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Longitudinal Variability of T/E Ratios in Australian Athletes

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Introduction

The analysis of routine urine samples provides information on the steroid profiles of the subjects. The Australian Sports Drug Testing Laboratory (ASDTL) has been collecting such data for several years but until last year there was no way of determining which of the samples came from the same subject. With the cooperation of the Australian Sports Drug Agency (ASDA) it was possible to link samples so that longitudinal data could be obtained. In the process the athlete's anonymity to the laboratory was maintained. In previous longitudinal studies Donike et al (1994) have reported T/E variability under a range of conditions for a relatively small number of athletes. The study conducted here was similar to that reported by Baenziger and Bowers (1994) except that more subjects were involved over a much longer period of time. So far only the data for the male subjects have been reviewed.

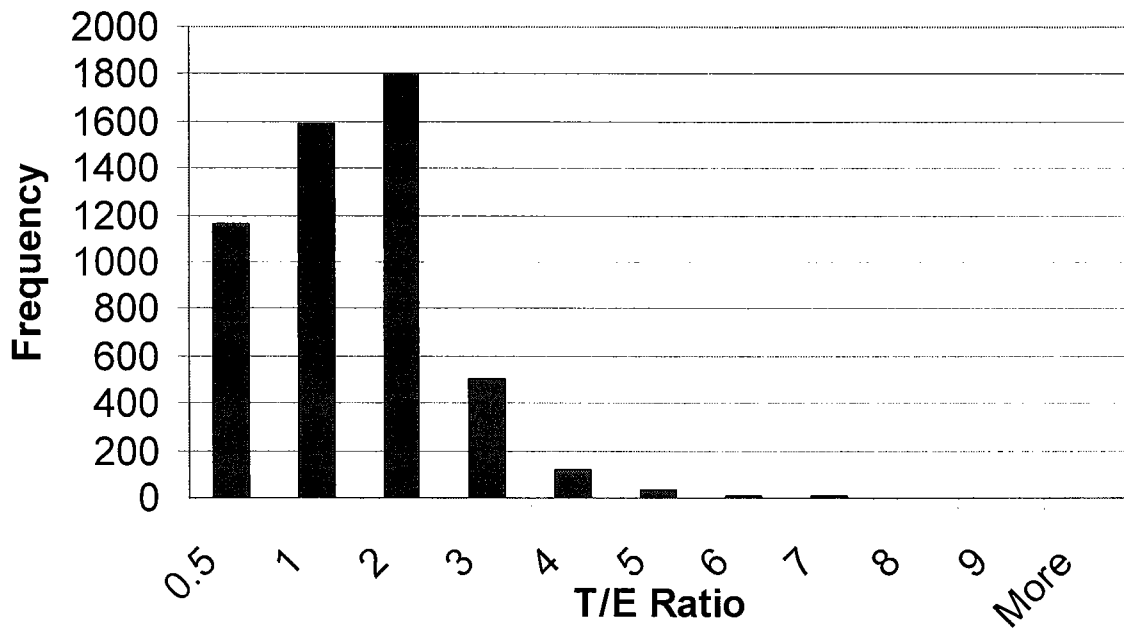
Method

The values of the T/E ratios used were those reported from the routine steroid screen. The steroid screen uses hydrolysis with β -glucuronidase from *E. Coli* followed by extraction of the steroids and analysis of the enolTMS derivatives on a 17m Ultra 1 column using a HP 5970 or 5973 MSD in selected ion monitoring mode. The chromatographic conditions have not changed in the period of the analyses reported. A standard is used to calibrate the testosterone and epitestosterone before each batch is analysed and a urine sample with a known elevated T/E ratio is extracted with each batch. When samples from the same athlete were seen to have a coefficient of variation of more than 25% the raw data was checked to eliminate the possibility of transcription or data integration errors. The data from 1159 male athletes having three or more tests in the period from 1995 to 1999 is presented. The mean number of collections per athlete was 4.5 with a maximum of 24.

Results

The distribution of T/E ratios is shown in Figure 1 and is similar to that typically found for a male population with the mean value at 1.15.

Figure 1 Histogram of Male T/E



Shown below in Figure 2 is a comparison between the percent coefficient of variation of the subject's T/E measurements versus their mean T/E. It can be seen that there is a clear tendency for the coefficient of variation to decrease as the T/E ratio increases. Table 1 summarises the variability data.

Figure 2 Variation of CV with T/E for all Males

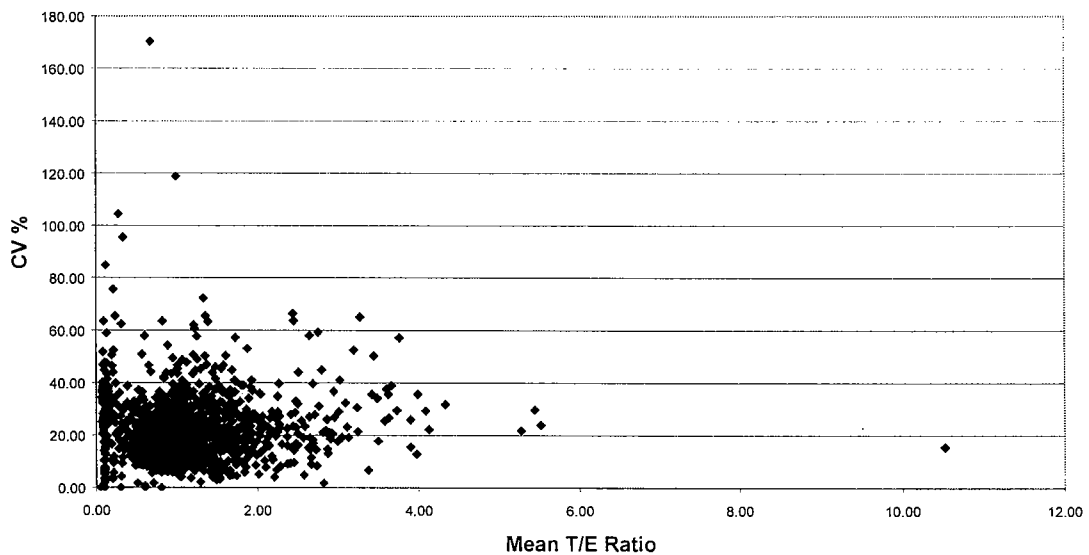
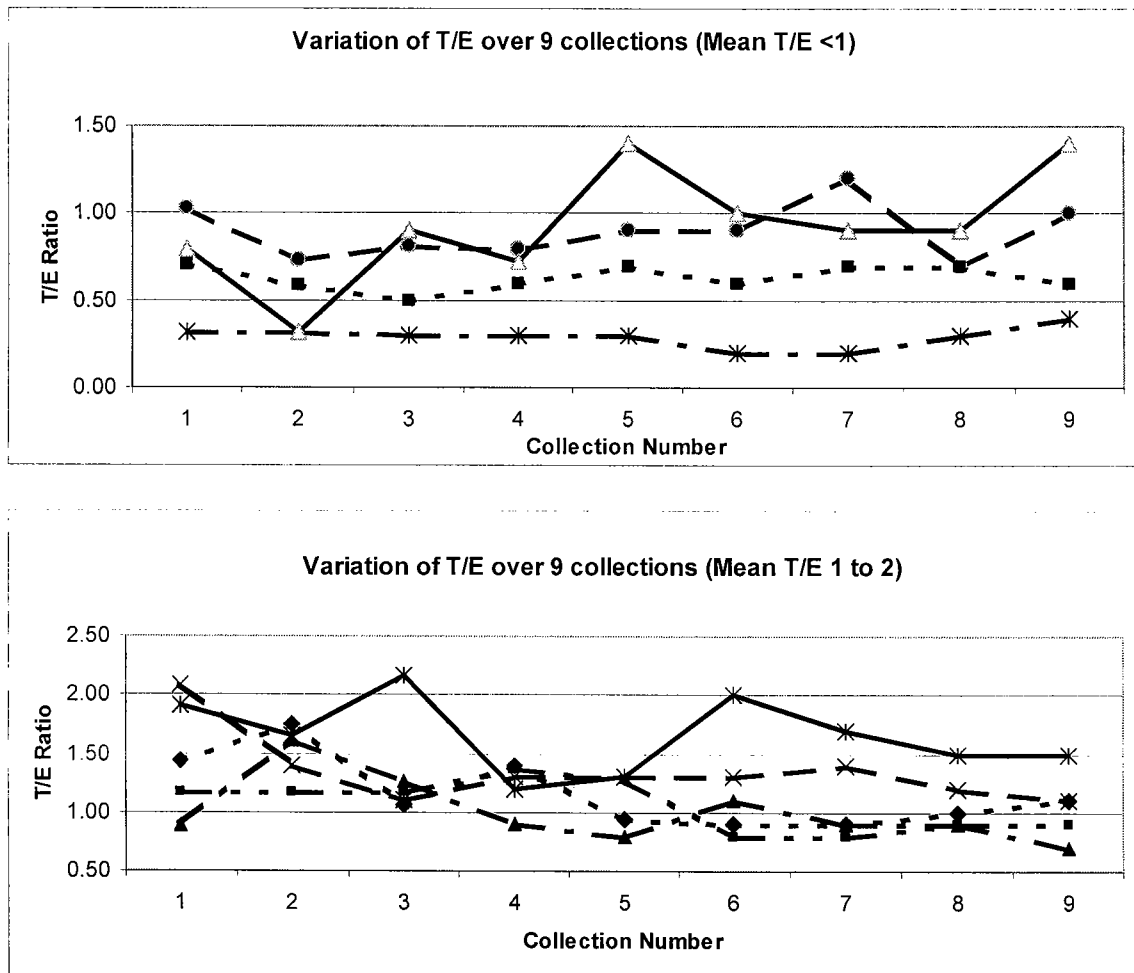


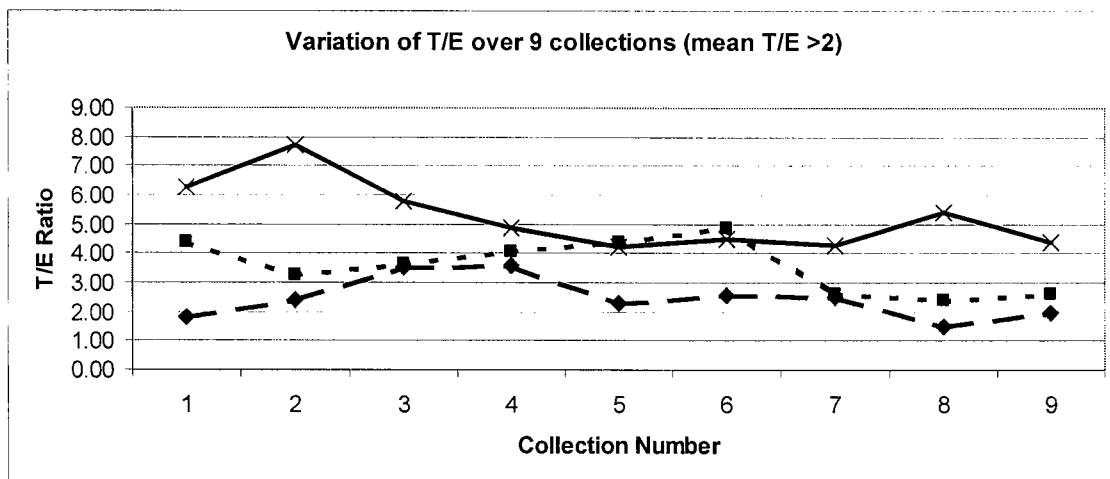
Table 1
Variation in Male T/E Ratios

T/E Ratio Range	Mean CV	Maximum CV	% of CVs above 60
> 3 (N= 34)	29.80	65.18	3
2 to 3 (N= 101)	22.43	66.50	2
1 to 2 (N= 456)	22.60	72.29	1
< 1 (N= 568)	22.44	170.37	1.7

Some typical variations in T/E ratio over time are shown in Figure 3 below for 12 subjects with 9 collections in the period from 1995 to 1999.

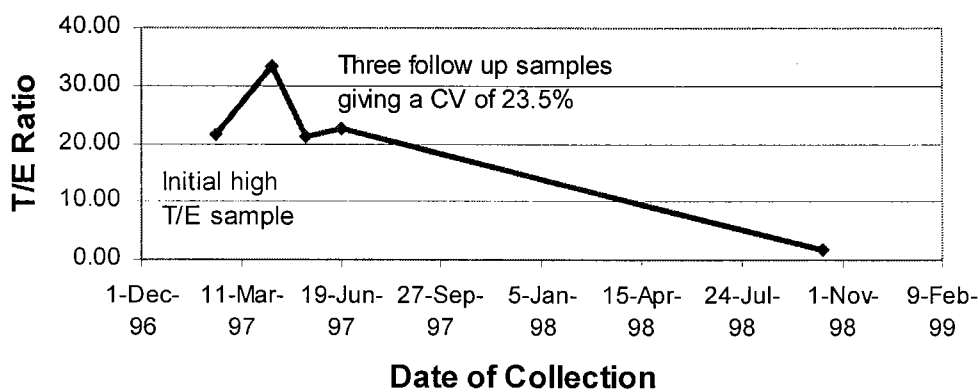
Figure 3





In reviewing the data a case was found (Figure 4) where an athlete who had an elevated T/E ratio was able to maintain this ratio at a consistent high level over the next three months when three more unannounced samples were taken. When another sample was taken over a year later the T/E ratio had dropped markedly. Such controlled doping would now be readily detected using carbon isotope ratio mass spectrometry.

Figure 4 Controlled doping



Conclusions

The long term data on T/E ratios has confirmed that this parameter is relatively stable over several years with no CVs greater than 40% being found for samples with a mean T/E of 4 or greater. All cases with CVs above 70% occurred at low T/E values where a relatively small change in either the T or E concentration can produce a large change in the T/E ratio.

The data is to be put into an updated database to enable more effective monitoring of steroid profiles to select those athletes which warrant further investigation using carbon isotope ratio mass spectrometry and additional sampling.

References

M. Donike, S. Rauth, U. Mareck-Engelke, H. Geyer and R. Nitschke, Evaluation of longitudinal studies, the determination of subject based reference ranges of the testosterone/epitestosterone ratio, Recent Advances in Doping Analysis, Proceedings of the 11th Cologne Workshop, 1994, 33-39.

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