Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (7)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

Sport und Buch Strauß, Köln, 1999

R. GRUCZA, J. SMORAWINSKI, D. KWIATKOWSKA:
Application of Ketoconazole Test in Doubtful Doping Cases of Polish Athletes
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (7). Sport und Buch Strauß, Köln, (1999) 347-352

R. Grucza, J. Smorawiński *, D. Kwiatkowska

Application of Ketoconazole Test in Doubtful Doping Cases of Polish Athletes

Department of Antidoping Research, Institute of Sport, Warsaw, Poland;

*Academy of Physical Education, Poznań, Poland

Introduction

In healthy men the ratio of testosterone (T) to epitestosterone (Et) has found to be relatively constant. According to Donike et al. (1993) the ratio of T/Et is about 1.37 SD 1.07 in physically active students and about 1.43 SD 1.00 in amateur cyclists. In polish elite athletes a decreasing tendency in T/Et ratio has been observed during the years 1991-1996, varying in men from 2.10 SD 1.40 to 1.60 SD 1.19 and in women from 1.38 SD 0.69 to 0.88 SD 0.64 (Grucza et al. 1997). The reference values of T/Et ratio are far below the level established by IOC Medical Commission (T/Et up to 6). There are only a few data available, however, suggesting a possibility of "naturally" elevated T/Et ratios (Oftebro 1992; Raynoud et al. 1992; Smorawiński and Grucza 1995). The reasons of the higher T/Et ratios might be some disturbances in steroid profile of an individual, usually expressed by an inhibited epitestosterone secretion, without measurable pathological changes in the organism..

Considering the above mentioned facts the IOC Medical Commission ordered performance a set of additional tests in the case of athletes exhibiting T/Et values between 6.0 and 10.0. One of the available method at this field is a ketoconazole test enabling to detect whether the increased T/Et ratio is endogenous or exogenous in origin. This paper presents a data collected from 9 polish athletes performed ketoconazole test during the years 1994-1997.

Materials and Methods

This study was performed in 9 athletes showing T/Et ratios between 6.0 and 10.0 and suspected to use anabolic steroids as a doping agent. The athletes were informed about possible side effects of the ketoconazole test and the experimental procedure. Written informed consent was obtained. The subjects represented the following disciplines of sport: track and field (3), handball (4) and ice hockey (2). The average age of the athletes was 20.2 years (SD 2.9), body mass 76.9 kg (SD 6.4) and height 184.6 cm (SD 7.9).

The subjects were closed in a medical clinic for 2 days: day 1- the control before the test; day 2 - the ketoconazole test. They were informed on the date of the test only one day before it started. The subjects were carefully checked before the entrance to the clinic. No private things

or clothes were allowed. Ketoconazole was applied in a single dose of 400 mg (Kicman et al. 1993) at . 9.00 a.m., with blood and urine samples taken at 9.00, 15.00 and 21.00 hours. The control sample of urine was taken at 20.00 hour the day preceding the test. The test was performed in the Clinic of Endocrinology Medical Academy in Poznań, under supervision of the Polish Commission Against Doping Use in Sport. The blood samples were analysed in the clinic whereas the urine samples were tested in Department of Antidoping Research the Institute of Sport in Warsaw.

Concentrations of testosterone (T), cortisol (Cl), lutropin (LH), follitropin (FSH) and sex-hormone-binding-globulin (SHGB) were analysed in plasma blood. In urine, concentrations of testosterone (T), epitestosterone (Et), androsterone (A), etiocholanolone (E) and lutropin (LH) were measured. The ratios of T/Et and A/E were calculated.

Results

During first 6 hours of the test the concentrations of T decreased almost 4 times while the concentrations of Et declined only by 50% (NS). Absolute values of A and E decreased significantly during the whole test (Fig.1).

T/Et ratio as well as A/E ratio decreased significantly in all subjects during first 6 hours of the experiment (Fig.2A and Fig.2B, respectively). The A/E decreased by 15% mostly during the first phase of the ketoconazole test.

The values of T/Et were individually different and represented an individual reactivity to ketoconazole (Fig.3). When changes in T/Et ratio after 6 hour of the test were normalised to the control values before the test the lowest reactivity was 0,75 whereas the highest found to be about 0,26. It seems, therefore, that the individual reactivity to ketoconazole test might be a useful factor in differentiation of the positive and negative cases in anti-doping procedure.

Time course of concentration of testosterone in blood plasma was similar as in urine. During the first phase of the test the concentrations o T decreased 4 times (Fig.4A). There was no change in concentrations of sex-hormone-binding-globulin (SHBG). The level of cortisol in blood plasma decreased during the ketoconazole test (Fig.4B). However, significant differences in the values of cortisol were observed only at the first hours of the test.

Concentrations of lutropin (LH) in urine decreased significantly during first 6 hours of the test. During that time the concentrations of LH in blood plasma did not change. After the 6 hours concentrations of LH in blood plasma increased insignificantly whereas LH in urine was stable at the low level (Fig.5).

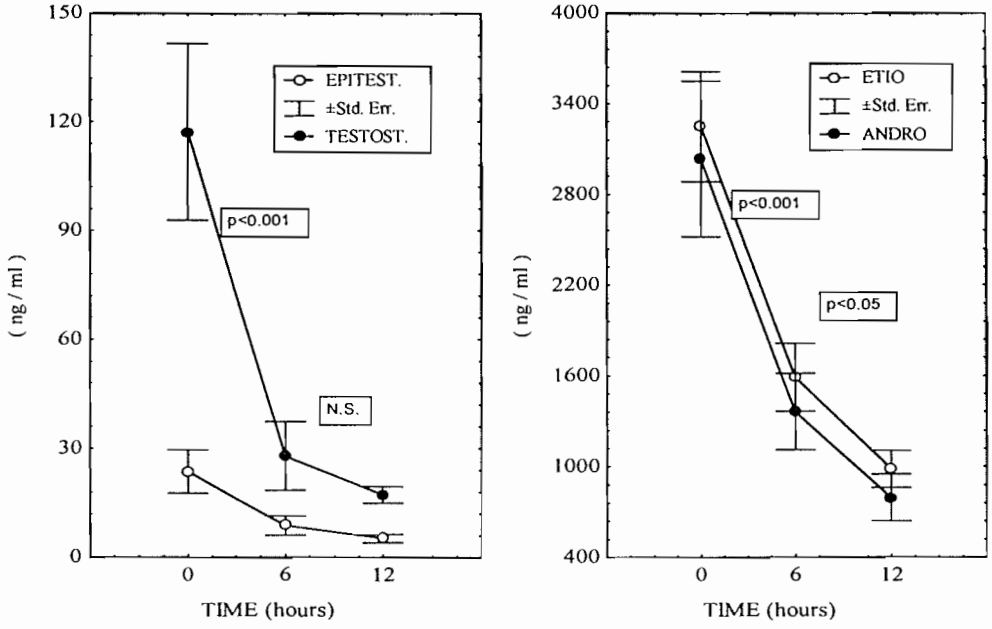

Fig.1. Changes in concentrations of testosterone, epitestosterone, androsterone and etiocholanolone in urine after ketoconazole intake.

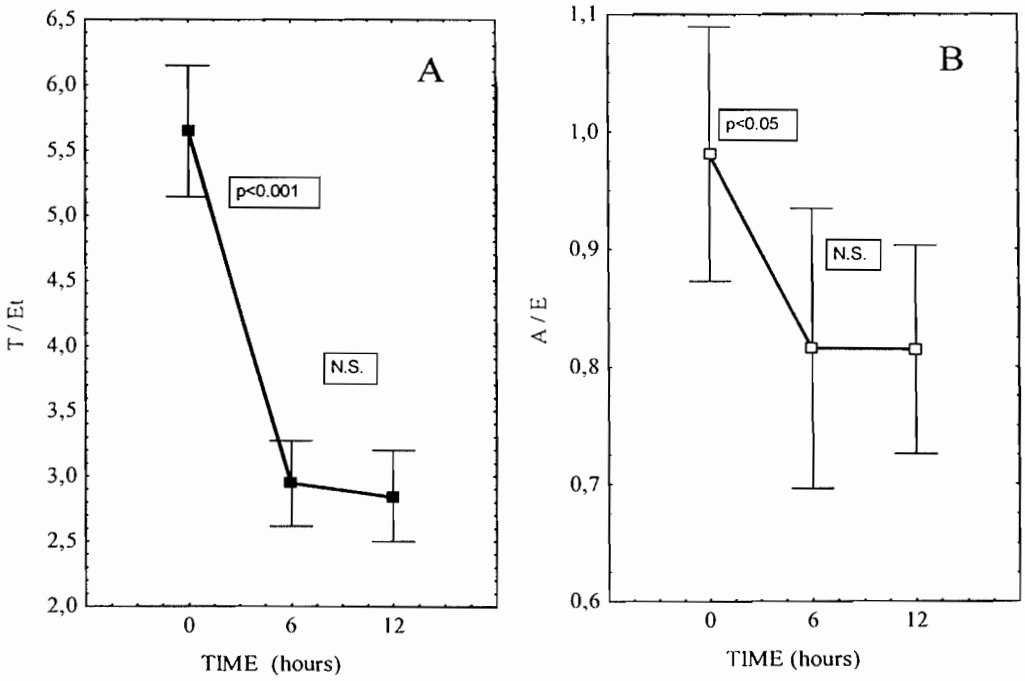

Fig.2. T/Et ratio (A) and A/E ratio (B) in subjects during ketoconazole test.

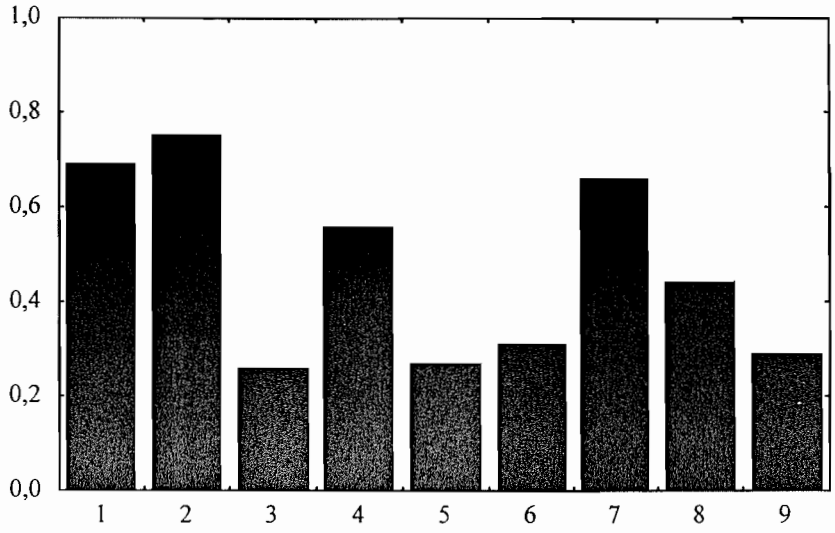

Fig.3. Individual reactivity to ketoconazole test expressed as T/Et change compared to the control value.

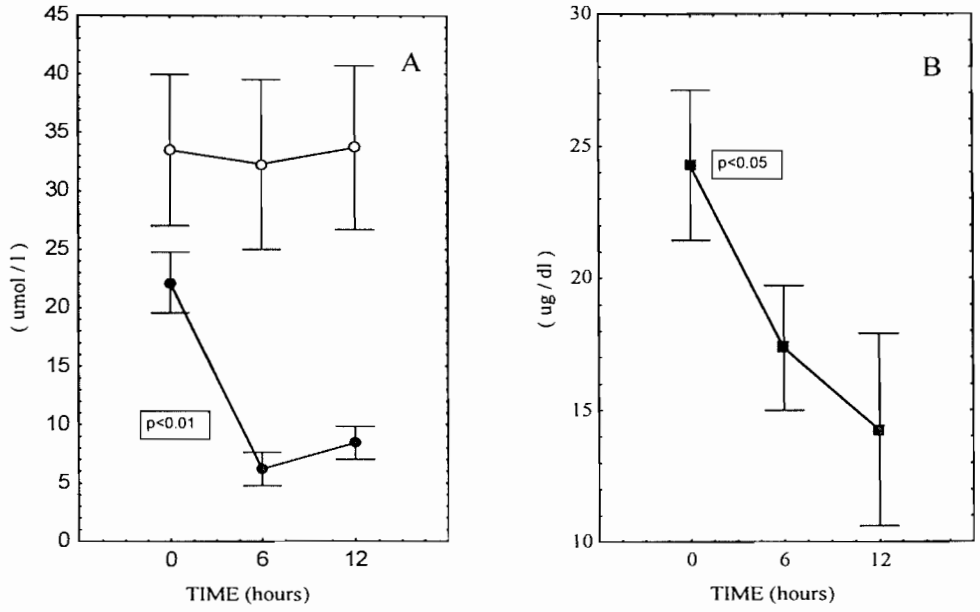

Fig.4. Changes of testosterone (A-closed circle), sex-hormone-binding globuline (A-open circle) and cortisol (B) in blood plasma during ketoconazole test.

The concentration of follitropin (FSH) did not change significantly and was 8,2 mIU/ml at the beginning and 9,7 mIU/ml at the end of the ketoconazole test. The ratio of testosterone to

lutropin (T/LH), calculated for urine, was stable during the test and was 12,63 SD 8,50 and 9,59 SD 14,10 (NS), respectively.

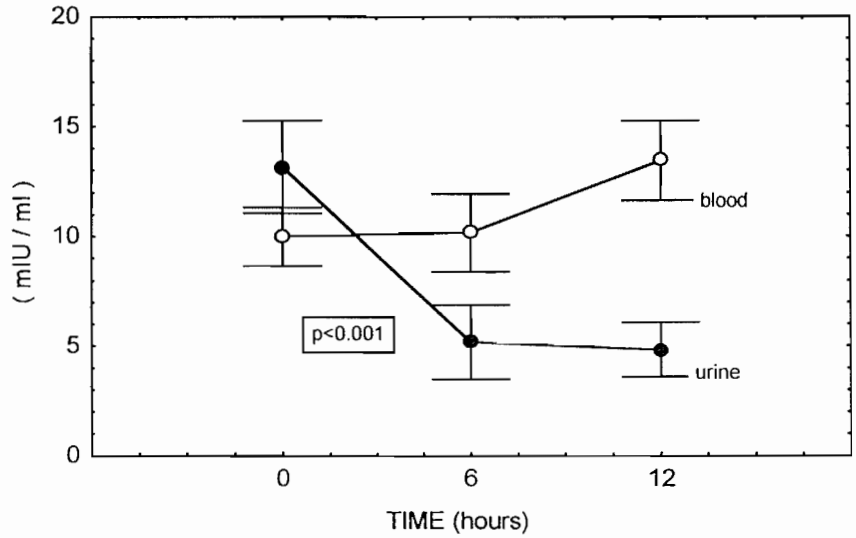

Fig. 5. Changes in lutropin (LH) in blood and urine.

Discussion

Kicman et al. (1993) found that in control subjects who had received testosterone, the resulting increase in T/Et ratio was further increased by the action of ketoconazole because of the additional suppression of epitestosterone production. Repeated administration of testosterone would have a cumulative effect, resulting in further suppression of the hypothalamic-pituitary-testicular axis and epitestosterone excretion. In such cases ketoconazole would be expected to cause little changes in an already high ratio.

No increase in T/Et ratio was observed in any subject under the ketoconazole test in the present study. From this result it might be concluded that hormonal responses to the ketoconazole test were endogenous in origin and that the subjects did not take testosterone doping in some short period before the test. However, the great individual variability in the reactivity of T/Et ratio to ketoconazole (from 0.26 to 0.75) could suggest that some of the subjects probably used testosterone doping in the past having , in result, a disturbed hormonal function, detected by the ketoconazole test. It is worth to mention that the reactivity did not correlated with the control values of testosterone ($r = -0.22$; NS) nor with control values of epitestosterone ($r = -0.04$; NS). The analysis of reactivity to ketoconazole can specially be useful in athletes with low level of epitestosterone. Since relation of decreases in absolute values of testosterone and epitestosterone is about 8:1, one can expect a greater reactivity to

ketoconazole in athletes not using testosterone doping, despite of the low level of epitestosterone.

Oftebro et al.(1994) observed that in untreated subjects the reduction of T/Et ratio after a double 400 mg dose of ketoconazole was substantially greater than that reported for a single 400 mg dose. It can not be excluded, therefore, that an individual reactivity to the ketoconazole might be a dose related. However, since one of the aim of this study was to detect the individual reactivity to ketoconazole we decided to apply the mentioned single dose and, in consequence, not to depress totally the male sex-hormone activity during the test. The ethical aspect of the experiment and diminishing the known side effects of the drug were also taken into account. In conclusion, we suppose that low reactivity to ketoconazole test would be an indices of using of testosterone doping far before the application of ketoconazole test. More research is necessary to implement the conclusion into practical procedure of doping control.

References

Donike M., S. Rauth, A. Wolansky (1993). Reference ranges of urinary endogenous steroids determined by gas-chromatography / mass spectrometry. Proc. 10th Cologne Workshop on Dope Analysis, Sport und Buch Strauss, pp.69 - 86.

Grucza R., K. Chrostowski, D. Kwiatkowska, A. Pokrywka, A. Chajewski (1997). Changes of steroid profiles in male and female athletes. Proc. 15th Cologne Workshop on Dope Analysis, Sport und Buch Strauss, 5:223-229.

Kicman A.T., H. Oftebro, Ch. Walker, N. Norman, D. Cowan (1993). Potential use of ketoconazole in dynamic endocrine test to differentiate between biological outliers and testosterone use by athletes. Clinical Chemistry, 39 (9): 1798-1803.

Oftebro H. (1992). Evaluating an abnormal urinary steroid profile. Lancet, 339: 941-942

Oftebro H., J. Jensen, F. Mowinckel, H.R. Norli (1994). Establishing a ketoconazole suppression test for verifying testosterone administration in the doping control of athletes. J. Clin. Endocrin. and Metab. 78: 973 - 977.

Raynaud E., M. Audran, J.F. Brun, C. Fedou, J.L. Chanal, A. Orsetti (1992). False positive cases in detection of testosterone doping. Lancet, 340: 1468-1469.

Smorawiński J., R. Grucza (1995). The use of ketoconazole test for estimation of abnormal steroid profile in an athlete. Proc. VIII FIMS European Congress of Sport Medicine, Granada, Spain, p. 10.