

Issues in detecting abuse of xenobiotic anabolic steroids and testosterone by analysis of athletes' urine

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Over the last decade the number of laboratories accredited by the International Olympic Committee (IOC) has grown to 25. Nearly half of the ~90 000 samples tested annually are collected on short notice—the most effective means to deter the use of anabolic androgenic steroids (AAS). The major urinary metabolites of AAS have been characterized and are identified by their chromatographic retention times and full or partial mass spectra. The process of determining if an athlete has used testosterone (T) begins with finding a T to epitestosterone (E) ratio >6 and continues with a review of the T/E–time profile. For the user who discontinues taking T, the T/E reverts to baseline (typically ~ 1.0). For the extremely rare athlete with a naturally increased T/E ratio, the T/E remains chronically increased. Short-acting formulations of T transiently increase T/E, and E administration lowers it. Among ancillary tests to help discriminate between naturally increased T/E values and those reflecting T use, the most promising is determination of the carbon isotope ratio.

INDEXING TERMS: sports medicine • epitestosterone

Testing for Anabolic Steroids

ACCREDITED LABORATORIES

In 1983 five laboratories were accredited by the International Olympic Committee (IOC)³ to perform national and

international sport drug testing. Now there are 25 accredited laboratories, and several more are training and preparing for the initial inspection and examination. The accreditation process is initiated by a recommendation from the National Olympic Committee and a commitment from the Committee or other relevant national authority to support the laboratory. The laboratories are reaccredited annually on the basis of performance on one annual examination with blind samples and several proficiency tests. In addition, the laboratories are obliged to adhere to rules and regulations laid down in the IOC Medical Code [1]. Of these, the most important is a provision that forbids testing samples from athletes outside of a regulated program that includes sanctions.

For a program to help deter athletes from using drugs, an important factor is geographical access to a laboratory; thus, the ideal distribution of laboratories would be in proportion to population. This is not yet the case, but it is improving. Seventeen of the laboratories are in Europe, three in North America, three in Asia, and one in Australia; South Africa is the most recent addition. There is still no laboratory in South America or the Middle East. A second, equally important factor is the number of tests performed on the national athletes. In some countries, Olympic-caliber athletes are tested many times a year by the national authority and may be tested other times by an International Federation. In other countries, where there is little or no national testing, an elite athlete may be tested only at major national or international competitions.

RESULTS REPORTED

The total number of tests conducted by IOC-accredited laboratories and the percentage of those positive for anabolic steroids are shown in Fig. 1. The total number of tests performed in 1994, the latest year for which data are available, was 93 680, compared with 33 982 in 1986. The graph shows rapid growth in the late 1980s, with a peak annual increase of 36% between 1989 and 1990. This corresponds to a time when sport organizations and the

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³Nonstandard abbreviations: AAS, anabolic androgenic steroids; T, testosterone; E, epitestosterone; IOC, International Olympic Committee; OOC, out-of-competition; HRMS, high-resolution mass spectrometry; SIM, selected-ion monitoring; CIR, carbon isotope ratio; LH, luteinizing hormone; TG, TS, T glucuronides and sulfates, respectively; EG, ES, E glucuronides and sulfates, respectively.

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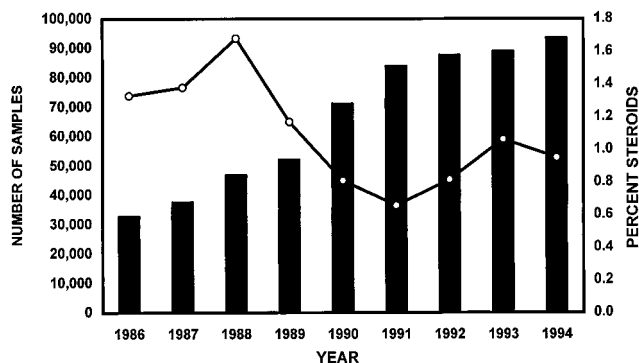


Fig. 1. Total number of urine samples tested in IOC-accredited laboratories by year (bars); the curve shows the percent of all samples that were reported positive for anabolic steroids.

media were particularly focused on the influence of drugs on sport. In the 1990s, the annual growth has tapered off, averaging 3.5% for the last 3 years shown in Fig. 1. Of greater importance than the number of tests is the proportion of tests performed on samples collected out-of-competition (OOC), i.e., samples collected on short notice or with no notice. OOC testing is the most effective deterrent to the use of anabolic androgenic steroids (AAS). In 1987, the percentage of total tests that was OOC testing was 17%, whereas in 1993 and 1994 this was 39% and 43%, respectively. It is encouraging that the percentage of OOC tests continues to increase at a rate equal to or greater than the total growth rate.

Laboratories that contribute data such as those reported in Fig. 1 are asked to classify the samples into the categories of major international competition, international competition, national competition, and OOC. Ideally, the OOC competition category should be further subdivided by the actual amount of time elapsed between notice to test and collection of the sample—because some steroids have effective half-lives of detection of just a few hours. Some of the OOC samples in Fig. 1 may represent samples collected after as much as 3 days' notice and others after virtually no notice. In some countries, virtually all OOC tests are no-notice tests.

The percentage of samples that test positive for AAS (Fig. 1) provides some information on the trend of usage; however, the lack of further detail precludes conclusions about individual sports or subsets of athletes according to level of performance. For example, an elite athlete who competes several times a year in track and field will probably give many samples, both in-competition and OOC, over the course of a year. Yet Fig. 1 gives only the total number of tests, not the number of athletes tested. Further, the data do not take into consideration the ultimate disposition of the case. For example, not all cases illustrated in Fig. 1 result in sanctions, for various factors that influence the adjudication process [2]. Despite the shortcomings in the available data, however, evidence is ample that a growing number of nations and sport federations are conducting credible testing.

Detection of Xenobiotic AAS

In 1994 about two-thirds of the steroid-positive cases shown in Fig. 1 were xenobiotic AAS. For these cases, detection is based on identifying the parent drug or metabolite(s) or both. Identification consists of obtaining the chromatographic retention time or relative retention time and the mass spectrum of the substance and showing that the (relative) retention time and spectrum match that of a reference compound. Fig. 2 shows the total ion chromatogram for an analyzed urine from a male who ingested boldenone. The peaks corresponding to boldenone (17β -hydroxyandrost-1,4-dien-3-one) and two major metabolites (17β -hydroxy-5 β -androst-1-en-3-one and 3 α -hydroxy-5 β -androst-1-en-17-one) are emphasized [3]. The spectra of these three peaks matched those of known (reference) compounds (data not shown). In this case the analysis identified not only a xenobiotic steroid but also two metabolites of the steroid. Thus the amount of analytical information obtained in this analysis is far beyond simple identification of a single substance; indeed, this analysis is helpful in responding to assertions that the sample might have been fortified, because the finding of metabolites is legal evidence that the individual who submitted the urine ingested the substance.

The ability of the laboratories to detect xenobiotic AAS continues to improve, given a growing body of knowledge of the metabolism of the compounds, method improvements, and instrument advances. The major metabolites of virtually all AAS are known and many have been synthesized [4]. For some drugs, as many as 11 metabolites have been detected [5]. The recent development of methods for analysis by high-resolution mass spectrometry (HRMS) is an important, but expensive, advance. HRMS operates in the selected-ion monitoring (SIM) mode to screen for xenobiotics and in the full-scan mode for confirmations [6]. This approach is particularly adept at screening for long-lasting metabolites of stanozolol and methandienone. As in all sports drug testing, a confirmation assay is performed on samples that screen positive. Note that the HRMS-based method described by Schänzer et al. [6] uses immunoextraction or HPLC to clean up the

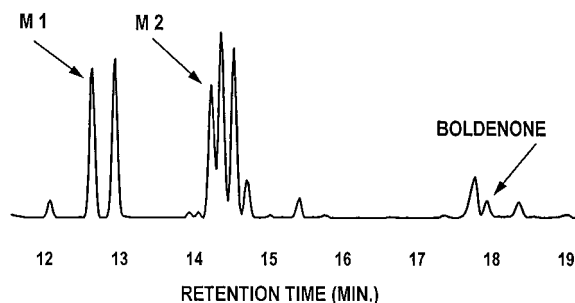


Fig. 2. Total ion current chromatogram of a urine sample collected from a subject who ingested boldenone.

The arrows point to boldenone (17β -hydroxyandrost-1,4-dien-3-one) and two major metabolites: 17β -hydroxy-5 β -androst-1-en-3-one (M1) and 3 α -hydroxy-5 β -androst-1-en-17-one (M2).

sample extracts before HRMS confirmation of stanozolol or methandienone metabolites, respectively.

One aspect of identification that continues to be debated is the absolute amount and nature of analytical information that must be obtained before a positive report is issued. Some advocate obtaining a full ion scan of at least one of the substances named in the positive report. Others find that SIM is sufficient, and still others use SIM with the major ions bracketed by the adjacent masses. Monitoring the preceding nominal mass allows demonstration that it is not substantially more intense than the ion of interest and thus excludes the possibility that the latter is an isotopic peak from some interference. The subsequent mass must have the expected response. Generally, drug-testing programs in the context of athletics ascribe to the full-scan approach for at least one of the substances reported. This is in contrast to the widely accepted practice in drugs-of-abuse testing of reporting positive cases on the basis of SIM data [7, 8].

Another aspect of xenobiotic steroid testing that merits review is the question of the origin of the steroid and metabolites. One report [9] describes the finding of small amounts of boldenone and two metabolites in the urine of a normal man who had not received boldenone. This case argues for caution in interpretation of positive tests for low quantities of boldenone, for additional studies of this issue, and for devising ways to discriminate between endogenous and exogenous sources of boldenone. It is illegal in most countries to treat or feed cattle with anabolic steroids, and European countries in particular vigorously monitor meat for contamination with anabolic steroids; nevertheless, evidence of contamination exists [10]. Debruyckere et al. [11], investigating the possibility that contaminated meat could result in steroids or their metabolites in human urine, found that some meat obtained from butcher shops contained clostebol acetate and that its ingestion led to excretion of a clostebol metabolite (4-chloro- Δ^4 -androstene-3- α -ol-17-one [9]) in urine. The possibility that urine from untreated subjects may contain very low concentrations of nandrolone metabolites has been discussed [12, 13]; however, the mass spectrum of these substances has not been presented, and others find no evidence for nandrolone metabolites in human urine after ingestion of meat from animals that were treated with nandrolone decanoate 28 and 61 days before slaughter [14]. Clenbuterol has been reported in very small amounts (<1 ng/L) in some human urines after ingestion of meat from clenbuterol-treated animals [14]. In an experiment designed to show human contamination, ingestion of meat from chickens deliberately treated with methenolone heptanoate led to finding methenolone and metabolites in urine [15].

Detection of Doping with Testosterone (T)

Doping with endogenous steroids is the most serious issue facing sport today; when cleverly administered, these are very difficult to detect. Quadrupole mass spec-

trometers cannot distinguish between pharmaceutical T and natural T because their spectra are identical. The average male produces 6–10 mg of T per day, of which only ~1% is excreted in urine. The urine concentration of T increases briefly after T administration; however, T has a short half-life (~1 h) and its concentrations fall rapidly [16]. Even T enanthate, an ester that increases concentrations of T in serum for ~2 weeks, often does not cause an obvious increase in urine T. Urinary androsterone and etiocholanolone account for ~70% of a dose of T [17]; however, many other endogenous steroids are metabolized to androsterone and etiocholanolone, and neither their concentrations nor their ratio is a useful marker of T administration. The ratio of androsterone to epitestosterone (E; 17 α -hydroxy-androst-4-en-3-one) has some utility as a marker of recent T use.

Various approaches to prove T administration have been considered within the practical constraint that only one untimed urine is available for the screening test. Brooks et al. [18] pointed out that urine concentrations vary over a very wide range such that defining T doping as a concentration that exceeds an upper limit of normal would be difficult. Instead, they advocated measuring the ratio of T to luteinizing hormone (LH) in urine, because chronic administration of T inhibits the production of LH and lowers the urine concentration of LH. Detailed studies of urine T/LH ratios are available [19]. Because the T/LH and other urine ratios are independent of urine volume and other factors that influence concentration, such ratios have been used extensively to detect doping with endogenous substances. One major drawback of T/LH is the lack of a reference method for LH [20].

Doping with T was reported in the 1950s [21], but not until 1982 did an effective test become available: Donike et al. [22] proposed to detect T doping by monitoring the T/E ratio. E is the 17 α epimer of T and is present in urine in concentrations similar to T. T and E differ chemically only in the configuration of the hydroxyl group on C-17. T is not metabolized to E [23], and T/E increases after T administration. Subsequently, Dehennin and Matsumoto pointed out that T administration lowers the concentration of E [24]; thus, the increase in T/E results from an increase in T and a decrease in E.

In 1982 the IOC Medical Commission defined a T/E >6:1 as the dividing line between the presumed upper limit of normal T/E values and doping with T. Since then, a small number of cases have been found with T/E >6:1 but without the subjects having been administered T [25–28]. Therefore, the definition of T doping has been changed: Individuals with T/E >6:1 undergo further testing before a conclusion is reached on the etiology of the increased T/E. The shape of the distribution of T/E values for 3710 male athletes (Fig. 3) is skewed, having a median of 1.1, and is 5.6 or less in 99% of the athletes tested. Of those with T/E >6:1, some admitted using T and others denied ever using it. Moreover, a T/E that is not >6 does not mean that the individual has not used T

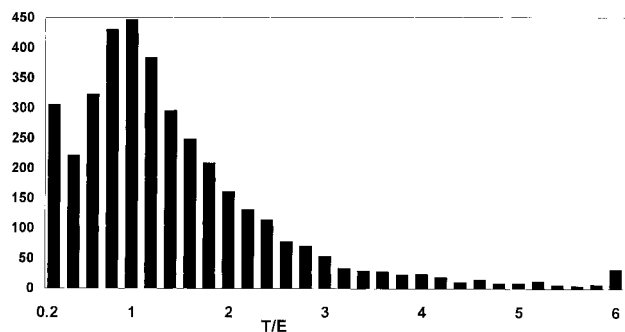


Fig. 3. Distribution of urine T/E in 3710 urines collected from male football players participating in a steroid detection program.

In most cases the athletes had <48 h notice of the test. The upper end of the distribution is such that the highest bin contains all T/E values >6:1. The highest three ratios were 30, 36, and 57. Ordinate, no. of samples.

recently; rather, it is consistent with recent use of short-acting T, use of T and E together, use of E to camouflage recent T use, or no use of T. When T/E is not >6, the laboratory issues a negative report, recognizing that it may be a describing a false-negative sample.

T/E-TIME PROFILE

In view of the many interpretations of an increased T/E value in a single urine, a strategy is needed for managing individual cases. If the athlete has never been tested before, one should try to obtain at least two additional samples at 3- to 6-week intervals, with as short a notice as possible. Next, plot the T/E results against time and calculate the mean, SD, and CV. Stable T/E values are interpreted as consistent with physiological or natural increases of T/E, and decreasing T/E values indicate that the high result reflected T administration. If the individual has been tested two or more times before the increased T/E result is obtained, and these T/E values are near or less than the median T/E for males (~1.0), then the high T/E is a strong indication of T administration. The actual value of the T/E is very helpful in differentiating natural increases from T administration, particularly if the T/E is 15 or higher. At those values, the likelihood of natural increases is remote. Conversely, the closer the high T/E is to 6:1, the more likely is the possibility of a physiological etiology.

Our data on urinary T/E ratios from drug-free males reveals that, for three or more samples taken at monthly or greater intervals, the CV will be <60%; 55% is the maximum we have observed. Fig. 4 shows the T/E-time profiles for three athletes. Athletes B and C have a mean T/E of 1.0 and 2.3 and CVs of 9.8% and 39.5%, respectively. Athlete A, whose initial T/E was 8.0, has a mean of 2.7 and CV of 105%; his pattern is typical of a T user who gets caught and discontinues T. In our experience, most T users who give three or more samples will have a CV >60%. This is in agreement with the data of Garle et al. [29] and appears to be consistent with the athletes who displayed a "T/E spike" pattern described by Baenziger and Bowers [30].

Many males will have a remarkably stable T/E over several months or years [31-33]. For example, the volunteer in Fig. 5 has a stable T/E over the first 9 consecutive days (CV 3.5%) and in intermittent assays over 8 months (CV 35%). Donike et al. reported [32] that the CV (in normal males) will not exceed 30%; however, in a study of 796 athletes who provided at least 3 urines over 2 years, Baenziger and Bowers [30] showed (their Figs. 8 and 9) that about one-third had CVs >30%, and that the 90th percentile for the CV of T/E ratios was 58%. Further, in a study [29] of 28 athletes with at least one T/E >6, 17 (61%) had CVs <30%, but 10 (36%) had CVs of 31-43%; the authors regarded the former group as "not likely to be testosterone users," and the latter as being in a "grey area." In the only study that covers several months of sampling in females (2 samples a month for 12 months, 5 subjects), Mareck-Engelke et al. [33] reported CVs of 15%, 51%, 25%, 31%, and 30%. Thus, although CVs are a useful guideline to understanding the T/E-time profile of an athlete, various factors may influence the T/E (see below, and [34]); therefore, the interpretation of a profile that includes one sample with T/E >6 should take into account all the available information for the subject tested.

The role of quantification of concentrations of T and E is important in the interpretation of T/E; however, the most useful information is the overall pattern of the T/E-time profile. Analytical and instrumental factors that influence the determination of T/E need to be taken into consideration [35, 37]. If the actual T/E value is reported, it is important to have documentation of the confidence interval of the measurement, in case that information is requested. Likewise, if the sample is reported as >6:1, it is important to have documentation of the statistical criteria used to make the decision.

The precise value of T/E is less critical if the value is two or more times the 6:1 threshold for reporting. The rationale for caution is that precise numbers are readily attacked by litigation attorneys, and lay-adjudicators often do not understand measurement variability. For example, the T/E on confirmation A will not be identical to the confirmation B value for T/E, and both will differ from the T/E reported in the screening assay.

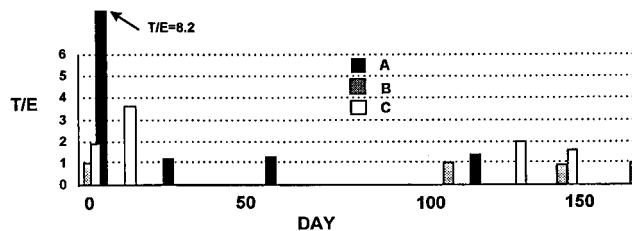


Fig. 4. T/E profiles of three athletes sampled four times over 170 days on relatively short notice.

The T/E of the first sample from athlete A was 8.2, followed by subsequent T/Es of 1.2, 1.3, and 1.4 (mean = 3.0, CV = 114%). The T/Es from athlete B showed minimal variability (mean = 0.97, CV = 9.8%); athlete C revealed greater variability (mean = 2.3, CV = 40%) but gave ratios still within our norms for non-T-using controls.

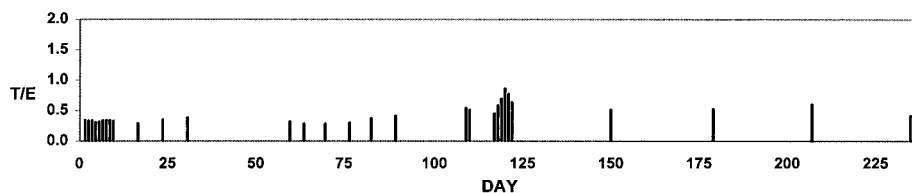


Fig. 5. T/E ratios of a drug-free volunteer who contributed 31 samples over 8 months.

For the first 9 days a sample was collected each day, all were analyzed in one batch, and the CV was only 3.5%. For the second period of consecutive daily samples shown, the CV was 21%. The CV for all samples was 35%.

In many cases of increased T/E, the concentration of E is low ($<5 \mu\text{g/L}$). Because the CV of the assay increases at low concentrations of T or E (or both), the corresponding CV of the T/E may be relatively high. Low concentrations of T are not an issue with T/E ratios; however, very high concentrations of T ($>300 \mu\text{g/L}$) are an indication of recent T administration. Expressing T and E concentrations in nanograms per milligram of creatinine is useful, but this approach has not been widely used, probably because of concerns that creatinine excretion rates are influenced by exercise and many other factors [26].

INCREASED T/E

T/E between 6:1 and 10:1. If an athlete produces three or more urine samples with T/E $>6:1$, none with T/E $<6:1$, and a CV $<60\%$, we tentatively classify the results as naturally increased and recommend additional tests. We also take into consideration the absolute value of the E and the amount of advance notice for the collections. If, at this point, a clinical examination and serum tests have not been performed, we recommend serum tests that are routinely available to the clinician: complete blood count; routine chemistries, including HDL and LDL cholesterol; free and total T, sex-hormone-binding globulin, dehydrotestosterone, follicle-stimulating hormone, and LH; urine LH; and a complete medical history and physical examination, with emphasis on the hypothalamic-pituitary-testicular axis and the adrenal. If these evaluations are normal, the likelihood of a T-secreting tumor is extremely remote. If the serum T is increased or if the follicle-stimulating hormone or LH is low, or both, it is important to extend the evaluation and determine if there is an endogenous source of T or the use of exogenous T. In difficult cases, a 24-h urine may be collected; analyzed for the total amount of T, androsterone, and etiocholanolone; and the results compared with published norms [39]. For a few of the athletes we monitor, serum tests and clinical evaluation have been performed and the results have been normal.

T/E $>10:1$. In this situation, we still recommend a minimum of two additional tests, even though we have never

encountered a case of potential physiologically increased T/E where the mean is $>15:1$. If the first sample tested from an athlete exceeds 15:1, we expect the subsequent T/E values to decrease to $<6:1$; in all cases where three additional samples have been obtained, T/E did fall to $<6:1$. If the index T/E is 10–15:1, a later T/E will decline to $<6:1$ in most, but not all, cases. When it does not, we recommend the clinical evaluation described above and, if possible, one or more of the ancillary tests described below.

Recently, we have encountered several cases where the T/E has remained in the 9–13:1 zone despite as many as five additional samples collected with <24 -h notice (Fig. 6). These cases are a growing concern because they raise the specter of sophisticated T delivery systems that can produce stable yet very high T/E values; moreover, they may be increasing in frequency.

ANCILLARY TESTS TO DETECT T ADMINISTRATION

Invasive tests. In case the above approach still does not lead to a definitive determination of whether or not T has been used, additional tests may be performed—with the understanding that documented experience with these tests is limited and that more-invasive techniques are involved.

The ketoconazole challenge test [26, 27, 40] involves collecting urines before and after administration of an oral dose of ketoconazole, which inhibits the synthesis of T. In a normal male, after administration of ketoconazole, urinary T declines, E is unchanged, and T/E decreases [27, 40]. Conversely, if the T/E is increased because of T administration, the T/E after ketoconazole administration increases. In individuals who probably have a naturally higher T/E, the ketoconazole test reveals a pattern similar to that of the normal male [27, 40]. Experience to date with the ketoconazole test indicates that it differentiates between T users and nonusers. Its greatest potential application is to determine whether individuals suspected of having physiologically increased T/E respond as expected. At this time, the test is not widely used in the US because it requires drug administration and entails the risks related thereto, as well as commitment and expense

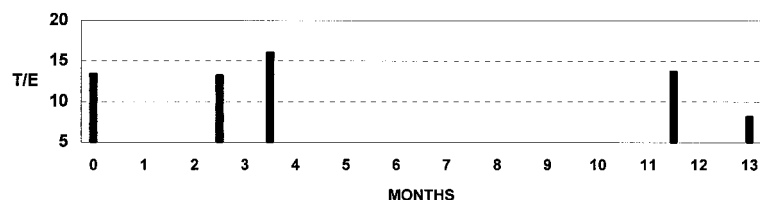


Fig. 6. T/E-time profile for one athlete who gave five samples on short notice over 13 months.

Although the mean of 13 is high, the CV is relatively low (22%).

on the part of the athlete or the sports organization. There is also a sense that athletes with naturally increased T/E should not be coerced into the test for the sake of proving their innocence.

Another approach is based on measuring the concentration in serum of any substance that precedes T in the biosynthetic pathway to T and calculating a ratio of that substance to T; in the presence of exogenous T, the production of the precursors will be suppressed and the ratio should be high. Carlström et al. [20] described the serum 17 α -hydroxyprogesterone/T ratio, presented the distribution of values for 12 males who did not use T, and showed that the ratio is increased after T administration; in the one subject with chronically increased T/E, the 17 α -hydroxyprogesterone/T ratio was within the normal range. More experience with this test is desired.

Noninvasive tests. In typical T/E analysis, the glucuronides and sulfates in the urine samples are hydrolyzed with β -glucuronidase from *Escherichia coli* or *Helix pomatia*. In the former case, the test measures the ratio of total T (unconjugated T plus the T deconjugated from the glucuronide) to total E. In the latter case, the unconjugated glucuronide and to some extent the sulfate fractions are taken into account. Dehennin [41] has taken the additional step of measuring the concentrations of T and E glucuronides (TG, EG) and sulfates (TS, ES) plus other precursors of T and E. He used β -glucuronidase to obtain the glucuronide fraction and DEAE-Sephadex to separate the sulfates from the glucuronides and the nonconjugated steroids. He has proposed [41, 42] that individuals with T/E >4:1 have a defect in E production, which results in decreased concentrations of EG and normal or increased concentrations of ES. Further, he finds that individuals with T/E >4:1 have low EG/ES ratios, and their TG/(EG+ES) values are less than the increase threshold (mean + 4.5 SD) of the reference group. Additional studies of these and other proposals [30] of Dehennin are underway. To that end, Sanaullah and Bowers [43] have synthesized deuterium-labeled T and E glucuronides and sulfates and have developed a liquid chromatography-tandem mass spectrometry method for directly quantifying these moieties.

Another noninvasive test is the determination of the carbon isotope ratio (CIR). Most carbon atoms are ^{12}C , and a very few are ^{13}C and ^{14}C . Becchi et al. [44] explored the hypothesis that the $^{13}\text{C}/^{12}\text{C}$ of synthetic T differs from the $^{13}\text{C}/^{12}\text{C}$ in endogenous T. Using a specialized GC-MS that measures this CIR, they found support for the hypothesis by showing that, after T administration, the $\delta^{13}\text{C}$ $^{\circ}/_{00}$ values for T were <-27 , whereas samples from normal controls had values that were less negative than -27 . In a subsequent study, the $\delta^{13}\text{C}$ $^{\circ}/_{00}$ values for T, cholesterol, and metabolites of T were determined for 25 samples from 8 apparently healthy volunteers before and after T administration [45]. The $\delta^{13}\text{C}$ $^{\circ}/_{00}$ values of T and metabolites were lower after T administration, and dis-

criminant analysis correctly identified the samples collected after T administration [45]. More recently, we have measured the $\delta^{13}\text{C}$ $^{\circ}/_{00}$ values of T in 14 urines from three individuals whom we expected to have physiological increases of T/E. The $\delta^{13}\text{C}$ $^{\circ}/_{00}$ values of T in all 14 was between -24 and -27 . At this juncture, the CIR studies consistently show that after T administration the $\delta^{13}\text{C}$ $^{\circ}/_{00}$ values of T are -30 to -36 if the T/E is >10 ; however, the studies to this point have not included enough samples from T users with T/E ratios in the critical range of 4:1 to 10:1, so the sensitivity of the CIR test is not known. Although it was encouraging to find high T $\delta^{13}\text{C}$ $^{\circ}/_{00}$ values in three subjects presumed to have physiologically increased T/E, more data are needed. Further on-going studies are anticipated to improve on the measurement of CIR and on the premeasurement analytical techniques.

OTHER VARIABLES INFLUENCING T/E

Ethanol. In 1992 Falk et al. [46] measured the T/E before and after administering ethanol (2 g/kg) over 4 h to four males. The ratio increased by 60% in one, $\sim 20\%$ in two, and no change in the fourth [46]. Subsequently, papers presented at a workshop on doping [47–49] found differing effects of ethanol on the T/E. Males showed no significant effect of ethanol on T/E at doses of 1.0–1.2 g/kg [46, 49], whereas the same dose produced large increases in another study [36]. At very high doses of ethanol (2 g/kg), the T/E ratios of males increased, although none exceeded 6:1 [46, 47, 49]. The T/E ratios in females appear to be more sensitive to ethanol. Doses of 1.0–1.2 g/kg produced either no effect on T/E [49] or increases [48], and high doses produced increases [47, 49], including two that exceeded 6:1 [48, 49]. The effect of ethanol on T/E appears to be limited to the 8 h after completing the ingestion. It will be important to advance these studies by adding placebo controls to factor out the effects of biorhythms and other sources of potential bias and to clarify dose-response relationships. Until further details are available, one may prudently consider that large and inebriating doses of ethanol may increase the T/E for several hours after ingestion and that some females may be particularly sensitive to the ethanol effect.

Gender. Little is published about gender differences in T/E; however, in our experience, the T/E distribution of a control group of female students, who are not at risk to take T, is shifted slightly to the left (lower values) of the distribution for control males. The reason for this is not known. We have found, however, that the menstrual cycle may influence T/E. We obtained daily T/E values on morning urines from three females throughout a total of five menstrual cycles; each showed a peak during menses, and the nadir-to-peak T/E ratio could vary by nearly threefold over the course of a cycle. Data on three cycles in one of these subjects have been presented [50]. In other studies that collected 24-h urines from four females on days 2, 7, 14, and 21 of the menstrual cycle [33], at the

beginning and at mid-cycle for 1 year ($n = 5$) [51], there was no evidence of a relationship between T/E and the menstrual cycle. Additional studies using identical protocols will help clarify the relation between T/E and the menstrual cycle.

Masking Testosterone Administration

Short half-life formulations of T. Currently, the drug of choice for the management of hypogonadal syndromes is T enanthate, typically given by injection every 2 or 3 weeks in doses of 100–200 mg. Because parenteral injections are inconvenient, the pharmaceutical industry has been developing alternative formulations and routes of administration. T cyclodextrin is a formulation administered by having the patient place a tablet in the buccal-sublingual pouch to be absorbed into the bloodstream. A dose of 5 mg produces serum concentrations of T within the reference range for normal [52] but, because of the very short half-life of T, the drug must be taken three times per day. This preparation leads to very high urinary ratios of T/E, but these typically fall to $<6:1$ within 4–6 h (Fig. 7) [55]. Two T formulations that utilize the transdermal route of administration became available recently and others are in development [53, 54]. One of these is a T gel that is administered by applying the gel on the skin. As expected, this formulation also increases the urine T/E (Fig. 7). (These studies were approved by the institutional review board.) The question remains whether or not these preparations can be administered in sufficient doses to enhance performance. The threshold dose for a pharmacological effect of T in men is not known, but 600 mg/week of T enanthate for 10 weeks is known to increase fat-free body mass and muscle size and strength in healthy men [56]. Given that T cyclodextrin is readily bioavailable, we can reasonably suspect that it would provide enhancement.

Administration of E. Anecdotal reports suggest that E is used as an emergency measure to rapidly lower a T/E that is above-normal as a result of T administration. E is not available in a pharmaceutical dosage form but it is available as a chemical. For this reason the IOC Medical Commission classified E as a urine-manipulating agent, set 150 $\mu\text{g/L}$ (520 nmol/L) as the threshold for reporting

cases, and later changed the threshold to 200 $\mu\text{g/L}$ (693 nmol/L). In our opinion the threshold should be higher: We often encounter cases in the 150–200 $\mu\text{g/L}$ range. In the future we expect that the CIR technique might be useful for detecting E administration. The two highest E concentrations we have encountered in urine samples were 1200 (4.16 $\mu\text{mol/L}$) and 1550 $\mu\text{g/L}$ (5.20 $\mu\text{mol/L}$).

Perhaps the ideal doping agent would be a combination of T and E designed to deliver T and E in a ratio of $\sim 25:1$, i.e., the ratio of production rates of T and E in men [42]. In theory, this might produce a T/E of 1:1 and therefore produce false-negative test results while allowing administration of T. In practice, the dosing regimens do not always lead to T/E $<6:1$, and the formulations are cumbersome to prepare. Moreover, Dehennin [42] proposes to detect this scheme by measuring the ratio of T and E to 5-androstene- $3\beta,17\alpha$ -diol, a precursor of E, and Kicman et al. [19] have shown that the T/LH ratio is high after administration of combined T and E.

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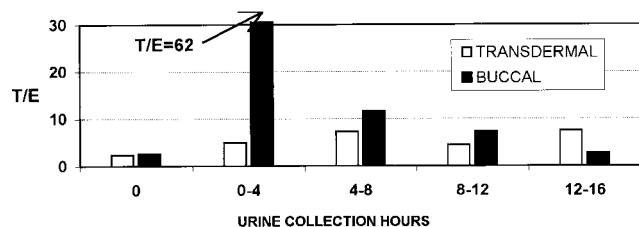


Fig. 7. T/E ratios in urines collected at 4-h intervals after transdermal and sublingual-buccal administration of T to two subjects.

The sublingual formulation contained 25 mg of T embedded in cyclodextrin. The transdermal formulation contained 100 mg of T.

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