

Screening Indicators of Dehydroepiandrosterone, Androstenedione, and Dihydrotestosterone Use: A Literature Review

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Abstract

Because of their perceived and reported effects on self-image, muscle development, performance, and similar factors, anabolic-androgenic steroids (AAS) and their precursors are among the most abused substances by professional, amateur, and recreational athletes. However, AAS abuse is not limited to athletes, but is also prevalent in the workplace, especially those professions in which image, strength, and endurance are coveted attributes. The detection of many steroids in biological specimens is analogous to the detection of an abused drug such as cocaine. Identification of the parent drug or its characteristic metabolite(s) in a donor's sample with a drug screening technique and confirmation of the drug/metabolite with a suitable alternative technology provides evidence of use. These analyses and subsequent interpretive scenarios become far more complex when the ingested AAS is an endogenous compound such as dehydroepiandrosterone (DHEA), androstenedione (Adione), or dihydrotestosterone (DHT). These compounds and their metabolites are present in specimens such as urine as a course of our natural endocrine function. Therefore, it becomes much more challenging for the laboratory to establish testing and interpretative paradigms that can distinguish "normal" urinary profiles of these steroids and their metabolites from profiles indicative of exogenous use. Distinguishing "normal" from "abnormal" urine profiles is particularly challenging during screening when literally tens of steroids and their metabolites may be tested simultaneously in a single chromatographic analysis. The purpose of this paper is to review the relevant literature about DHEA, Adione, and DHT administration, detection, and interpretation specifically as it relates to changes in the urinary AAS profile that may be identified during the routine laboratory screening of donor urine specimens.

Introduction

The use of anabolic-androgenic steroids (AAS) by competitive athletes has escalated since their first reported use in the 1954 World Weightlifting Championships (1). The International Olympic Committee (IOC) added AAS to its list of prohibited substances in 1975, and testing was first introduced at the 1976 Olympic Games (1,2). Because of increasing abuse, AAS testing has expanded into all levels of professional and amateur sports. In the 2009 World Anti-Doping Agency (WADA) report of Adverse Analytical Findings and Atypical Findings, AAS accounted for 64.9% of the detected substances by WADA accredited laboratories (3). However, AAS use and abuse are not limited to athletes and have become more common even in individuals engaged in sensitive positions such as the military, security services, and law enforcement.

The availability of "nutritional" supplements containing AAS or AAS precursors over-the-counter (OTC) or via the internet is a contributing factor in the abuse of these compounds. Numerous investigators have reported detecting the presence of AAS, or their precursors, such as dehydroepiandrosterone (DHEA), androstenedione (Adione) and androstenediol in nutritional supplements (4–12). A study of 634 supplements described as "non-hormonal" purchased between 2000 and 2001 revealed that approximately 15% of the products contained AAS or AAS precursors not declared on the label (9). The Anabolic Steroid Control Act of 2004 attempted to curb the availability of these tainted products in the U.S. (13). However, a recent study of 58 nutritional supplements purchased OTC in the U.S. or from the internet demonstrated that Adione and DHEA were detected in 27% and 23% of the products, respectively (14). The study's authors concluded that despite regulatory efforts to control the content of these products, the presence of AAS and AAS precursors in nutritional supplements remains a concern.

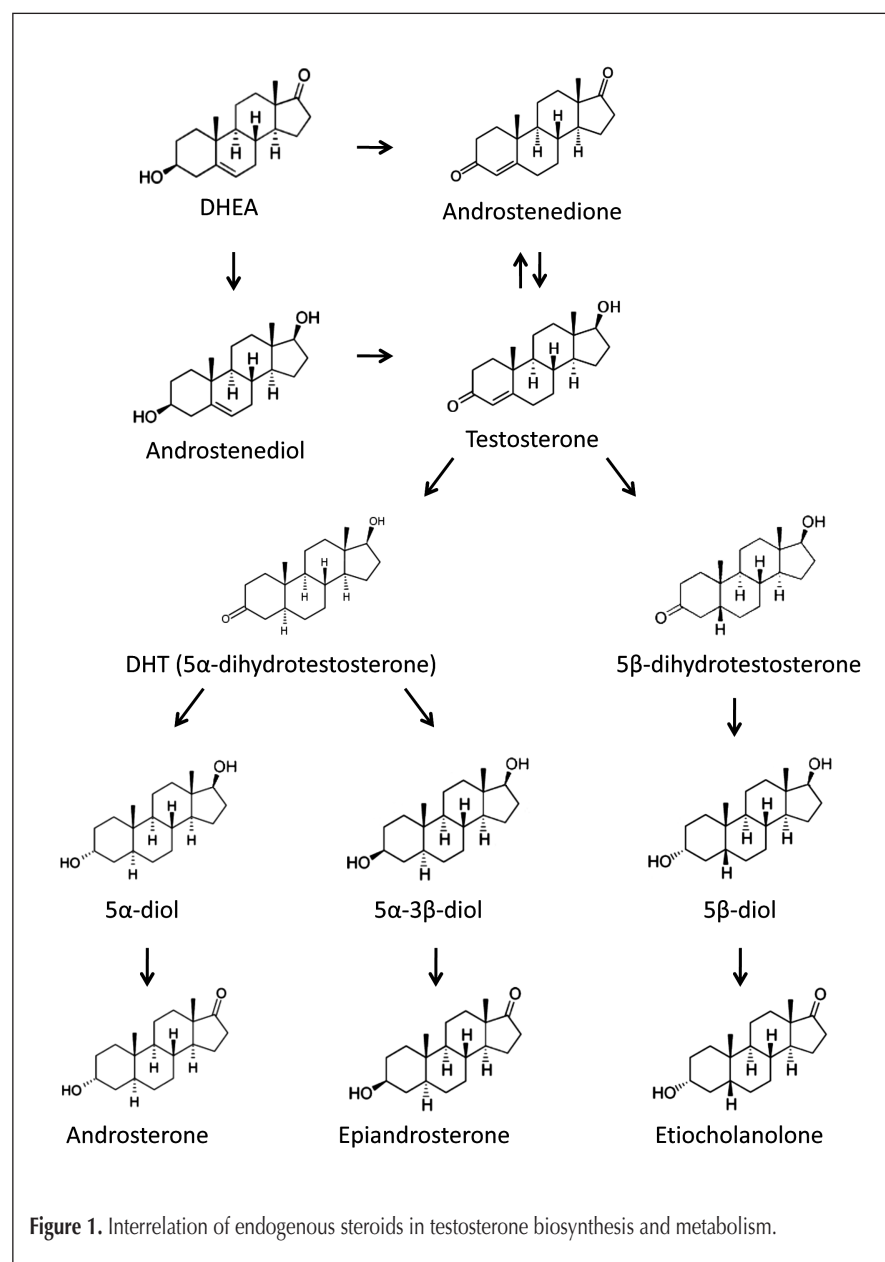
One of the more challenging aspects of AAS testing has been identifying the use of naturally occurring or endogenous AAS

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such as testosterone (T), Adione, DHEA, and dihydrotestosterone (DHT). Figure 1 shows the interrelation of these substances during T biosynthesis and metabolism. The use of synthetic AAS (such as methandienone, stanozolol, or methyltestosterone) can be determined by the mere presence of the drug or its metabolites in the donor's urine. However, use of endogenous AAS is much more difficult to detect and substantiate because the presence of a naturally occurring AAS, or its metabolites, in the donor's urine is anticipated and not diagnostic of exogenous use. Historically, to screen for use of naturally occurring AAS, investigators have examined changes in the urinary "profile" of T, epitestosterone (E), androsterone (Andro), etiocholanolone (Etio), DHEA, DHT, 5 α -androst-3 α ,17 β -diol (5 α -diol), and 5 β -androst-3 α ,17 β -diol (5 β -diol) that may occur following exogenous use. Changes in the concentrations and various ratios of these compounds have been proposed as potential "markers" of use that could be moni-

tored during screening analyses (15). Recently, the presence and/or changes in the concentrations or ratios of minor metabolites of endogenous AAS have also been advocated as providing evidence of use of naturally occurring AAS (16,17).

Several studies have reported success in detecting synthetic endogenous AAS use by gas chromatography with isotope ratio mass spectrometry (GC-IRMS) analyses (18–21). (For a review of this application, see references 22,23.) However, this technology has been described as "too delicate and fastidious to be used in routine screening" (23). Therefore, it remains a significant challenge for laboratories to identify donor samples with urinary "profiles" or "markers" indicative of administration of naturally occurring AAS during screening. This paper reviews the literature relevant to changes in the urinary AAS profiles following administration of DHEA, Adione or DHT with the primary focus on AAS profile patterns, or other indicators that may be used during screening to detect use.



Dehydroepiandrosterone (DHEA)

As shown in Figure 1, DHEA (3 β -hydroxy-androst-5-ene-17-one) is a precursor of steroid biosynthesis (24,25). DHEA is readily interconverted to its 3 β -sulfate form (DHEAS) (26,27). In blood, DHEA circulates both in its free and sulfate forms but is converted to DHEA in target tissues. Approximately 80% DHEA and up to 95% DHEAS are of adrenal origin (27). The body produces 15 to 30 mg of DHEA/S per day making them by far the most abundant sex-hormone precursors found in human serum (24,28,29). DHEA/S are estimated to make up 30 to 50% in men and up to 75% in premenopausal women, respectively, of the total androgens (27). Both DHEA and DHEAS are weak androgens with a low conversion rate to T (<1%), but also may be converted peripherally to Adione, T, and DHT (24,29). DHEA/S concentrations peak in early adulthood and, beginning at about age 30, decline in an age-dependent fashion at a rate approaching 25%/decade (27).

DHEA has been advocated for steroid replacement therapy in part because animal and in vitro tissue studies have demonstrated several potentially beneficial effects from DHEA such as lowering body fat; modulating obesity, diabetes, and arthritis; stimulating the immune system; inhibiting carcinogenesis and providing cardiovascular protection (27,30). DHEA enjoys a unique status in the U.S. because it is one of the few an-

abolic steroid precursors that is not banned by the Anabolic Steroid Control Act of 2004. Therefore, DHEA is still legally available in OTC dietary/nutritional supplements. However, DHEA has been banned by the IOC/WADA since 1997 (29) and is prohibited in most anti-doping programs. The DHEA content of supplements varies considerably, but generally is in the range of 25 to 200 mg/dose (24,28).

Urine concentrations of T, E, Andro, Etio, 5 α -diol, 5 β -diol, DHEA-glucuronide, and DHEAS have been evaluated as potential indicators of exogenous DHEA use. WADA has suggested that urine samples containing specific gravity (SG) corrected DHEA concentrations (equivalent to the glucuronide) of > 100 ng/mL be selected for further testing (15). This concentration may be too low and, therefore, an ineffective criterion for the presumptive identification of exogenous DHEA use and for selecting samples for IRMS confirmation as suggested in the Technical Document. A recent publication suggests that a SG-corrected DHEA concentration of 100 ng/mL may be "normal" (31). In addition to concentrations of various AAS, it has been suggested that ratios of T/E and Andro/Etio may provide insight into DHEA use. Like other 3 β -OH steroids, DHEA is preferentially excreted as sulfate rather than as glucuronide conjugates, and therefore, the sulfate to glucuronide urinary

ratios of DHEA and its metabolites have also been investigated as markers of use (Table I).

Several studies have been published in which DHEA was administered to humans and changes in the subject's urinary AAS profiles have been evaluated for indicators of the administration. In a study by Uralets and Gillette (32), three subjects were administered a single 200-mg dose of DHEA in supplement form. Shortly after administration, urinary Andro and Etio concentrations increased dramatically and DHEA concentrations rose either moderately (2 subjects) or dramatically (1 subject). The Andro, Etio, and DHEA concentration essentially returned to near predose values within 20 to 36 h. However, in two of the three subjects, the Etio concentrations remained above predose values for more than 50 h. The 5 α - and 5 β -diol excretion patterns followed those of Andro and Etio with rapid increases in their concentrations. Further, like Andro, 5 α -diol concentrations returned to baseline within 20 to 30 h, whereas the peak 5 β -diol concentrations were reached later and persisted longer than the 5 α -diol concentrations. Two subjects with predose T/E ratios of < 1 had moderate ratio increases (two- to threefold) for less than 10 h, and then the ratios fell below baseline. The investigators also observed that the Andro/Etio and the 5 α -diol/5 β -diol ratios fell below predose

Table I. Changes in Urinary Concentrations of Glucuronide and Sulfate Metabolites of Endogenous Steroids Following DHEA Administration*

Steroid	Predose		Postdose Increase over Predose		Postdose Increase over Predose	
	24-h Mean (μ g/h)	Range (μ g/h)	0–2 h (μ g/h)		24-h Mean (μ g/h)	
DHEA						
Glucuronide	1.8	(1.6–2.4)	12	Significant†	3.3	
Sulfate	82	(64–245)	453	Significant	249	Significant
Testosterone						
Glucuronide	2.2	(2.1–2.9)	3.1	Significant	0.3	
Sulfate	0.4	(0.3–0.6)	2	Significant	0.5	
Epitestosterone						
Glucuronide	2.1	(1.8–2.6)	Not detected		Not detected	
Sulfate	0.7	(0.7–1.0)	1.1		Not detected	
Androsterone						
Glucuronide	125	(114–168)	286	Significant	104	Significant
Sulfate	191	(139–406)	794	Significant	243	Significant
Etiocolanolone						
Glucuronide	116	(109–150)	312	Significant	153	Significant
Sulfate	152	(131–281)	598	Significant	203	Significant
5 α -Androstane-3 α ,17 β -diol						
Glucuronide	2.5	(2.4–3.2)	Not detected		Not detected	
Sulfate	1	(0.6–1.7)	4.4	Significant	2.9	Significant
5 β -Androstane-3 α ,17 β -diol						
Glucuronide	6.9	(6.6–8.4)	5.1	Significant	8.6	Significant
Sulfate	4.9	(3.1–5.7)	13.1	Significant	15	

* Dehennin et al. (27).

† Significance $p < 0.05$.

values at about 20 h postdose and persisted below the baseline for up to 50 h.

Dehennin and colleagues published an oral DHEA administration study that included nine male subjects with a mean age of 30.8 years (range 22–45) (27). Seven of the subjects received 50 mg of DHEA, and two received deuterium-labeled DHEA containing either 20.4 or 25.1 mg of drug. Urine specimens were collected for 24 h prior to dosing and showed a decided circadian rhythm for several urinary AAS (Table I). Predose maximum concentrations of the urinary T, E, Andro, Etio, 5 α -diol, and 5 β -diol all occurred between 10 a.m. to 12 p.m. Also shown in Table I are the changes in glucuronide and sulfate concentrations of DHEA, T, E, Andro, Etio, 5 α -diol, and 5 β -diol from predose levels following DHEA administration. Statistically significant concentration changes are noted in the table. The magnitude of the concentration increases was generally greatest during the first 2 h postdose and declined to insignificant changes by 24 h for some steroids (T and E). Mean concentration changes remained significantly higher than predose for 24 h for Andro, Etio, 5 β -diol glucuronide and sulfate, DHEA, and 5 α -diol sulfate. The authors estimated that approximately 12% of the administered drug was recovered as DHEA, 17% as Andro, 17% as Etio, 1% as 5 β -diol, < 1% as T, and < 1% as 5 α -diol. Data from the deuterium-labeled administrations showed that 50 to 75% of the dose was excreted within the first 24 h and 20 to 25% was recovered between 24 and 36 h after dosing.

In a 1998 study designed to determine the effect(s) of DHEA on the T/E ratio, 7 subjects (ages 36 to 49) were administered 50 mg of DHEA orally each day for 30 days (33). Prestudy, 3 h predose and 3 h postdose urine samples were collected. Varying effects on the T/E ratio were observed. One subject's T/E ratio increased slightly from its predose mean of 0.34 to an in-study mean of 0.43. Another subject's mean ratio nearly doubled from 1.2 predose, to 2.11 during administration. Although immediate "increases" in the subject's T/E ratios were observed following the first day of dosing, no statistically significant changes were seen during the study. These investigators also administered a single "high acute" dose of 250 mg of DHEA to a single subject. This subject's T/E ratio increased from 0.8 predose to 1.2, but returned to predose levels by 7 h.

In 2004, Inthong and colleagues (34) performed a study to determine the effects of multiple doses of DHEA on urinary AAS profiles. Three male subjects (ages 22 to 40) were given 25 mg doses of DHEA twice daily for seven days. Free and glucuronide metabolites of T, Andro, Etio, 5 α -diol, and 5 β -diol were measured in specimens collected during the study and for two weeks postadministration. The authors concluded that there were "significant" increases in the urinary excretion rates of DHEA, Andro and Etio during administration. However, "statistical significance" was not presented in the paper. The excretion rates of T and E and the Andro/Etio ratio were unchanged during the study with the exception of one volunteer who had an elevated predose T. There were also slight increases in the 5 α -diol and 5 β -diol concentrations with no change in the ratio.

Setiawati and colleagues (26) published a multiple dose study in 2001, in which 13 healthy male subjects (ages 20 to

30) were administered 50 mg of DHEA orally each morning for five days. Urine specimens were collected predose and periodically for 24 h following administration. Postadministration urinary profile data showed a high degree of intersubject variability. In general, there were small and insignificant increases in the subjects' T/E ratios between 8 and 12 h postadministration. However, the T/E ratio of one subject, who had a baseline ratio of 3.5, reached approximately 9 during the study. The ratios of Andro/Etio were marginally depressed following administration, and there were no statistical differences between the 5 α -diol/5 β -diol ratios. However, statistically significant differences were observed between the sulfate/glucuronide ratios of DHEA at most postadministration time points.

Despite the cited research and the availability of DHEA, interpreting use from changes in the urinary excretion profile of DHEA-related AAS remains problematic for several reasons. Although some studies have included female subjects most have not, therefore, data to assist in interpreting use by females and female athletes are exceedingly limited. Most studies used a small number of subjects who often demonstrated large age ranges (24,28,29,32,34,35). Because of this, and similar limitations in the study designs, statistical comparisons of predose or control group urinary concentrations and ratios to postdose concentrations and ratios have been inadequate. However, some trends were identified in the studies. Following single doses, T, E, Andro, Etio, 5 α -diol, and 5 β -diol concentrations appear to increase over baseline for a period of time. T, E, Andro, and 5 α -diol usually returned to baseline within 24 h, whereas Etio and 5 β -diol concentrations may be elevated for longer periods. The excretion of DHEA and its sulfate metabolites were also increased following single doses (Table I) (29). The T/E ratios were moderately and transiently increased for most subjects, and some subjects demonstrated increases of threefold (or more) in the ratio (29,32,34). Levesque and Ayotte (29) suggested that the variability in increases in the T/E ratios following administration of DHEA in men may be dependent upon their baseline T/E ratio. Considerable intersubject variability was a consistent trend in both single and multiple dose studies. Multiple dose studies demonstrated moderate and usually short-lived increases in DHEA, T, E, Andro, Etio, 5 α -diol, and 5 β -diol urinary concentrations. However, Setiawati et al. (26) reported a statistically significant increase in the ratio of DHEAS to DHEA-glucuronide at most time points.

Investigators continue to search for additional urinary indicators of exogenous DHEA use. 7 α and 7 β -OH-DHEA have been identified as endogenous DHEA metabolites, and their concentrations may be affected by exogenous DHEA use (29). However, they are not unique indicators of DHEA use and have been reported as metabolites of 7-Oxo-DHEA as well (36,37). Levesque and Ayotte (29) proposed using the ratio 7 β -OH-DHEA/16 α -OH-androsterone as an indicator of DHEA administration. 7 β -OH-DHEA and 16 α -OH-androsterone are recognized in WADA Technical Document TD2009EAAS as DHEA metabolites (15). However, the Technical Document does not provide instructions for how these metabolites should be considered, nor does it provide criteria with which they may be evaluated during screening as "presumptive" indicators of use. Although reference ranges for urinary concentrations of these

metabolites have been reported, similar ranges for their ratios have not (31). Recently the following “good candidate markers” of DHEA administration were proposed from a single 50-mg dose study ($n = 6$ males): DHEA/E, 16 α -OH-DHEA/E, 7 β -OH-DHEA/E, and 5 β -diol/5 α -diol (38).

Shackleton et al. (18) and others (39) have advocated the use of IRMS to distinguish administered from endogenous DHEA. These investigators demonstrated increased concentrations and reduced ^{13}C content of 5-androstene-3 β ,17 β -diol following administration of DHEA. No changes were observed in con-

centration or ^{13}C content of 5-androstene-3 β , 17 α -diol. However, both 5 α -diol and 5 β -diol showed diminished ^{13}C content. Researchers in Australia have also used IRMS to investigate potential AAS profile changes following DHEA use. These investigators reported an increase in 3 α ,5cyclo-5 α -androstane-6 β -ol-17-one (3 α ,5cyclo) concentration as a potential indicator of use (17). Results from administering a 100-mg dose of DHEA to a single volunteer showed increases in the urinary concentration of 3 α ,5cyclo for approximately 24 h and a peak change from baseline at 5 h postdose. In a parallel study, 100 mg of

Table II. Summary of Potential Markers for DHEA Use

Marker	Oral Dose (mg)	Dosing Frequency	<i>n</i>	Reference
Concentrations				
[DHEA (equivalent to glucuronide)]	NA	NA	NA	WADA (15)
[DHEA]	50	single administration	7	Dehennin et al. (27)
	50	7 days	3	Inthong et al. (34)
	50	single administration	1	Kazlauskas (24)
[Andro]	50	single administration	7	Dehennin et al. (27)
	200	single administration	3	Uralets and Gillette (32)
	200	single administration	4	Levesque and Ayotte (29)
	50	7 days	3	Inthong et al. (34)
[Etio]	50	single administration	7	Dehennin et al. (27)
	200	single administration	3	Uralets and Gillette (32)
	200	single administration	4	Levesque and Ayotte (29)
	50	7 days	3	Inthong et al. (34)
[5 α - 5 β - diols]	50	single administration	7	Dehennin et al. (27)
	200	single administration	3	Uralets and Gillette (32)
	50	7 days	3	Inthong et al. (34)
[3 α ,5cyclo-5 β -androstane-6 β -ol-17-one (3 α ,5cyclo)]	100	single administration	1	Cawley et al. (17)
Ratios				
DHEAS/DHEA glucuronide	50	5 days	13	Setiawati et al. (26)
DHEA/E	50	single administration	6	Van Renterghem et al. (38)
T/E	50/250	30 days/single	7/1	Bosy et al. (33)
	200	single administration	3	Uralets and Gillette (32)
	50	5 days	13	Setiawati et al. (26)
Andro/Etio	50	single administration	1	Kazlauskas (24)
	200	single administration	3	Uralets and Gillette (32)
	50	5 days	13	Setiawati et al. (26)
5 α -diol/5 β -diol or 5 β -diol/5 α -diol	50	single administration	1	Kazlauskas (24)
	200	single administration	3	Uralets and Gillette (32)
	50	single administration	6	Van Renterghem et al. (38)
7 β -OH-DHEA/E	50	single administration	6	Van Renterghem et al. (38)
7 β -OH-DHEA/16 α -OH-androsterone	200	single administration	4	Levesque and Ayotte (29)
16 α -OH-DHEA/E	50	single administration	6	Van Renterghem et al. (38)
Others				
increase in 7 α -OH and 7 β -OH DHEA	200	single administration	4	Levesque and Ayotte (29)
16 α -OH-DHEA, 16 α -OH-androsterone, and 16 α -OH-etiocholanolone suppression	200	single administration	4	Levesque and Ayotte (29)

DHEA administered twice/day for seven days resulted in an increase of the $3\alpha,5\text{cyclo}$ concentration for the duration of the study with a peak concentration at approximately $15\times$ the baseline. IRMS analysis demonstrated ^{13}C depletion in $3\alpha,5\text{cyclo}$ following single and multiple administrations. Based on reference ranges of $3\alpha,5\text{cyclo}$ generated from a population of 632 athletes, the authors proposed a screening cutoff of 140 ng/mL. However, in a later study, 19 doping control samples that contained $3\alpha,5\text{cyclo}$ concentrations > 140 ng/mL tested negative for exogenous DHEA use by IRMS (40). In addition, 6 of these 19 samples also had DHEA concentrations in excess of the WADA suggested threshold of 100 ng/mL. The utility of $3\alpha,5\text{cyclo}$ as a marker of DHEA use has been further questioned in a 2010 publication in which six healthy males were administered a single 50-mg dose of DHEA, and transient and inconsistent changes in $3\alpha,5\text{cyclo}$ were observed (41). A summary of potential markers of DHEA administration is shown in Table II.

Androstenedione (Adione)

Adione (androstenedione; 4-androstene-3,17-dione) is a product of DHEA metabolism and a precursor of T (Figure 1) (24,25). Adione is produced by the adrenal gland and gonads and metabolized to T by 17β -hydroxysteroid dehydrogenase. However, Adione may also be converted directly to estrone by the enzyme aromatase (42). Peripheral aromatization of Adione is greater than that of T and also occurs to a greater extent in males than females (42). Because Adione is a precursor of T, theoretically its use should increase the urinary excretion of T and its metabolites Andro, Etio, 5α -diol, and 5β -diol.

Numerous studies have examined urinary AAS profile changes following the administration of Adione. Van Eenoo and colleagues (43) studied the effects of oral administration of 25 and 50 mg of Adione on urinary T concentrations and subsequent T/E ratios of four male subjects (ages not reported). For data comparison, routine doping control samples from 377 male athletes were used to calculate "far outside limits" (calculated by adding three times the interquartile range to the 75th percentile) for urinary Adione concentrations as well as Adione/E ratios. Following administration, urinary Adione concentrations increased in a dose-dependent manner, and maximum concentrations were reached between 2 and 4 h postdose. The authors reported that the Adione concentrations were below the calculated far outside limit (23 ng/mL) for all subjects by 9 h postadministration. However, results from only two subjects were shown for each dose and only one subject's Adione concentrations exceeded the far outside limit at 6 h postadministration. Following the 50-mg dose, urinary T concentrations increased rapidly, but returned to predose levels by approximately 6 h postadministration (data from 2 subjects). Although the authors reported that Adione administration increased urinary concentrations of Andro, Etio, 11β -OH Andro, 11β -OH Etio, and DHT (which returned to preadministration values after approximately 9 h), far outside limits were not calculated for these compounds and the observed increases

were not statistically significant when compared to an alternate control population presented in the article. From their study, the authors concluded that urinary concentrations of Adione, as well as the ratios of Adione/E and T/E, could be used to detect use of single doses of 25 and 50 mg of Adione for approximately 9 h (43).

In 1999, Uralets and Gillette reported an excretion study designed to determine the effects of Adione administration on urinary AAS profiles. Six male subjects (ages not reported) were administered 50 mg of Adione (data shown for three subjects with varying predose T/E ratios [low (~ 0.1), normal (~ 1.2), and high (~ 4)]). Following Adione administration, the urinary concentrations of Andro and Etio increased in all subjects. T concentrations increased in the (non-Asian) subjects who had normal and high predose T/E ratios, reaching maxima of 6 and greater than 20, respectively. However, T concentrations were not changed in the third (Asian) subject who had a low predose T/E ratio. The T/E ratios of this subject actually decreased because of increased E concentrations. Concentrations of the urinary AAS returned to predose values within 24 h of the administration. From their study, the authors suggested that Adione use might be detected in routine AAS screening by abnormally high Andro and Etio concentrations. However, they also detected a hydroxylated metabolite of Adione that increased sharply following administration and suggested that this metabolite might also be a useful marker of Adione administration (44).

Levesque and Ayotte (16) evaluated the urinary AAS profiles of one female and three male subjects following oral dosing with 100 mg of Adione. Urine samples were collected for 24 h prior to and up to 48 h after the administration. Each subject's results were compared to their predose values and to reference ranges calculated from two databases. After dosing, urinary concentrations of T, and consequently, the T/E ratios increased rapidly for the female and two of the male subjects. However, these parameters were not changed significantly in the third male subject who had a low predose T/E (< 1). Although the authors stated that increased concentrations of Andro and/or Etio were the only parameters of the AAS profile that were altered for all subjects, the pattern of these changes varied by subject. The female subject's maximum Andro and Etio concentrations occurred between 4 and 9 h and at approximately 19 h postadministration, respectively. Additionally, the magnitude of her Etio concentration increase was much greater than that of Andro. In contrast, one male subject's urinary Andro and Etio concentrations both peaked at approximately 5 h postadministration and returned to baseline by about 10 h after administration. Although his peak concentrations of Andro exceeded that of Etio, the difference was not substantial. The second male subject's urinary concentrations of Etio did not increase until 4 h after administration. The concentrations then reached a plateau for several hours, and subsequently, increased substantially reaching a maximum between 14 and 24 h postadministration. This subject's urinary Andro concentrations increased after 4 h, returned to predose levels by 14 h, increased again between 14 and 24 h and returned to predose values within 24 h. The third male subject's urinary Andro and Etio concentrations increased sharply at

approximately 19 h postdose, reached a peak around 24 h, and returned to baseline by 29 h. There were unremarkable differences in this subject's Andro and Etio concentrations. In the same study, the investigators also identified 6 α -hydroxyandrostenedione (6 α -OH Adione), 6 β -hydroxyandrosterone (6 β -OH Andro), 6 β -hydroxyetiocholanolone (6 β -OH Etio), and 6 β -hydroxyepiandrosterone (6 β -OH Epiandro) as metabolites of Adione (16). The authors reported that, following a single 100-mg dose of Adione, 6 α -OH Adione could be detected in urine between 9 and 11 h postadministration and 6 β -OH Andro and 6 β -OH Etio for up to 13 h, whereas 6 β -OH Epiandro persisted for 24 h (data not shown) (16). From their studies, the authors concluded that T/E ratios were not always elevated following Adione administration. However, they suggested that elevated concentrations of Andro and Etio in combination with detection of the characteristic 6 β -hydroxy metabolites were diagnostic of Adione administration.

Goudrealt et al. (45) administered a single 100-mg dose of Adione to two male subjects (ages 23 and 30 years) and studied the resulting 6 α - and 6 β -hydroxylated Adione urinary metabolites. The concentrations of 6 α -OH Adione and the 6 β -hydroxy metabolites, 6 β -OH Andro, 6 β -OH Etio, and 6 β -OH Epiandro, were estimated in the glucuronide and sulfate fractions following the administration. The authors reported that 6 β -OH Andro, 6 β -OH Etio, and 6 β -OH Epiandro were present in higher concentrations and detectable for longer periods as sulfate versus glucuronide metabolites. Further, the investigators reported that 6 β -OH Epiandro was the only Adione metabolite that was detectable for more than 24 h (one of two subjects) (45). The authors concluded that 6 α -OH Adione and the 6 β -hydroxy metabolites were indicative of Adione administration and should be examined when abnormal AAS profiles are obtained during screening (45).

Catlin and colleagues, examined E, its putative precursor 5-androsten-3 β ,17 α -diol (E-pre) and metabolites 5 β -androst-3 α ,17 α -diol (EM-1) and 5 α -androst-3 α ,17 α -diol (EM-2) as potential markers of exogenous Adione use (46). Male subjects were randomly assigned to treatment groups (100 mg Adione $n = 13$; 300 mg Adione, $n = 11$; or control, $n = 13$) and administration occurred daily for 7 days. Urine samples were collected on the day prior to administration as well as on day 1 and 7 of treatment. Following administration of 100 or 300 mg of Adione per day for 7 days, mean E excretion rates increased three- and eightfold, respectively. Excretion rates for EM-1 and EM-2 were also significantly increased following both doses. However, the excretion of E-pre decreased significantly following the 300 mg dose. The ratios of EM-1/E-pre and EM-2/E-pre increased in a dose dependent manner following the administrations. However, the authors mentioned that if cutoffs of the pretreatment mean EM-1/E-pre and EM-2/E-pre ratios plus four standard deviations were established for suspicion of Adione use, only 27–42% of the postadministration urine samples in this study would have exceeded the respective cutoffs. The authors concluded that the EM-1/E-pre and EM-2/E-pre ratios might be used as potential markers of Adione administration, but acknowledged that the ratios lacked sensitivity. The authors also examined 6 α -OH Adione as a marker of Adione administration. 6 α -OH Adione was not detected in any

of the control group urine specimens, but was present in all urine specimens collected postadministration. Based on this finding, the authors suggested that 6 α -OH Adione could be used as a sensitive and specific marker of Adione administration (46).

In a 2001 study, Leder and colleagues (47) reported the effects of oral Adione administration on serum T glucuronide, and urinary excretion rates of T, Andro, Etio, and DHT. Male subjects (ages 20–40) were administered placebo, 100, or 300 mg of Adione ($n = 13$, $n = 13$, or $n = 11$, respectively) for 7 days. Urine samples were collected on the day prior to administration and on days 1 and 7 during administration. Marked, and dose-dependent, increases in the urinary excretion rates of free and glucuronide-conjugated T, Andro, Etio, and DHT were observed following dosing. However, there was considerable intersubject variability in T excretion. For example, baseline testosterone glucuronide excretion rates were lower in two Asian subjects receiving 300 mg/day Adione than all other subjects in the treatment group. Even though their T glucuronide excretion rates increased over baseline during Adione administration, their mean T glucuronide excretion rates were less than one-tenth of the mean excretion rate of the entire 300 mg-dosed group. Despite the intersubject variability, the authors concluded that administration of Adione increased urinary excretion rates of T-glucuronide, Andro, Etio, and DHT in a dose-dependent manner (47).

In one of the few studies that included female subjects, Bassindale et al. (48) administered a single 100-mg dose of Adione to three female subjects of ages 20, 23, and 25 years. Urine samples were collected at 24 h and 10 min prior to administration and in 2-h intervals for up to 12 h postadministration. Mean excretion rates for T and E increased following administration. The mean T/E ratios increased from approximately 1 predose to a maximum of 15 at 6 h postdose and, subsequently, returned to predose levels by 30 h postdose. The authors also examined the peak heights of 6 β -OH Andro, 6 β -OH Etio, and 6 β -OH Epiandro relative to an internal standard used in the analyses as an indication of concentration changes for these metabolites. An increased response was observed for each metabolite relative to the internal standard for approximately 12 h following administration. However, the authors suggested that these metabolites required further examination before they could be considered diagnostic of Adione administration. The authors concluded that using changes in the urinary T/E ratio as a marker of Adione administration had a limited detection window of approximately 6 h and, therefore, was of limited utility for routine detection of use during screening (48).

Brown et al. (49) studied changes in the urinary AAS profiles of 20 male subjects (ages 30–39) who were either administered placebo ($n = 10$) or 100-mg doses of Adione ($n = 10$) orally three times per day for 28 days. One subject in the Adione treatment group was Asian, and all other subjects were Caucasian. Urine samples were collected on day 0 and day 28 approximately 8–10 h after the last dose. Following administration, urinary concentrations of T glucuronide, E, Andro, and Etio were increased by 839, 256, 925, and 567%, respectively, in the Caucasian subjects' AAS profiles. In general, the

T/E ratios were not significantly increased. However, one Caucasian subject's T/E ratio increased to 17. In contrast, the Asian subject's T/E ratio decreased. His increase in E excretion was approximately twofold greater than that of the Caucasian subjects, whereas his increase in T excretion was approximately one-fourth that of the Caucasian subjects. As a consequence of the intersubject variability, the authors concluded that Adione administration did not always increase T/E and alternate marker(s) of exogenous use should be explored (49).

Cawley et al. (50) acknowledged the utility of using 6-hydroxylated metabolites of Adione as markers of exogenous administration in routine GC-MS screening. However, they suggested that the low urinary concentrations of the 6-hydroxylated metabolites limited their use to confirm Adione use by methods such as GC-IRMS. Alternatively, the authors proposed using 4-hydroxyandrostenedione (4-OH Adione) as a marker of exogenous use because its urinary concentrations were sufficient to be detected during both screening and confirmation analyses. Their conclusions were based on the results of single (100 mg) and multiple dose studies (200 mg/day for 3 days) using two male subjects (ages 21 and 30). Results were compared to reference ranges established from a population of male athletes from Australia ($n = 74$), New Zealand ($n = 76$),

and Malaysia ($n = 50$). From this population, the interquartile range for 4-OH Adione was between 1 and 7 ng/mL and the upper limit was determined to be 37 ng/mL. Consequently, the authors proposed a GC-MS screening cutoff of 40 ng/mL for 4-OH-Adione. Preadministration 4-OH Adione concentrations for the study subjects were between 20 and 30 ng/mL. Following Adione administration, the subjects' 4-OH Adione urine concentrations exceeded the upper reference limit of 37 ng/mL for 12 to 24 h. However, the authors acknowledged that during the administration, the concentrations of 4-OH Adione sometimes precluded GC-IRMS confirmation (50).

Research continues in the challenge to identify robust urinary markers of exogenous Adione use because currently there is no consensus in the literature as to which changes in the urinary AAS profile may be diagnostic. A summary of potential markers of Adione administration are shown in Table III. Some current studies were inconclusive because the reported AAS profile changes after Adione administrations were insignificant relative to population-based or predose ratios or concentrations. Although limited data are available, potential differences in Adione metabolism and its urinary AAS excretion profile based on ethnicity were observed. Extrapolation from some existing Adione administration studies should be done

Table III. Summary of Potential Markers for Adione Use

Marker	Route of Administration	Dose (mg)	Dosing Frequency	<i>n</i>	Reference
Concentrations					
[Adione]	oral	25 and 50	single administration	4	Van Eenoo et al. (43)
[Andro]	oral	50	single administration	6	Uralets and Gillette (44)
	oral	100	single administration	4	Levesque and Ayotte (16)
[Etio]	oral	50	single administration	6	Uralets and Gillette (44)
	oral	100	single administration	4	Levesque and Ayotte (16)
Ratios					
Adione/E	oral	25 and 50	single administration	4	Van Eenoo et al. (43)
T/E	oral	25 and 50	single administration	4	Van Eenoo et al. (43)
EM-1/E-pre	oral	100 and 300	7 days	24	Catlin et al. (46)
EM-2/E-pre	oral	100 and 300	7 days	24	Catlin et al. (46)
Others					
6 α -OH Androstenedione	oral	100	single administration	4	Levesque and Ayotte (16)
	oral	100	single administration	2	Goudrealt et al. (45)
	oral	100 and 300	7 days	24	Catlin et al. (46)
6 β -OH Androsterone	oral	100	single administration	4	Levesque and Ayotte (16)
	oral	100	single administration	2	Goudrealt et al. (45)
6 β -OH Etiocholanolone	oral	100	single administration	4	Levesque and Ayotte (16)
	oral	100	single administration	2	Goudrealt et al. (45)
6 β -OH Epiandrosterone	oral	100	single administration	4	Levesque and Ayotte (16)
	oral	100	single administration	2	Goudrealt et al. (45)
4-OH Androstenedione	oral	100	single administration	2	Cawley et al. (50)
	oral	200	3 days	2	Cawley et al. (50)

cautiously because they included a small number of subjects (16,43–45,48), and few have included female subjects. Trends from the Adione literature presented include

1. The urinary T/E ratios increased in some Caucasian men after administration. However, the T/E ratios did not always increase in Caucasians and decreased in Asian subjects (44,47,49).
2. Urinary Andro and Etio concentrations increased, but there was no consensus cutoff diagnostic of use.
3. 6-Hydroxylated metabolites including 6 α -OH Adione, 6 β -OH Andro, 6 β -OH Etio, and 6 β -OH Epiandro have been suggested as characteristic metabolites of Adione (16). Also, reports have indicated that 6 α -OH Adione was not detected in preadministration or control group urine samples (46). Further reference ranges for 6-hydroxylated metabolites of Adione were recently reported (Tables IV and V) (31). However, of the samples used to establish the reference ranges many contained detectable 6 β -OH metabolites and only approximately 2% contained 6 α -OH Adione above the limit of quantitation of the methods used for detection.
4. Recently, screening concentrations of 4-OH Adione above 40 ng/mL have been proposed as indicative of Adione administration (50). Further, Cawley and colleagues (50) suggested that 4-OH Adione was better suited for confir-

mation by GC–IRMS than are the 6-hydroxylated metabolites because 4-OH Adione is normally present in higher concentrations. However, 4-OH Adione (an aromatase inhibitor) may be found in nutritional supplements and may not be a specific marker of Adione administration.

Dihydrotestosterone (DHT)

5 α -Dihydrotestosterone (DHT) is a biologically active metabolite of T resulting from the irreversible 5 α -reduction of T by 5 α -reductase. DHT has greater affinity for the androgen receptor than T and, therefore, is considered a more potent androgen (51). Unlike T, DHT does not undergo aromatization, thus supraphysiological doses are not predicted to produce the undesirable “estrogenic” side effects associated with T abuse. However, like T, supraphysiological DHT doses inhibit the release of luteinizing hormone (LH) (52), resulting in suppression of the hypothalamic-pituitary-gonadal axis (HPGA). As a consequence of this suppression, T production and urinary excretion of both T and E may be reduced (53), leading investigators to predict that DHT administration will not alter T/E (54,55).

The metabolism of DHT is shown in Figure 1 (24,25). Reduction of DHT by 3 α -hydroxysteroid dehydrogenase (3 α -HSD) produces a 5 α -androstane-3 α ,17 β -diol (5 α -diol) metabolite whereas reduction by 3 β -hydroxysteroid dehydrogenase (3 β -HSD) produces a 5 α -androstane-3 β ,17 β -diol (5 α 3 β -diol) (56). The rate of DHT reduction by 3 α -HSD in the liver is approximately 3 times that of 3 β -HSD (56). In vitro studies suggest the major metabolic pathway for DHT is reduction by 3 α -HSD to 5 α -diol and subsequent glucuronidation (56).

Southan and colleagues (54) assessed the effects of intramuscular administration of 150 mg of DHT heptanoate on the urinary AAS profile of two male subjects (ages 28 and 65). Their findings were compared to subject predose values and “normal” urinary AAS ranges established from a population of 36 athletes (data not reported). Urinary excretion rates of T were suppressed relative to subject predose values for at least 15 days following administration. As predicted, T/E ratios were not elevated following administration. However, the authors reported that the urinary ratios of 5 α -diol or 5 α 3 β -diol to T, E, LH, or 5 β -diol increased following administration (data for all ratios not shown). The ratios of DHT/E, DHT/5 β -diol, 5 α -diol/T, 5 α -diol/E, and 5 α 3 β -diol/T were elevated for both subjects for approximately 10 days. From their findings,

Table IV. Reference Ranges of Concentrations and Ratios of Urinary Steroids in Men (*n* = 2027)*

Compound	% Results Above LOQ	Median (ng/mL)	Mean (ng/mL)	Upper RL (%)	Upper RL (ng/mL)	Upper Limit 95% CI (ng/mL)
Adione	25.4	6.90	8.19	97.5	22.0	17.5–28.3
6 α -OH-Adione	2.37	4.86	5.60	97.5	18.3	8.03–19.01
6 β -OH-Andro	41.9	8.29	9.52	97.5	20.6	19.5–22.1
6 β -OH-Etio	75	22.7	29.6	97.5	90.1	82.3–99.6
Adione/E	24.8	0.24	0.34	97.5	1.09	0.89–1.63

* Doping control urines, ~99.5% Caucasian. Data taken from Van Renterghem et al. (31).

Table V. Reference Ranges of Concentrations and Ratios of Urinary Steroids in Women (*n* = 1004)*

Compound	% Results Above LOQ	Median (ng/mL)	Mean (ng/mL)	Upper RL (%)	Upper RL (ng/mL)	Upper Limit 95% CI (ng/mL)
Adione	19.9	7.18	8.31	97.5	19.7	16.0–25.6
6 α -OH-Adione	2.00	5.27	5.28			
6 β -OH-Andro	22.1	7.91	10.8	97.5	19.3	16.5–32.6
6 β -OH-Etio	71.9	20.8	27	97.5	92.9	74.1–108.6
Adione/E	13.9	0.60	0.76	97.5	1.68	1.43–2.23

* Doping control urines, ~99.5% Caucasian. Data taken from Van Renterghem et al. (31).

the authors suggested that monitoring multiple AAS ratios such as those listed might be beneficial in detecting DHT abuse (54).

Donike and colleagues (57) examined the metabolism of DHT after the oral administration of 10 mg of deuterium-labeled drug to a single male subject (age 43). Additionally, they studied changes in the urinary AAS profile following sublingual administration of 25 mg DHT to four subjects (ages 33 to 43). Following hydrolysis with β -glucuronidase isolated from *E. coli*, approximately 44% (7.2% DHT, 33.2% Andro, 2.5% 5 α -diol, and 0.9% 5 α 3 β -diol) of the deuterium labeled DHT was recovered within 24 h. When samples were hydrolyzed with *Helix pomatia*, which includes both β -glucuronidase and aryl-sulfatase activity, recovery was increased to approximately 48% of the dose. From these data, the authors concluded that sulfation was a minor metabolic pathway for DHT and most of its metabolites. However, following hydrolysis with *Helix pomatia* the percent of the 5 α 3 β -diol metabolite recovered was increased fivefold from 0.04% to 2.0% (57). The investigators reported that sublingual administration of 25 mg DHT increased the urinary concentrations of DHT, Andro, 5 α -diol, and 5 α 3 β -diol as well as their ratios to Etio, 5 β -diol, and E for up to 48 h (data not shown for all ratios). Of the potential markers investigated, urinary DHT concentrations showed the greatest changes increasing as much as 150 times over predose levels. The authors suggested that DHT, 5 α -diol, and 5 α 3 β -diol concentrations as well as the ratios DHT/E, DHT/Etio, 5 α -diol/5 β -diol, 5 α 3 β -diol/5 β -diol, and Andro/Etio could be used as indicators of DHT administration. They concluded that the most significant indicators of use were increases in DHT concentration and the changes in the ratios of 5 α -diol/5 β -diol and Andro/Etio (57).

Geyer et al. (58) performed a study to identify potential urinary markers of sublingual DHT administration and to evaluate the utility of using subject-based versus population-based reference ranges to detect AAS profile changes after DHT use. Subject-based reference ranges were calculated from samples collected during the 24-h period prior to dosing. The upper limit of the subject's reference range for each parameter was defined as the parameter mean plus three standard deviations. The population-based AAS reference ranges were calculated from 4631 doping control samples collected from male athletes. Population-based 95% reference limits were calculated from the data using the non-parametric method of the REFVAL program and previously published (57). In the study, four volunteers were given 25 mg of DHT and their urine samples were collected for 90 h after dosing. Following the drug administration, DHT, Andro, 5 α -diol, 5 α 3 β -diol, and Epiandrostosterone concentrations increased and peaked between 2 and 4 h postdose. Urinary Etio, 5 β -diol, and E concentrations were not changed. The ratios of DHT/Etio and DHT/E were most affected by the administration and the Andro/Etio ratios were the least influenced. Despite this observation, the authors suggested that it was important to monitor the Andro/Etio ratio because it had previously been reported as one of the most stable parameters of the urinary AAS profile (58–60). The advantages of subject-based versus population-based reference ranges to detect DHT doping were demonstrated by changes in

the ratios of Andro/Etio, 5 α -diol/5 β -diol and DHT/Etio that were identified for longer periods when compared to the subject-based limits. Further, the subject's 5 α -diol/5 β -diol ratio was greater than the population-based reference limit for approximately 20 h, but exceeded his subject-based limit for approximately 40 h (58).

Kicman et al. (55) administered 125 mg of DHT percutaneously, twice daily for 4 days to 10 male subjects (ages 23–32). The subject's urinary AAS excretion rates were compared to their own predose values obtained from a 0–12 h collection that occurred 24 h prior to dosing. Additionally, potential markers of DHT use were compared to AAS “discrimination limits” calculated from single and untimed urine specimens collected from 120 healthy males of varied ethnic origins (55). Discrimination limits for 5 α -diol/E, 5 α -diol/5 β -diol, and 5 α -diol/LH were calculated as the mean plus 3 times the standard deviation (for data sets that transformed to Gaussian distribution), or as twice the far-outside limit for other ratios from those data sets that could not be transformed to Gaussian distribution. Following DHT administration, the mean urinary excretion rates of DHT and 5 α -diol increased to a maximum of approximately 5 times their predose values. Mean urinary excretion rates of T, E, and LH decreased, but did not differ significantly from their predose rates. However, when individual subject excretion rates were compared to their respective predose rates, the decreases were significant and both T and E concentrations fell to approximately 50% of their respective predose rates. Mean ratios of 5 α -diol/E, DHT/E, and 5 α -diol/LH exceeded the discrimination limits after the third day of dosing. At 40 h after the last dose, the 5 α -diol/E ratios remained above the calculated discrimination limits. However, the DHT/E and 5 α -diol/LH ratios decreased below their calculated discrimination limits, but exceed the subject-based predose values. The authors suggested that 5 α -diol/E, DHT/E, and 5 α -diol/LH ratios might be useful urinary markers of percutaneous DHT administration and recommended DHT/E be used as the principle marker. Although the subjects' 5 α -diol/5 β -diol ratios did not exceed the discrimination limits, the authors suggested that this ratio might also be a useful marker of DHT use in part because they hypothesized that when abused, doses of DHT would likely exceed the “modest” dose administered in their study. Andro/Etio ratios were increased after four days of administration, but the authors did not recommend use of this ratio as a marker of short-term percutaneous DHT administration because they considered it insensitive. However, they suggested that Andro/Etio ratio might be a useful marker for other routes of administration. Because of large variability in T excretion, the 5 α -diol/T ratio was not proposed for use as a marker of DHT administration. The usefulness of the 5 α 3 β -diol metabolite of DHT was not examined in this study (55).

Coutts and colleagues (61) evaluated whether the markers (DHT/E, 5 α -diol/E, 5 α -diol/LH, and 5 α -diol/5 β -diol) proposed by Kicman et al. (55) for assessing percutaneous DHT administration could also be used to detect intramuscular (IM) administration (61). Six male subjects were administered a single 250-mg IM injection of DHT heptanoate. The subjects' urinary AAS profile results for the selected ratios were compared to the same reference group discrimination limits used by

Table VI. Reference Ranges of Concentrations and Ratios of Urinary Steroids Used as Markers for DHT Administration in Men

Parameter	N	Lower RL (%)	Lower RL (ng/mL)	Lower Limit 90% CI (ng/mL)	Upper RL (%)	Upper RL (ng/mL)	Upper Limit 90% CI (ng/mL)	Upper Limit 95% CI (ng/mL)	Nonparametric 95% Reference Range	Population Type	Reference
[DHT]	388	2.5	0.50	0.36–0.55	97.5	21.75	18.73–26.14			Doping control urines, Asian games 1990	57
	342	2.5	0.45	0.20–0.70	97.5	26.03	21.06–28.78			Doping control urines, Asian games 1994*	57
	4631	2.5	0.23	0.21–0.24	97.5	20.55	10.17–22.45			Doping control urines, 1994 Cologne, mostly Caucasian	57
	2027				97.5	21.5		15.2–26.1		Doping control urines, ~99.5% Caucasian	31
[5 α -diol]	391	2.5	9.0	6.0–12	97.5	282.00	225–398			Doping control urines, Asian games 1990	57
	106	2.5	5.36		97.5	102.57	–			Doping control urines, Asian games 1994*	57
	4631	2.5	17.44	16.69–18.17	97.5	203.87	196.78–210.88			Doping control urines, 1994 Cologne, mostly Caucasian	57
	5101	2.5	13.85	13.15–14.72	97.5	166.5	161.4–173.3		13.85–166.5	Doping control urines	58
	2027				97.5	155		143.5–169		Doping control urines, ~99.5% Caucasian	31
[Andro]	391	2.5	580	372–697	97.5	7525.40	7003–8528			Doping control urines, Asian games 1990	57
	344	2.5	314.11	195.40–391.23	97.5	5631.51	4744–6750			Doping control urines, Asian games 1994*	57
	4631	2.5	961.88	934.01–1015.02	97.5	9103.04	8602.37–9503.68			Doping control urines, 1994 Cologne, mostly Caucasian	57
	5101	2.5	867.2	834.5–894.3	97.5	6703	6533–6881		867.2–6703	Doping control urines	58
	482	2.5	766.4	690.3–857.2	97.5	6673	5475.8–6758.7		766.4–6673	Amateur cyclists	57
	2027				97.5	6700		6390–6860		Doping control urines, ~99.5% Caucasian	31
DHT/Etio [†]	388	2.5	0.24	0.19–0.36	97.5	10.81	8.76–17.04			Doping control urines, Asian games 1990	57
	343	2.5	0.85	0.48–1.05	97.5	28.62	23.74–32.96			Doping control urines, Asian games 1994*	57
	4631	2.5	0.10	0.09–0.10	97.5	8.22	7.73–8.81			Doping control urines, 1994 Cologne, mostly Caucasian	57
DHT/E	387	2.5	0.02	0.01–0.02	97.5	0.63	0.45–0.85			Doping control urines, Asian games 1990	57
	342	2.5	0.05	0.03–0.06	97.5	1.63	1.27–2.45			Doping control urines, Asian games 1994*	57
	4559	2.5	0.01	0.01–0.01	97.5	0.73	0.69–0.79			Doping control urines, 1994 Cologne, mostly Caucasian	57
	2027				97.5	1.03		0.82–1.47		Doping control urines, ~99.5% Caucasian	31
	2027				99	2.53		1.88–3.59		Doping control urines, ~99.5% Caucasian	31

* No Chinese athletes.

[†] Multiplied by 1000.

Table VI (continued). Reference Ranges of Concentrations and Ratios of Urinary Steroids Used as Markers for DHT Administration in Men

Parameter	N	Lower RL (%)	Lower RL (ng/mL)	Lower Limit 90% CI (ng/mL)	Upper RL (%)	Upper RL (ng/mL)	Upper Limit 90% CI (ng/mL)	Upper Limit 95% CI (ng/mL)	Nonparametric 95% Reference Range	Population Type	Reference
And/Etio	391	2.5	0.57	0.46–0.65	97.5	2.94	2.79–3.37			Doping control urines, Asian games 1990	57
	344	2.5	0.67	0.54–0.70	97.5	2.85	2.53–3.41			Doping control urines, Asian games 1994*	57
	4631	2.5	0.52	0.50–0.54	97.5	2.86	2.82–2.96			Doping control urines, 1994 Cologne, mostly Caucasian	57
	5283	2.5	0.55	0.53–0.57	97.5	2.869	2.81–2.93			Doping control urines	58
	482	2.5	0.45	0.41–0.51	97.5	2.38	2.23–2.67			Amateur cyclists	57
	2027				97.5	3.64		3.38–3.75		Doping control urines, ~99.5% Caucasian	31
	194	2.5	0.77	0.67–0.93	97.5	3.78	3.12–4.41			Chinese athletes	62
	630								0.504–3.302	Cuban athletes	63
5 α -diol/ 5 β -diol	391	2.5	0.17	0.15–0.19	97.5	1.84	1.56–2.49			Doping control urines, Asian games 1990	57
	344	2.5	0.22	0.16–0.26	97.5	2.58	2.43–3.70			Doping control urines, Asian games 1994*	57
	4631	2.5	0.12	0.12–0.13	97.5	1.53	1.48–1.57			Doping control urines, 1994 Cologne, mostly Caucasian	57
	5283	2.5	0.11	0.11–0.12	97.5	1.56	1.51–1.61			Doping control urines	58
	2027				97.5	1.69		1.55–1.88		Doping control urines, ~99.5% Caucasian	31
	194	2.5	0.26	0.12–0.34	97.5	1.58	1.36–2.00			Chinese athletes	62

* No Chinese athletes.

Table VII. Reference Ranges of Concentrations and Ratios of Urinary Steroids Used as Markers for DHT Administration in Women

Parameter	N	Lower RL (%)	Lower RL (ng/mL)	Lower Limit 90% CI (ng/mL)	Upper RL (%)	Upper RL (ng/mL)	Upper Limit 90% CI (ng/mL)	Upper Limit 95% CI (ng/mL)	Nonparametric 95% Reference Range	Population Type	Reference
[DHT]	208	2.5	0.23	0.13–0.28	97.5	8.33	5.88–11.67			Doping control urines, Asian games 1990	57
	155	2.5	0.37	0.04–0.56	97.5	12.13	9.63–17.03			Doping control urines, Asian games 1994*	57
	1341	2.5	0.17	0.14–0.20	97.5	18.43	16.51–23.77			Doping control urines, 1994 Cologne, mostly Caucasian	57
	1004				97.5	20.5		15.1–21.1		Doping control urines, ~99.5% Caucasian	31
[5 α -diol]	208	2.5	4.00	2.00–5.00	97.5	107.20	87.00–127.00			Doping control urines, Asian games 1990	57
	59	2.5	1.49		97.5	58.46				Doping control urines, Asian games 1994*	57

* No Chinese athletes.

Table VII (continued). Reference Ranges of Concentrations and Ratios of Urinary Steroids Used as Markers for DHT Administration in Women

Parameter	N	Lower RL (%)	Lower RL (ng/mL)	Lower Limit 90% CI (ng/mL)	Upper RL (%)	Upper RL (ng/mL)	Upper Limit 90% CI (ng/mL)	Upper Limit 95% CI (ng/mL)	Nonparametric 95% Reference Range	Population Type	Reference
[5 α -diol]	1340	2.5	6.37	5.98–6.78	97.5	88.67	79.91–96.95			Doping control urines, 1994 Cologne, mostly Caucasian	57
	1694	2.5	4.74	4.12–5.14	97.5	91	83.1–98.3		4.74–91	Doping control urines	58
	1004				97.5	69.7		56.5–80.9		Doping control urines, ~99.5% Caucasian	31
[Andro]	208	2.5	508.95	423.00–680.00	97.5	6757.45	6100.00–10289.00			Doping control urines, Asian games 1990	57
	156	2.5	306.38	209.30–387.52	97.5	3397.97	2812.12–5298.33			Doping control urines, Asian games 1994*	57
	1340	2.5	466.51	435.62–508.00	97.5	7562.08	6947.09–8134.88			Doping control urines, 1994 Cologne, mostly Caucasian	57
	1694	2.5	404.1	368.8–458.4	97.5	6439	6135–6964		404.1–6439	Doping control urines	58
	1004				97.5	5920		4960–6610		Doping control urines, ~99.5% Caucasian	31
DHT/Etio [†]	207	2.5	0.088	0.045–0.11	97.5	3.98	3.45–5.25			Doping control urines, Asian games 1990	57
	156	2.5	0.47	0.12–0.77	97.5	10.76	9.16–15.31			Doping control urines, Asian games 1994*	57
	1341	2.5	0.08	0.07–0.10	97.5	8.54	7.65–10.04			Doping control urines, 1994 Cologne, mostly Caucasian	57
DHT/E	208	2.5	0.012	0.009–0.014	97.5	0.71	0.52–0.81			Doping control urines, Asian games 1990	57
	156	2.5	0.08	0.05–0.10	97.5	2.72	1.92–5.40			Doping control urines, Asian games 1994*	57
	1340	2.5	0.02	0.02–0.02	97.5	2.27	2.03–2.56			Doping control urines, 1994 Cologne, mostly Caucasian	57
Andro/Etio	208	2.5	0.36	0.28–0.45	97.5	2.44	2.06–2.95			Doping control urines, Asian games 1990	57
	156	2.5	0.41	0.19–0.48	97.5	2.20	2.02–2.58			Doping control urines, Asian games 1994*	57
	1341	2.5	0.39	0.35–0.40	97.5	2.14	2.05–2.30			Doping control urines, 1994 Cologne, mostly Caucasian	57
	1742	2.5	0.42	0.39–0.44	97.5	2.15	2.03–2.22			Doping control urines	58
	1004				97.5	2.70		2.38–2.83		Doping control urines, ~99.5% Caucasian	31
	172	2.5	0.37	0.30–0.48	97.5	2.08	1.89–3.27			Chinese athletes	62
	259								0.425–2.236	Cuban athletes	63
5 α -diol/ 5 β -diol	207	2.5	0.12	0.07–0.17	97.5	2.04	1.58–5.63			Doping control urines, Asian games 1990	57
	155	2.5	0.21	0.06–0.24	97.5	1.88	1.73–2.92			Doping control urines, Asian games 1994*	57

* No Chinese athletes.

[†] Multiplied by 1000.

Table VII (continued). Reference Ranges of Concentrations and Ratios of Urinary Steroids Used as Markers for DHT Administration in Women

Parameter	N	Lower RL (%)	Lower RL (ng/mL)	Lower Limit 90% CI (ng/mL)	Upper RL (%)	Upper RL (ng/mL)	Upper Limit 90% CI (ng/mL)	Upper Limit 95% CI (ng/mL)	Nonparametric 95% Reference Range	Population Type	Reference
5 α -diol/5 β -diol	1340	2.5	0.10	0.09–0.10	97.5	1.25	1.20–1.33			Doping control urines, 1994 Cologne, mostly Caucasian	57
	1742	2.5	0.07	0.07–0.08	97.5	1.24	1.16–1.28			Doping control urines	58
	1004				97.5	1.33		1.09–1.45		Doping control urines, ~99.5% Caucasian	31
	172	2.5	0.08	0.07–0.10	97.5	1.22	1.12–1.44			Chinese athletes	62

Table VIII. Markers of DHT Administration and “Positive” Male Athletes from the 1994 Asian Games*

Date	cDHT (ng/mL)	5 α -diol/5 β -diol	Andro/Etio	DHT/Etio [†]	DHT/E	Athlete
97.5% Ref Limit	26.0	2.58	2.85	28.62	1.63	
10/3/94	83.7	49.46	3.56	59.26	14.23	1
10/7/94	86.0	70.51	5.52	75.98	22.43	1
10/8/94	95.5	41.94	4.21	97.29	36.82	1
9/30/94	2.5	0.43	1.26	2.62	3.21	2
10/4/94	75.9	56.12	3.54	77.09	30.78	2
10/10/94	38.6	17.34	2.98	60.35	9.32	3
10/9/94	36.2	53.37	4.62	53.69	8.97	4
10/7/94	32.8	65.21	5.32	77.73	24.15	5
10/4/94	34.7	131.26	3.97	36.32	N.A.	6

* Data from Donike et al. (57).
[†] Multiplied by 1000.

Table IX. Markers of DHT Administration and “Positive” Female Athletes from the 1994 Asian Games*

Date	cDHT (ng/mL)	5 α -diol/5 β -diol	Andro/Etio	DHT/Etio [†]	DHT/E	Athlete
97.5% Ref Limit	12.13	1.88	2.20	10.76	2.72	
9/30/94	7.15	1.04	0.92	5.58	3.70	1
10/5/94	388.67	56.61	5.70	252.83	83.14	1
9/30/94	77.40	10.62	1.99	39.63	29.07	2
10/4/94	89.54	12.65	1.99	41.93	24.77	2
10/7/94	47.93	17.75	2.26	26.91	17.43	2
10/8/94	60.73	10.21	1.92	31.69	13.22	2
10/14/94	18.63	14.02	2.53	18.91	4.73	3
10/14/94	16.38	67.88	2.91	20.46	9.38	4
9/30/94	3.15	12.81	1.11	1.13	NR [‡]	5
10/5/94	28.70	62.45	2.52	18.80	6.42	5
10/7/94	15.68	70.52	2.51	13.32	7.80	5

* Data from Donike et al. (57).
[†] Multiplied by 1000.
[‡] Not reported.

Kicman et al. (55). Although there was considerable intersubject variation in the data, the DHT/E, 5 α -diol/E, 5 α -diol/LH, and 5 α -diol/5 β -diol ratios increased following administration and the subjects' group mean of each ratio (with the exception of 5 α -diol/5 β -diol) exceeded the discrimination limits for approximately 10 days postadministration. The 5 α -diol/5 β -diol ratio was the least sensitive marker, but also was variable. Two subject's 5 α -diol/5 β -diol ratios did not exceed the discrimination limit which the authors attributed to high predose 5 β -diol concentrations for these subjects. However, for another subject, the 5 α -diol/5 β -diol ratio was the only marker that exceeded discrimination limits. In contrast to Kicman and co-workers' (55) percutaneous administration study results, the T/E ratios for five of the six subjects were approximately twice their predose values by three days postadministration. However, the subjects' group-mean changes in T/E ratios were not significant. From their work, the authors concluded that the proposed marker ratios (DHT/E, 5 α -diol/E, 5 α -diol/LH, and 5 α -diol/5 β -diol) were suitable for detection of IM doping with DHT esters (61).

Although there have been anecdotal reports of DHT use by athletes, the only well-documented case investigation was that of a group of Chinese athletes at the 1994 Asian Games (57). Urine samples collected from some of the Chinese athletes during the games contained very high Andro concentrations. A further investigation of these samples revealed that they also had elevated concentrations of DHT and 5 α -diol. To assist in determining

whether these findings could be attributed to DHT administration, three different sets of reference ranges for potential markers of DHT use were calculated (Tables VI and VII). Ultimately, the criteria used to determine if the athletes were doping, were based on the 97.5% upper reference limits of ranges calculated from samples collected during the 1994 Asian Games (57). These data were chosen because they minimized interlaboratory analytical variability and potential ethnic differences. Using the 97.5% upper reference limits (Tables VIII and IX), both male and female athletes were confronted with DHT use. The following data from athletes considered "positive" for DHT doping demonstrate the variability of their AAS parameters.

All parameters considered indicative of DHT doping were drastically increased in male athlete #2 when his out of competition results (9/30/1994) were compared to his in-competition results (10/4/1994). Similar conclusions were made for female athlete #1 when her out-of versus in-competition results were compared (Table IX).

For female athlete #5, all reported out of competition values (9/30/1994) were within the 97.5% reference limits with ex-

ception of 5 α -diol/5 β -diol that was approximately 6 times the reference limit (Table IX). In contrast, female athlete #2, who was later suspected of doping with DHT out of competition, had an Andro/Etio ratio within the 97.5% reference limits in 3 of 4 samples, but values of all other markers exceeded the 97.5% reference limits in all of her samples.

For both male and female athletes, the Andro/Etio ratio was the least responsive to DHT administration (Tables VIII and IX).

Donike and colleagues (57) concluded that the most significant indicators of DHT administration in this cohort of athletes were 1. DHT concentration after correction for specific gravity; 2. 5 α -diol/5 β -diol; and 3. Andro/Etio (57). Although the Andro/Etio ratios appeared to be the least sensitive marker of use, a comparison of data in Tables VI and VII, suggests that the Andro/Etio ratios were the least influenced by interlaboratory analytical variation.

Similar to the DHEA and Adione literature, well-designed and controlled studies of the effects of DHT administration on the urinary AAS profile are limited. Most DHT studies have included a small number of subjects making the results suggestive, easily over-interpreted and at risk for over extrapolation.

Table X. Summary of Potential Markers for DHT Use

Marker	Route of Administration	Dose (mg)	Dosing Frequency	n	Reference
Concentrations					
[DHT]	Sublingual	25	single administration	4	Donike et al. (57)
[5 α -diol]	Sublingual	25	single administration	4	Donike et al. (57)
[5 α 3 β -diol]	Sublingual	25	single administration	4	Donike et al. (57)
Ratios					
DHT/E	Intramuscular injection*	150	single administration	2	Southan et al. (54)
	Intramuscular injection*	250	single administration	6	Coutts et al. (61)
	Sublingual	25	single administration	4	Donike et al. (57)
	Sublingual	25	single administration	4	Geyer et al. (58)
	Percutaneous	125	2x/day for 4 days	10	Kicman et al. (55)
	Percutaneous	250	single administration	6	Van Renterghem et al. (38)
DHT/5 β -diol	Intramuscular injection*	150	single administration	2	Southan et al. (54)
	Percutaneous	250	single administration	6	Van Renterghem et al. (38)
DHT/Etio	Sublingual	25	single administration	4	Donike et al. (57)
	Sublingual	25	single administration	4	Geyer et al. (58)
5 α -diol/T	Intramuscular injection*	150	single administration	2	Southan et al. (54)
5 α -diol/E	Intramuscular injection*	150	single administration	2	Southan et al. (54)
	Intramuscular injection*	250	single administration	6	Coutts et al. (61)
	Percutaneous	125	2x/day for 4 days	10	Kicman et al. (55)
5 α -diol/5 β -diol	Sublingual	25	single administration	4	Donike et al. (57)
	Intramuscular injection*	250	single administration	6	Coutts et al. (61)
	Percutaneous	250	single administration	6	Van Renterghem et al. (38)
5 α -diol/LH	Percutaneous	125	2x/day for 4 days	10	Kicman et al. (55)
5 α 3 β -diol/T	Intramuscular injection*	250	single administration	6	Coutts et al. (61)
	Intramuscular injection*	150	single administration	2	Southan et al. (54)
5 α 3 β -diol/5 β -diol	Sublingual	25	single administration	4	Donike et al. (57)
Andro/Etio	Sublingual	25	single administration	4	Donike et al. (57)
	Sublingual	25	single administration	4	Geyer et al. (58)

* DHT heptanoate.

tion. Additionally, the subject's urinary AAS profiles showed considerable intersubject variability even when the same route of DHT administration was used. Numerous markers including concentrations ([DHT], [Andro], [5 α -diol], [5 α 3 β diol]) and ratios (DHT/E, DHT/Etio, DHT/5 β -diol, 5 α -diol/T, 5 α -diol/E, 5 α -diol/5 β -diol, 5 α -diol/LH, 5 α 3 β diol/T, 5 α 3 β diol/5 β -diol, and Andro/Etio) have been proposed for detecting DHT use. Although there does not appear to be a consensus in the literature or substantiation of the most diagnostic or predictive marker(s) of DHT use. However, in a 2010 study ($n = 6$, 250 mg dermal application of DHT) in which numerous ($n = 576$) potential indicators of DHT use were evaluated, DHT/E, DHT/5 β -diol, and 5 α -diol/5 β -diol were advocated as "good candidate markers" (41). Proposed indicators for DHT administration are summarized in Table X.

Conclusions

The purpose of this review was to report screening indicators of DHEA, Adione, and DHT administration identified in the literature. Although IRMS is frequently used to confirm or distinguish administration of naturally produced AAS, only samples identified through screening markers will proceed to confirmation by IRMS. Therefore, it is extremely important to know what markers can be used to identify use and their limitations.

There were several recurring themes in our review of DHEA, Adione, and DHT literature for the purpose of identifying urinary AAS profile changes indicative of exogenous use of these steroids. For all three steroids, the number of studies was limited and in some instances the study methods were not fully described. The studies frequently used small subject cohorts/treatment paradigm, and few included female subjects or effectively evaluated the potential effects of ethnicity or age. Often considerable intersubject variability was observed in the postdose urine AAS profiles which, when combined with the limited number of study subjects, precluded statistical comparisons of the data. As a consequence, it is difficult from the literature to identify urinary markers of DHEA, Adione and DHT that are diagnostic of use. Tables II, III, and X summarize potential screening markers to indicate DHEA, Adione and DHT administration respectively. To address the current limitation in the scientific literature, studies with statistically relevant control and test groups are needed to discern the qualitative and quantitative alterations in urinary AAS profiles following acute and chronic use of DHEA, Adione, and DHT.

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