

Reprint from

RECENT ADVANCES  
IN DOPING ANALYSIS  
(6)

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## **Excretion studies with 4-androstene-3,17-dione**

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### **Introduction**

4-Androstene-3,17-dione (Adione) is a precursor of testosterone in humans. An increase in the urinary testosterone concentration can therefore be expected after administration of this endogenous steroid. Rumours are widespread that Adione is abused by bodybuilders. On the world wide web (WWW) numerous companies offer Adione and promise an increase in testosterone levels of up to 337%, one and a half hour after oral administration of 100 mg. Stacking regimens are also readily available on the internet including the combination of Adione and dehydroepiandrosterone (DHEA). \*

In order to detect the abuse of Adione and because of the “promising” results, the influence of this steroid on testosterone concentrations and the testosterone to epitestosterone ratio was investigated. As Adione is an endogenous steroid, a statistically based decision limit will probably have to be determined in order to detect its abuse.

### **Material and methods**

#### *Administration*

A self-prepared capsule containing 25 mg of Adione was taken orally by four healthy male volunteers. Urine was collected before administration and quantitatively during the first 12 h, i.e. 1, 2, 4, 6, 9, 12 h post administration. An additional urine sample was collected 24 h post

administration. During the experiment the volunteers were allowed to drink ad libitum. One month later the same volunteers took a capsule AndrostenePower® (Olympian Labs, USA), containing 50 mg (tested with GC/MS) of Adione. The same scheme of sample collection was followed as in the previous experiment.

### *Analysis*

Endogenous steroids were quantified by GC-MS (SIM), as routinely done for samples collected for doping analysis. Briefly, 1 mL of sodium acetate buffer (pH 5.2) and 50 µL β-glucuronidase (100000 U/mL β-glucuronidase activity and 5000 U/mL sulfatase activity) were added to 2 mL of urine and the mixture was hydrolysed for 2.5 h at 56°C. After addition of the internal standard (100 ng 17α-methyltestosterone), extraction was performed by rolling (20 min) with 5 mL freshly distilled diethylether, followed by centrifugation. The organic layer was separated, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under OFN. The residue was derivatised with 100 µL MSTFA/NH<sub>4</sub>I/ethanethiol (380:1:2 vol/wt/vol) for 2.5 h at 80°C.

Mass spectrometric analysis was performed on an HP-MSD 5970 instrument equipped with a 17 m HP-Ultra 1 column (internal diameter 0.2 mm, film thickness 0.11 µm). The GC temperature program was as follows: 120°C (1 min) - 70°C/min → 182°C (0 min) - 4°C/min → 235°C (0 min) - 30°C/min → 300°C (3 min). Injected sample volume was 0.5 µL, splitless. The instrument was operated in SIM-mode. The relative retention time (R.R.T.) and monitored m/z-values for the endogenous steroids are given in Table 1.

### *Statistical analysis:*

The concentration of Adione and the ratio of Adione to epitestosterone in all samples, routinely collected for doping analysis in Flanders during the period December 1997 to February 1998, were statistically processed using the Statview software package (Abacus Concepts Inc, Berkeley, CA, USA). Far outside values were determined by adding three times the interquartile range to the 75<sup>th</sup> percentile<sup>1</sup>.

## Results

The change in urinary concentration of Adione after oral intake of different amounts of this steroid is shown in Fig. 1. The increase of the urinary testosterone concentration for two volunteers after intake of Androstene-power® is illustrated in Fig. 2. The measured urinary testosterone to epitestosterone ratio in two volunteers after taking 25 mg of Adione is shown in Fig. 3, while the change in Adione to epitestosterone ratio after intake of Androstene-power is given in Fig. 4.

The median value, the 10- and 90-percentile and the far outside value for Adione and the ratio Adione to epitestosterone (Adion/EpiT) in 377 male subjects are summarized in Table 2 .

## Discussion

Preliminary statistical analysis of urinary concentrations of Adione and other endogenous steroids, in samples collected for doping analysis in Flanders, resulted in not-normally distributed data sets for all endogenous steroids and their ratios. For Adione, neither logarithmical nor square root transformations led to a gaussian distribution. Therefore, a non-parametrical approach was preferred to determine preliminary threshold levels for the concentration of Adione and for the ratio of Adione to epitestosterone. A far outside value for the urinary concentration of Adione and the ratio of Adione to epitestosterone was calculated (Table 2.). Any value exceeding such a far outside value can be regarded as extremely unusual. The far outside value can be regarded as a decision limit in doping analysis<sup>2,3</sup>.

Adione is a precursor of testosterone<sup>4</sup>. The increase in urinary testosterone concentration after intake of 25 mg of Adione is shown in Fig. 2. Urinary concentrations of testosterone and Adione reached a maximum value 2 - 4 h post administration (Fig. 1 and Fig. 2). Obviously, the increase in concentration of Adione was larger after the intake of 50 mg. However, in both experiments the concentration was below the preliminary decision limit of 23 ng/ml for all volunteers 9 hours post administration (Fig. 1).

The testosterone to epitestosterone ratio can be used as a parameter to detect testosterone abuse. In Flanders<sup>5</sup>, the decision limit for this ratio is still set a 6. Using this threshold level, oral administration of 25 mg or 50 mg Adione can be detected for 9 hours (Fig. 3).

From preliminary statistical analysis a far outside value of 1.2 for the ratio of Adione to epitestosterone was calculated (Table 2). Values below this threshold level were obtained after 9 hours in each volunteer for both dosages (Fig. 4).

Adione plays a major role in the biosynthesis and metabolism of testosterone and other endogenous steroids<sup>4</sup>. Consequently, minor changes were observed in the steroid profile of the volunteers. Urinary androsterone, etiocholanolone, 11 $\beta$ -hydroxy-androsterone, 11 $\beta$ -hydroxy-etiocholanolone and dihydrotestosterone concentrations increased after Adione intake. However, no statistically significant differences with the concentrations in a control population of about 3900 male athletes were observed during both experiments. The urinary concentrations of these endogenous steroids returned to pre-administration levels after 9 hours. Furthermore, an unidentified GC peak (R.R.T. = 0.921) was observed during the analysis. Further work is in progress in order to identify this substance and to determine its potential usefulness in detecting Adione abuse.

## **Conclusion**

Using a routine GC/MS method and threshold levels for the Adione concentration and the ratios Adione/EpiT and T/EpiT, the abuse of 25 and 50 mg of Adione could be detected for about 9 hours.

## **Acknowledgements**

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## References

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3. Delbeke F.T., Van Eenoo P., De Backer P.: Detection of human chorionic gonadotrophin misuse in sports. *Int. J. Sports Med.* 19: 287-290, 1998.
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5. Order of the Flemish Government N92-1150 of 23 October 1991 (B.S. 10.04.1992)

## Footnotes

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<http://www.vitamin-disc.com/articles/stack1.html>

<http://www.cyberiron.com/supplement/androstenedione.html>

<http://www.powershack.com/androgen.htm>

<http://www.mesomorphosis.com/androsteneFAQ.htm>

<http://www1.viaweb.com/an50mg60cap1.html>

<http://idt.net/~knpo5719/andro.html>

Table 1. Relative retention time (RRT) and monitored m/z-values for the endogenous steroids monitored during steroid profiling (all steroids as per-trimethylsilyl derivatives).

steroid	RRT	m/z
androsterone	0.745	419-434
etiocholanolone	0.756	419-434
5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol	0.766	241
5 $\beta$ -androstane-3 $\alpha$ ,17 $\beta$ -diol	0.773	241
5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol	0.851	421
epitestosterone	0.851	432 - 417
dihydrotestosterone	0.865	434
4-androstene-3,17-dione	0.879	430
testosterone	0.901	432-417
11 $\beta$ -hydroxy-androsterone	0.919	522
11 $\beta$ -hydroxy-etiocholanolone	0.930	522
17 $\alpha$ -methyltestosterone (I.S.)	1.000	446-301

Table 2. Results (mean, median, 10<sup>th</sup>-percentile, 90<sup>th</sup> percentile and far outside value) of a preliminary statistical analysis (n=305).

	mean	median	10 <sup>th</sup> -percentile	90 <sup>th</sup> percentile	Far outside value
4-androstene-3,17-dione*	6.43	5.00	2.00	12.00	23
Adione/E	0.35	0.23	0.08	0.71	1.2

\* concentrations in ng/ml

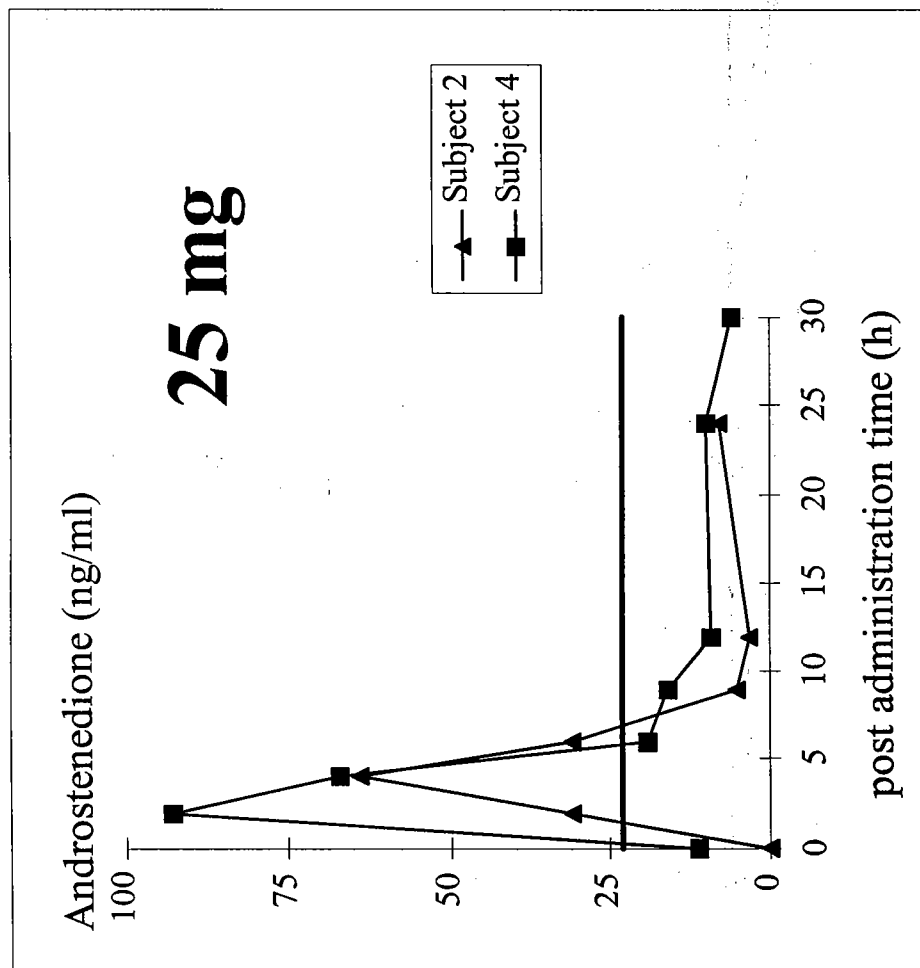
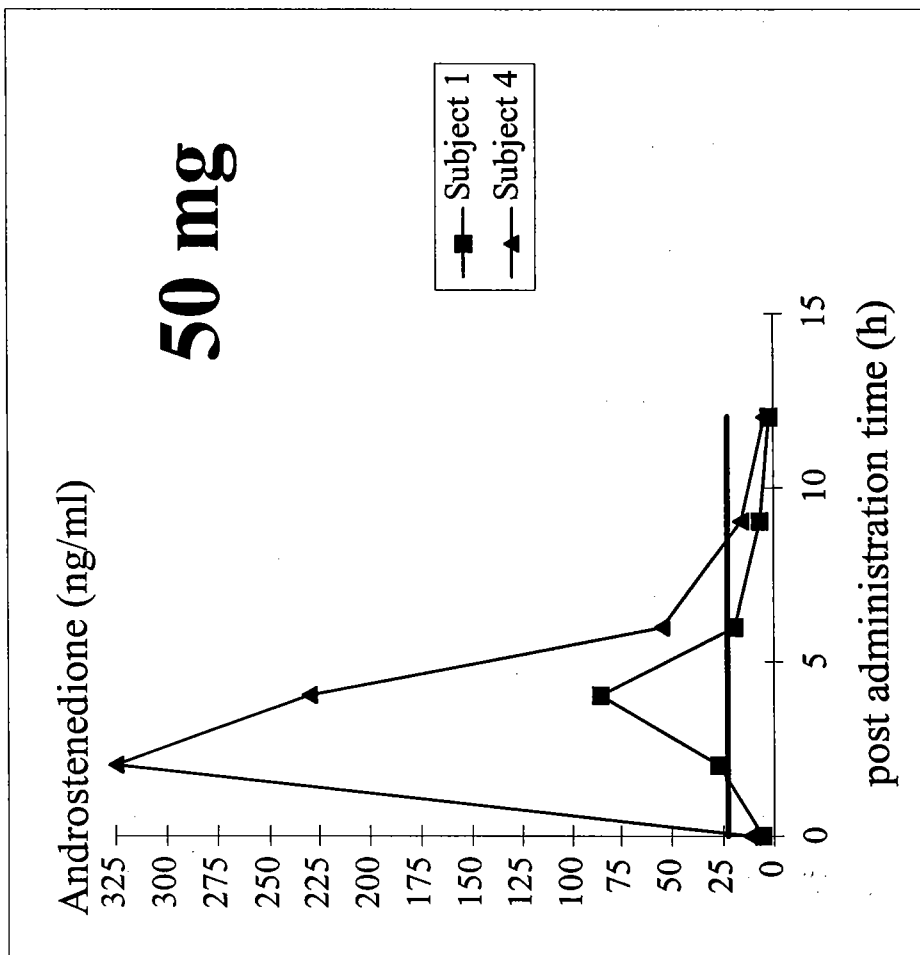


Fig. 1. Profile of the urinary Adione concentration after oral intake of both 25 and 50 mg of this steroid.



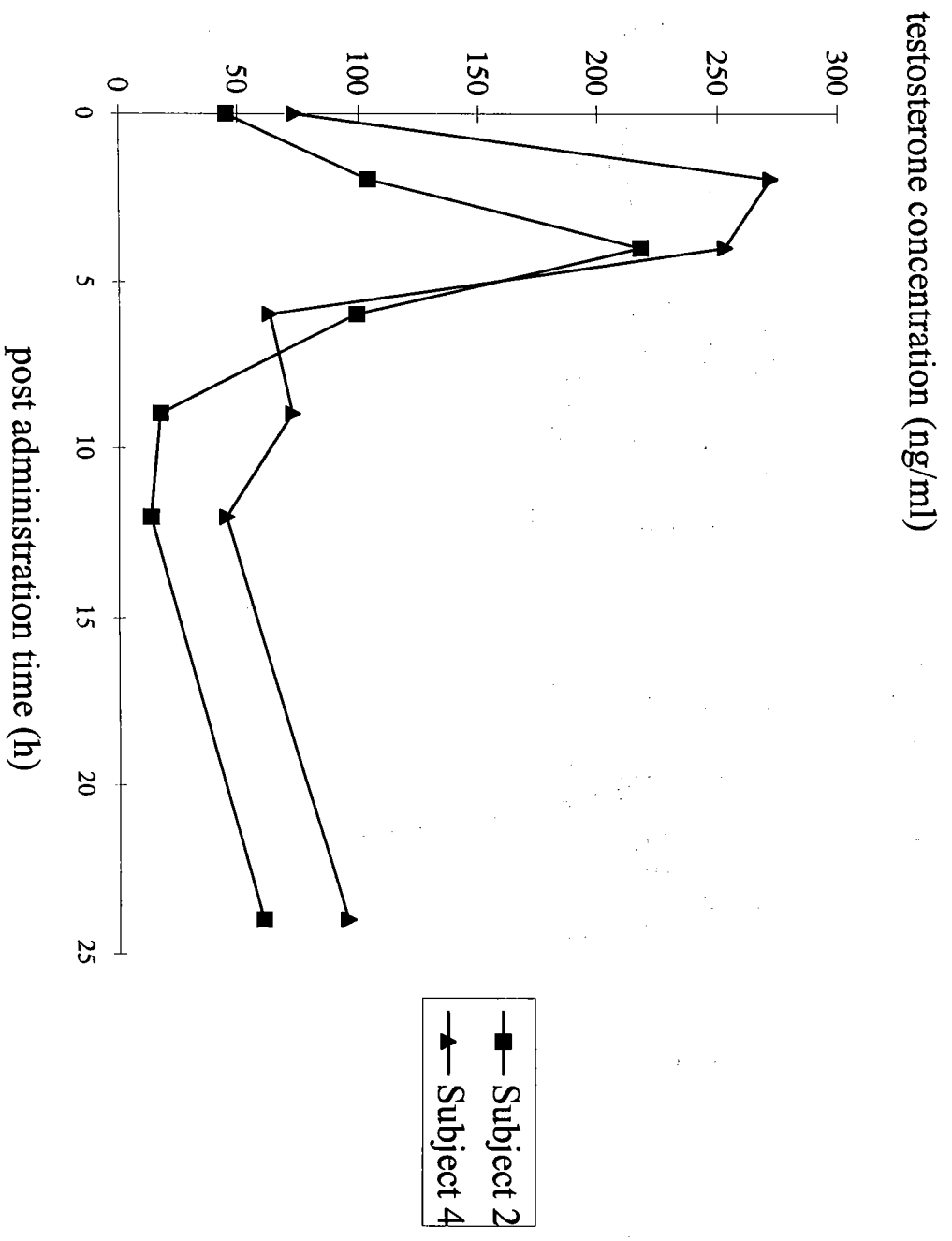


Fig. 2. Change in urinary testosterone concentration after oral administration of 50 mg Adione.

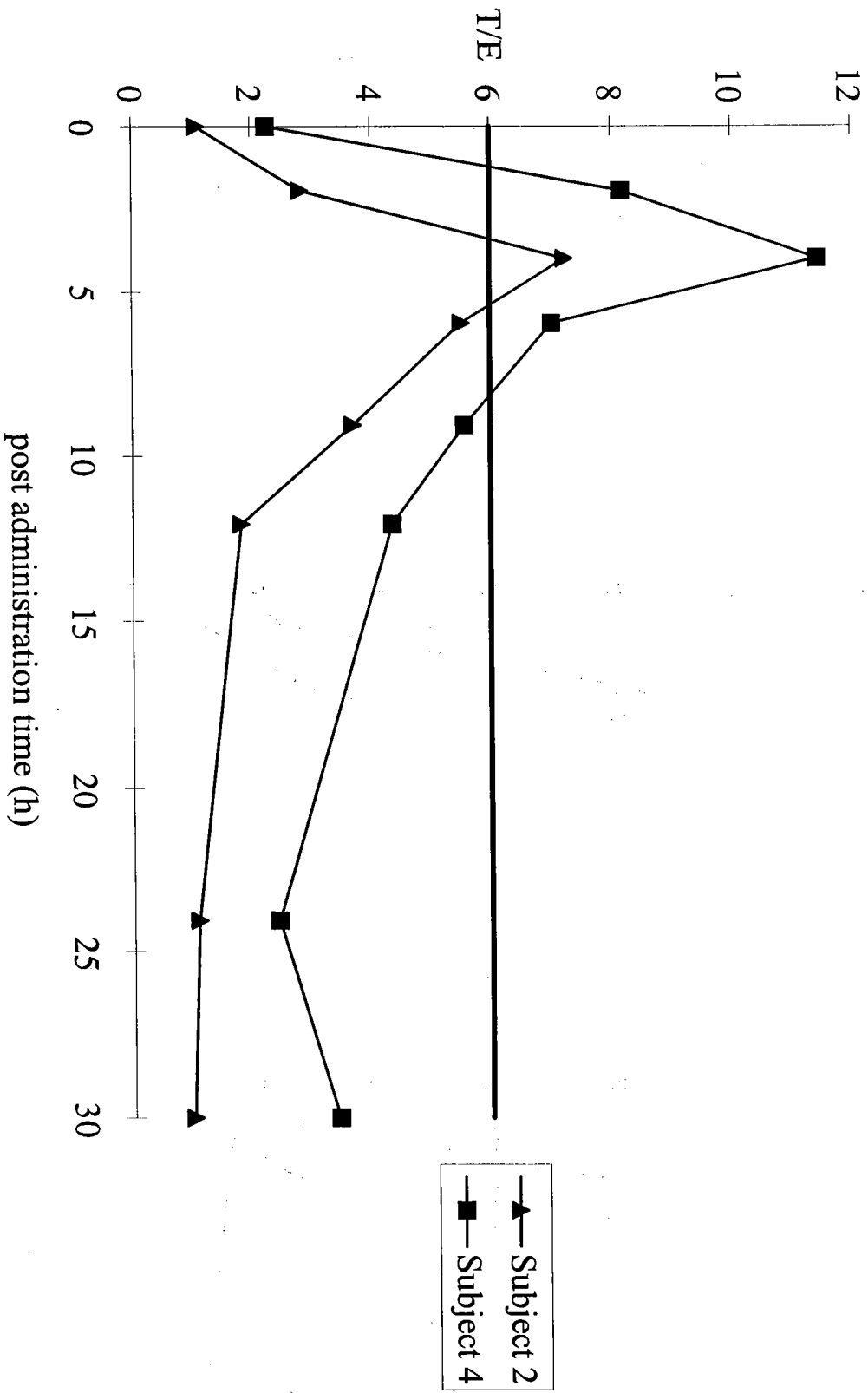


Fig. 3. Profile of the T/E ratio after intake of 25 mg Adione.

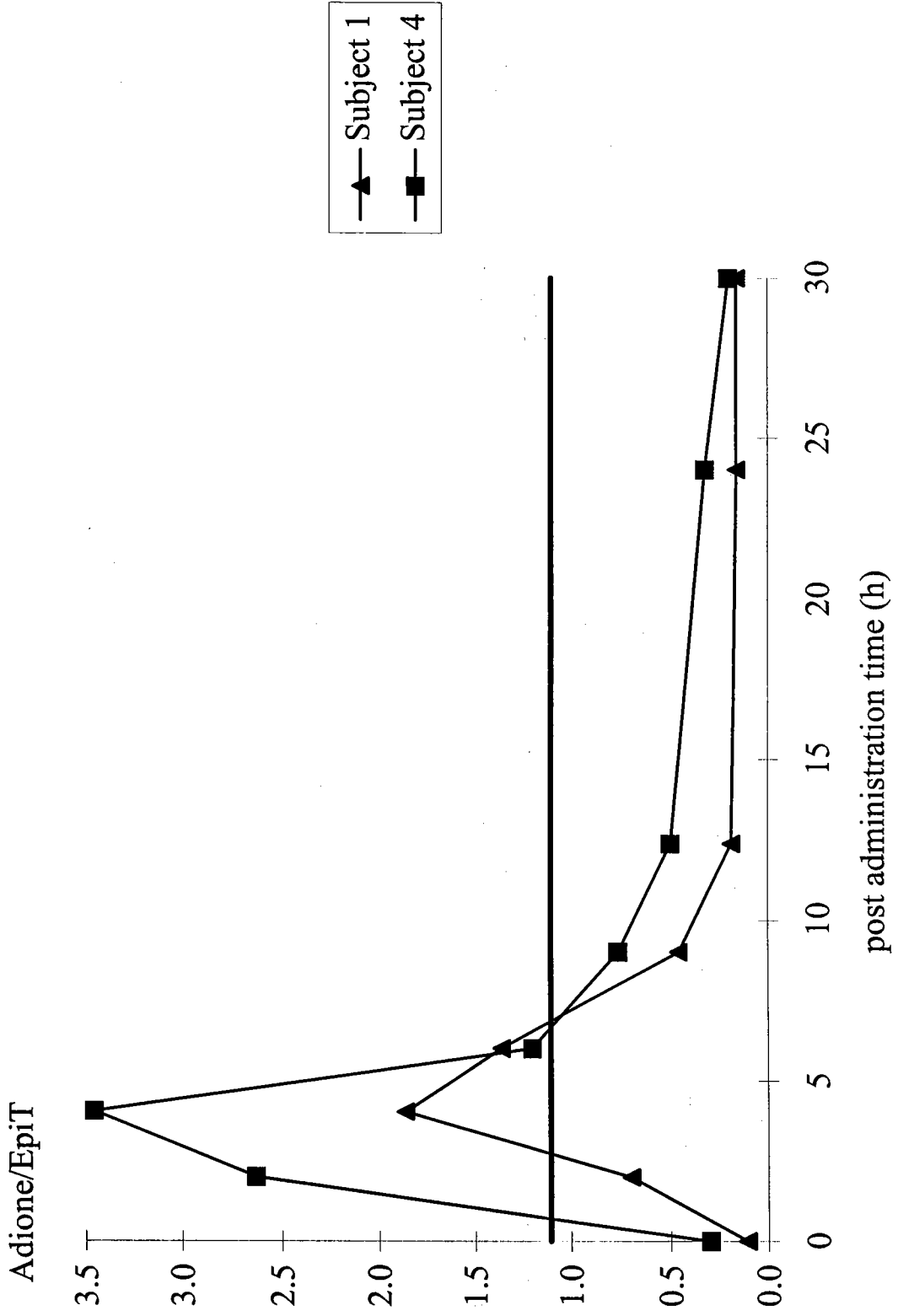


Fig.4. Change in the Adione/E ratio after intake of 50 mg of Adione.