In: Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (eds.)
Recent Advances in Doping Analysis (1). Sport und Buch Strauß, Köln 1994

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Variability of T/E Ratios in Athletes

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INTRODUCTION:

The use of the Testosterone(T)/Epitestosterone(E) Ratio (T/E) has been the primary method used to determine whether an athlete has used exogenous testosterone. The finding of T/E results greater than 6:1 has been considered as a positive anti-doping test by the IOC since 1982. Since this time a few athletes were identified which had elevated T/E ratios and after extensive evaluation this elevated T/E ratio was considered to be due to a normal physiologic variation. Many athletic programs will now more frequently test those individuals who have a T/E ratio between 6-10:1 before considering taking punitive actions[1]. However, it is also known that some athletes are using exogenous testosterone or other manipulations of the endocrine system by various substances, yet their T/E ratios never exceed 6:1. Several methods are being investigated to help resolve the above problems[2] , and we now present preliminary data on the use of T/E Ratio variability as a tool to help resolve some of these problems.

SUBJECTS:

Urine samples were collected from a group of male athletes (subjects) as part of their athletic organization's Anti-doping Program during 1990-1991. Multiple samples from the subjects were received over this time period and were uniquely identified by a sample number so that the analyses of the T/E ratios were performed blind to the relationship between samples. The actual identity of the subject is only known to the athletic organization with each subject having a unique player number assigned. Subsequent to the analysis, the athletic organization generated a list of sample numbers that were associated with the unique player number for our use in matching sample results for each subject.

METHODS:

Anabolic Steroid Analysis:

The T/E Ratio was determined on each subject's sample(s) using our laboratory's routine procedure for conjugated steroids[3]. In brief, 5 ml of urine is extracted using a C-18 solid phase column. The column is eluted with methanol, evaporated, and then hydrolyzed using Helix pomatia. The hydrolysate is extracted with ether and then derivitized with MSTFA/TMIS to silinate all hydroxyl and keto functions. Analysis is performed using one of 5 Hewlett/Packard 5890 GC's equipped with a capillary column and attached to 5970B MSD detectors operated in the SIM mode. The T/E ratio's were determined by comparing the peak area of testosterone and epitestosterone using the $M^{+\bullet}$ (432 m/z) ion signal. Additional analytical data from endogenous urinary steroids is also routinely collected and recorded along with other sample data as follows:

Specimen Data	Analytical Data
Subject's specimen ID#	Androsterone(A) & Etiocholanolone(Et) areas
Collection date of specimen	11-ßOH A & Et areas
Receipt date of specimen	Testosterone & Epitestosterone areas
Subject's sport	Internal Standard area
Subject's sex	Creatinine concentration
Laboratory Accession #	Specific gravity
Laboratory Batch #	Urine pH

Within each analytical batch analysis (20 subject samples) the following controls and standards are simultaneously analyzed: a conjugated control (both abnormal and normal); plus an endogenous standard which includes A, Et, 11-OH A, 11-OH Et, T, and E. Thus, semi-quantitative concentrations for the endogenous steroids can be determined on each subject by comparing area ratios for each steroid with the endogenous standard. These concentrations are currently being entered into a comprehensive subject database.

Data Evaluation:

The results of the T/E ratio determinations for each subject were evaluated and the mean, standard error of the mean (SEM), standard deviation (SD), percent coefficient of

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variation (%CV), and percent standard error of the mean (%SEM) were calculated using routine statistical methods. A subset of subjects were selected for further evaluation of T/E variability and consisted of those subjects who had at least one T/E result greater than 3 and who had at least 3 samples analyzed.

RESULTS:

Analytical Performance:

The analytical precision of the T/E ratio for the screening procedure has been determined by recording the T/E ratio from both the abnormal (mean = 7.4, %CV = 8) and normal (mean = 1.4, %CV = 15) conjugated controls over multiple batch assays and across 5 instruments.

Subject Population Characteristics:

A total of 1285 subjects were tested with 4973 T/E results measured. During this same time period a total of 22,806 T/E ratios were performed on athletes from all sports in the laboratory. The two populations' T/E ratios are compared in Table 1 and Figures 1 and 2, which show that the two populations are similar.

The mean T/E was determined in a subset of 796 subjects who had at least 3 values recorded and compared to the mean of the 1285 subjects. These populations' T/E ratios are compared in Table 1 and in Figures 3 and 4, which again show that the populations are similar. The populations are further characterized by determining the values at the 90th, 95th, and 99th percentiles for these non-gaussian population distributions.

Subject Variability of T/E Ratios:

The variability of each subject's T/E determinations were calculated on the population subset of 796 subjects who had 3 or more values recorded. Figure 5 shows the distribution of the number of values that have been recorded for each subject. The results of these variability determinations are summarized in Tables 2-3 and in Figures 6-9. For each variability statistic a plot of the distribution frequency as well as a plot versus the mean T/E ratio was made. The variability determinations demonstrated a non-gaussian distribution and so the 90th, 95th, and 99th percentile values are listed in the Tables.

Subject Screening Procedure:

A second subset of the total 1285 subject population was selected on the basis of whether a subject had at least one T/E result > 3:1 (approximately the 90th percentile for all athletes). This subset was further evaluated to determine the variability in their T/E results. A total of 122 subjects with 629 T/E measurements were identified using the above criteria. Of these 122 subjects, 97 subjects (8% of total subjects) also had at least 3 T/E values to use for variability calculations. These 97 subjects could be divided into three basic patterns of T/E variability:

Pattern Type 1: elevated T/E ratio but with low variability

Pattern Type 2: high variability as the result of a T/E "spike"

Pattern Type 3: high variability without a distinct T/E "spike"

Of these 97 subjects, 33 subjects had Pattern Type 1, 38 subjects had Pattern Type 2, 21 subjects had Pattern Type 3, and 5 subjects could not be classified into either Pattern 2 or 3. Graphical representations of a selected number of each Pattern Type are shown in Figures 10-12.

DISCUSSION/CONCLUSIONS:

These preliminary results of the variability of T/E ratios on this population of athlete subjects shows that the vast majority of subjects have low T/E ratios (ie clustered around the population mean of 1.2) and demonstrate little variability in their T/E ratios over time (ie %CV < 30%). Comparing the T/E variability (%CV) between the subjects and the analytical precision it is concluded that most of a subject's T/E variability is a determinate of the analytical imprecision. However, a small fraction of the subjects do show large variability in their T/E measurements over time and thus warrant additional evaluation.

The finding of a small fraction of subjects with relatively high T/E ratios (ie > 3:1) and with low variability (ie Pattern Type 1) may be due to a normal physiologic variation between subjects. In fact, our laboratory has documented several individuals who have had T/E ratios > 6:1 and who have demonstrated relatively consistent T/E ratios over several years and who have been further investigated using serum hormonal studies to document that the pituitary-testicular endocrine system is not suppressed or abnormal. The specific explanation for why T/E ratios may be elevated normally in some individuals is still not available, but research is currently underway that may help determine how this finding relates to an individual's production or metabolism of androgenic steroids.

The use by athletes of aqueous based testosterone with its relatively short half-life, epitestosterone use, and other techniques that alter the endocrine system makes timing of specimen collection critical to detect an elevated T/E ratio on a single determination. To detect this type of anabolic steroid misuse it has been suggested that the T/E ratio cut-off be lowered (ie 3:1) and that serial specimens be collected and used to evaluate the variability of an athlete's T/E measurements. The data presented in this study suggests that this may indeed be a viable method. These results indicate that the finding of an elevated T/E ratio (ie > 3:1) and a large T/E variability (ie > 30 %CV) is statistically uncommon in an athletic population. Thus those subjects with Pattern Type 3 are of interest since the large variability in T/E ratio results indicates variable excretion of T and E which has been seen in persons who are receiving exogenous aqueous testosterone or in persons whose pituitary-testicular endocrine system has been variably suppressed by anabolic steroids[4 5]. In addition, the finding of an isolated "T/E spike" as is seen in the Pattern Type 2 cases is strong evidence that exogenous testosterone use has occurred.

FUTURE PLANS:

Investigations are underway in various laboratories that are evaluating the endocrine and metabolic changes that result from the use of exogenous testosterone or other synthetic anabolic steroids. Donike[6] has described the use of urine "steroid profiles" to detect previous exogenous testosterone or anabolic steroid use and consists of comparing the relative quantities of various androgenic steroid metabolites in urine. Donike has found that the excretion of various androgenic steroids is altered in individuals who have used synthetic anabolic steroids or exogenous testosterone. This current study is presented as a preliminary evaluation of the intra-individual variability of T/E ratios in a large population. All data from these subjects are stored on tape and subsequent evaluation will consist of the determination of the quantitative levels of testosterone, epitestosterone, and other endogenous androgenic steroids present in each sample. Thus, a combined evaluation of both the "steroid profile" and T/E variability over time will be investigated, and may be of additional benefit for the detection of anabolic steroid doping. Additional samples from these subjects are continually being analyzed and thus future decoding of these specimens will allow for this database to be continuously evaluated over time.

Additional measurements of urinary luteinizing hormone (LH) levels, plus serum hormone determinations when available will also be incorporated into the on-going study of these subjects.

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3. Sample, RHB and Baenziger, JC. Gas Chromatography/Mass Spectrometric Detection of Anabolic Steroids. In, Analytical Aspects of Toxicology, edited by Deutsch, John R. Wiley and Sons, Inc., New York, 1989.

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Figure 1: T/E ratios from all athletes' samples received during 1990-91.

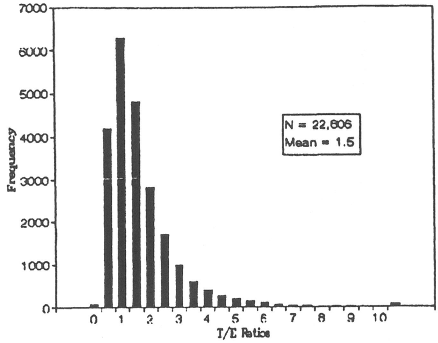

Figure 2: Distribution of T/E ratios from 796 subject athletes (who had 3 or more T/E values) received during 1990-91, and associated with 4,310 individual results.

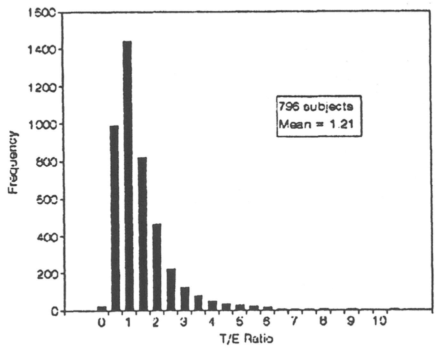

Table 1: Percentile cut-off levels for T/E ratios from the populations of all athletes, subjects from this study, and subsets of study subjects.

Statistic/Percentile Cut-off	#	90%	95%	99%
T/E's of all athletes 1990-91	22806	3-3.5		6.5-7
T/E's of 1285 subjects	4973	2.3	3.2	5.2
T/E Mean's of subjects	1285	2.2	2.9	5.0
T/E Mean's of subjects with 3 or > values	796	2.2	2.9	4.3

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Figure 3: Distribution of T/E Means from the 1285 subjects.

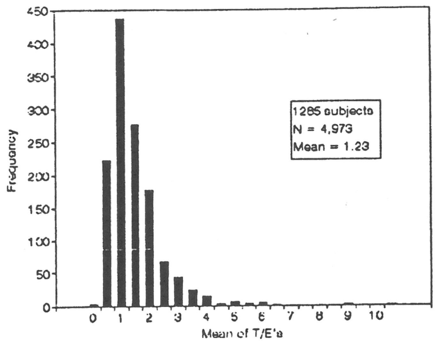

Figure 4: Distribution of T/E Means from those subjects with at least 3 values.

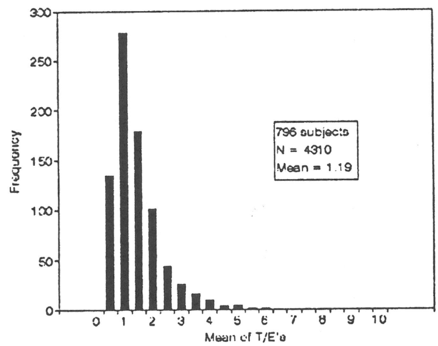

Figure 5: Distribution of the number of T/E values on the 1285 subjects.

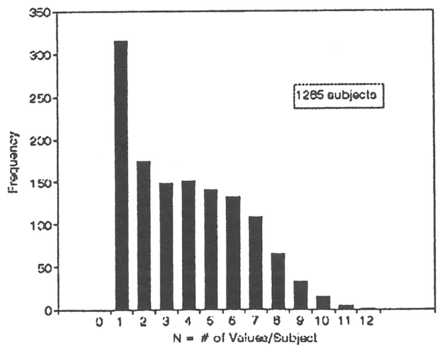

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Figure 6: Distribution of subjects' standard deviations of T/E ratios.

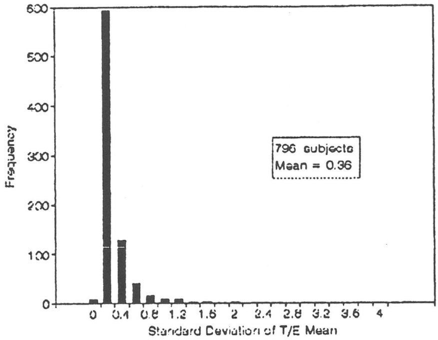

Figure 7: Comparison of subjects' T/E mean versus the standard deviation of the T/E measurements.

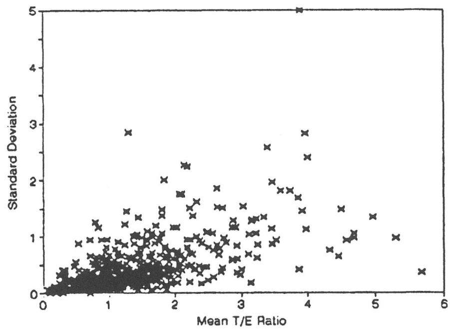

Table 2: Percentile cut-off levels for the standard deviation (SD) determinations grouped by T/E (mean) ranges.

Statistic/Percentile Cut-off	#	90%	95%	99%
SD of T/E's 0-1	405	0.38	0.52	0.95
SD of T/E's 1-2	284	0.81	1.01	1.45
SD of T/E's 2-3	68	1.5	1.76	2.24
SD of T/E's > 3	35	1.96	2.16	2.8
SD of T/E's of all subjects	796	0.87	1.16	1.96

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Figure 8: Distribution of Percent Coefficient of Variation of subjects' T/E measurements.

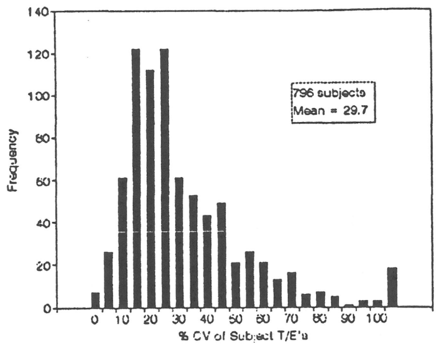

Figure 9: Comparison between the Percent Coefficient of Variation (%CV) of the subjects' T/E measurements versus the subject's mean T/E.

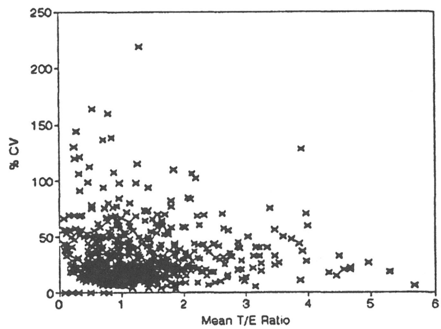

Table 4: Percentile cut-off level for the Percent Coefficient of Variation (%CV) of the subjects' T/E measurements grouped by T/E (mean) ranges.

Statistic/Percentile Cut-off	#	90%	95%	99%
%CV of T/E's 0-1	405	57	75	136
%CV of T/E's 1-2	284	55	68	98
%CV of T/E's 2-3	68	58	70	102
%CV of T/E's > 3	35	56	71	75
%CV of T/E's of all subjects	796	58	72	122

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Figure 10: Type 1 pattern of T/E variability (33 of 97 subjects) examples showing low variability; ie %CV < 30, and T/E > 3. (A = 6 %CV; B = 20 %CV; C = 19 %CV)

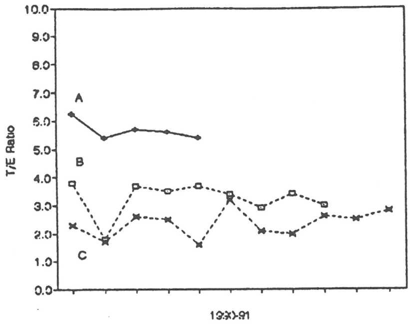


Figure 11: Type 2 pattern of T/E variability (38 of 97 subjects) examples showing a "spike" change in an athlete's T/E ratios.

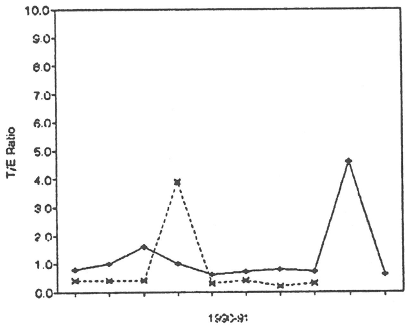
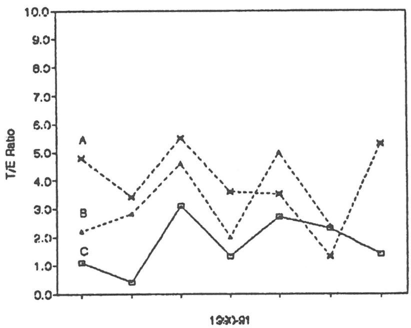


Figure 12: Type 3 pattern of T/E variability (21 of 97 subjects) examples showing a high degree of variability;5ie %CV > 30 and T/E above 3. (A = 37 %CV; B = 55 %CV; C = 41 %CV)



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