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An increased testosterone to epitestosterone ratio due to high doses of ethanol - a case report on a female powerlifter

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Introduction

Determination of the ratio of testosterone and epitestosterone (T/E) in urine is used to detect testosterone administration in athletes. According to the rules of International Olympic Committee a T/E ratio greater than 6 constitutes an offence, unless there is evidence that an unusually high ratio could be due to a physiological or pathological condition, e.g. low epitestosterone excretion, enzyme deficiencies, tumour androgen production or dietary factors (2-6).

In Finland, a female powerlifter gave a positive doping test with the T/E ratio 8.6. As a consequence the national anti-doping committee required additional experiments which indicated that the high T/E ratio was preferably due to the consumption of high amounts of ethanol an evening before the out of competition test. Therefore, the effect of ethanol intake on urinary T/E ratio was investigated experimentally in 4 male and 4 female volunteers. The results obtained gave further evidence on the increasing effects of high amounts of ethanol on the urinary T/E ratio especially in females.

Materials and methods

Case of a female powerlifter suspected of doping.

A female powerlifter (38 years old, 48 kg body weight) gave a positive out of competition test with a high T/E ratio. In this sample the urinary T/E ratio was 8.6 and testosterone concentration 82 ng/ml. Records of the Finnish Anti-Doping Committee showed that the athlete had been tested three times previously. In these tests carried out 3 months, 5 years 2 months and 5 years 3 months earlier, the urinary T/E ratios had been at normal level: 0.6, 0.8 and 0.5, respectively. The athlete strongly denied the use of testosterone or other prohibited substances. She told to have had a party and become intoxicated the night preceding the test occasion. She was tested again one week later the T/E ratio being now 0.7.

The Supervisory Group of the Finnish Anti-Doping Committee decided to investigate the urinary T/E ratio of the athlete during one menstrual cycle. Urine and serum samples were collected three times a week (on every Monday, Wednesday and Friday) for four weeks. All samples were kept frozen until analysis. The athlete was asked to keep a diary about her food intake, alcohol consumption, use of medicine etc. during the study. The samples were analysed for serum testosterone, urinary testosterone, epitestosterone and creatinine by routine methods in an IOC accredited laboratory. Since the results suggested that heavy drinking of alcohol in the night preceding the test might have resulted in a high T/E ratio, another follow-up period (13 days) was started. The results of this period confirmed those of the first one. Thereafter, the Finnish doping authorities felt it necessary to carry out an experiment in which the urinary T/E ratio after ethanol intake was studied in healthy females and males.

Experiment on the effect of ethanol intake on urinary and serum hormone levels in males and females.

Four female (20-24 years old, mean 22) and four male (19-22 years old, mean 20.5) subjects volunteered in the study. The volunteers consumed first 1.2 g of ethanol per kg of body weight during 5 hours starting at 5 p.m. One month later the procedure was repeated using 2.0 g of ethanol per kg of body weight.

Urine samples were collected daily for 4 days starting one day before the day of ethanol-intake. Samples were collected each day from 8 a.m. to 12 noon. Serum samples were taken

one day before and up to two days after the day of ethanol-intake, always at 12 a.m. All samples were kept frozen until analysis.

Serum testosterone, urinary testosterone, epitestosterone, androsterone, etiocholanolone, 11β-hydroxyandrosterone and 11β-hydroxyetiocholanolone and creatinine were assayed.

Analytical procedures.

All assays were carried out in duplicates. The quantitation of testosterone, epitestosterone, androsterone, etiocholanolone, 11β–hydroxyandrosterone and 11β-hydroxyetiocholanolone in urine was performed by gas chromatography - mass spectrometry selected ion monitoring (GC/MS SIM). The samples were prepared as presented previously (7, 8) with some modifications. Briefly, the procedure consisted of extraction of urine samples on Sep-Pak C18 columns, enzymatic hydrolysis with β-glucuronidase from E. coli, extraction with n-pentane and derivatisation with MSTFA/TMSI/dithioerythritol. Methyltestosterone, d_3-testosterone and d_5-epitestosterone (9) were used as internal standards. Five levels of standards were prepared in water and treated in the same way as other samples. Samples were analysed on HP 5890E / HP 5972A GC/MS (Hewlett-Packard) using a fused silica capillary column (HP 1, 16 m, 0.2 mm i.d., film thickness 0.11 μm). Helium was used as a carrier gas (constant flow 0.5 ml/min). Oven was first programmed from 180 to 230°C at 3°C/min and finally to 310°C at 30°C/min. Split injection (3 μl, 1:28) was done at 280°C. Ions m/z 301.1, 430.3, 431.3, 432.3, 434.4, 435.3, 436.3 and 522.45 were monitored. The inter-assay CVs were < 9 % for testosterone and epitestosterone and < 14 % for all other steroids.

Urinary creatinine was determined with a commercial colour reagent kit (Bayer Diagnostics Manufacturing S.A., Belgium). The inter-assay CV was < 4 % over the whole concentration range.

Serum total testosterone was measured by a RIA kit (Diagnostic Products Corporation, USA). The inter-assay CV was < 7 %.

Results

Findings from the samples taken from the powerlifter suspected of doping.

During the first follow-up period the urinary T/E ratio of the athlete was relative constant (T/E ratios varied from 0.4 to 0.8) except on two days when ratios 3.0 and 4.2 were measured. Similar observations were made when urinary testosterone was related to creatinine but urinary epitestosterone to creatinine ratio was relatively constant during the whole period (Figure 1.). Serum testosterone concentrations varied from 0.8 to 2.4 nmol/l, the mean being 1.4 nmol/l. The highest values (2.4 and 2.0 nmol/l) were measured on those days when urinary T/E ratios were increased. Heavy alcohol intake preceded both of these days. No evidence of the alteration of the T/E ratio due to the phase of menstrual cycle was observed. During the second follow-up period remarkably high urinary T/E ratio, urinary testosterone concentration and serum testosterone concentration were seen only on one day (4.5, 49.8 ng/ml and 2.9 nmol/l, respectively). On the other days the corresponding values varied from 0.4 to 1.0, from 0.6. to 7.5 ng/ml and from 0.8 to 1.2 nmol/l, respectively. It was seen from the diary kept by the athlete that she had been drinking again alcohol heavily (1-2 g of ethanol per kg of body weight) during the night before high T/E ratio and high testosterone concentration were measured.

Findings from the experimental study.

Intake of ethanol in amounts 1.2 g per kg of body weight in male and female volunteers did not cause remarkable changes in urinary or serum hormone concentrations. However, a dose of 2 g of ethanol per kg of body weight caused an increase of the T/E ratio in the samples collected day after ethanol consumption especially in females (Figure 2.). Similar changes were observed in urinary testosterone/creatinine ratio but not in epitestosterone/creatinine ratio (Figure 3.). On the other hand, ethanol caused a decrease in urinary androsterone/creatinine and etiocholanolone/creatinine ratio but did not affect the 11β-hydroxyandrosterone/creatinine and 11β-hydroxyetiocholanolone/creatinine ratio. Testosterone concentration in serum varied widely. The highest values were reached in samples taken just after ethanol-intake. The detailed results of the study will be published elsewhere.

Discussion and conclusions

The effect of ethanol on the urinary T/E ratio in male subjects has been investigated previously by Falk et al. (5). They found out that doses of ethanol greater than 1 g per kg of body weight resulted in a significant increase in the T/E ratio. The increase ranged from 30 % to 90 % in different subjects and was observed up to 22 hours. In our experimental study 2 g of ethanol per kg of body weight affected strongly the T/E ratio in females. The average increase of this ratio was 392 %. The increased T/E ratio was due to elevated testosterone concentration in urine. On the other hand, in our study ethanol did not show any significant effect on the T/E ratio in males even at a dose of 2 g/kg. In fact the T/E ratio was slightly elevated (mean 50 %) also in males but due to the low number of subjects and large individual variations in hormone concentrations and timing of sampling this increase did not reach the statistical significance. All data presented suggests that ethanol effects more strongly on the T/E ratio in females than in males. As discussed by Falk et al., it is well documented that ethanol can affect oxido-reduction of steroids by increasing the ratio between NADH and NAD^+ in plasma. This fact may at least in part explain the strong effect of ethanol on the T/E ratio in females because in females large amounts of testosterone are formed via peripheral conversion from androgen precursors (10).

In conclusion, ethanol can affect the urinary T/E ratio. However, the doses of ethanol have to be high in order to increase this ratio markedly. The effect of ethanol on the T/E ratio is more obvious in females than in males. Consumption of high doses of ethanol on the day before the doping test may result in a T/E ratio > 6 and it should be taken into account especially when performing out of competition tests. In the case of the female powerlifter, the Finnish doping authorities did not consider her case as doping.

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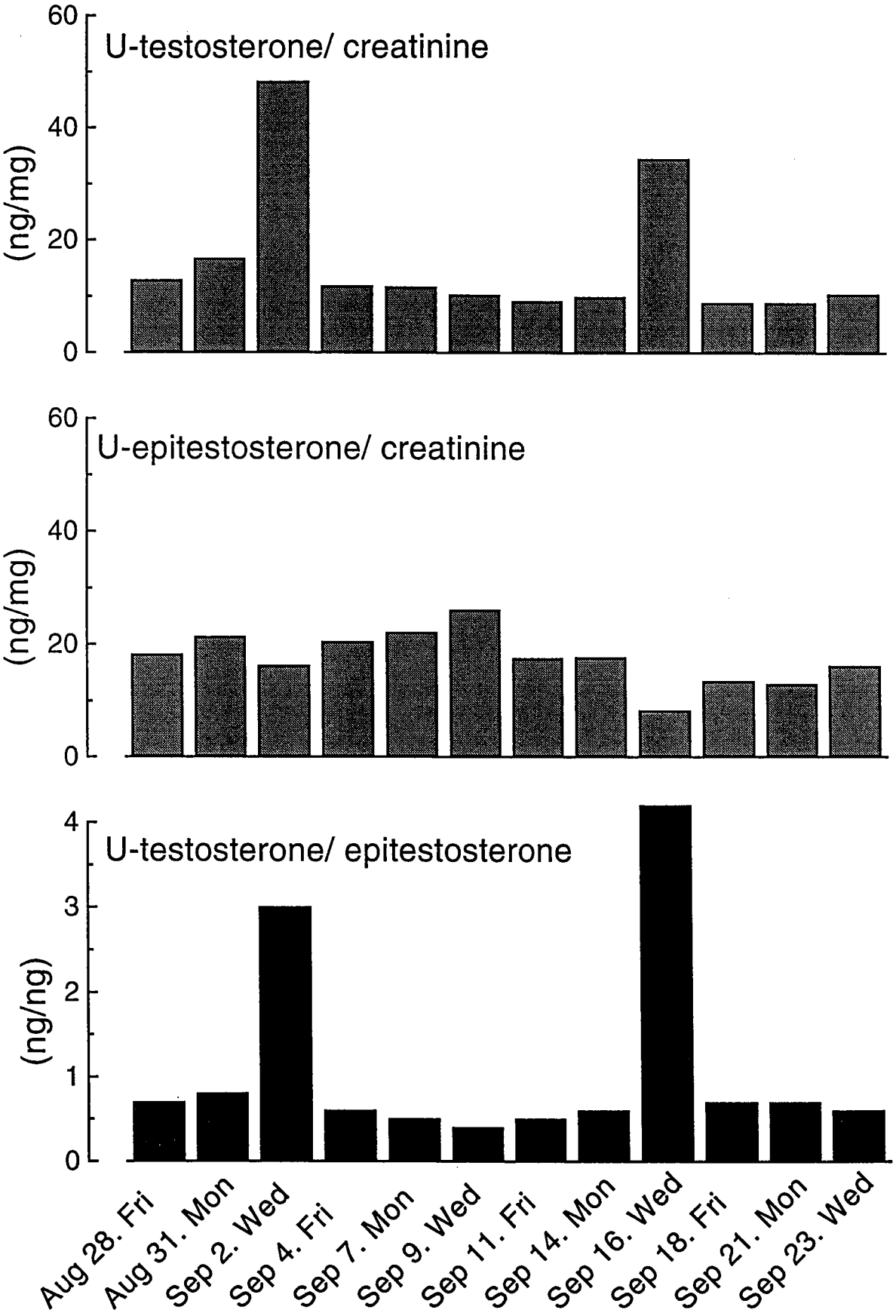
FIGURE 1. One month follow-up of urinary testosterone and epitestosterone in a female powerlifter. Urine samples were collected three times a week. Testosterone and epitestosterone concentrations are related to creatinine. The athlete had been drinking heavily (1-2 g of ethanol per kg of body weight) nights preceding the two days with high values of T/E .

FIGURE 2. Effect of ethanol ingestion on urinary T/E ratio. All subjects received 2.0 g/kg of ethanol during 5 hours starting at 5 p.m. Urines were collected for 4 days starting at 8 p.m. for

4 hours. Day 0 refers to the day of ethanol intake. The results are means ± SEM for four female and male subjects. * = $p < 0.05$ vs. baseline (paired t-test).

FIGURE 3. Urinary testosterone and epitestosterone related to creatinine under the same experimental conditions as in Figure 2. * = $p < 0.05$ vs. baseline.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Aug 28. Fri
Aug 31. Mon
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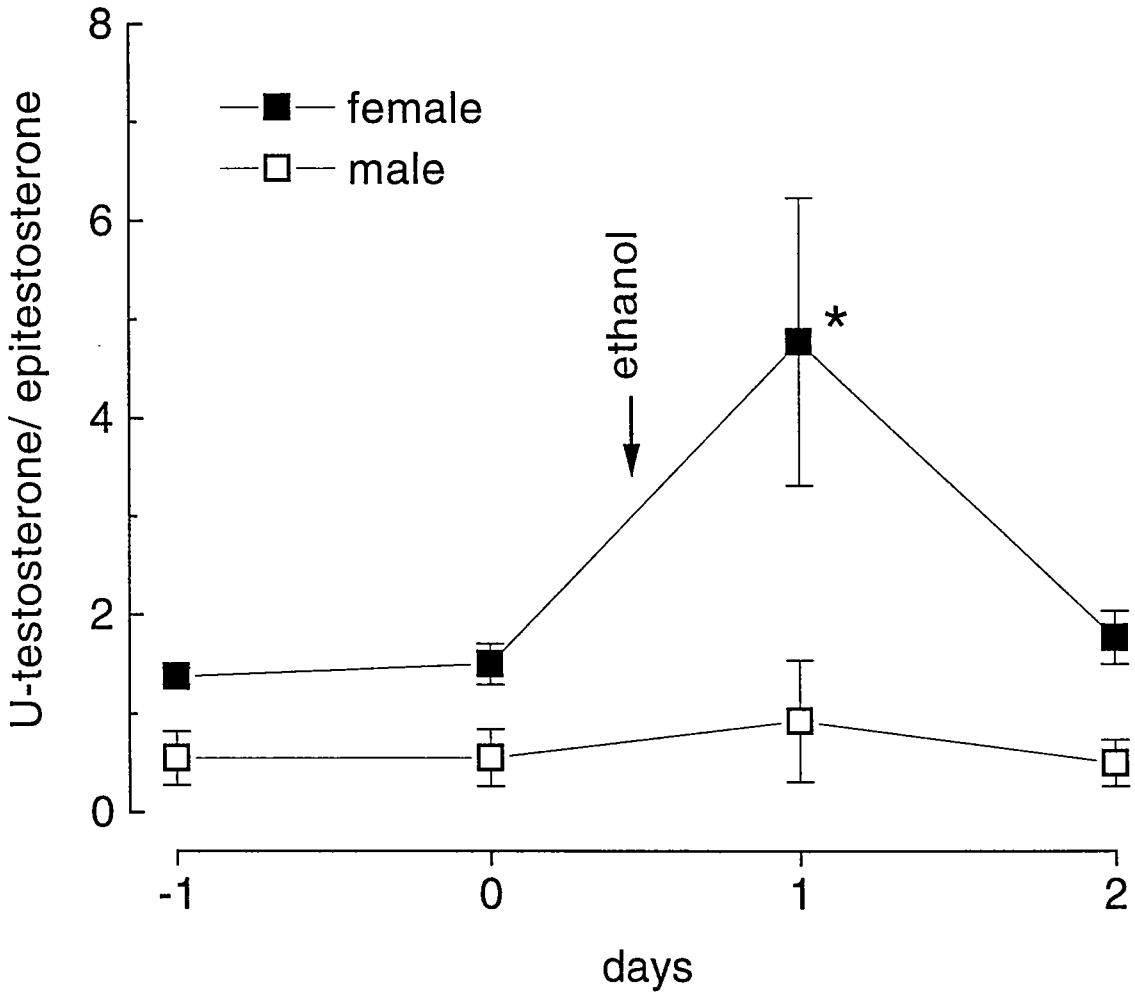
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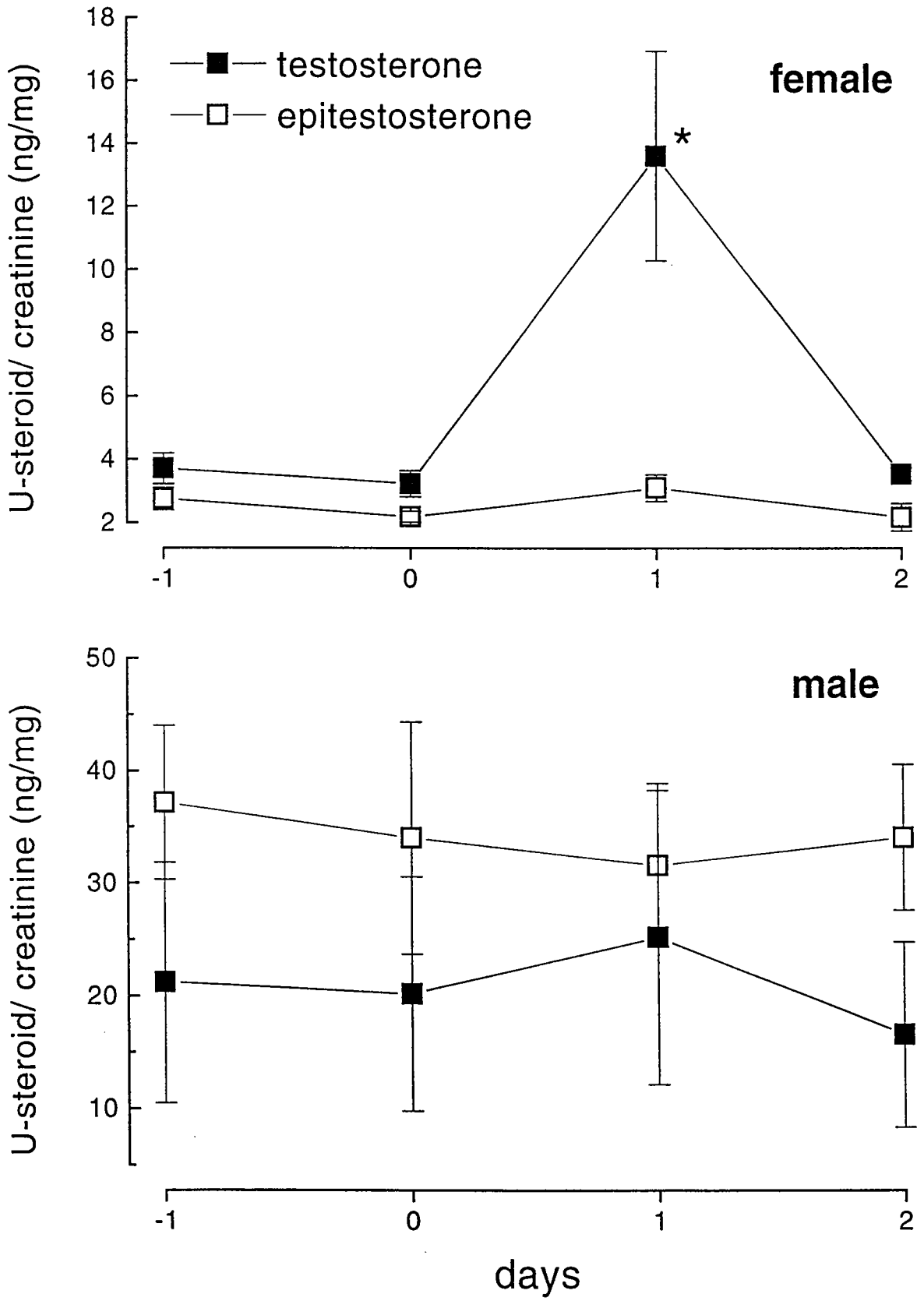
testosterone
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