Detecting Testosterone Administration

Controlling drug abuse in sports requires effective means to detect the administration of banned substances. For synthetic anabolic androgenic steroids, the identification of the parent steroid, or one or more diagnostic metabolites, in a sample of the athlete's urine is evidence that the offense of drug abuse has taken place.

For substances that are produced endogenously, the mere presence of the substance in the urine obviously cannot constitute proof of an offense. Nevertheless, sport authorities may wish to ban the administration of the substance; this is the situation with respect to testosterone (T). Various approaches to prove administration have been considered within the practical constraint that only a single un timed urine sample is available for the screening test. The notion of setting a concentration cutoff for urinary T was abandoned because the range of values in normal subjects is very wide and the cutoff would have to be set so large that the ability of the test to identify users would be poor and therefore unfair to athletes who do not use T.

A more realistic approach would be a ratio of substances that are independent of urine flow. Since the early 1980s, the two candidate methods for this involve determining the ratios of T to epitestosterone (E) (1) and of T to luteinizing hormone (LH) (2). The former is based on the findings that T administration results in an increase in the T/E ratio (1, 2), and that administration of labeled T does not result in substantial incorporation of the label into E (1). The latter is based on endocrine control mechanisms that lead to a decrease in LH consequent to use of anabolic androgenic steroids; however, the T/LH ratio is not practical for urine samples from females, who are permitted to suppress LH with oral contraceptives. Definitive methods such as detecting a synthetic T ester in blood or finding an unnatural $^{12}$C/$^{13}$C isotope ratio are being investigated.

In recognition of this situation, the International Olympic Committee (IOC) and many national and international sport authorities have worded their rules to state not simply that T administration is banned but that the T/E ratio may not exceed 6. When T was banned in 1982, the IOC rule (3) was "... the administration of testosterone or the use of any other manipulation having the result of increasing the ratio in urine of testosterone-epitestosterone to above 6." The most recent IOC rule (4) goes one step further by stating that a T/E >6 "... constitutes an offence unless there is evidence that this ratio is due to a physiological or pathological condition."

In this issue of Clinical Chemistry, Carlström et al. (5) report administering T to seven normal subjects, with the ensuing changes in several urine and serum androgens, androgen precursors, estrogens, and LH. They confirmed that the urine T/E ratio is a valuable indicator of T administration. They also discovered that the ratio of serum T to 17α-hydroxyprogesterone (17OHP) was increased for several days in all subjects and may be a more sensitive indicator of T administration. Although they proposed estimating the T/17OHP ratio in all suspected cases of T doping, just as for T/LH the T/17OHP ratio will not be useful for females because 17OHP concentrations vary with the menstrual cycle (6).

The urine T/LH ratio was increased in only one of seven subjects in their study. This is unexpected because T is well known to suppress serum LH concentrations, and others have reported an increase in the urine T/LH after T administration (2, 7). The most likely explanation is a change in the characteristics of the LH assay, resulting from modifying the kit procedure by greatly increasing the sample volume and altering the ionic strength of the buffer. This represents substantial changes that require revalidation of the assay.

Pituitary LH stimulates steroidogenesis in the Leydig cell. The administration of LH or human chorionic gonadotropin (8) to men increases plasma concentrations of 17OHP and T. Thus, the administration of chorionic gonadotropin may mask any detection of T administration that is based on T/E or T/17OHP. However, the ratio of T to LH in urine is a valuable criterion in detecting administration of both T and chorionic gonadotropin (9).

Carlström et al. criticize the use of the simple T/E ratio, suggesting that a few athletes may be falsely penalized; on the other hand, the guilty could escape by contaminating their urine with E or by taking E. The IOC, in an attempt to prevent misuse of E, now recommends an investigation whenever the concentration of E in urine exceeds 150 µg/L (4).

Missing from the literature so far is a detailed description of the distribution of T/E in a large population known not to have used T. Carlström et al. cite a study (10) that estimated the frequency of T/E >6 at 1.5 per thousand; this was based on fitting a curve to data from only 11 adolescents. The difficulty is that a study designed to accurately estimate the incidence of such a low-frequency event would require several thousand

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1 Nonstandard abbreviations: T, testosterone; E, epitestosterone; LH, luteinizing hormone; 17OHP, 17α-hydroxyprogesterone; and IOC, International Olympic Committee.
subjects. Neither the adolescent study nor the other paper (11) they cited as containing cases of false-positive results actually present analytical data in support of such a case.

Carlstöm et al. also present preliminary data on an athlete with a urine T/E ratio of 5.8 and 6.3, a normal T/170HP ratio, and an increased concentration of urine LH. They refer to data obtained after administering ketoconazole (T/E decreased) and indicate an intent to publish the full details of the case. These details are awaited with interest. They should include a complete history, verification that neither T nor another anabolic androgenic steroid was administered, the results of hormone tests, a description of the methods, and a discussion of the increased LH. Until then, the comments regarding T and E production rates and genetic etiology should be cautiously interpreted.

For athletes with urine T/E ratios between 6 and 10, many sport authorities have adopted the approach of reviewing T/E ratios from previous tests and (or) analyzing additional urine samples, as well as reviewing all other data before making a decision to sanction an athlete. Although Carlström et al. find the multiple urine method to be "time-consuming" and "less safe," this approach does provide the maximum benefit to all athletes, users and nonusers, and it is embraced by the most recent IOC rule (4), which, in cases of T/E between 6 and 10, recommends: "...that further tests be conducted before considering the result as positive or negative." Examples of additional studies include review of previous tests, longitudinal studies, unannounced urine collections, and endocrine investigations.

Estimation of the T/170HP ratio is a logical candidate for the list of additional tests; however, this ratio and other endocrine tests require blood samples and the selection of an analytical method for confirming the ratio. Currently, the IOC is reviewing a proposal to allow blood to be taken from athletes in the context of the Olympic Games. If approved, this would also provide an entry into the important area of detecting blood doping and the administration of hormones such as erythropoietin and human growth hormone (somatotropin). However, current IOC regulations require that all positive cases be confirmed by gas chromatography-mass spectrometry, although at this time there is no practical means for identifying such peptides in urine or blood by this method. For the T/170HP ratio, published gas chromatography-mass spectrometry methods exist for both T (as noted) and 170HP (12); however, for investigators to implement this test, the methods would have to be fully validated in each laboratory.

References

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