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Excretion of Ephedrine and Endogenous Steroids under Conditions of Controlled Water Intake and of Water Diuresis

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1. Introduction

At the occasion of the 9th Cologne Workshop in Dope Analysis, 8 participants volunteered in an excretion study with ephedrine in order to evaluate the extent water diuresis influences the detection of ephedrine under routine doping analysis conditions.

The results of this study also give valuable information on the excretion of the endogenous androgenic steroids under the influence of water diuresis. Further it was of interest if ratios like the testosterone / epitestosterone ratio remain constant under such conditions.

For dope analysis only spot samples collected after an event or out of competition are available. Normally, no information detailing the exact volume and time interval in which the urine was collected is available. Therefore, urine concentrations of doping agents are the only measureable parameter. However, these are influenced by renal function and urine flow. In spot urine samples, renal function may be assessed by specific gravity and creatinine concentration. This study was performed in order to determine which of these two parameters is more appropriate to correct the urine concentration for differences in renal function and urine flow.

2. Experimental

2.1 Design

8 participants of the workshop volunteered to participate in the study. One person was excluded due to inconsistent urine collection. The protocol for fluid intake, the oral application of 25 mg ephedrine, and the sample collection scheme is shown in Table 1. The first day with a controlled fluid intake serves as control for the situation of water diuresis on the second day.

Table 1: Protocol for drinking, application of ephedrine and sample collection.

1st day - Wednesday 20th March 1991			
time	samples	ephedrine	drinking
7:45	empty the bladder		
8:00	breakfast		300 ml
9:00	1st sample		
11:00	2nd sample	**25 mg** (orally)	200 ml
13:00	3rd sample		300 ml
14:00	4th sample		
15:00	5th sample		
16:00	6th sample		
18:00	7th sample		free
20:00	8th sample		
2nd day - Thursday 21th March 1991			
time	samples	ephedrine	drinking
7:45	empty the bladder		
8:00	breakfast		300 ml
9:00	1st sample		
11:00	2nd sample	**25 mg** (orally)	200 ml
13:00	3rd sample		10 ml/kg*
14:00	4th sample		
15:00	5th sample		
16:00	6th sample		
18:00	7th sample		
20:00	8th sample		

* 10 ml/kg bodyweight every 15 min.

2.2 Analysis

The following data were obtained:

1. volume, time, pH, specific gravity (Cologne)
2. ephedrine and creatinine (Indianapolis),
3. steroid profile (Cologne).

2.2.1 Procedure for Ephedrine Quantification

The concentration of ephedrine in the urine samples was determined using a modification of the selective derivatization procedure utilized by most antidoping laboratories for the screening of non-conjugated drugs [1]. Briefly, 200 μl of calibrator (1, 5, 10, 20 μg/ml), blank, or unknown were added to a Toxi-Lab A extraction tube. 100 μl of pholedrine (10 μg/ml) was added to each tube as an internal standard. 4 ml of distilled water was then added to each tube to make up the volume for the extraction. The tubes were capped and extracted by inversion for 2 minutes and centrifuged for 5 minutes. The top, organic, layer was transferred to a disposable centrifuge tube, to which 50 μl of TMCS was added. The contents were evaporated to dryness under nitrogen. The residue was reconstituted with 50 ml of a mixture of acetonitrile and trifluoracetic acid (60/40), which contained 200 mg/l of methyl orange as a pH indicator. To this solution MSTFA was added dropwise until the color changed from red to yellow. The tubes were allowed to cool and 10 μl of MBTFA was added to each tube. The tubes were heated for an additional 15 minutes at 80°C. The derivatized extract was transferred to a GC vial and analyzed by GC/MS operated in the selective ion monitoring mode. The area ratio of the quantification ion (179 m/z) for ephedrine versus the quantification ion (179 m/z) for the pholedrine internal standard was calculated for each sample. The calibration curve was constructed by performing a linear regression of the area ration versus the calibrators. The ephedrine study samples were then quantified versus this calibration curve.

2.2.2 Creatinine Concentration

The determination of the creatinine concentration in the urine sample was performed on an Abbott TDX according to manufacturer's protocols.

2.2.3 Steroid Profile

The concentrations of the endogenous steroids of the steroid profile were determined following the routine screening procedure IV for the detection of anabolic steroids [2].

2.3 Correction of urinary concentrations

Urinary concentrations were corrected to a specific gravity of 1.020 g/ml, if the specific gravity was lower than 1.020 g/ml.

The formula for correction is:

$$(1)\quad c_{corr} = f * c_m$$

$$(2)\quad f = \frac{1.020 - 0.998}{d - 0.998}$$

f = factor for correction
c_{corr} = corrected concentration
d = measured specific gravity
c_m = measured concentration
0.988 = s.g. of water

The creatinine correction is calculated as substance to creatinine ratio e.g.

$$(3)\quad \frac{\mu g \text{ ephedrine}}{mg \text{ creatinine}}$$

3. Results and Discussion

3.1 Diuresis

Table 2 shows the descriptive statistics for specific gravity and urine flow. All participants complied with the protocol and reached the maximum urine flow on the 2nd day between 14 and 16 hours.

Table 2:
Descriptive statistics of specific gravity (s.g.in g/ml) and urine flow (flow in ml/min) for the first day with normal water intake and the second day with water diuresis (n=7).

		mean	median	percentile 10th	percentile 90th
s.g.	1st day	1.021	1.022	1.016	1.026
	2nd day	1.010	1.010	0.9998	1.023
flow	1st day	1.03	0.95	0.30	1.72
	2nd day	6.27	4.00	0.73	15.32

3.2 Ephedrine

Figure 1.1 shows the mean of the ephedrine concentration on the first and second day. On the 2nd day, the extensive drinking started 2 hours after the oral application of 25 mg ephedrine. As these data show, it is possible to manipulate a urine sample so that the ephedrine concentration falls below the cut-off limit of 5 μg/ml set by the IOC Medical Commission.

Figures 1.2 and 1.3 show the correction by specific gravity and creatinine respectively. Both corrections compensate for the dilution. Comparing the values of the 1st to those of the 2nd day, the correction by creatinine gives higher values on the 2nd day whereas the correction by specific gravity does not compensate for the dilution.

The cumulative excretion of ephedrine (fig. 2) is significantly higher on the 2nd day than on the first, which is reflected in the values of the ephedrine/creatinine ratio, but not in the specific gravity corrected concentrations.

If the ephedrine/creatinine ratio shall be used, a new cut-off limit must be defined. The average of this ratio in the morning of the 2nd day is about 2 μg ephedrine / mg creatinine so that based on these results a reasonable cut-off limit might be 3 μg ephedrine /mg creatinine .

These results show that the correction by specific gravity will be a correction in the right direction, but it does not fully compensate for the dilution by water diuresis.

On the other hand it is well known that the creatinine concentration is related to muscle mass and increased by high workload (physical activity), so the creatinine excretion and by this the concentration may be higher than under resting conditions. This will lead to lower values of the ratio of ephedrine to creatinine as well as other creatinine based ratios after physical activity.

Both corrections by specific gravity and by creatinine will thus be in "favour" of the athlete as lower concentrations and lower substance to creatinine ratios will result.

3.3 Endogenous Androgenic Steroids

Figure 3 shows the results when the two correction methods are applied to the androsterone concentration for person 7.

In order to compare the goodness of correction of endogenous androgenic steroid concentrations, the first day was used as reference (100%) and the absolute difference (in %) between first and second day was calculated for the androsterone concentration corrected for specific gravity respectively corrected by the creatinine concentration. Both differences were compared with a t-test (Table 3.1). The mean difference between first and second day is significantly lower when correcting by creatinine than by specific gravity.

Table 3.1:
Comparison of the difference in % between first and second day of the androsterone concentration corrected by specific gravity ($and_{s.g.}$) and of the androsterone/creatinine ratio (and_{crt})
(T-test for dependent samples).

	diff ($and_{s.g.}$) [%]	diff (and_{crt}) [%]
mean	55.1	16.7
stdv	65.15	15.05
n	55	55
p	< .001	

Supposing that very dilute urines caused the problem, a piecewise linear regression (4) between androsterone concentration and specific gravity was calculated. The lower breakpoint was 1.007 g/ml. Then the comparison of the differences was made without urine samples with a specific gravity below this breakpoint of 1.007 g/ml, but both differences decreased and the difference of the creatinine ratio remained significantly lower (Table 3.2).

Table 3.2:
Comparison of the difference in % between first and second day of the androsterone concentration corrected by specific gravity ($and_{s.g.}$) and of the androsterone/creatinine ratio (and_{crt}) (T-test for dependent samples) without samples with a specific gravity lower than 1.007 g/ml.

	diff ($and_{s.g.}$) [%]	diff (and_{crt}) [%]
mean	34.4	12.9
stdv	23.62	11.18
n	31	31
p	< .001	

3.4 Androgen Ratios

As androgen ratios, especially the testosterone / epitestosterone ratio, serve in dope control as indicators for application of testosterone or any manipulation which leads to an increased testosterone production, the question whether this and other ratios could be influenced by water diuresis is of great interest. In Table 4 the mean and coefficient of variation of the ratios androsterone/etiocholanolone (A/Etio), testosterone / epitestosterone (T/E), and androsterone / testosterone (A/T) are summarized for each person. All three ratios are not influenced by the water diuresis. If the coefficient of variation is taken as a measure, they show the same stability as a reference group (5).

Table 4:
Coefficient of variation of the ratios androsterone/etiocholanolone (A/E),
testosterone/epitestosterone (T/E),
androsterone/testosterone (A/T) for each person (V) (n=16).

	A/E		T/E		A/T	
V.	Mean	vc%	Mean	vc%	Mean	vc%
1	2.12	8	.80	10	65.6	25
2	1.08	17	1.13	13	42.3	28
3	1.40	9	1.18	8	87.8	15
5	.84	15	.97	16	70.3	11
6	1.31	10	.49	14	70.9	20
7	1.43	13	.97	14	75.5	20
8	1.00	7	.09	33	360.9	15

4. Conclusions

In this study the correction of the urinary concentrations of ephedrine as well as of endogenous androgenic steroids relative to creatinine gives better results than that by specific gravity. The questions regarding the influence of high physical activity on the creatinine excretion remains open.

If only the specific gravity is available for correction, this kind of correction should only be applied for samples with a specific gravity between 1.007 and 1.020 g/ml. Samples with a specific gravity lower than 1.007 should be excluded and samples with a specific gravity higher than 1.020 g/ml not corrected.

5. References

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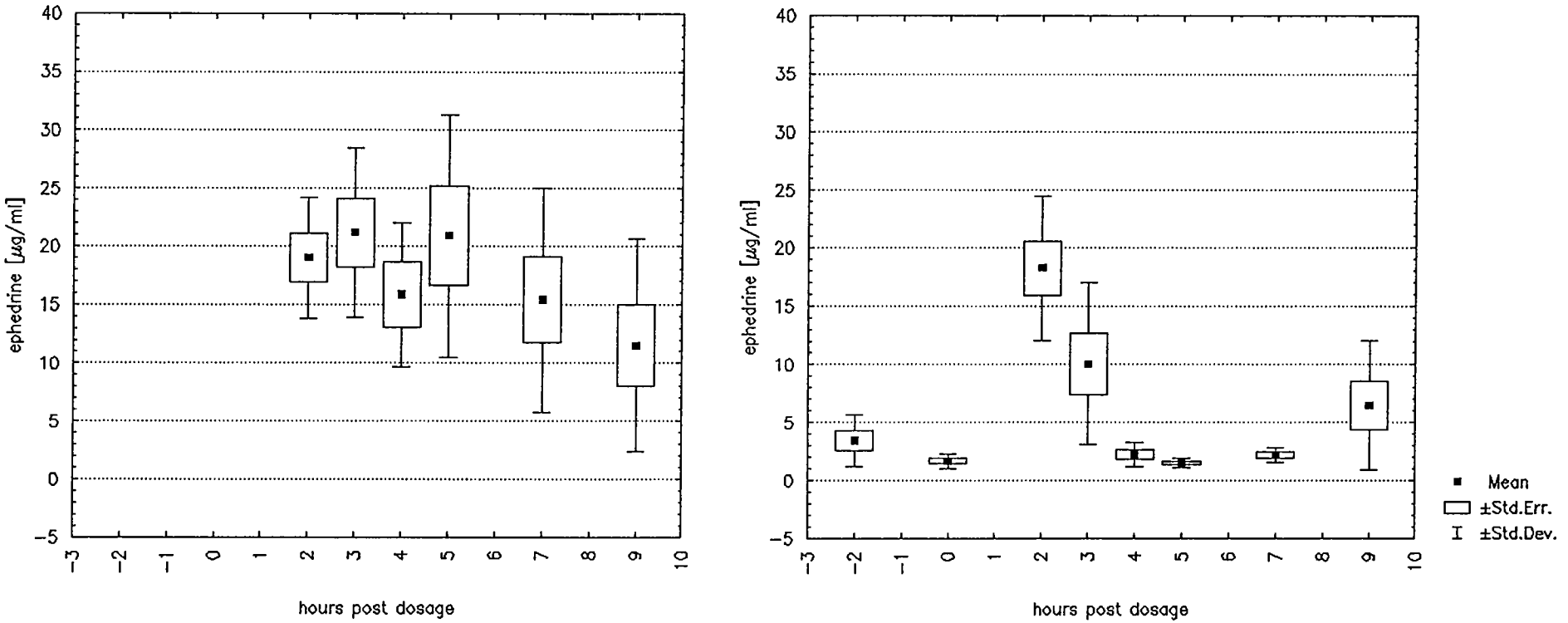

Figure 1.1:
Average concentration of ephedrine on the day 1 with normal water intake and on day 2 under water diuresis (n=7).

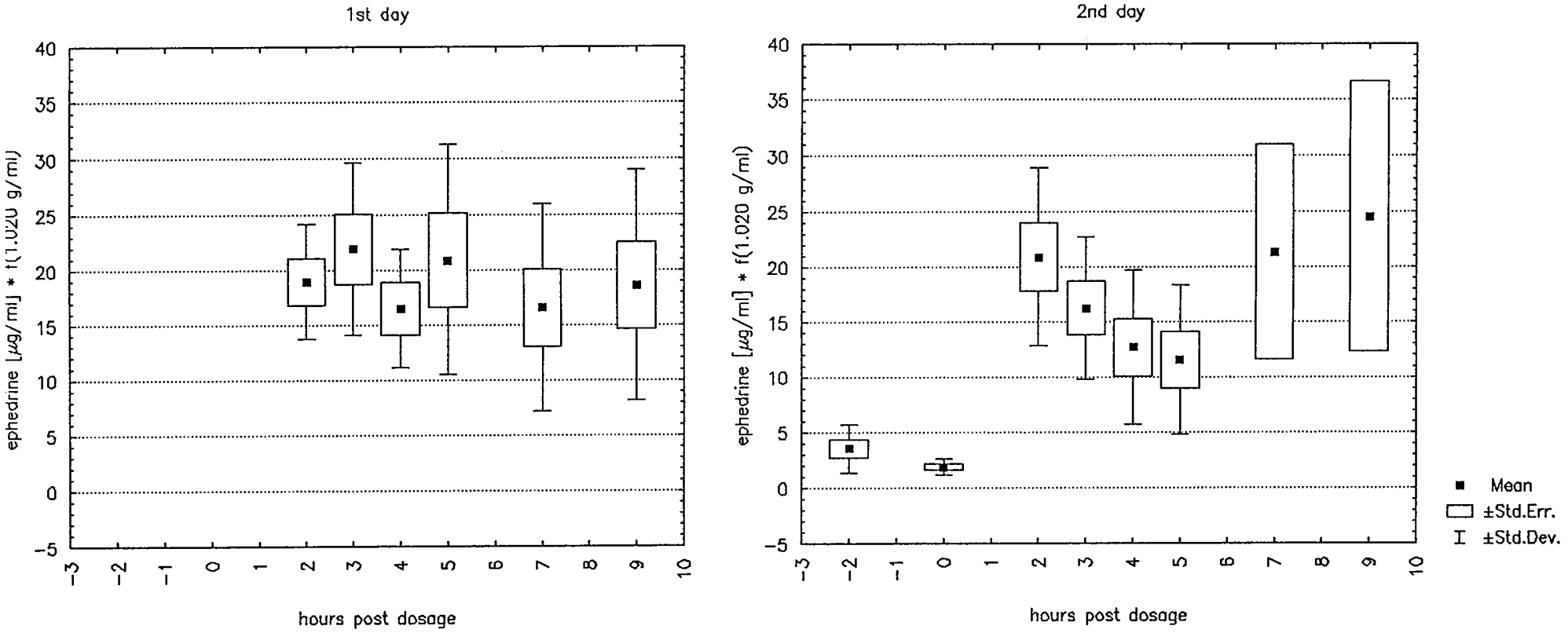

Figure 1.2:
Average concentration of ephedrine corrected to the specific gravity of 1.020 g/ml day 1 and day 2 (n=7).

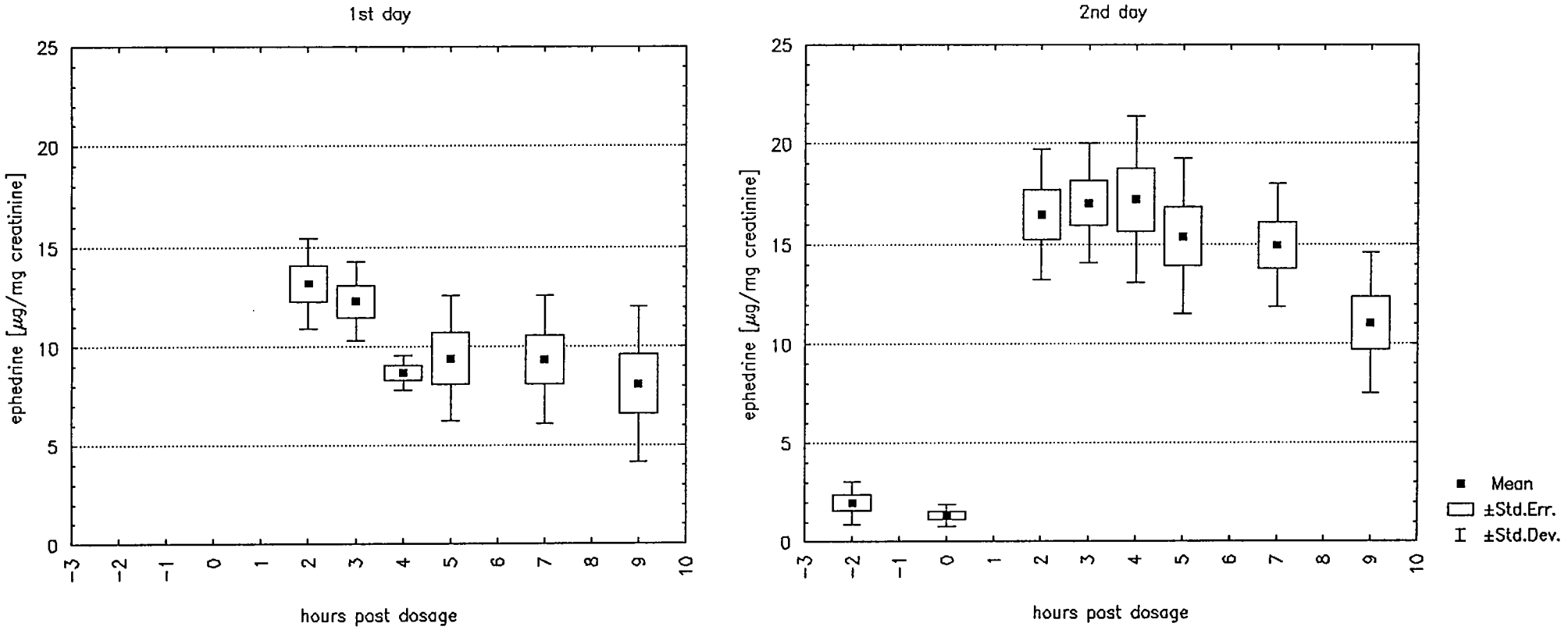

Figure 1.3:
Average ratio ephedrine/creatinine on day 1 and day 2 (n=7).

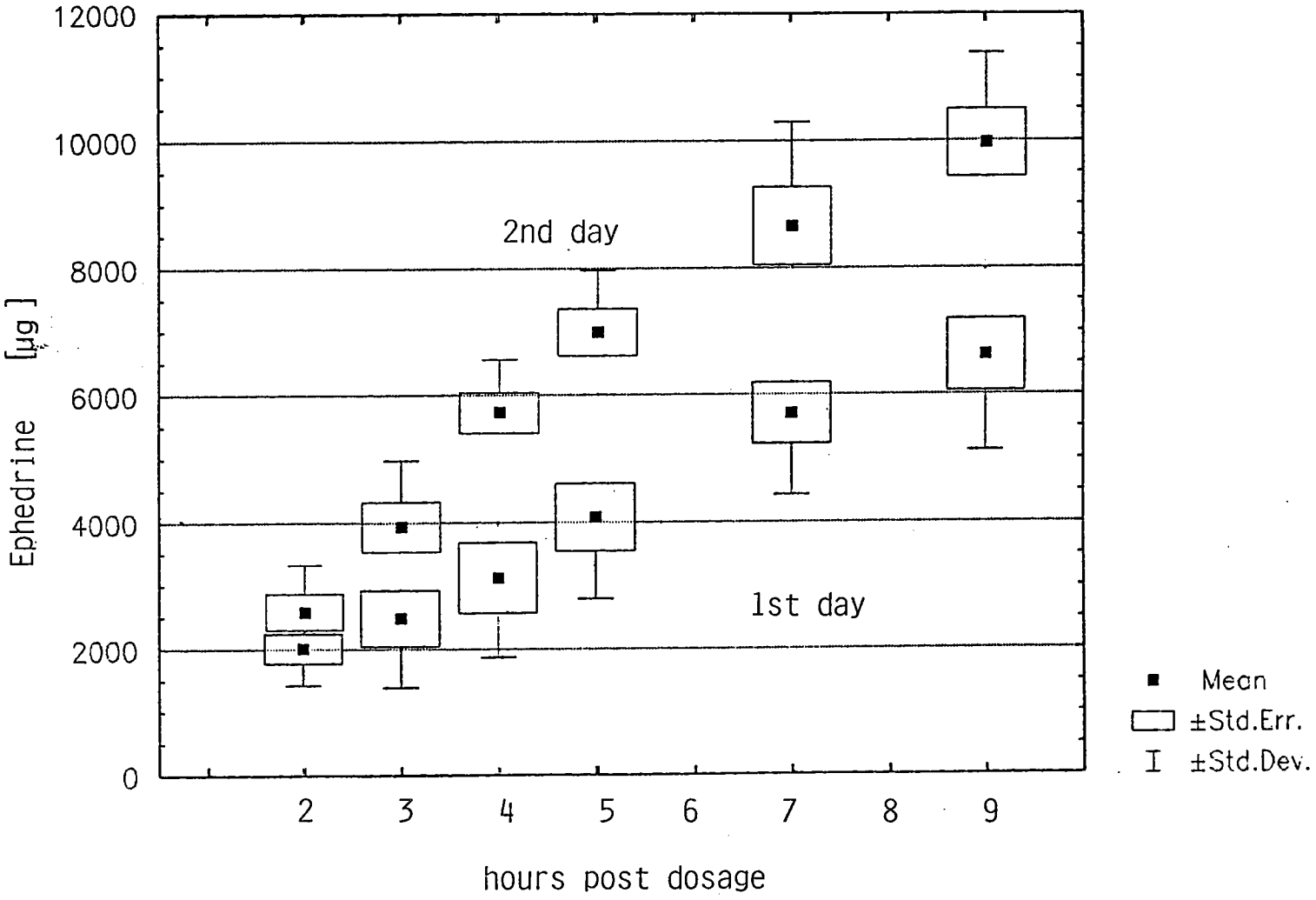

Figure 2:
Average cumulative ephedrine excretion on day 1 and day 2 (n=7).

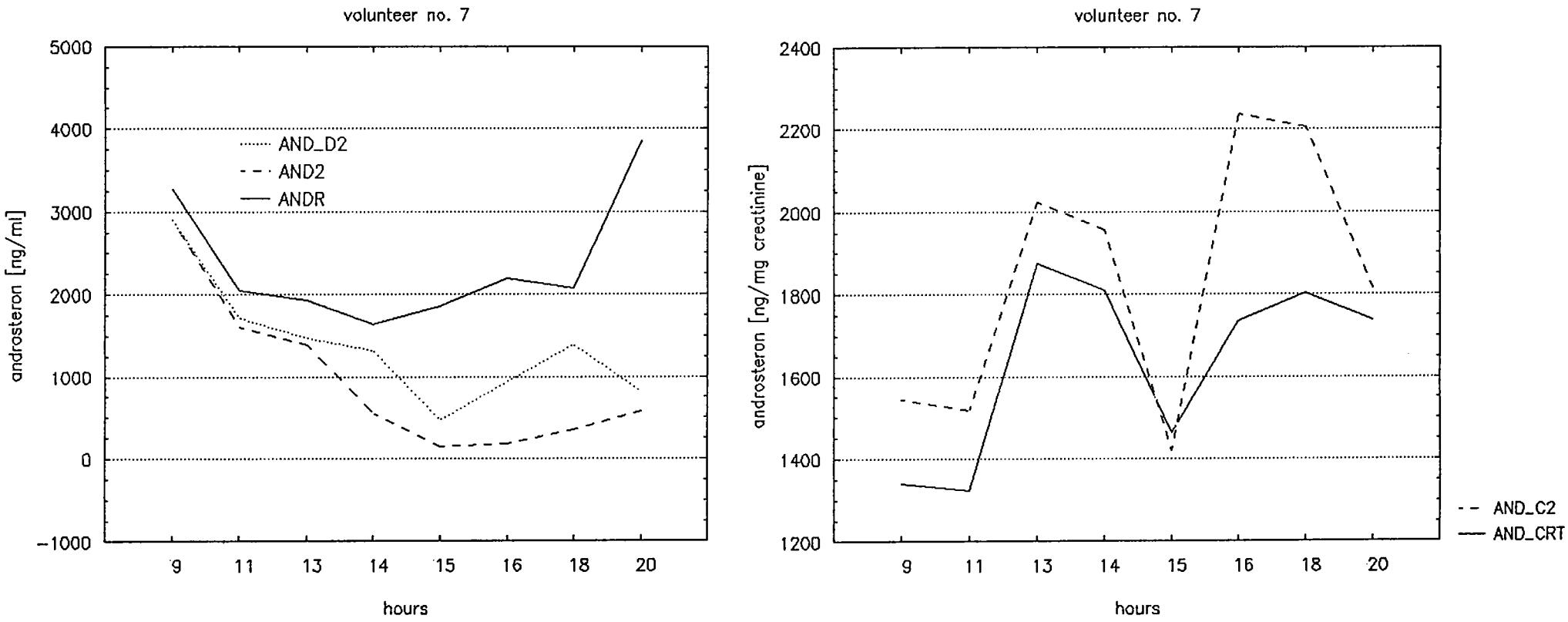

Figure 3:
Comparison of the androsterone concentration of volunteer number 7 with and without correction to a specific gravity of 1.020 g/ml and of the androsterone/creatinine ratio.
ANDR = androsterone concentration on day 1;
AND2 = androsterone concentration on day 2;
AND_D2 = androsterone concentration on day 2 corrected to a s.g. of 1.020 g/ml.
AND_CRT = ratio of the 1st day;
AND_C2 = ratio of the 2nd day;