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RECENT ADVANCES IN DOPING ANALYSIS (8)

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P.VAN EENOO, F.T.DELBEKE, N.DESMET, P.DE BACKER:
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Excretion studies with *Tribulus terrestris*

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Abstract

Tribulus terrestris is marketed as a natural testosterone booster and is rumouredly used by athletes. According to internet sources, *Tribulus terrestris* stimulates LH and consequently testosterone production.

In this study the effect of *Tribulus terrestris* on urinary concentrations of lutheinising hormone (LH), testosterone and other endogenous steroids, monitored in doping control, was investigated. Four volunteers took two tablets of Tribulin®, each containing 250 mg of *Tribulus terrestris*, three times a day for four and a half days. The urinary steroid and LH profile of the volunteers was monitored before and after intake of the *Tribulus terrestris* supplement. No significant changes in endogenous steroid concentrations were observed.

Introduction

Rumours on the use of *Tribulus Terrestris* are widespread. On the internet this substance is offered under various trade names from different companies. In newsgroups several so-called body building experts advise sportsmen to use *Tribulus terrestris* as a muscle growth promoter. *Tribulus* is freely available in the US and in Europe where it is sold as a food supplement.

In Belgium, a number of high ranked athletes , both nationally and internationally, admitted in the press that they were taking this product and consequently probably stimulated the use of *Tribulus* among other Belgian athletes.

Advertisements state that the use of *Tribulus terrestris* increases lutheinising hormone and boosts testosterone levels. Although manufacturers of this kind of supplements are mostly more interested in the profits they make rather than in the scientific value of their promises, it seems necessary for doping control to test whether the characteristics attributed to Tribulus are (partially) true.
Very little scientific data on the effects of *Tribulus* are published and the advertisements only state that testosterone levels increase. No indication is given about the changes of other endogenous steroids and there is no knowledge about possible alterations of the testosterone to epitestosterone ratio. If *Tribulus* increases LH concentrations and testosterone levels, the implications for current and future doping analysis techniques could be tremendous.
Because of the proposed mechanism of *Tribulus*, totally differing from other currently very popular testosterone boosters including androstenedione or DHEA, it seems that use of Tribulus would remain undetected with carbon isotope ratio mass spectrometry.

In order to test if *Tribulus* really constitutes a threat for doping control procedures, an excretion study experiment was established.

Material and methods

Administration
Preceeding the administration experiment, basal steroid profiles of the male volunteers were determined by monitoring the urinary concentrations of the endogenous steroids, routinely screened for during doping analysis.

The *Tribulus* supplement was analyzed for the presence of different anabolic steroids by GC-MS as described elsewhere (1).

The volunteers ingested two tablets of Tribulin® (Goodlife Power Sports, the Netherlands), each containing 250 mg of Tribulus, three times a day. Urine was collected three times a day, i.e. in the morning, at noon and in the evening.

After four and a half day the volunteers ceased to take Tribulin® and urine was collected three times a day for another 5 and a half days.

Analysis

LH- levels were measured with an Abbott IMX (Abbott Park, Ill, USA) using the MEIA technique.

Endogenous steroids were quantified by GC-MS in selected ion monitoring mode (SIM), as routinely done for samples collected for doping analysis (2).
Briefly, 1 mL of sodium acetate buffer (pH 5.2) and 50 μL β-glucuronidase (type HP-2, Sigma Co, St-Louis, MO, USA) 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 (50 μL of a 2 μg/mL 17α-methyltestosterone solution in methanol), 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_2SO_4 and dried under OFN. The residue was derivatised with 100 μL MSTFA/NH4I/ethanethiol (380:1:2 vol/wt/vol) for 2 h at 80°C.
Mass spectrometric analysis for steroid profiling was performed on a HP-MSD 5973 instrument (Hewlett-Packard, Waldbron, Germany) equipped with a 17 m HP-Ultra 1 column (internal diameter 0.2 mm, film thickness 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). Injection 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.

Results

Analysis of the *Tribulus terrestris* food supplement did not reveal the presence of anabolic steroids.

The urinary LH and testosterone profiles of two volunteers are shown in Fig. 1 and Fig. 2, respectively. The profile of the urinary testosterone to epitestosterone ratio for the same volunteers is shown in Fig. 3.

Discussion

As shown in Fig. 1 and Fig. 2, no marked alterations neither in testosterone nor in LH concentrations were observed. The testosterone to epitestosterone ratio also remained unchanged (Fig. 3). No changes were detected in the urinary concentrations of the other endogenous steroids. Previous reports on the increase of testosterone levels after ingestion of *Tribulus* were based on Bulgarian and Russian studies with male subjects suffering from reproductive disorders (3). Manufacturers of Tribulus supplements might argue that our administration period was too short, the dose too small or that changes in testosterone concentration are most likely to occur when the natural testosterone production has dropped due to ageing. However, our results indicate that *Tribulus terrestris* is not effective in normal healthy males. It has been suggested previously that the effects of *Tribulus* in normal males might be minimal and probably only effective if stacked with anabolic steroids (3).

If athletes claim observing physical benefits from this supplement it will probably be because of psychological reasons or because of "extra ingredients" added to Tribulus. Recent investigations (3, 4) have shown that some manufacturers of nutritrional supplements do not hesitate to add anabolic steroids without any indications on the label. It is therefore likely that food supplements such as *Tribulus*, claiming to increase testosterone concentrations or promote muscle growth, are high risk products.

Athletes should be aware of the risks associated with the intake of nutritional supplements.

Acknowledgements

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References

1. De Cock, K., Delbeke, F.T., Van Eenoo, P., Desmet, N., Roels, K. & De Backer, P. Abstract: Detection of anabolic steroids in nutritional supplements. IV Latin American Workshop in Doping Analysis. Buenos Aires, Argentina, 2000, Acta Toxicologica Argentina, 2000.

2. Van Eenoo, P. Delbeke, F.T., Desmet, N. & De Backer, P. (1998). Excretion studies with 4 - androstene-3,17-dione. In: Schänzer, W., Geyer, H., Gotzmann, A. & Mareck-Engelke, U. (Eds). Recent Advances in doping Analysis (6). Proceedings of the Manfred Donike Workshop. 16th Cologne Workshop on Dope Analysis, 15th to 20th March 1998., 171-180.

3. Burke, E. (1999) Fad or Fact? A Look at *Tribulus terrestris*. Nutrition Science News, 03. (http://exchange.healthwell.com/nutritionsciencenews/NSN_backs/Mar_99/tribulus.cfm)

4. Geyer H., Mareck-Engelke, U., Sigmund, G. & Schänzer, W. Positive dope control cases after application of nutritional supplements. In: Schänzer, W., Geyer, H., Gotzmann, A. & Mareck-Engelke, U. (Eds). Recent Advances in doping Analysis (6). Proceedings of the Manfred Donike Workshop. 18th Cologne Workshop on Dope Analysis, 20th to 25th February. 2000.

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)

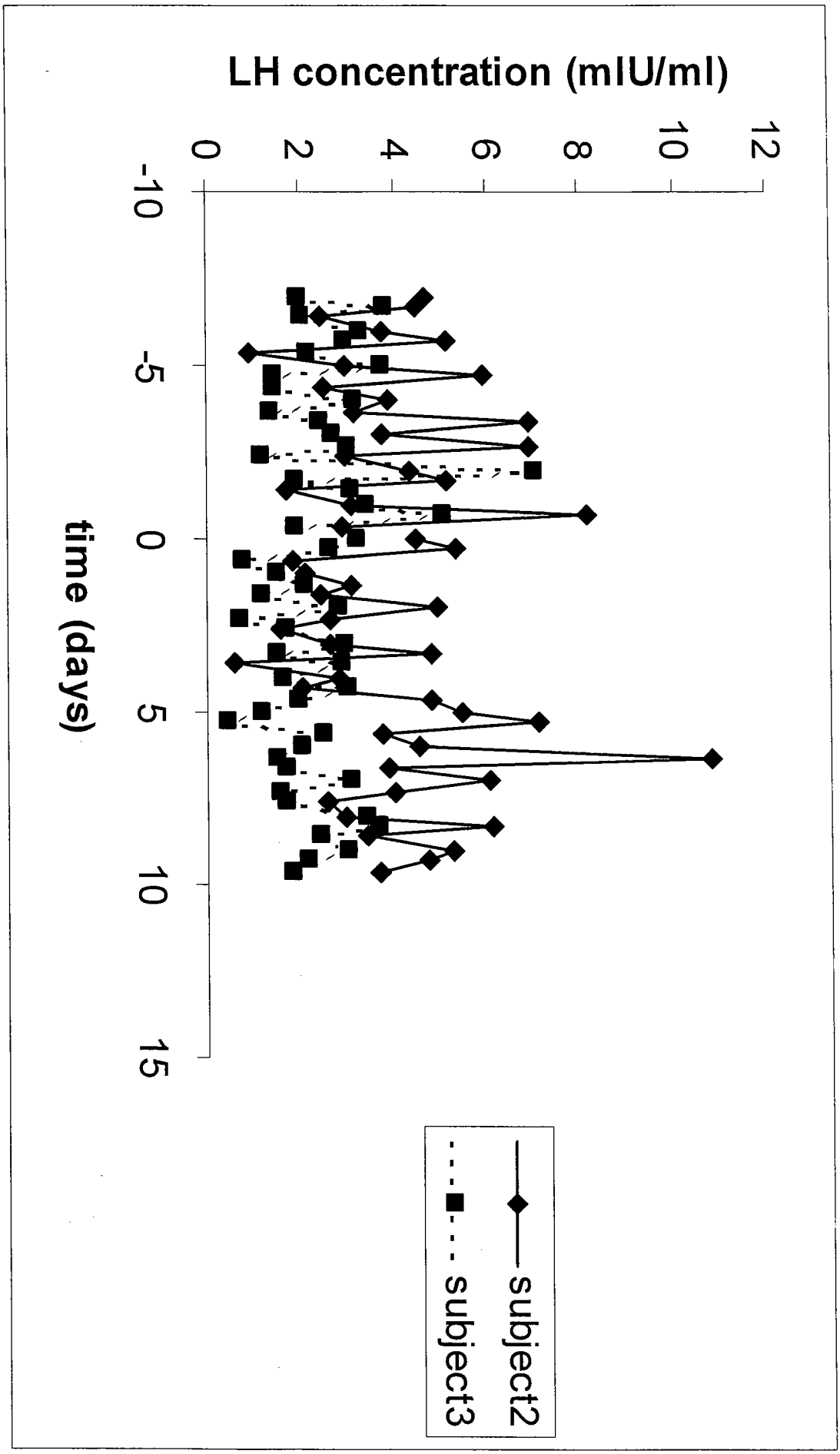
Steroid	RRT	m/z
Androsterone	0.745	419-434
Etiocholanolone	0.756	419-434
5α-Androstane-3α,17β-diol	0.766	241
5β-Androstane-3α,17β-diol	0.773	241
Dehydroepiandrosterone	0.820	417-432
5α-Androstane-3β,17β-diol	0.851	421
Epitestosterone	0.851	432-417
Dihydrotestosterone	0.865	434
4-Androstene-3,17-dione	0.879	430
Testosterone	0.901	432-417
11β-Hydroxyandrosterone	0.919	522
11β-Hydroxyetiocholanolone	0.930	522
17α-Methyltestosterone (IS)	1.000	446-301

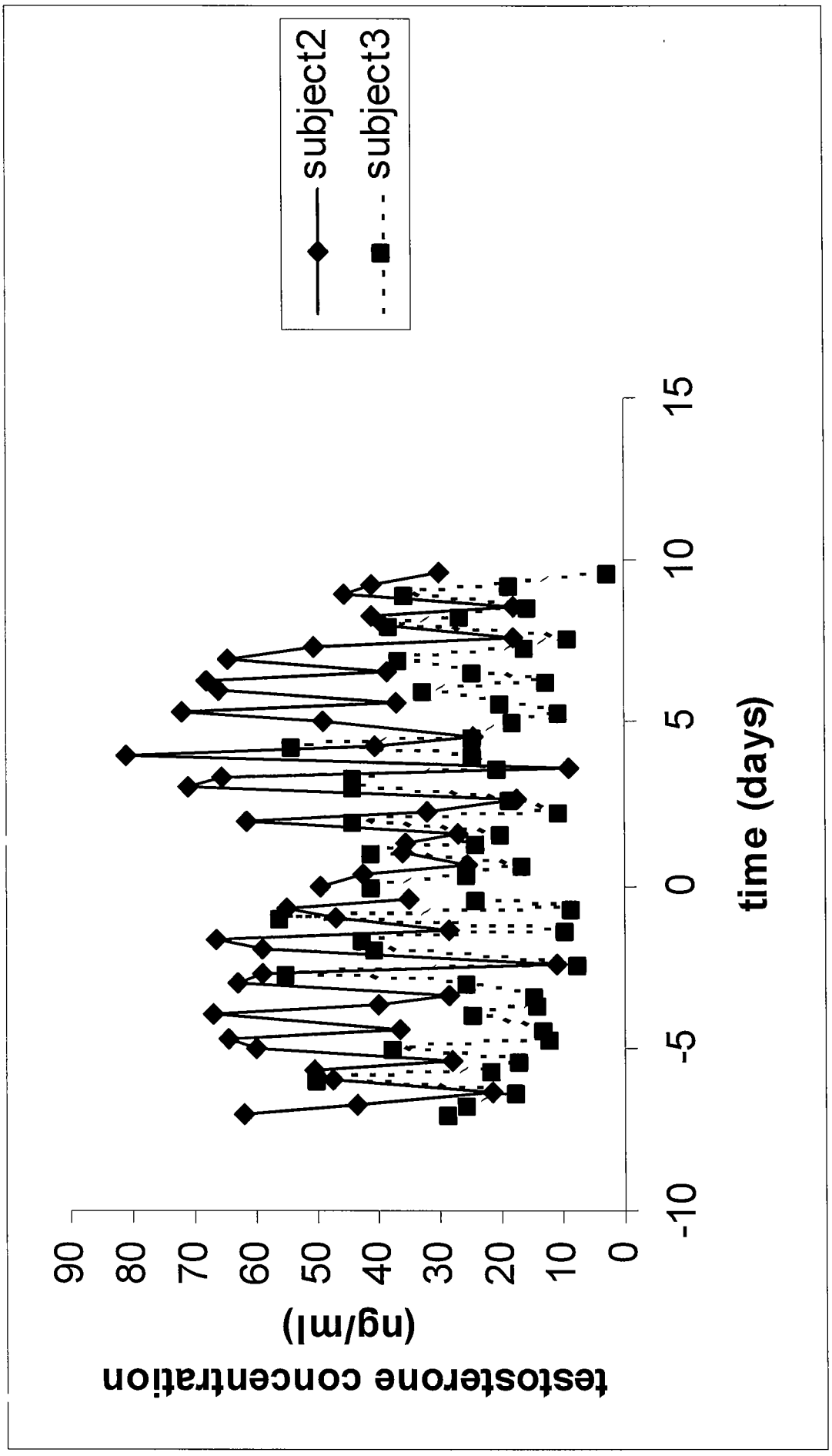
Legend to Figures:

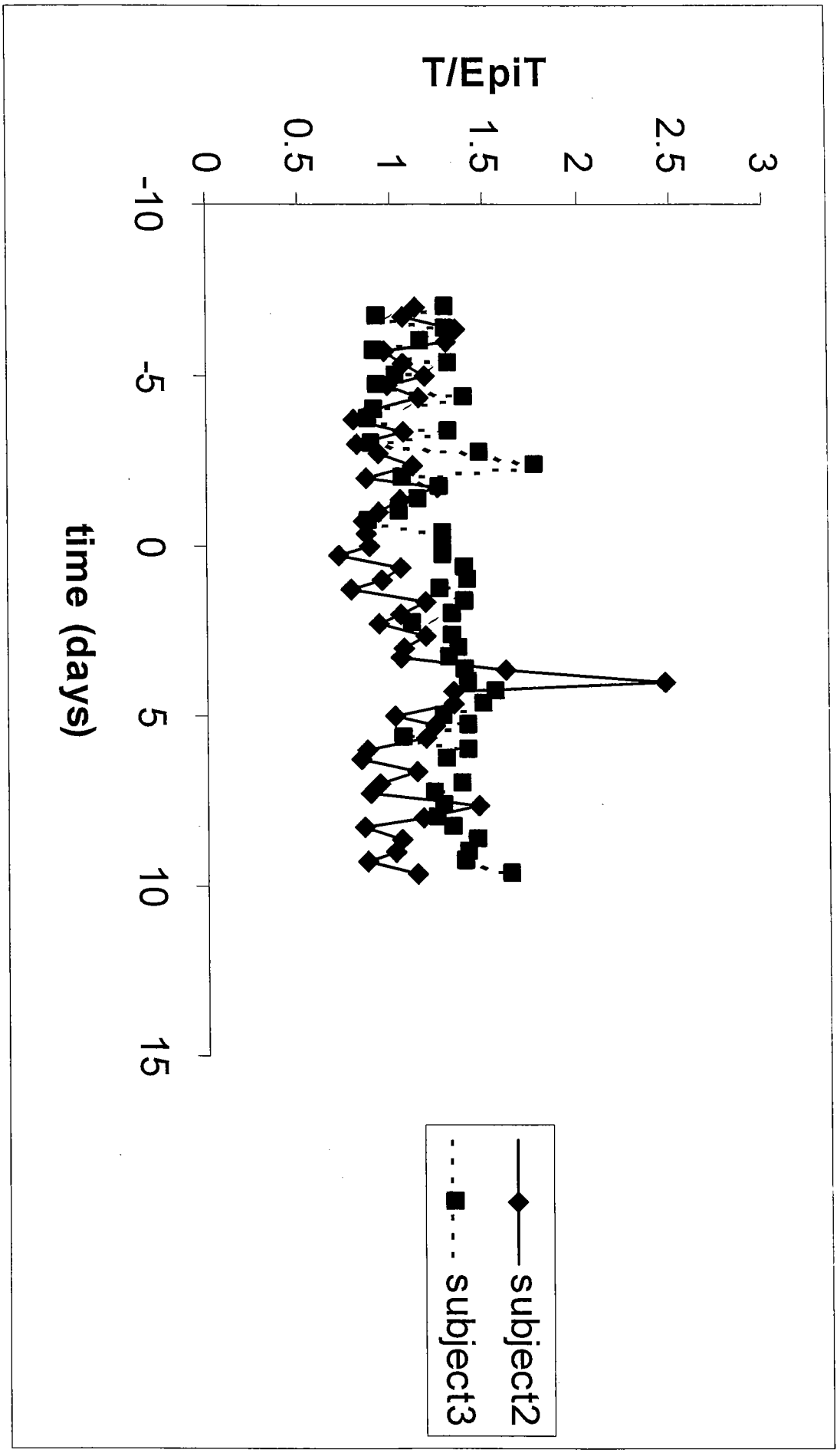
Fig.1 Urinary LH concentrations (mIU/ml) prior to and after intake of Tribulin® for two subjects. The intake of the first Tribulin® tablet is day 0.

Fig.2 Urinary testosterone concentrations (ng/ml) prior to and after intake of Tribulin® for two subjects. The intake of the first Tribulin® tablet is day 0.

Fig.3 Urinary testosterone to epitestosterone ratio prior to and after intake of Tribulin® for two subjects. The intake of the first Tribulin® tablet is day 0.


LH concentration (mIU/ml)
0
2
4
6
8
10
12
-10
-5
0
5
10
15
time (days)
subject2
subject3


subject2
subject3
testosterone concentration
(ng/ml)
0
10
20
30
40
50
60
70
80
90
-10
-5
0
5
10
15
time (days)


T/EpiT
0
0.5
1
1.5
2
2.5
3
-10
-5
0
5
10
15
time (days)
subject2
subject3