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# Genetic Variations in UDP-Glucuronosyl Transferase 2B17: Implications for Testosterone Excretion Profiling and Doping Control Programs

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The use of drugs and ergogenic substances to augment athletic performance, commonly referred to as doping, has evolved along with sporting events. Ancient Olympic athletes consumed mushrooms, plants, and herbs in an attempt to gain a competitive edge. The modern Olympic Games made their debut in 1896, and mixtures of cocaine, ephedrine, and strychnine were used to enhance performance. Anabolic androgenic steroids (substances similar to the hormone testosterone) were used after World War II by Soviet athletes to increase muscle mass and power in weightlifting and bodybuilding events. Anabolic androgenic steroids rapidly spread to athletes in other sporting events and are still a problem in today's sports world. To deal with the problem of doping in sports, the International Olympic Committee established a Medical Commission. The first list of prohibited substances was created in 1967; drug testing was implemented at Olympic Games the following year. In 1999, an independent international organization, the World Anti-Doping Agency (WADA), was created to combat doping in sports and provide unified standards for doping control.

## The T/E Ratio to Screen for Testosterone Use

Anabolic androgenic steroids are the most abused class of prohibited substances, with testosterone accounting for many positive cases. Testosterone abuse is problematic because synthetic testosterone is indistinguishable from endogenous testosterone by routine screening methods such as gas chromatography–mass spectrometry. In the 1980s, it was discovered that testosterone use alters the ratio of testosterone glucuronide to epitestosterone glucuronide (T/E ratio)[2] in urine. Epitestosterone is a naturally occurring biologically inactive epimer of testosterone that remains relatively constant in urine. A population-based T/E ratio cutoff of 6.0 was initially used to indicate synthetic testosterone use; the T/E ratio cutoff was lowered to 4.0 in 2005. Based on data from several laboratories, the average T/E ratio ranges from 0.9 to 1.6 for healthy male adolescents and men. At the UCLA Olympic Analytical Laboratory, we found that the average T/E ratio during a 31-month period was 1.1 (median 0.9%). The distribution of T/E ratios is shown in Fig. 1. Approximately 99.6% of urine samples have a T/E ratio $<4.0$, and 99.8% have a T/E ratio $<6.0$.

## Confirmation Testing for Testosterone Use

The T/E ratio is typically used as a screening test, and urine samples with a ratio $>4.0$ are submitted for confirmation testing by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS). GC/C/IRMS has excellent specificity and can measure very small differences in the $^{13}C$ to $^{12}C$ ratio of testosterone and steroid metabolites. The $^{13}C$ content of natural (endogenously produced) testosterone is influenced by plant and animal sources consumed in food. In contrast, synthetic (pharmaceutical) testosterone is produced from plant precursors (stigmasterol) containing less $^{13}C$. This results in smaller $^{13}C/^{12}C$ ratios for synthetic testosterone, compared to natural testosterone. $^{13}C/^{12}C$ ratios are expressed in parts per thousand relative to a reference gas as a $\delta$ notation. The $\delta$ value is compared to that obtained for a compound in the steroid pathway such as pregnanediol, whose $^{13}C/^{12}C$ ratio is unchanged by testosterone use. If the $\delta$ value for the metabolite differs from the reference compound (pregnanediol) by 3 or more units, synthetic steroid use is indicated. Although GC/C/IRMS is used to confirm testosterone use, the technique has low

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[2] Nonstandard abbreviations: T/E ratio, ratio of testosterone glucuronide to epitestosterone glucuronide; GC/C/IRMS, gas chromatography/combustion/isotope ratio mass spectrometry; UGT2B, UDP-glucuronosyl transferase 2B.

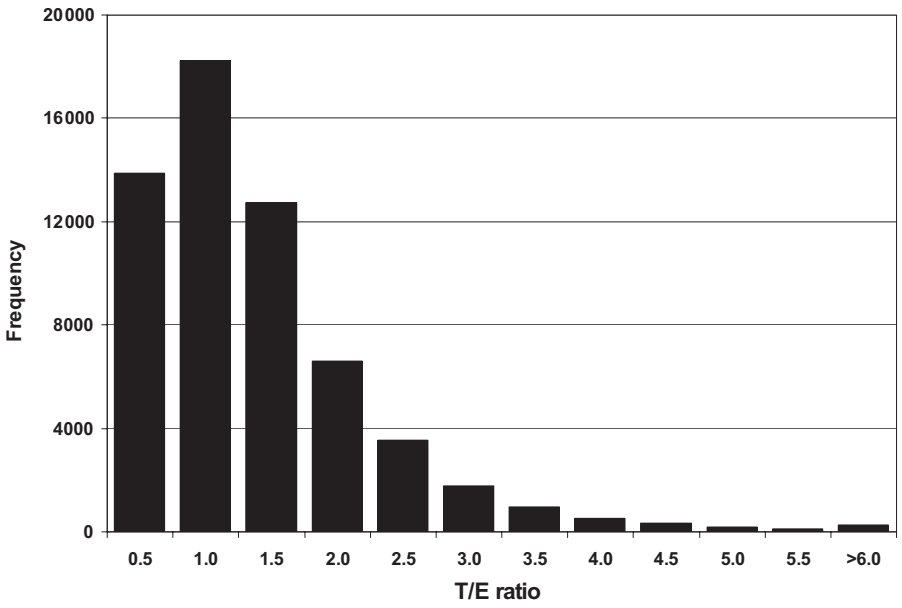


**Fig. 1. Distribution of T/E ratios for 59 095 athlete urine samples (78.5% male and 21.5% female) measured at the UCLA Olympic Analytical Laboratory during a 31-month period (January 2006 to August 2008).**

T/E ratios on the *x* axis represent cutoff values. A T/E ratio of 1 includes all urine samples with a value >0.5 and <1.0.

analytical sensitivity and is labor-intensive (owing to extensive sample cleanup) and costly to perform, and thus cannot be used as a screening test.

### The Influence of Genetic Polymorphisms on the T/E Ratio

T/E ratio testing provided a solution for detecting synthetic testosterone use until Asian men were found to have a lower urinary T/E ratio (compared with whites) more than a decade ago. Circulating concentrations of steroid hormones are controlled by the UDP-glucuronosyl transferase 2B (UGT2B) subfamily of uridine diphospho-glucuronosyl transferases, which facilitate urinary excretion by glucuronidation reactions that make steroid molecules more hydrophilic. UGT2B17 is the major enzyme in the UGT2B subfamily that conjugates glucuronide to testosterone, dihydrotestosterone, and androsterone in the liver and tissues. A common deletion polymorphism in the UGT2B17 gene was recently shown to differ among ethnic groups, being more common in whites than in African Americans. Further studies revealed that large differences in urinary testosterone concentrations are associated with a deletion polymorphism in the UGT2B17 gene *(1)*. Men homozygous for the UGT2B17 deletion polymorphism have extremely low or undetectable urinary testosterone concentrations, and this genotype is 7 times more common in Korean men (66.7%) than Swedish men (9.3%). Epitestosterone concentrations are similar in the 2 ethnic groups, regardless of whether they have low or high urinary testosterone concentrations.

The strong association of the UGT2B17 deletion polymorphism with testosterone excretion brings into question the ability of a population-based T/E ratio to detect testosterone use. To better define T/E ratios based on genotypes, Schulze et al. *(2)* identified men with 2 (ins/ins), 1 (ins/del), or 0 (del/del) copies of the UGT2B17 gene and examined urinary steroid profiles after an intramuscular injection of testosterone enanthate. As expected, baseline urinary testosterone concentrations and T/E ratios were significantly lower for the del/del group. Mean T/E ratios were 0.14, 1.4, and 2.3 for the del/del, ins/del, and ins/ins (control) groups, respectively. Baseline epitestosterone concentrations did not differ between groups. After testosterone administration, the mean T/E ratio for the del/del group increased to 5.3; however, 40% of the participants failed to reach a T/E ratio of 4.0. In contrast, the mean T/E ratio increased to 50.4 and 100 for the ins/del and ins/ins groups, respectively. Urinary epitestosterone concentrations were lower in all groups but did not differ between groups. When applying the WADA T/E ratio cutoff of 4.0, 94% of the del/del subjects produced a false-negative test result on day 2, 41% on day 6, and 71% on day 11 after testosterone administration. A

genotype-specific cutoff of 1 for del/del subjects produced a false-negative rate of 43% at day 2 and eliminated all false negatives at days 6 and 11. These findings suggest that genotype-specific cutoffs could enhance the performance of the T/E ratio test. Unfortunately, it would be difficult at this time for laboratories to incorporate genetic testing into their routine test menu and screen each athlete for UGT2B17 deletions. An alternative would be to use a low urinary T/E ratio ($<0.2$) as evidence for the del/del polymorphism. However, this would be problematic since it would incorrectly classify athletes that are doping with a combination of testosterone and epitestosterone (to lower their T/E ratio) as having the del/del polymorphism. In these cases, either genotyping or other tests to detect doping with epitestosterone would be required, such as the epitestosterone to 5-androstene-3$\beta$,17$\alpha$-diol ratio *(3)* or $^{13}C/^{12}C$ ratio of epitestosterone *(4)*.

## Longitudinal Steroid Profiling

Another approach to detect testosterone use that is gaining widespread acceptance is longitudinal studies of urinary steroid concentrations. The concept is based on the observation that the T/E ratio for a single individual male typically varies by $<30\%$, whereas between-individual variability is considerably larger. Individual T/E values from at least 3 test results are used to establish a baseline, and suspicious results that differ significantly from baseline are proof of synthetic testosterone use. Several statistical approaches have been used to detect outliers in longitudinal data. A Bayesian test using both population data and individual athlete test results appears to be superior to other statistical tests for detecting T/E ratio outliers. A Bayesian interpretation of T/E test results has been shown to produce 0 false-positive results for 43 true positives using a *P* value of 0.1% *(5)*. For the same data set, a population-based T/E cutoff of 4.0 resulted in 24 false positives and 34 true positives. Urine samples producing a test result significantly higher than baseline could then be submitted for GC/C/IRMS confirmation testing. This approach can be used to detect testosterone use in individuals with the UGT2B17 deletion polymorphism, possibly negating the need for genetic testing. Athlete-specific baseline T/E ratios, along with GC/C/IRMS testing on random and suspicious urine samples, were used at the Olympic Games this year to catch athletes that use synthetic testosterone and have low a T/E ratio. Longitudinal studies can also be applied to other urinary steroid metabolites to further enhance detection of synthetic steroid use.

## Concluding Remarks

It has been known for more than a decade that urinary T/E ratios are significantly lower in certain ethnic groups. This observation has limited the effectiveness of a population-based T/E ratio as a screening test for testosterone use. Recent evidence has demonstrated that a deletion polymorphism in the UGT2B17 gene is responsible for reduced urinary testosterone levels. UGT2B17 deletion polymorphism testing would be difficult to perform on every athlete at this time, and GC/C/IRMS is not a practical screening test for testosterone use. Individual-based T/E ratio baseline values derived from longitudinal steroid profiles is the most promising approach to detect testosterone use. As deletion polymorphism testing evolves, it is conceivable that longitudinal steroid profiling in conjunction with genotyping may prove to be the optimal test combination for detecting testosterone use. Genotype-specific T/E ratio cutoffs will be especially useful while individual baseline values are being established. Clearly, further studies are needed to validate the usefulness of these strategies in different ethnic groups.

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