Journal of Steroid Biochemistry, 1974, Vol. 5, pp. 167–171. Pergamon Press. Printed in Great Britain

CIRCADIAN VARIATIONS OF PLASMA TESTOSTERONE AND ESTROGENS IN NORMAL MEN. A STUDY BY FREQUENT SAMPLING

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(*Received* 8 *August* 1973)

SUMMARY

Plasma levels of testosterone, estrone and estradiol were measured by radioimmunoassay every 30 min for 25 h in four young adult males. A coincident though unidentical circadian rhythm of plasma testosterone was demonstrated in all cases. Rapid variations which were consistent with a pulsatile pattern of testosterone secretion were seen in each subject. In one of them an identical rhythm was shown again when reinvestigated 3 months later. Plasma estradiol level was constant throughout the day, but the estrone curve exhibited a phase of lower levels during the sleep while testosterone concentration was gradually increasing.

INTRODUCTION

The study of the circadian rhythm of plasma testosterone in normal men has been the subject of many investigations in recent years following the first publication by Dray *et al.* [11]. In most cases the existence of a circadian rhythm with a maximum in the morning was reported [6, 8, 11, 14, 20, 23, 28–31, 33, 36, 38]. However contradictory observations do exist [2, 5, 16, 19, 22]. The intermittent sampling technique could possibly account for these discrepancies since in most experiments, two successive blood samplings were separated by a 3–12 h interval. Such a time interval, because it is much longer than the testosterone half-life [15, 35, 37], does not allow a study to show eventual rapid variations superimposed on a diurnal rhythm. An alternative explanation could be that some subjects present very small variations, if any at all.

The purpose of this work was to follow closely the testosterone variations in four subjects by frequent blood sampling and to look for eventual variations of estrone and estradiol-17β which have never been thoroughly investigated.

SUBJECTS AND METHODS

A. Subjects

Four medical students, 24–25 years old, were examined. Three of them were strictly normal, the fourth had had one testis removed at the age of 12 following acute torsion, but his sexual development and activity were normal; he was married and the father of a child.

The spermogram and the excretion of urinary steroids (total and fractionated 17-oxo-steroids, 17-hydroxy-corticosteroids, pregnanediol pregnanetriol, estrone + estradiol and estriol) were normal. The same was true for red and white blood cell counts, sedimentation rate, fasting blood sugar and urea nitrogen.

B. Blood sampling

The four men were subjected to medical observation during 25 h, from 4:30 p.m. on the first day to 5:30 p.m. on the next one, without disrupting their usual rhythm of daily activity, consisting of rest and intellectual work. They were fed a normal diet of approximately 2500 calories a day.

Ten millilitres of heparinized blood was obtained every 30 min for 25 h starting at 5 p.m. by using a percutaneous Teflon catheter without disrupting the night's sleep. The blood was immediately cooled at 0°C., centrifuged, and the plasma stored at −20°C. until analyzed.

C. Radioimmunoassays

Testosterone was measured according to Leymarie *et al.* [21]. 40,000 d.p.m. of [1,2,6,7,-^{3}H]-testosterone (NEN-Specific activity 87 Ci/mM) was added to 0·1 ml of plasma diluted to 1 ml. This tritiated testosterone served both for recovery measurements and as a tracer in the final competition reaction. After extraction by ether (Merck purified just before use), testosterone was

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partially purified by chromatography on a Sephadex LH 20 column in benzene–ethanol 95/5. The immunological reaction was performed in duplicate on two aliquots of the eluate evaporated to dryness under nitrogen. The conditions for equilibration and separation of bound from unbound steroids conformed to those proposed by Castanier and Scholler for estrogen measurements [7]. The antiserum was raised in rabbits immunized against testosterone-3-(0-Carboxy) methyloxime–BSA.

The specificity of this method is good and only 5α-dihydrotestosterone can interfere in the assay, but the overestimation of the results is practically negligible as was shown by comparative measurements with mass spectrometry [9].

The reproducibility or interassay precision, at levels between 2 and 5 ng/ml, was 7·3% for duplicate assays. In 88 normal men, aged from 21 to 38, the average concentration was found to be 5·88 ng/ml (2·7 to 11·8 ng/ml) when measured between 9 a.m. and 10 a.m.

Estrone and estradiol-17β were measured according to Castanier and Scholler [7]. In order to reduce the blank value, the purification step was modified as follows: the height of the Sephadex LH 20 column was reduced to 40 mm, its dia. to 3 mm and the dye indicator was taken out. The antiserum was raised in rabbits immunized against estrone-17-(0-Carboxy) methyloxime–BSA.

The reproducibility for estradiol, at a concentration of 25 pg/ml, was characterized by a coefficient of variation of 14% when 2 ml of plasma samples were used. For duplicate determinations, in two different series, the coefficient of variation was 9·6%. The detection limit was 8 pg/ml.

Precision for estrone at the level of 36 pg/ml was characterized by a coefficient of variation of 28% with 2 ml plasma samples. For measurement in duplicate, the coefficient of variation was 20%. The detection limit was 17 pg/ml.

For both testosterone and estrogen assays, the binding measurements were performed using two aliquots of the Sephadex column eluates. Moreover, each

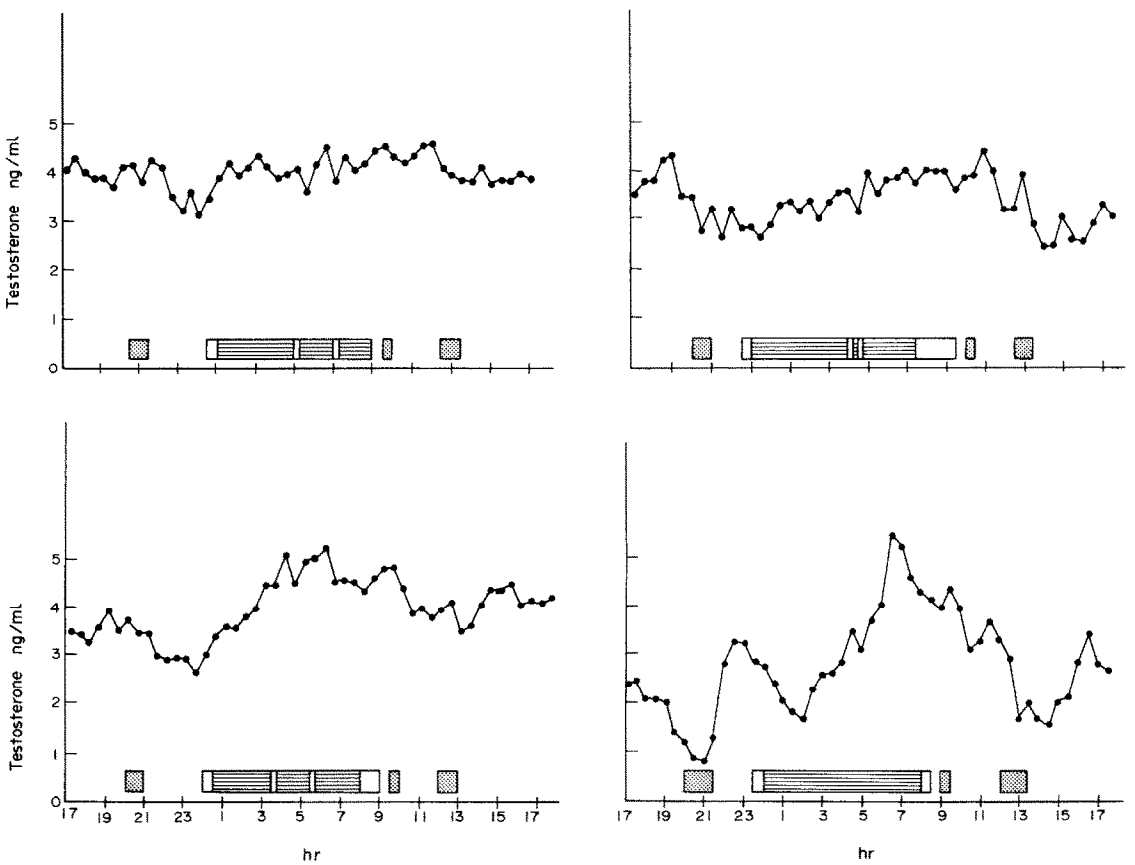

Fig. 1. 24 h variations of plasma testosterone in four men. Meal time, sleep and decubitus without sleeping are indicated by dotted, hatched and empty rectangles respectively.

plasma sample was processed in two different series which means four competition reactions per result. The elimination of outlying values was done according to the usual statistical methods [1, 10].

RESULTS

I. *Variations of the testosterone plasma level*

A circadian rhythm was obvious in subjects 3 and 4 (Fig. 1). It was not as evident in subjects 1 and 2 who exhibited, however, a phase of maximal concentrations between 9 and 11 a.m. and a phase of minimal concentrations between 11 p.m. and 1 a.m. The difference between these two levels is statistically significant ($P < 0{\cdot}05$). Moreover the value observed at 9 a.m. was, in all subjects, higher than the individual mean calculated from all samples (Table 1).

In all subjects, phases of transitory increase existed which were superimposed on the circadian rhythm.

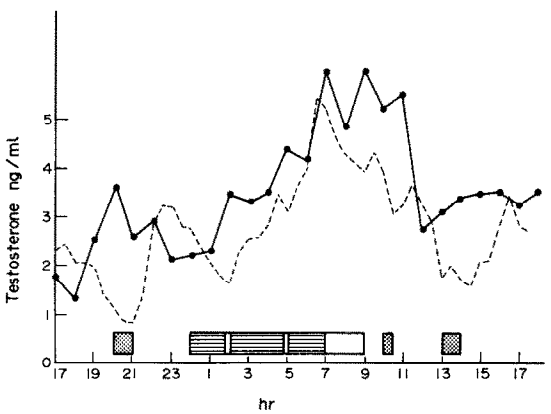

Fig. 2. Reproducibility of the testosterone circadian rhythm in subject 4. The dotted line represents the pattern obtained 3 months earlier as shown in Fig. 1. (Symbols as in Fig. 1).

One can also observe rapid rises (100% between 9:30 p.m. and 10 p.m. in subject 4; 30% between 12 p.m. and 1:30 a.m. in subject 1) and rapid falls (40% between 12:30 p.m. and 1 p.m. in subject 4; 36% between 1 p.m. and 2 p.m. in subject 2; 20% between 9:30 a.m. and 10:30 a.m. in subject 3).

In all subjects, the level at the end of the observation period was close to the starting level.

II. *Constancy of the circadian rhythm*

Because of the large variations shown in subject 4, he was selected for a second analogous observation three months later (observation 4B). For practical reasons, blood was collected every hour, but the variation curve (Fig. 2) was very similar to the first one, and the amplitude of the variations was comparable (Table 1.). However a 2 h shift of the evening peak was observed.

III. *Variations of plasma estrogens*

The levels of estrone and estradiol-17β, measured by hourly sampling in observation 4B are shown in Fig. 3. Estrone concentrations, measured during sleep (from 11 p.m. to 8 a.m.), were significantly different from those measured during periods of activity ($P < 0{\cdot}01$).

In the other subjects under observation, these variations existing between the period of decubitus and the period of activity are not so evident. However, in subjects 2, 3 and 4A, the levels measured between 8 a.m. and 5 p.m. were significantly higher than the levels measured during sleep.

Only in subject 1 was there no significant variation. The estradiol curve in observation 4B (Fig. 3) showed a slight rise during the supine period but this rise was not significant, taking into account the statistical error of the assays. The same was true for subjects 2, 3 and 4A.

Table 1. Mean features of plasma testosterone variations in each individual

	General Mean (ng/ml) and Coefficient of Variation %	Deviation from the mean (%)			Urinary 17-oxo-steroids mg/24h
		at 9 a.m.	at maximum level	at minimum level	
1	4·00 (7·8%)	+11	+15	−22	26·5
2	3·37 (15·4%)	+18	+30	−28	18
3	3·92 (16·4%)	+21	+21	−34	15
4A	2·78 (38%)	+43	+97	−70	9
4B	3·49 (36%)	+73	+73	−62	

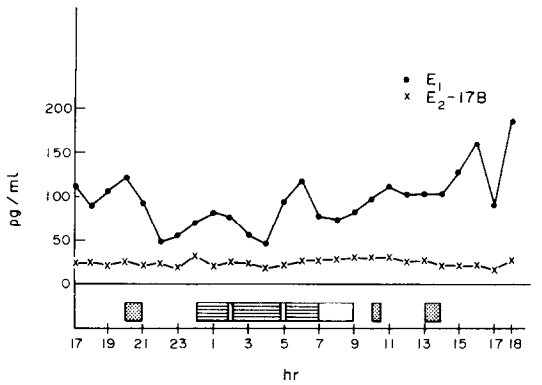

Fig. 3. 24 h variations of plasma estradiol-17β and estrone in observation 4B. (Symbols as in Fig. 1).

DISCUSSION

I. As reported by many others, this work shows the existence of a circadian rhythm in plasma testosterone. However, it is evident here that its range may be extremely variable from one subject to the other. It may be interesting to note that this variation was widest in subject 4, who had had a testis removed at the age of 12, and whose mean testosterone values were the lowest (Table), whereas subject 1, who exhibited the smallest circadian variation was also the one whose testosterone values were the highest (Table). A similar phenomenon has been observed concerning the plasma cortisol circadian rhythm in normal men and in men with Cushing syndrome.

The pattern of variations reported in the four subjects of this paper is in disagreement with some recent works. Boon *et al.* [5], by collecting blood every 6 h in 10 subjects, observed asynchronous variations of plasma testosterone. The mean curve of the 10 subjects had two minima, one at 9 a.m. and the other at 9 p.m. Alford *et al.* [2], in studying 7 subjects, found a maximum concentration during the morning in four of them and a maximum during the evening in the other three. The method they used in collecting blood continuously during periods of 3–4 h lead to an integration of the instantaneous levels, masking rapid variations. For instance in subject 4A of our study, it would not have shown the two minima at 9 p.m. and 12:30 a.m. enclosing a wave of elevated levels. In the same manner, it is easy to verify on the curves of Figs. 1 and 2, that a blood sampling every 6 h, starting at 5 p.m. would not show a synchronous rhythm in the four subjects.

The technique of sampling every 30 min prevents the detection of acute secretion peaks analogous to those described for cortisol [39] dehydroepiandrosterone [32] or aldosterone [17]. However, it does show some sharp variations, comparable to some of the waves reported by Naftolin *et al.* [27] by sampling every 10 min between 10 a.m. and 6 p.m. and compatible with a pulsatile secretion of testosterone. Evans *et al.* [13] had already observed a pulsatile secretion of testosterone during the sleep, leading to a progressive rise of the plasma level similar to the one observed here in experiments 3, 4A and 4B.

II. The repetition of the same variation pattern in the same individual is a remarkable phenomenon which has not been reported (excluding the cases of Crafts *et al.* [8] where blood was collected only every 12 h). The similarity of curves 4A and 4B, obtained 3 months apart, indicates that this pattern was consistent in one particular individual.

The metabolic clearance rate of testosterone in males does not vary significantly at various times of the day [38] and neither does the plasma testosterone binding activity [12]. Thus, the variations of total testosterone concentration should probably be interpreted as a consequence of variations of the secretion rate, the determinism of which remains to be investigated.

In this view, subjects were selected because of their large plasma testosterone variations, with the intention of comparing in a further study the patterns of plasma testosterone, LH and FSH.

III. The contrast between the stability of the estradiol plasma concentration and the large fluctuations in testosterone levels is very surprising in view of the fact that estradiol comes both from peripheral testosterone conversion and from direct testicular secretion [4, 18, 25, 34].

This phenomenom could be partly due to an asynchronism of both types of production and to the fact that the estradiol concentration gradient between the peripheral blood and the spermatic vein blood is 3–5 times lower than the testosterone gradient [25, 34].

Concerning the estrone level stability, it may be explained by the multiple origins of this steroid: direct secretion by the adrenal [3] and the testis [4, 25, 34] and conversion from androstenedione [20].

Lastly, the increased metabolic clearance rate of estrone in supine subjects compared to standing subjects as reported by Longcope [24] could explain the slight decrease of the estrone level observed here during the night.

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