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STATISTICAL EVALUATION OF LONGITUDINAL STUDIES, PART 2: THE USEFULNESS OF SUBJECT BASED REFERENCE RANGES IN STEROID PROFILING

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ABSTRACT

The results presented in this paper demonstrate that the subject-based reference ranges react more sensitive to variations than the population-based reference ranges, so that subject-based reference ranges on endogenous hormone concentrations or ratios e.g. the T/E ratio are reliable tools to monitor this kind of doping. The results of longitudinal studies of the urinary steroid profile underline that the biosynthesis of the endogenous steroids is tightly controlled and that the metabolism is so constant that the stationary, homeostatic model is appropriate for calculating subject-based reference ranges.

INTRODUCTION

Because the misuse of anabolic androgenic steroids is detectable by screening with GC/MS for the parent compounds or their metabolites **[1]**, endogenous hormones as testosterone or dihydrotestosterone or peptide hormones are used to increase performance.

When searching for endogenous substances in urine as well as in blood we enface first a general problem that the substance(s) administered, the exogenous substance(s), is/are identical or very similar in chemical structure to that produced by the body so that the analytical methods cannot differentiate between applied and endogenous product.

The second problem is a clear differentiation between the concentration found normally in body fluids and the increased level after application of endogneous hormones. The approach used in monitoring the widely used testosterone application is to determine the relation to an endogenous substance not affected by the application i.e. the testosterone/epitestosterone ratio. In both cases, concentrations and ratios, an interpretation is necessary whether the measured value is "normal". The decision limit set by the IOC Medical Commission 1982 for the testosterone/epitestosterone ratio is based on a population-based reference range **[2]**.

APPROPRIATENESS OF POPULATION-BASED REFERENCE RANGES

The appropriateness of a population-based reference range for the interpretation of an individual measurement can be judged by the ratio of intra- to inter-individual variation of the parameter (ratio r) **[3]**. Steroid profiling from research samples **[4,5]** and out of competition controls of the German decathlon team allow to calculate the ratio r for all parameters of the steroid profile (Table 1).

(1) $$r = \sqrt{\frac{E\sigma^2}{Var\mu_i}}$$

$E\sigma^2$: average of the intra-individual variances ($\approx \overline{s_i^2}$)

$Var\mu_i$: variance of the individual mean values ($= s^2_{\bar{x}_i}$)

Table 1: Relation of the intra- to the interindividual variance (ratio r according to formula 1) of the concentrations [ng/ml] of the endogenous steroids and the concentration ratios (20 members of the German decathlon team, data base 1992) **[6]**
$E\sigma^2$: average of the intra-individual variances
$Var\mu_i$: variance of the individual mean values

	$E\sigma^2$	$Var\mu_i$	Quotient r
AND	0.023	0.018	1.25
ETIO	0.030	0.037	0.79
EPIT	0.028	0.061	0.45
TEST	0.030	0.134	0.22
Adiol	0.033	0.026	1.26
Bdiol	0.073	0.120	0.61
Pregnd	0.032	0.036	0.88
AND/ETIO	0.005	0.036	0.15
TEST/EPIT	0.008	0.179	0.04
AND/TEST	0.010	0.078	0.12
AND/EPIT	0.013	0.146	0.09
ETIO/TEST	0.010	0.132	0.08
ETIO/EPIT	0.010	0.065	0.15
Adiol/Bdiol	0.009	0.076	0.12

In summary, Harris **[3]** gives the following interpretations for the ratio r:

1. $r \leq 0.6$: the subject based reference range reacts more sensitive to variations of the steroid concentrations and the steroid ratios than the population based reference range.
2. $r > 0.6$ und $r < 1.4$: subject based reference range and population based reference range are in the first approximation equivalent.
3. $r > 1.4$: the population based reference range is more reliable than the subject based reference range, at least for individuals whose variance is equal to the average variance ($E\sigma^2$).

For the steroid concentrations, except for testosterone and epitestosterone, the ratio r lies between 0.6 and 1.4 so that the population based reference range is a useful tool. For the testosterone and epitestosterone concentration and especially for the steroid ratios the ratio r is lower than 0.6 which demonstrates that the population based reference range is quite insensitive for monitoring variations in a person.

In Figure 1 the means of the T/E ratio plus/minus two times the standard deviations of 20 members of the German decathlon team are plotted as well as the population based reference limits (95% range). The means of all athletes are lying within the population based reference range. Only the lower end of two person's distributions are below the lower reference limit. From the Figure 1 it is obvious that the population based reference range is very insensitive to individual variations (compare also the ratio r=0.04). Most of the athletes could have undetected increases of their T/E ratio of more than 4 times their individual standard deviations.

Apart from the variances, the magnitude of the intra-indivudual mean compared to the inter-individual mean is of importance. In doping control, this fact has been considered when monitoring T/E ratios between 6 and 10 which are due to low epitestosterone concentrations. The means of such athletes are lying apart from the population mean at the upper end or outside the distribution of the population. Having only a single measurement there is no way to interpret such a value as physiologically. Only a series of measurements can confirm the measurement as being in agreement with the individual's mean and variance.

In doping control the major advantage of sequential data of athletes's steroid profiles are a more precise interpretation of changes in the steroid ratios than the comparison to population based reference ranges. A prediction of the range in which the next value will fall can be made under the model of homeostasis of the steroid production and excretion. The use of subject

APPROPRIATE STATISTICAL MODEL

In the literature different statistical approaches are described to detect an "outlier" value out of an individual's series of data. Two main models are the homeostasis model and the "random walk" model. The first model presumes a stationary, strictly homeostatic variation so that a current observation be compared with the mean of all previous observations. The nonstationary model uses weighting of the consecutive measurements (time series) so that a current observation be compared with an exponentially smoothed average **[7]**.

The experiences gathered from research projects and from out of competition controls show that the ratios T/E, A/E in individuals are very constant due to the homeostasis of the endocrine steroid biosynthesis **[8]**. Longitudinal studies on the stability of the steroid profile **[4,5]** allow a comparison of the variance "between" and "within" a sampling period. 6 male volunteers collected urine samples over night for a period of one month. The first three days within a week were regarded as sampling period so that 4 sampling periods can be compared by ANOVA. In case of a significant F-statistics, a component of variance is attributable to the greater time spans between sampling periods, meaning that a time series model is more appropriate to the problem.

In Table 2 the summarizing descriptive statistics and the results of the analysis of variance for the T/E ratios are given for each volunteer. Figure 2 shows the means and standard deviations of the T/E ratio of 6 male volunteers in each week of the experiment. The means are calculated from the first three days of each week.

Table 2a: T/E ratio of men, sampling interval 4 weeks. Summarizing statistics and results of analysis of variance (ANOVA) for each of the volunteers 1, 2 and 3. (SS: sum of squares, df: degrees of freedom, MSS: mean sum of squares)

Person 1						
Weeks	*n*	*Sum*	*Mean*	*Variance*	*Stdev*	*Cv%*
1	3	5.38	1.79	0.02	0.15	8.36
2	3	5.98	1.99	0.00	0.05	2.43
3	3	5.31	1.77	0.05	0.22	12.50
4	3	4.89	1.63	0.02	0.14	8.65
ANOVA						
	SS	*df*	*MSS*	*F*	*P*	*critical F*
"between weeks"	0.20	3	0.07	2.88	0.10	4.07
"within weeks"	0.19	8	0.02			
Total	0.39	11				
Person 2						
Weeks	*n*	*Sum*	*Mean*	*Variance*	*Stdev*	*Cv%*
1	3	0.39	0.13	0.0003	0.02	13.86
2	3	0.37	0.12	0.0001	0.01	8.95
3	3	0.40	0.13	0.0004	0.02	15.05
4	3	0.40	0.13	0.0002	0.01	10.34
ANOVA						
	SS	*df*	*MSS*	*F*	*P*	*critical F*
"between weeks"	0.00	3	0.00	0.17	0.92	4.07
"within weeks"	0.00	8	0.00			
Total	0.00	11				
Person 3						
Weeks	*n*	*Sum*	*Mean*	*Variance*	*Stdev*	*Cv%*
1	3	2.95	0.98	0.042	0.20	20.83
2	3	2.73	0.91	0.001	0.03	2.88
3	3	2.58	0.86	0.001	0.03	3.51
4	3	2.86	0.95	0.000	0.02	2.22
ANOVA						
	SS	*df*	*MSS*	*F*	*P*	*critical F*
"between weeks"	0.03	3	0.01	0.77	0.54	4.07
"within weeks"	0.09	8	0.01			
Total	0.11	11				

Table 2b: T/E ratio of men, sampling interval 4 weeks. Summarizing statistics and results of analysis of variance (ANOVA) for each of the volunteers 4, 5 and 6. (SS: sum of squares, df: degrees of freedom, MSS: mean sum of squares)

Person 4						
Weeks	*n*	*Sum*	*Mean*	*Variance*	*Stdev*	*Cv%*
1	3	2.58	0.86	0.00	0.03	3.34
2	3	2.56	0.85	0.00	0.06	7.05
3	3	2.61	0.87	0.01	0.07	8.18
4	3	2.79	0.93	0.03	0.17	18.31
ANOVA						
	SS	*df*	*MSS*	*F*	*P*	*critical F*
"between weeks"	0.01	3	0.00	0.40	0.76	4.07
"within weeks"	0.08	8	0.01			
Total	0.09	11				
Person 5						
Weeks	*n*	*Sum*	*Mean*	*Variance*	*Stdev*	*Cv%*
1	3	3.18	1.06	0.01	0.10	9.16
2	3	3.55	1.18	0.09	0.30	25.66
3	3	2.76	0.92	0.01	0.11	12.10
4	3	2.88	0.96	0.03	0.19	19.38
ANOVA						
	SS	*df*	*MSS*	*F*	*P*	*critical F*
"between weeks"	0.12	3	0.04	1.11	0.40	4.07
"within weeks"	0.30	8	0.04			
Total	0.42	11				
Person 6						
Weeks	*n*	*Sum*	*Mean*	*Variance*	*Stdev*	*Cv%*
1	3	18.08	6.03	0.22	0.47	7.84
2	3	14.35	4.78	0.51	0.71	14.89
3	3	15.62	5.21	0.41	0.64	12.35
4	3	17.16	5.72	0.13	0.36	6.37
ANOVA						
	SS	*df*	*MSS*	*F*	*P*	*critical F*
"between weeks"	2.73	3	0.91	2.85	0.11	4.07
"within weeks"	2.55	8	0.32			
Total	5.28	11				

None of the F-values is significant, i.e. the assumption of the homeostatic model for the T/E ratio is strenthened. One month is a short time period and further investigations are necessary to confirm this result.

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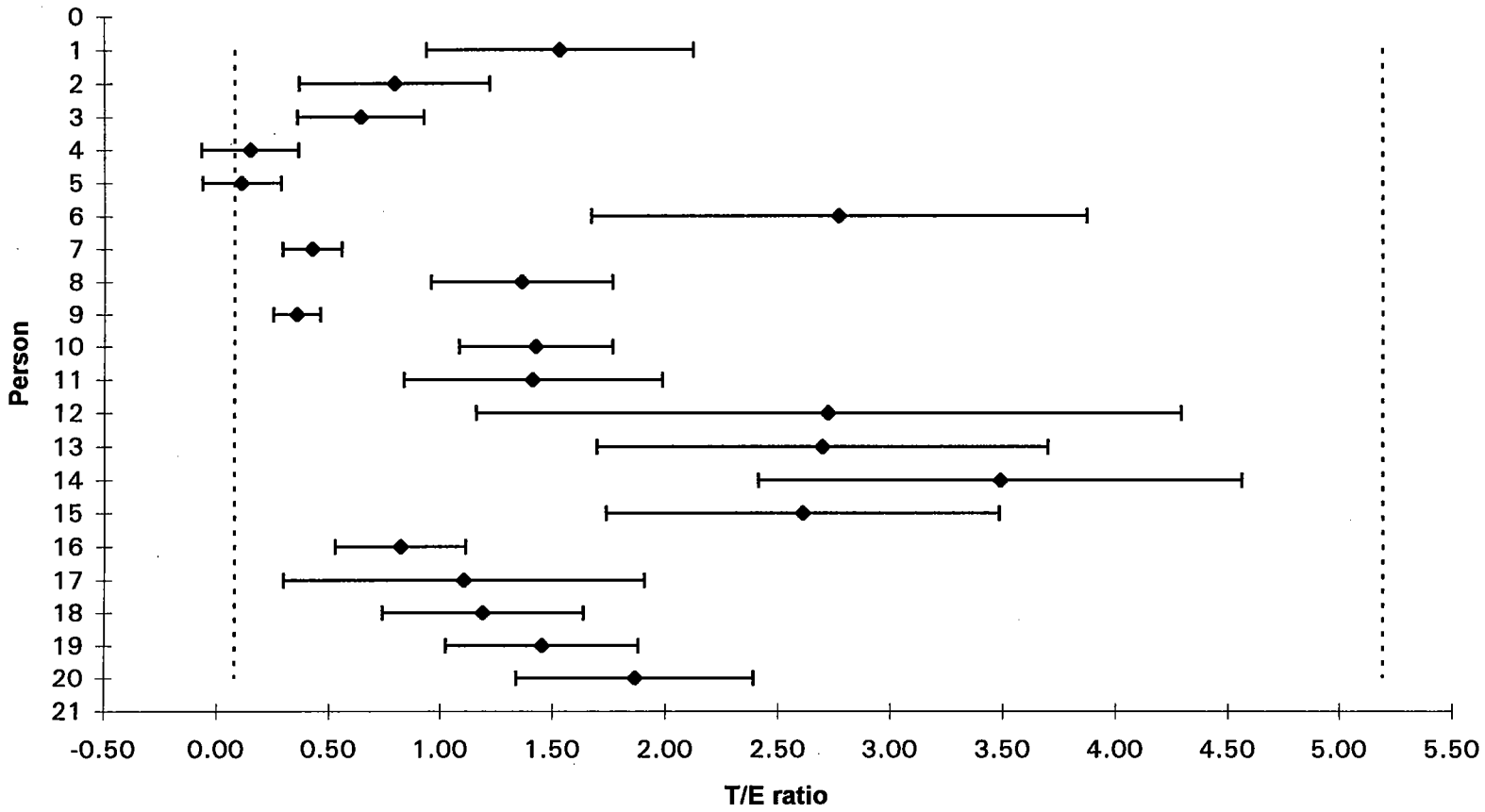

Figure 1: Means (filled diamond) and standard deviations (vertical bars) of the T/E ratio of 20 members of the German decathlon team; lower (0.08) and upper (5.19) reference limits of the population-based reference range [4] are shown as dotted lines.

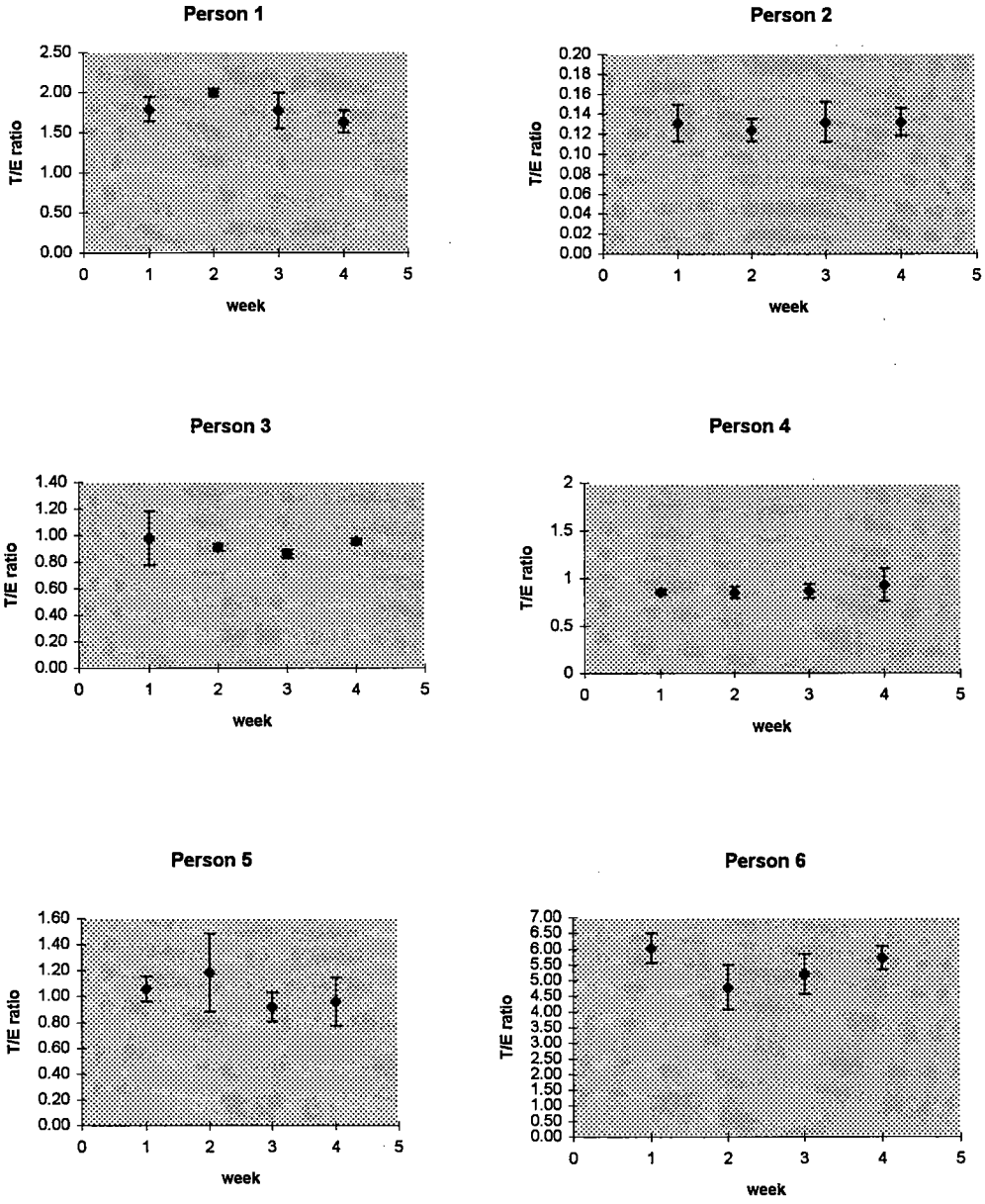

Figure 2: Means and standard deviations of the T/E ratio of each volunteer in each sampling period (week).