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# RECENT ADVANCES IN DOPING ANALYSIS (2)

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# Evaluation of endogenous steroid profiles in urine (1) effects of illness and the use of antibiotics

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## Introduction

Steroid profiles can be succesfully used as a tool to detect the misuse of anabolic steroids [1]. Although significant variation has been found for the stability of steroid profiles [2], reference ranges of urinary endogenous steroids have already been proposed [3].
To obtain more experience with steroid profiling in Utrecht, a small trial has been set up. Morning urine samples from men (during 30 days), from women taking oral contraceptives (during 28 days) and from women taking no contraceptives (during one menstrual cycle) are being collected. At this moment, the collection is still in progress. However, some preliminary findings are presented, as they seem very interesting according to steroid profiling.
It quitely happened that one of the male subjects got ill (flu) during the period of collection of the respective urine samples, and his clinical situation induced a therapeutic dose of amoxycillin, a broad-spectrum penicillin antibiotic. Some endogenous steroids within the steroid profile were obviously affected by the use of the antibiotic. Typical changes in the steroid profile compared to that of a healthy subject are discussed.

## Subjects and methods

During one month, morning urine samples were collected by two male subjects. Subject K.B. is a "healthy" young man (24 years old). Subject L.H. (22 years old) got ill (flu) in the second week and then decided to take once a dose of amoxycillin.
The urine samples were analyzed according the standard IOC screening procedure for the detection of anabolic steroids. Enzymatic hydrolysis of the glucuronides was performed with

ß-glucuronidase from E.Coli (Boehringer Mannheim). The deconjugated steroids were measured by gas chromatography-mass spectrometry. The following steroids were quantified, using methyltestosterone as internal standard: testosterone, epitestosterone, androsterone, etiocholanolone and dehydroepiandrosterone. All steroid concentrations were corrected for respective creatinine concentrations. Creatinine was measured spectrophotometrically at 500 nm according to the Jaffe reaction.

## Results

The steroid excretion profiles expressed as change from average (calculated over the whole period) are seen in Figure 1. The ratios of the respective steroids are shown in Figures 2 and 3. The concentrations of testosterone (T) and epitestosterone (E) increased slightly when subject L.H. got ill. An obvious decrease followed after amoxycillin intake. For androsterone (AO), etiocholanolone (EO) and dehydroepiandrosterone (DHEA) no changes were found during illness or subsequent medication therapy. Therefore, obvious increases were found for the AO/T, AO/E, EO/T, EO/E and DHEA/T ratios. The AO/EO ratio was not affected by the medication intake, but the T/E ratio seemed to increase significantly after amoxycillin administration.

## Conclusion

Effects of illness and antibiotic therapy must be taken into account while interpreting steroid profiles. Further conclusions will be drawn as soon as the proposed study has been completed.

## References

1. M. Donike. Steroid profiling in Cologne. In: 10th Cologne Workshop on Dope Analysis, 7th till 12th June 1992, Proceedings. Eds.: M.Donike, H. Geier, A. Gotzmann, U. Mareck-Engelke and S. Rauth. Sport und Buch Strauß, Köln, 1993. pp. 47 - 68.

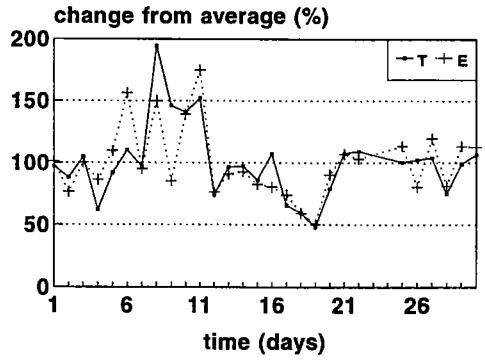
3. U. Mareck-Engelke, H. Geyer and M. Donike. Stability of steroid profiles. In: 10th Cologne Workshop on Dope Analysis, 7th till 12th June 1992, Proceedings. Eds.: M.Donike, H. Geier, A. Gotzmann, U. Mareck-Engelke and S. Rauth. Sport und Buch Strauβ, Köln, 1993. pp. 87 - 89.

2. M. Donike, S. Rauth and A. Wolansky. Reference ranges of urinary endogenous steroids determined by gas-chromatography/mass spectrometry. In: 10th Cologne Workshop on Dope Analysis, 7th till 12th June 1992, Proceedings. Eds.: M.Donike, H. Geier, A. Gotzmann, U. Mareck-Engelke and S. Rauth. Sport und Buch Strauβ, Köln, 1993. pp. 69 - 86.

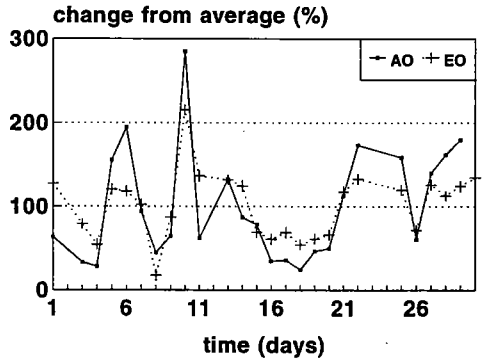
*Figure 1:* *Changes in endogenous steroid excretions in morning urine samples collected during one month: An ill subject versus a healthy volunteer. At the end of the second week subject L.H. took a therapeutic dose of amoxycilline.*

**SUBJECT K.B. "healthy"**

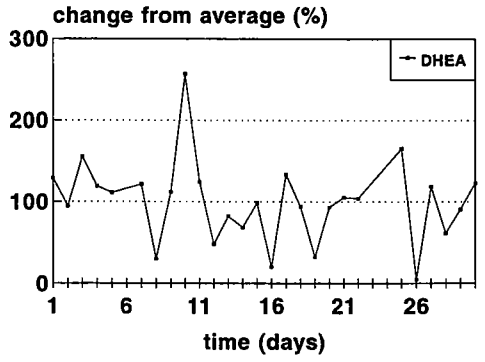
**Testosterone (T) and Epitestosterone (E)**




**Androsterone (AO) and Etiocholanolone (EO)**

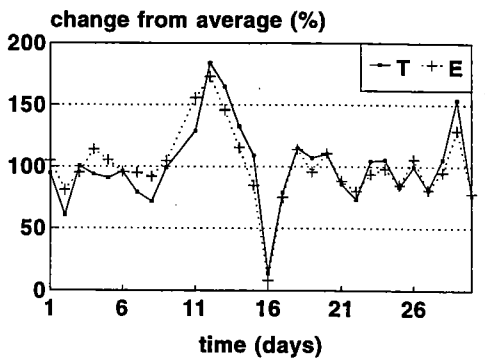



**Dehydroepiandrosterone (DHEA)**

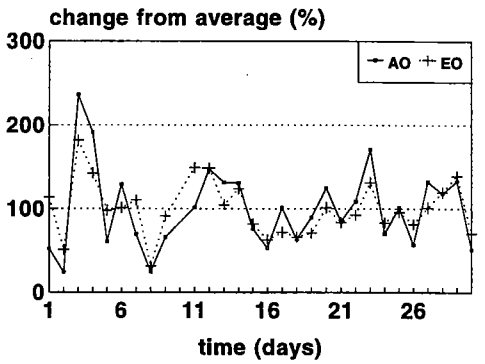



**SUBJECT L.H. "ill"**

**Testosterone (T) and Epitestosterone (E)**




**Androsterone (AO) and Etiocholanolone (EO)**




**Dehydroepiandrosterone (DHEA)**

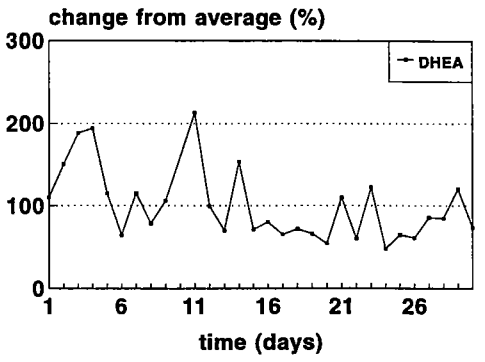
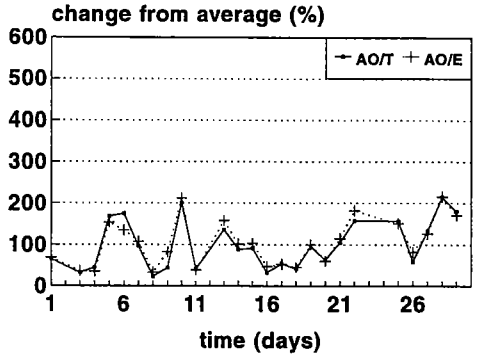


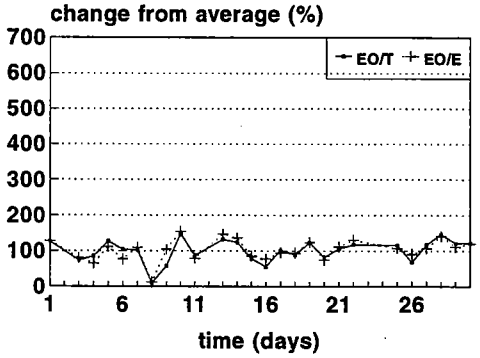
*Figure 2:* *Changes in endogenous steroid ratios in morning urine samples collected during one month: An ill subject versus a healthy volunteer. At the end of the second week subject L.H. took a therapeutic dose of amoxycilline.*

**SUBJECT K.B. "healthy"**

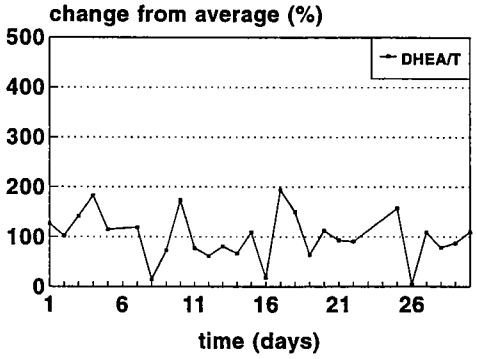
**AO/T and AO/E**




**EO/T and EO/E**

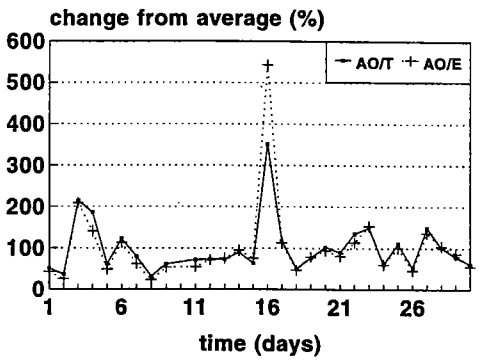



**DHEA/T**

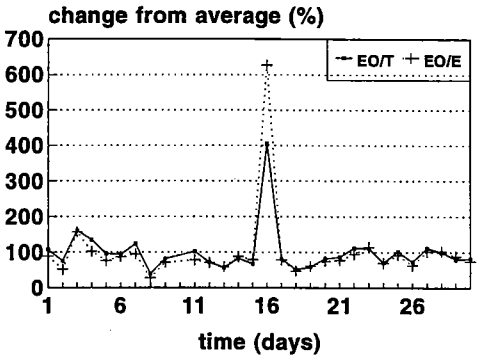



**SUBJECT L.H. "ill"**

**AO/T and AO/E**




**EO/T and EO/E**




**DHEA/T**

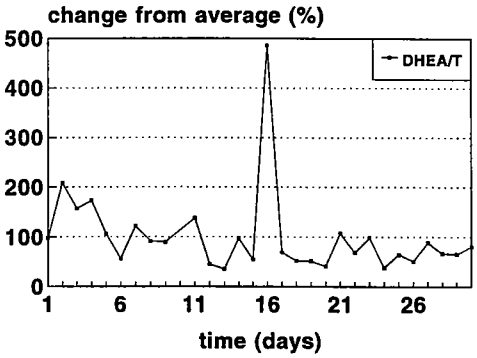


*Figure 3:* *Changes in endogenous steroid ratios in morning urine samples collected during one month: An ill subject versus a healthy volunteer. At the end of the second week subject L.H. took a therapeutic dose of amoxycilline.*