, Becchi M, Phillips A, Wang C, Swerdloff RS, Pope HG, Hatton CK. Screening urine for exogenous testosterone by isotope ratio mass spectrometric analysis on one pregnanediol and two androstanediols. *Journal of Chromatography B* 1999; **727**: 95.

58. Aguilera R, Chapman TE, Catlin DH. A rapid screening assay for measuring urinary androsterone and etiocholanolone $\delta^{13}C$ (%) values by gas chromatography/combustion/isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* 2000; **14**: 2294.
59. Aguilera R, Chapman TE, Starcevic B, Hatton CK, Catlin DH. Performance characteristics of a carbon isotope ratio method for detecting doping with testosterone based on urine diols: controls and athletes with elevated testosterone/epitestosterone ratios. *Clinical Chemistry* 2001; **47**: 292.
60. Aguilera R, Hatton CK, Catlin DH. Detection of epitestosterone doping by isotope ratio mass spectrometry. *Clinical Chemistry* 2002; **48**: 629.
61. Ueki M, Okano M. Analysis of exogenous dehydroepiandrosterone excretion in urine by gas chromatography/combustion/isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* 1999; **13**: 2237.
62. Flenker U, Güntner U, Schänzer W. $\delta^{13}C$-values of endogenous urinary Steroids. *Steroids* 2008; **73**: 408.
63. Kinniburgh D, Zhu H, Cheng L, Kicman AT, Baird DT, Anderson RA. Oral desogestrel with testoterone pellets induces consistent suppression of spermatogenesis to azoospermia in both Caucasian and Chinese men. *Human Reproduction* 2002; **17**: 1490.
64. Brady BM, Walton M, Hollow N, Kicman AT, Baird DT, Anderson RA. Depot testosterone with etonogestrel implants result in induction of azoospermia in all men for long-term contraception. *Human Reproduction* 2004; **19**: 2658.
65. Walton MJ, Anderson RA, Kicman AT, Elton RA, Ossowska K, Baird DT. A diurnal variation in testicular hormone production is maintained following gonadotrophin suppression in normal men. *Clinical Chemistry* 2007; **66**: 123.
66. Occhiato EG, Guarna A, Danza G, Serio M. Selective non-steroidal inhibitors of 5α-reductase type 1. *Journal of Steroid Biochemistry and Molecular Biology* 2004; **88**: 1.
67. Southan GJ, Brooks RV, Cowan DA, Kicman AT, Unnadkat N, Walker CJ. Possible indices for the detection of the administration of dihydrotestosterone to athletes. *Journal of Steroid Biochemistry and Molecular Biology* 1992; **42**: 87.
68. Kicman AT, Coutts SB, Walker CJ, Cowan DA. Proposed confirmatory procedure for detecting 5α-dihydrotestosterone doping in male athletes. *Clinical Chemistry* 1995; **41**: 1617.
69. Coutts SB, Kicman AT, Hurst DT, Cowan DA. Intramuscular administration of 5α-dihydrotestosterone heptanoate: changes in urinary hormone profile. *Clinical Chemistry* 1997; **43**: 2091.
70. Garle M, Palonek E. Androstenedione: excretion studies from single and multiple dose experiments. In *Recent Advances in Doping Analysis (6)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1998; 181.
71. Uralets VP, Gilette PA. Over-the-counter anabolic steroids 4-androsten-3,17-dione; 4-androsten-3β,17β-diol, and 19-nor-4-androsten-3,17-dione: excretion studies in men. In *Recent Advances in Doping Analysis (6)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1998; 147.
72. World Anti-Doping Agency. The 2008 prohibited list. International standard. Available: http://www.wada-ama.org/rtecontent/document/2008_List_En.pdf (accessed 27.09.2007).
73. Franke WW, Berendonk B. Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic government. *Clinical Chemistry* 1997; **43**: 1262.
74. Brooks RV, Giuliani G. Epitestosterone: isolation from human urine and experiments on possible precursors. *Steroids* 1964; **4**: 101.
75. Cawley A, Hine ER, Trout GJ, George AV, Kazlauskas R. Searching for new markers of endogenous steroid administration in athletes: ''looking outside the metabolic box''. *Forensic Science International* 2004; **143**: 103.
76. Donike M, Geyer H, Schänzer W, Zimmermann J. Die suppression der endogenen androgenproduktion durch metandienon. In *Training und Sport zur Prävention und Rehabilitation in der technisierten Umwelt*, Franz I, Mellerowicz H, Noack W (eds). Springer-Verlag: Berlin-Heidelberg, 1985.
77. Donike M, Geyer H, Kraft M, Rauth S. Longterm influence of anabolic steroid misuse on the steroid profile. In *Official Proceedings. IInd International Athletic Foundation World Symposium on Doping in Sport Monte Carlo*, Bellotti P, Benzi G, Ljungqvist A (eds), International Athletic Foundation: Italy, 1989; 107.
78. Catlin DH, Ahrens BD, Kucherova Y. Detection of norbolethone, an anabolic steroid never markted, in athletes' urine. *Rapid Communications in Mass Spectrometry* 2002; **16**: 1273.
79. Catlin DH, Sekera MH, Ahrens BD, Starcevic B, Chang YC, Hatton CK. Tetrahydrogestrinone: discovery, synthesis, and detection in urine. *Rapid Communications in Mass Spectrometry* 2004; **18**: 1245.
80. Sekera MH, Ahrens BD, Chang YC, Starcevic B, Georgakopoulos C, Catlin DH. Another designer steroid: discovery, synthesis, and detection of ''madol'' in urine. *Rapid Communications in Mass Spectrometry* 2005; **19**: 781.
81. Gotzmann A: *Nachweis biologisch aktiver Substanzen mit Hilfe der Hochdruckflüssigkeits-Chromatographie und Normbereichsbestimmungen von Corticosteroiden*. Thesis, Deutsche Sporthochschule Köln, 1991.
82. Tharp GD. The role of glucocorticoids in exercise. *Medicine and Science in Sports* 1975; **7**: 6.
83. dpa. Cortison-Spritzen für Sinkewitz. Spiegel Online, 2007; http://www.spiegel.de/sport/sonst/0,1518,514819,00.html (accessed 08.04.2008).
84. Schulze D. ''Ich bin Bella'' – Das Doping-Geständnis des Jörg Jaksche. Spiegel TV Exclusiv, 2007; http://www.spiegel.de/sptv/magazin/0,1518,490531,00.html (accessed 08.04.2008).
85. Geyer H, Schänzer W, Donike M. Probenecid as masking agent in dope control – inhibition of the urinary excretion of steroid glucuronides. In *10th Cologne Workshop on Dope Analysis*, 7th to 12th June 1992, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, Rauth S (eds). Sport und Buch Strauß: Köln: 1992; 141.
86. Donike M, Rauth S, Sample B. Excretion of ephedrine and endogenous steroids under conditions of controlles water intake and of water diuresis. In *10th Cologne Workshop on Dope Analysis*, 7th to 12th June 1992, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, Rauth S (eds). Sport und Buch Strauß: Köln: 1992; 163.
87. Falk O, Palonek E, Björkhem I. Effect of ethanol on the ratio between testosterone and epitestosterone in urine. *Clinical Chemistry* 1988; **34**: 1462.
88. Karila T, Konsunen V, Leinonen A, Tähtelä R, Seppälä T. High doses of alcohol increase urinary testosterone-to-epitestosterone ratio in females. *Journal of Chromatography B, Biomedical Applications* 1996; **687**: 109.
89. Mareck-Engelke U, Geyer H, Schindler U, Flenker U, Iffland R, Donike M. Influence of ethanol on steroid profile parameters. In *Recent Advances in Doping Analysis (3)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1995; 143.
90. Simões SMS, Vitoriano B, Manzoni C, de la Torre X. 4-Alpha reductase inhibitors detection in doping analysis. In *Recent Advances in Doping Analysis (13)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2005; 199.
91. Mareck-Engelke U, Flenker U, Schänzer W. Stability of steroid profiles (6): the influence of oral contraceptives on steroid profiles. In *Recent Advances in Doping Analysis (4)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1996; 139.
92. Geyer H, Mareck-Engelke U, Schänzer W, Donike M. Simple purification of urine samples for improved detection of anabolic and endogenous steroids. In *Recent Advances in Doping Analysis*,

Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, Rauth S (eds). Sport und Buch Strauß: Köln, 1993; 97.
93. Guddat S, Thevis M, Mareck U, Geyer H, Schänzer W. Identification of cyclodextrins by liquid chromatography – ESI-tandem mass spectrometry. In *Recent Advances in Doping Analysis (13)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2005; 419.
94. Kicman AT, Coutts SB, Cowan DA, Handelsman DJ, Howe CJ, Burring S, Wu FCW. Adrenal and gonadal contributions to urinary excretion and plasma concentration of epitestosterone in men – effect of adrenal stimulation and implications for detection of testosterone abuse. *Clinical Endocrinology* 1999; **50**: 661.
95. Kicman AT, Gower DB. Anabolic steroids in sport: biochemical, clinical and analytical perspectives. *Annals of Clinical Biochemistry* 2003; **40**: 321.
96. Cowan DA, Kicman AT, Walker CJ, Wheeler MJ. Effects of administration of human chorionic gonadotropin on criteria used to assess testosterone administration in athletes. *Journal of Endocrinology* 1991; **131**: 147.
97. de Boer D, de Jong EG, van Rossum JM, Maes RA. Doping control of testosterone and human chorionic gonadotrophin: a case study. *International Journal of Sports Medicine* 1991; **12**: 46.
98. Löffler G, Petrides PE. *Biochemie and Pathobiochemie*. Springer-Verlag: Berlin-Heidelberg-New York, 2003.
99. Voss S, Gotzmann A, Geyer H, Mareck U, Schänzer W. Evaluation of LH concentration in male and female urines. Effects of a single LH application on the steroid profile. In *Recent Advances in Doping Analysis (13)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). SPORT und BUCH Strauss: Köln, 2005; 423.
100. Kicman AT, Oftebro H, Walker C, Norman N, Cowan DA. Potential use of ketoconazole in a dynamic endocrine test to differentiate between biological outliers and testosterone use by athletes. *Clinical Chemistry* 1993; **39**: 1798.
101. Oftebro H, Jensen J, Mowinckel P, Norli HR. Establishing a ketoconazole suppression test for verifying testosterone administration in the doping control of athletes. *Journal of Clinical Endocrinology and Metabolism* 1994; **78**: 973.
102. Saugy M, Cardis C, Robinson N, Schweizer C. Test methods: anabolics. *Baillière's Clinical Endocrinology and Metabolism* 2000; **14**: 111.
103. Van Eenoo P, Delbeke FT, Desmet N, De Backer P. Excretion studies with tribulus terrestris. In *Recent Advances in Doping Analysis (8)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). SPORT und BUCH Strauss: Köln, 2000; 13.
104. Rogerson S, Riches CJ, Jennings C, Weatherby RP, Meir RA, Marshall-Gradisnik SM. The effect of five weeks of tribulus terrestris supplementation on muscle strength and body composition during preseason training in elite rugby league players. *Journal of Strength and Conditioning Research* 2007; **21**: 348.
105. Saudan C, Baume N, Emery C, Strahm E, Saugy M. Short term impact of tribulus terrestris intake on doping control analysis of endogenous steroids. *Forensic Science International* 2008; **178**: e7.
106. Koehler K, Parr MK, Geyer H, Mester J, Schänzer W. Serum testosterone and urinary excretion of steroid hormone metabolites after administration of a high-dose zinc supplement. *European Journal of Clinical Nutrition* 2007; 1, DOI: 10.1038/sj.ejcn.1602899.
107. Schänzer W, Donike M. Synthesis of deuterated steroids for GC/MS quantification of endogenous steroids. In *Recent Advances in Doping Analysis (2)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1994; 93.
108. Thevis M, Opfermann G, Schmickler H, Schänzer W. Mass spectrometry of steroid glucuronide conjugates. I.Electron impact fragmentation of 5α-/5β-androstan-3α-ol-17-one glucuronides, 5α-estran-3α-ol-17-one glucuronide and deuterium-labelled analogues. *Journal of Mass Spectrometry* 2001; **36**: 159.
109. Donike M, Geyer H, Gotzmann A, Kraft M, Mandel F, Nolteernsting E, Opfermann G, Sigmund G, Schänzer W, Zimmermann J. Dope analysis. In *Official Proceedings. International Athletic Foundation World Symposium on Doping in Sport*, Bellotti P, Benzi G, Ljungqvist A (eds). International Athletic Foundation: Florence, 1987; 53.
110. Mareck U, Thevis M, Guddat S, Gotzmann A, Bredehöft M, Geyer H, Schänzer W. Comprehensive sample preparation for anabolic steroids, glucocorticosteroids, beta-receptor blocking agents, selected anabolic androgenic steroids and buprenorphine in human urine. In *Recent Advances in Doping Analysis (12)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2004; 65.
111. 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 *Recent Advances in Doping Analysis (5)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1997; 99.
112. Donike M, Zimmermann J. Zur Dastellung von Trimethylsilyl-, Triethylsilyl- und tert.-Butyldimethylsilyl-enoläthern von Ketosteroiden für gas-chromatographische und massenspektrometrische Bestimmungen. *Journal of Chromtography* 1980; **202**: 483.
113. Geyer H, Mareck-Engelke U, Schänzer W, Donike M. The Cologne protocol to follow up high testosterone/epitestosterone ratios. In *Recent Advances in Doping Analysis (4)*, Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1996; 107.
114. Linnet K. Effect of urine matrix on the testosterone/epitestosterone calibration curve. In *10th Cologne Workshop on Dope Analysis*, 7th to 12th June 1992, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, Rauth S (eds). Sport und Buch Strauß: Köln, 1992; 125.
115. Vobejda C. *Zur Frage der Linearität von Eichkurven für gaschromatographisch-massenspektrometrische Untersuchungen*. Thesis, Deutsche Sporthochschule, 1990.
116. Geyer H, Mareck-Engelke U, Nolteernsting E, Opfermann G, Donike M. The matrix problem in calibration of the GC/MS for endogenous steroids. The search for an artificial urine. In *Recent Advances in Doping Analysis (2)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1994; 199.
117. Nolteernsting E, Opfermann G, Donike M. 1-(N,N-diisopropylamino)-n-alkanes: a new reference system for systematical identification of nitrogen containing substances by gas-chromatography and nitrogen specific or mass spectrometrical detection. In *Recent Advances in Doping Analysis (3)*, Donike M, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 1995; 369.
118. Kuoppasalmi K, Leinonen A, Kajalainen U. Detection of exogenous testosterone in doping analysis: methodological aspects. In *Sports Medicine and Exercise Science. Proceedings of the Olympic scientific congress*, Pieron M, Graham G (eds), Human Kinetics Publishers: Eugene, 1984.
119. Hebestreit M, Flenker U, Fußhöller G, Geyer H, Güntner U, Mareck U, Piper T, Thevis M, Ayotte C, Schänzer W. Determination of the origin of urinary norandrosterone traces by gas chromatography combustion isotope ratio mass spectrometry. *The Analyst* 2006; **131**: 1021.
120. Leinonen A, Apajalahti J, Moisander T, Siikanen O, Kuuranne T, Lauraeus M. Experiments on production of ''endogenous boldenone''. In *Recent Advances in Doping Analysis (15)*, Schänzer W, Geyer H, Gotzmann A, Mareck U (eds). Sport und Buch Strauß: Köln, 2007; 163.