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RECENT ADVANCES IN DOPING ANALYSIS

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Evaluation of Longitudinal Studies, the Determination of Subject Based Reference Ranges of the Testosterone / Epitestosterone Ratio

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The observations made in the past in measuring the steroid profile prove that for a given individual the excreted quantities of endogenous steroids are more or less constant [1]. The reason for this is the homeostasis of biosynthesis and metabolism of endogenous steroids [2, 3]. The concentrations of the eliminated endogenous steroids vary according to the urinary flow, whereas the concentration ratios of steroids are more constant [4].

The homeostasis of the endocrine steroid biosynthesis and metabolism is not disturbed by physical workload at least not to such an extend that the T/E ratio of an individual is altered [5]. The T/E ratios in longitudinal studies fluctuate around the mean with a CV which will not exceed 30%.In table 1 the T/E ratio of sport students with moderate physical activity are summarized. High work load will also not influence the T/E ratio to a greater extend. The T/E ratios of a professional cycling team participating in the Tour de France 1992 with 9 riders are presented in table 2. Training in high altitude had also no influence on the T/E ratio as we observed in following up the German rowing team before the 1988 Olympic Games in Seoul.

In case of testosterone application, the concentration of testosterone glucuronide will be increased more than any of the other testosterone metabolites leading to a significant increase of the individuals ratios of testosterone to epitestosterone [6, 7]. Depending on the route of administration, the dose, chemical structure of the ester etc. the T/E ratio will be elevated for some time before it returns to normal.

The increase of the T/E ratio is more prominent than the increase of other ratios within the steroid profile because testosterone is not metabolized to epitestosterone but contributes to androsterone and etiocholanolone [6, 7]. But nevertheless, also the ratios of testosterone to androsterone or etiocholanolone are increased and maybe used as additional parameters.

To monitor testosterone application the question to answer is, if the ratio observed in an athlete's urine is higher than "normal".

Two possible approaches to answer this question are:
1. Comparison with a **population-based** reference range [8]
2. Comparison with the **subject-based** reference range.[8, 9]

The first approach has been chosen 1982 by the IOC Medical Commission to define a decision limit for testosterone application (T/E=6) [10, 11]. This ratio discriminates testosterone users from non-users only when the epitestosterone concentration is proportional to the concentrations of the other endogenous steroids. In case of a low epitestosterone concentration a few individuals may have a T/E ratio of higher than 6. This observation led to the decision of the IOC Medical Commission to recommend a follow-up of T/E ratios between 6 and 10 by longitudinal studies, to exclude physiological reasons for an elevated T/E ratio [12].

The proposal of the IOC Medical Commission to collect data in longitudinal investigations for an athlete found to have a T/E-ratio higher than 6 and lower than 10 provides the data to calculate a subject-based reference range [9].

The necessary data may be obtained:
1. by requesting from the authorities documented T/E ratios of previous tests - retrospective data
2. by additional unannounced in and/or out of competition doping controls - prospective data
3. a study under quarantaine-like conditions.

Evaluation of the longitudinal approach

The problem can be statistically defined as the discrimination of an outlier from the series of T/E-ratios measured on the same individual. An outlier can be defined e.g. as a value lying outside the mean ±4 times the standard deviation calculated without the suspicious value.

For a consensus approach the following parameters must be defined:
1. the number of T/E ratios (in addition to the suspicious value)
2. the statistical probability
3. the time interval in which additional samples shall be collected
4. the acceptance of retrospective values
5. the definition of an outlier.

Proposal for the calculation of the subject-based reference range [9]:
1. calculate the mean out of at least 3 observations
2. calculate the limit for the outliers (l_o) following the formula below:

$$l_o = \text{mean} + t(p,n) * \text{stdev}$$

Note: All factors which may influence the analytical measurements will lead to a greater standard deviation, e.g. values determined in different laboratories or extent of physical workload. The greater standard deviation will give a higher limit for outliers and by this be in favor of the athlete.

Table 1: T/E-ratio of 5 male volunteers under resting conditions; urine samples taken in the morning [1].

cyclist	n	mean	s	c.v.%	min	max
1	30	1.82	0.21	11.4	1.41	2.20
2	30	0.13	0.01	11.0	0.11	0.16
3	30	0.91	0.10	11.0	0.79	1.22
4	30	0.90	0.15	16.2	0.69	1.46
5	30	1.04	0.17	16.0	0.76	1.53

Table 2a: T/E-ratio of 9 cyclists participating in the Tour de France 1992; urine samples collected over night.

cyclist	n	mean	s	c.v.%	min	max
1	9	1,81	0,34	18,82	1,36	2,45
2	14	0,22	0,06	27,69	0,10	0,34
3	23	2,02	0,15	7,47	1,72	2,38
5	22	1,85	0,24	13,02	1,36	2,44
6	11	0,12	0,04	38,00	0,09	0,24
7	21	1,22	0,16	12,67	0,78	1,48
8	15	0,84	0,08	9,24	0,69	0,96
9	21	1,87	0,34	18,24	1,28	2,55

Table 2b: T/E-ratio of 9 cyclists participating in the Tour de France 1992; urine samples taken after each stage.

cyclist	n	mean	s	c.v.%	min	max
1	7	2,34	0,28	12,11	1,90	2,72
2	11	0,24	0,08	31,88	0,10	0,35
3	18	2,34	0,36	15,25	1,56	2,78
5	21	2,38	0,59	24,80	1,21	3,55
6	9	0,13	0,02	19,08	0,10	0,18
7	18	1,32	0,21	15,82	0,95	1,60
8	12	0,76	0,14	18,25	0,62	1,07
9	19	1,80	0,32	17,77	1,36	2,61

Table 3: T/E-ratio of the German rowing team (males) in a training camp in high altitude preparing the Olympic Summer Games in Seoul from the 18th August till the 7th September 1988.

Urine samples have collected over a time period of about 12 hours overnight.

rower	N	mean	stdev	vc %	min	max
1	7	1,99	0,14	7,09	1,76	2,11
2	6	4,04	0,53	13,21	3,20	4,81
3	6	0,87	0,20	23,41	0,68	1,24
4	5	0,93	0,14	14,99	0,80	1,17
5	5	2,79	0,30	10,88	2,42	3,10
6	6	0,08	0,02	24,79	0,06	0,11
7	7	1,06	0,22	20,81	0,81	1,38
8	5	1,39	0,17	12,36	1,20	1,63
9	4	1,79	0,40	22,55	1,40	2,27
10	6	2,63	0,57	21,50	1,59	3,25
11	6	0,72	0,07	9,32	0,63	0,82
12	0	no data	no data	no data		
13	5	0,74	0,07	9,18	0,63	0,79
14	7	0,51	0,13	26,29	0,34	0,67
15	7	0,93	0,19	20,68	0,68	1,23
16	6	0,20	0,03	13,87	0,17	0,24
17	7	1,77	0,29	16,38	1,27	2,10
18	6	3,93	0,97	24,77	2,92	5,38
19	7	0,76	0,16	20,69	0,52	1,00
20	6	0,84	0,14	16,56	0,70	1,08
21	6	0,17	0,04	20,93	0,13	0,22
22	7	1,15	0,19	16,48	0,86	1,43
23	7	0,81	0,24	29,61	0,50	1,11
24	6	0,10	0,05	52,47	0,06	0,20

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