

Reprint from

RECENT ADVANCES  
IN DOPING ANALYSIS  
(6)

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(Editors)

Sport und Buch Strauß, Köln, 1999

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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in  
doping analysis (6). Sport und Buch Strauß, Köln, (1999) 237-242

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## **KETOCONAZOLE TEST - A CASE STUDY**

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### **ABSTRACT**

High urinary testosterone to epitestosterone ( T/Et ) ratio was detected in an athlete during routine antidoping test. The athlete was punished by the sport federation and appealed to the Polish Commission Against Doping Use in Sport. Further five tests showed that the elevated T/Et ratio was associated with fluctuating level of T and relatively low and fluctuating level of Et. Luteinising hormone (LH) was in norm. To clarify the reason of the elevated T/Et ratios ketoconazole test (Test1) was performed. In urine, the test showed typical, decreasing response of T and Et to ketoconazole. T in plasma blood also decreased. Ketoconazole test was repeated in the same athlete four months later (Test2) in a closed clinic. T, Et and LH in urine decreased. In plasma, T decreased while LH showed tendency to increase. Follicle-stimulating hormones (FSH) and sex -hormone-binding globulin (SHGB) were unchanged. It was concluded that the high and unstable T/Et ratio was endogenous in origin and that the athlete should be free from suspicion of testosterone abuse. Since other medical tests performed by methods of radiology, ultrasonography and computer tomography did not reveal any pathological changes in the athlete organism , the reason of fluctuation of T and E associated with low level of Et remained unclear.

## **INTRODUCTION**

Medical Commission of the International Olympic Committee and the monitoring Group of Anti-Doping Convention of the Council of Europe commonly accepted that the presence of testosterone (T) to epitestosterone (Et) ratio greater than six (6) to one (1) in urine of competitor constitutes an offence unless there is evidence that this ratio is due to a physiological or pathological conditions e.g. low epitestosterone excretion, androgen producing tumour or enzyme deficiencies. In the case of T/Et ratio higher than 6, it is mandatory that the relevant medical authority conduct an investigation before the sample is declared positive. A full report will be written and will include a review of previous tests, subsequent tests and any results of endocrine investigations. In the event that previous tests are not available, the athlete should be tested unannounced at least once per month for three months. The results of these investigations should be included in the report.

The Polish Commission Against Doping Use in Sport has been applying this approach since the year 1993. Among some doubtful cases collected from that time the one, presented in this paper, seems to be especially interesting because of double ketoconazole tests applied during clarification procedure.

## **MATERIALS AND METHODS**

The study was performed on one male athlete (track and field) in whom a high urinary testosterone to epitestosterone ratio (T/Et) was detected during routine antidoping test. The athlete was, therefore, punished by the national sport federation and appealed to the Polish Commission Against Doping Use in Sport to consider the fact that he never used any anabolic steroids. The Commission analysed of available, retrospective data and found that a low level of epitestosterone would be reason of the increased T/Et ratio. Five further antidoping tests performed in the athlete did not bring any clear solution but confirmed the low level of epitestosterone. In order to exclude influence of masking agents the Commission decided to perform two unannounced ketoconazole tests within the period of 4 months. During the second test the ketoconazole was given to the athlete twice in order to check the sensitivity of steroidogenesis inhibition and the restitution times. The subject was fully informed on the testing procedures and possible side effects of the ketoconazole application. Informed, written consent was obtained and the athlete co-operated well during the whole experiment.

Ketoconazole is a pharmaceutically licensed antifungal agent that also inhibits testosterone biosynthesis in men and can be used, therefore, in differentiating between athletes using exogenous testosterone and those having naturally elevated T/Et ratio [ 1, 2 ]. It is supposed that in the case of naturally elevated T/Et ratio the intake of ketoconazole will decrease testosterone concentration and T/Et value in urine. If elevated T/Et ratio is due to testosterone doping the intake of ketoconazole will not affect testosterone concentration and T/Et ratio or may increase both values in urine sample [ 3 ].

The experiments were performed in a closed clinic of the Medical Academy in Poznań. Test 1 lasted two days - control and ketoconazole dynamic test. Test 2, carried out 4 months later and preceded by 2 days of quarantine, lasted 3 days - control and two subsequent ketoconazole dynamic tests. The hours of taking of urine and blood samples are depicted on the Figures 3 and 4. Ketoconazole was given in dose of 400 mg at the morning of the test days [ 1 ]. Blood samples were analysed in the Clinic of Endocrinology of the Medical Academy. Urine samples, shipped in random order, were analysed in the Department of Antidoping Research, Institute of Sport in Warsaw.

Concentrations of testosterone (T), epitestosterone (Et), androsterone (A), etiocholanolone (E) and lutropin (LH) were measured in the urine samples. T/Et and A/E ratios were calculated. In blood plasma, concentrations of testosterone, cortisol (Ct), lutropin, follitropin (FSH) and sex-hormone-binding globulin (SHBG) were measured. At the end of the experiment an additional medical checking by the methods of radiology, ultrasonography and computer tomography was performed in the athlete.

## RESULTS

Antidoping tests revealed that urinary T/Et ratio varied between 6 and 16 with a median value of about 7.8 (Fig.1). The values, as well as their distribution, were far away from any accepted level enabling samples to consider as a negative.

Analysis of absolute values of testosterone and epitestosterone showed that both hormones fluctuated within the range of 60-130 ng/ml and 8-16 ng/ml, respectively (Fig. 2). The unstable and fluctuating level of Et seemed to be a reason of the elevated T/Et ratios.

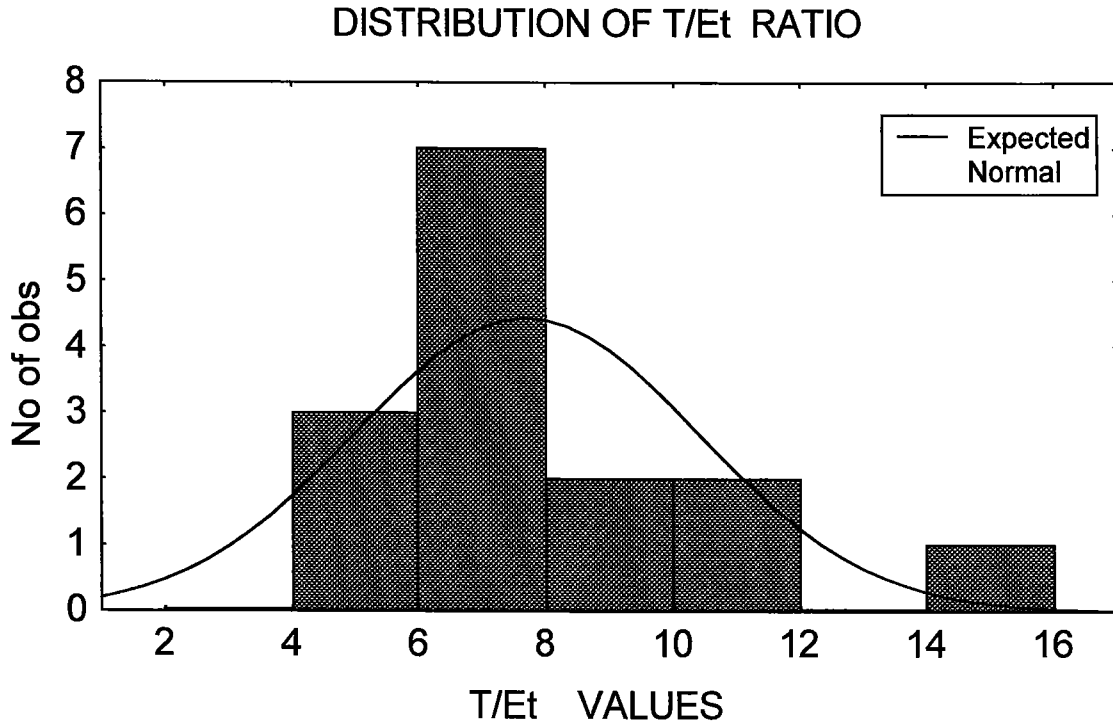


Figure 1. Retrospective and current values of T/Et ratio in an athlete accused of using of testosterone doping.

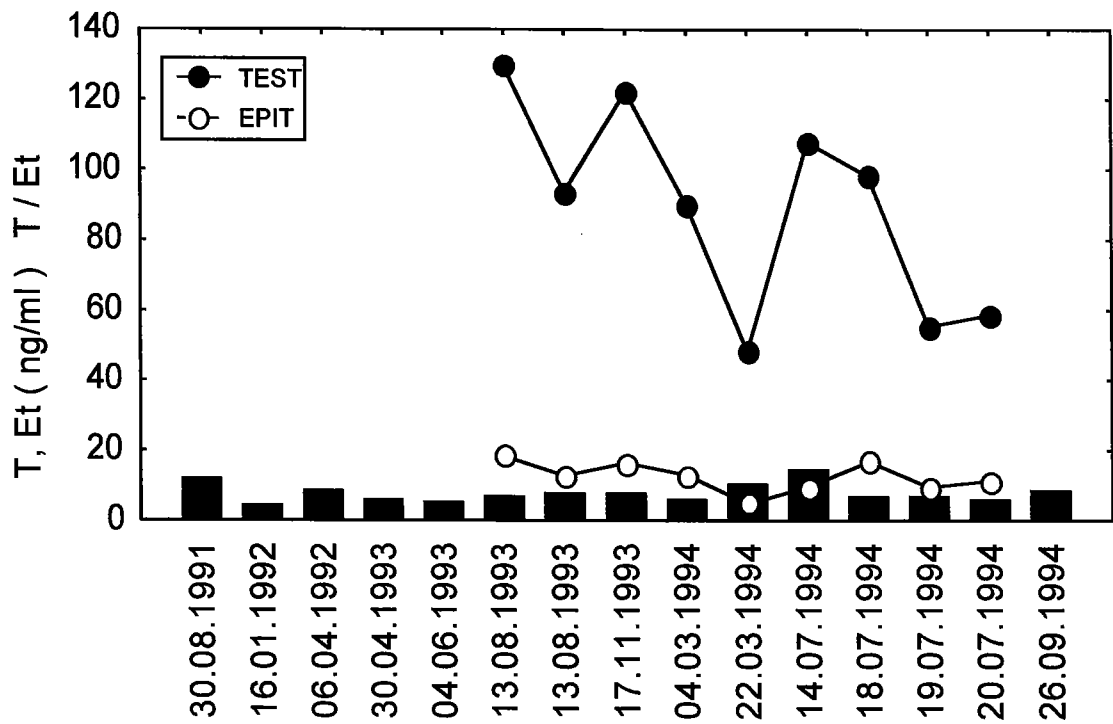


Figure 2. Absolute values of testosterone (T), epitestosterone (Et) and T/Et ratios in available retrospective data.

During Test1 testosterone and epitestosterone in urine showed typical response to ketoconazole decreasing from 89.0 to 15.9 ng/ml and from 32.3 to 3,3 ng/ml, respectively (Fig.3). Plasma blood T decreased from 22.3 to 8.0 nmol/l. LH in urine and blood was not affected by the ketoconazole but LH tended to decrease in urine and to increase in blood.

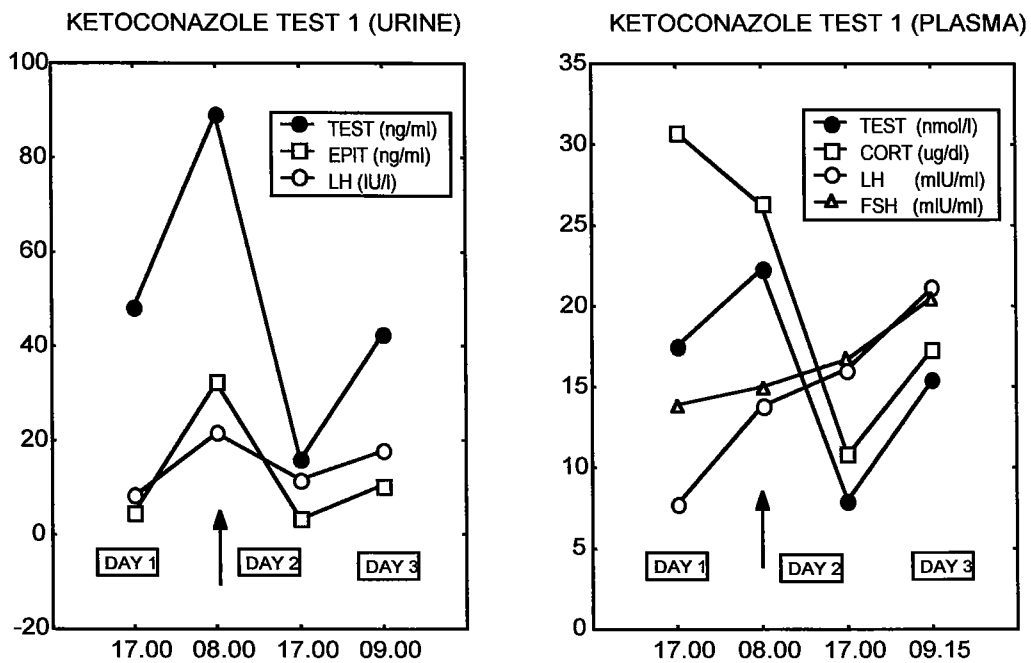


Figure 3. Changes in testosterone, epitestosterone, lutropin, cortisol and follitropin in athlete during ketoconazole Test1.

Data collected during ketoconazole Test2 are presented in Fig.4. In urine, T, Et and LH decreased after first application of ketoconazole (day 2) as follows: from 69.0 to 9.0 ng/ml, from 10.0 to 2.0 ng/ml and from 11.0 to 1.8 IU/l. T in plasma blood decreased from 18.1 to 3.5 nmol/l while LH showed tendency to increase from 9.5 to 12.6 mIU/ml. Follitropin (FSH) and sex-hormone-binding globulin (SHBG) were unchanged (12.3 vs. 12.6 mIU/ml and 32.6 vs. 32.9 nmol/l). The second phase of the experiment (day 3) did not bring qualitative differences in hormonal responses to ketoconazole except the tendency to decrease in LH and FSH observed in plasma.

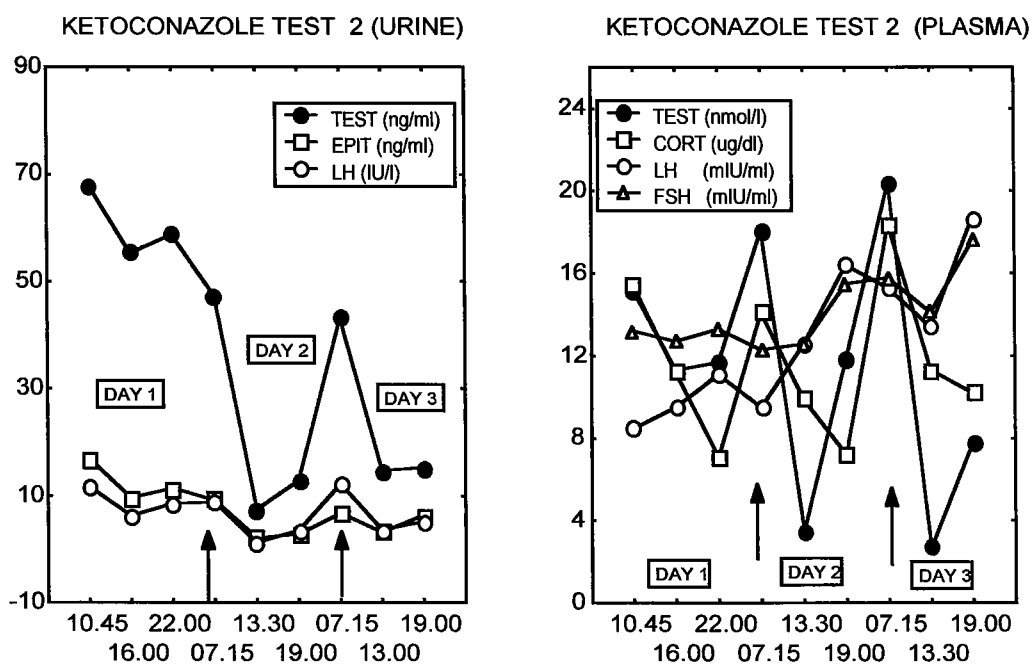


Figure 4. Hormonal responses to ketoconazole Test 2 in urine and blood plasma.

## CONCLUSIONS

1. It was concluded that the high and unstable T/Et ratio is endogenous in origin and that the athlete should be free from suspicion of testosterone abuse. Since other medical tests did not revealed any pathological changes in the athlete organism the reason of fluctuations in T and Et remained unclear.
2. The application of ketoconazole test may be useful in clarification procedures of some doubtful doping cases.
3. The recovery after ketoconazole is relatively fast. After 24 hours of its application organism is able to response to the new dose of ketoconazole with similar hormonal sensitivity .

## REFERENCES

1. Kicman A.T., H. Oftebro, Ch. Walker, N. Norman, D. Cowan (1993). Potential use of ketoconazole in a dynamic endocrine test to differentiate between biological outliers and testosterone use by athletes. *Clinical Chem.*, 39:1798-1803.
2. De Boer D., T.J.A. Waasdorp, P. Schoone, J.H.H. Thijssen (1994). The power of ketoconazole test. *Proc. 8<sup>th</sup> Cologne Workshop on Dope Analysis, Sport und Buch Strauss.*
3. Oftebro H. (1992). Evaluating an abnormal urinary steroid profile. *Lancet*, 339:941-942.