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Daily Rhythmic and Non-Rhythmic Variations of Follitropin, Lutropin, Testosterone, and Sex-Hormone-Binding Globulin in Men

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Summary: The circadian rhythmic variations of the serum concentrations of follitropin, lutropin, sex-hormone-binding globulin and testosterone, the ratio between the serum concentrations of testosterone and sex-hormone-binding globulin, and the salivary concentration of testosterone were investigated in a group of 13 apparently healthy men. Venous blood and salivary specimens were collected at 4-h intervals over a 24-h period. The circadian rhythms were studied by using a periodic function resulting from the sum of two cosine functions with periods of 24 and 12 h. The serum concentrations of follitropin and lutropin showed no significant rhythmic variations. For the salivary concentration of testosterone and for the ratio between the serum concentrations of testosterone and sex-hormone-binding globulin, only the cosine function with a period of 24 h was significant. Serum concentrations of sex-hormone binding globulin and testosterone were significantly affected by 24- and 12-h rhythmic components. Of the quantities studied, the salivary concentration of testosterone showed the greatest daily rhythmic variation (28.8% of the mean estimated over rhythm).

Introduction

Many clinical biochemical quantities are affected by circadian rhythms. The knowledge of such biological rhythms is of physiological and pathophysiological interest and has been utilised in the diagnosis, treatment and clinical management of a variety of diseases (1). In the clinical laboratory, the knowledge of the rhythmic variations can be mainly used to produce time-qualified reference values when the amplitude of the circadian rhythm is large enough, and to establish a priority between biochemical quantities with similar semeiologic value according to their rhythmic amplitude, choosing the biochemical quantity having the narrowest amplitude (2–4).

The aim of this paper is the study, in 13 apparently healthy men, of the daily within-subject variability in serum concentrations of follitropin, lutropin, sex-hormone-binding globulin and testosterone, the ratio between the serum concentrations of testosterone and sex-hormone-binding globulin, and the salivary concentration of testosterone, especially the detection and description of their circadian rhythms, if any.

Several studies have been published on the circadian rhythmic variations of serum or plasma concentrations of testosterone (5–25), follitropin (5, 6, 8–11, 13, 25–30) and lutropin (5, 6, 8–14, 25–30) in men, while fewer studies exist on the circadian rhythms of salivary testosterone concentration (7, 31–35), serum or plasma sex-hormone-binding globulin concentration (6, 12, 21, 22, 24), and the ratio between the serum concentrations of testosterone and sex-hormone-binding globulin in men (22).

To our knowledge, no previous data have been published on the circadian rhythms of these quantities using a periodic function resulting from the sum of two cosine functions of 24 and 12 h.

Materials and Methods

Subjects and protocol

The study was performed from April to June on 13 apparently healthy men, ages 25 to 42 years ($\bar{x} = 29.5$; $s = 4.3$). These subjects maintained a homogeneous pattern of meals and activity, and their habitual daily routine before the study began; none of them was taking any medication. The study was performed in the hospital under standardised premetrological and metrological conditions. Each volunteer had full freedom of movement and ate three daily meals from the hospital kitchen; ingestion of alcoholic drinks was restricted. Breakfast was between 10:15 and 11:00, lunch was between 14:15 and 15:00, and dinner between 22:15 and 23:00. The subjects had 5 to 6 h to sleep.

Venous blood and salivary (unstimulated) specimens were collected every 4 h over a 24-h period, starting at 10:00. The 10:00, 14:00 and 22:00 specimens were collected just before the imminent meal. Venous blood specimens were drawn with the volunteers in a sitting position and with minimal stasis. Blood and saliva were centrifuged at 1400 *g* for 10 min and the resulting serum and salivary specimens were stored at −80 °C until assayed. Using this protocol, the premetrological variation was considered negligible.

All procedures follwed were in accordance with ethical standards of the hospital where the work was done.

Measurements

Concentrations of follitropin and lutropin were measured by fluoroenzymoimmunoassay (Stratus Immunoassay System; Baxter Diag-

nostics Inc., Miami, FL), and concentrations of testosterone and sex-hormone-binding globulin were measured in duplicate by radioimmunoassay (Extraction Testosterone [^{125}I] radioimmunoassay kit, and Sex Hormone Binding Globulin [^{125}I] immunoradiometric assay kit; both from Farmos Diagnostica, Oulunsalo, Finland). All specimens from one individual were analysed within the same run, in order to avoid between-run variations.

The quality of the measurements was controlled by using the following control materials: Dade® Immunoassay Control Comprehensive Tri-Level (lot No. ACK-14; Baxter Diagnostics Inc.) for measurements of serum concentrations of follitropin and lutropin; control material included in Sex Hormone Binding Globulin [^{125}I] immunoradiometric assay kit (Farmos Diagnostica) for measurements of serum concentrations of sex-hormone-binding globulin; and Lyphochek® Immunoassay Control Serum (lot No. 6000; Bio-Rad, Anaheim, CA) for measurements of serum and salivary concentrations of testosterone. To estimate the within-run metrological variance by *Snedecor*'s formula (36) we used the differences between duplicates of Dade control material with "physiological" concentrations of follitropin and lutropin, and between duplicates of serum concentrations of sex-hormone-binding globulin, and of serum and salivary concentrations of testosterone from the participants. For the ratio between the serum concentrations of testosterone and sex-hormone-binding globulin, we used the results of these quantities from the same specimen.

Mathematical analysis

For each quantity we estimated:

– the daily within-subject biological variance (s^2_{Bw}) of each volunteer, using the equation (37)

$$s^2_{Bw} = s^2_{Tw} - s^2_{Mw} - s^2_{PM}$$

where

s^2_{Tw} is the overall daily within-subject variation, which can be calculated from measurements;

s^2_{Mw} is the within-run metrological variance; and

s^2_{PM} is the premetrological variance, considered negligible.

– the daily within-subject biological coefficients of variation of each volunteer (with respect to the "homeostatic values") (CV_{Bw}), as well as the median of all them, which can be considered the best estimation of the daily within-subject biological variability in this group (37).

The circadian rhythmic variations of each quantity were characterised by a periodic function (38) resulting from the sum of two cosine functions of 24 and 12 h, respectively:

$$Y_t = M + A_1 \cos\left(\frac{2\pi}{1440}t + \varphi_1\right) + A_2 \cos\left(\frac{2\pi}{720}t + \varphi_2\right)$$

where

Y_t is the value of the quantity at time t (in minutes with respect to local midnight);

M is the mesor (mean estimated statistic over rhythm);

A_1 and A_2 are the amplitudes (half of the variability due to the rhythm), and

φ_1 and φ_2 are the acrophases (time of maximum value of the quantity in the cosine function) of 24-h and 12-h cosine functions, respectively.

In our case, because we used regular sampling, the mesor and mean values can be considered equal.

To compensate for the small number of specimens obtained from each individual, we supposed that the 78 experimental data obtained for each biochemical quantity (six from each of the 13 subjects) came from a single hypothetical subject who represents the central tendency of the population studied. The data were expressed as a percentage of the individual mesor in order to correct interindividual differences.

The cosine functions were estimated for each quantity by fitting the experimental data through linear least-squares regression analysis, with the previous linearization of the periodic function by single cosinor procedure (38).

Results

The results of the "homeostatic" mean, the within-run metrological variability and the daily within-subject biological variability are shown in table 1.

Tab. 1 "Homeostatic" mean, within-run imprecision (CV_{Mw}) of each measurement procedure, and daily median within-subject biological variation (med CV_{Bw}) observed for each quantity.

Analyte[a]	Unit	Mean concentrations	CV_{Mw} (%)	med CV_{Bw} (%)
Follitropin	IU/l	3.64	4.7	5.5
Lutropin	IU/l	4.47	4.3	31.9
Sex-hormone-binding globulin	nmol/l	27.0	3.7	4.9
Testosterone	nmol/l	22.7	6.0	14.9
Testosterone (saliva)	nmol/l	0.38	11.1	36.3
Testosterone/Sex-hormone-binding globulin		0.99	8.1	14.2

[a] In serum except where noted.

Tables 2, 3 and 4 show the estimated quantities characterising the circadian rhythmic variations of the quantities studied: the mesor, amplitudes, and acrophases of cosine functions with periods of 24 and 12 h, and their respective 95% confidence intervals when the rhythmic variation is statistically significant. Table 2 also shows

Tab. 2 Properties of periodic function that characterise the daily rhythmic variation of each quantity: Mesor (M) and percent of total daily variation that is attributable to circadian rhythm (%V).

Analyte[a]	Unit	M[b] concentration	%V
Follitropin	IU/l	0.2	
Lutropin	IU/l	0.6	
Sex-hormone-binding globulin	mmol/l	0.0 (−1.4; 1.4)	21.3
Testosterone	nmol/l	−0.2 (−3.8; 3.8)	28.0
Testosterone (saliva)	nmol/l	0.4 (−6.4; 7.2)	33.3
Testosterone/Sex-hormone-binding globulin		0.0 (−3.5; 3.4)	17.1

[a] In serum except where noted.
[b] 95% confidence limits listed in parentheses when the rhythmic variation is statistically significant.

Tab. 3 Properties of periodic function that characterise the daily rhythmic variation of each quantity: Amplitudes of 24-h (A_1) and 12-h (A_2) cosine functions, respectively, expressed as a percentage of change with respect to the mesor.

Analyte[a]	A_1[b]	A_2[b]
Follitropin	2.6	2.0
Lutropin	12.2	9.1
Sex-hormone-binding globulin	3.4 (1.5; 5.4)	2.7 (0.6; 4.7)
Testosterone	10.0 (4.7; 15.4)	8.8 (3.5; 14.1)
Testosterone (saliva)	28.8 (19.1; 38.5)	5.6
Testosterone/Sex-hormone-binding globulin	9.5 (4.6; 14.5)	6.1

[a] In serum except where noted.
[b] 95% confidence limits listed in parentheses when the rhythmic variation is statistically significant.

Tab. 4 Properties of periodic function that characterise the daily rhythmic variation of each quantity: Acrophases of 24-h (φ_1) and 12-h (φ_2) cosine functions, respectively (hours:minutes after local midnight).

Analyte[a]	φ_1[b]	φ_2[b]
Follitropin	02:32	04:55
Lutropin	06:41	04:57
Sex-hormone-binding globulin	15:23 (12:20–18:25)	11:00 (08:45–13:14)
Testosterone	10:24 (07:47–13:03)	10:29 (08:50–12:07)
Testosterone (saliva)	10:14 (08:48–11:41)	07:13
Testosterone/Sex-hormone-binding globulin	08:59 (06:27–11.31)	10:12

[a] In serum except where noted.
[b] 95% confidence limits listed in parentheses when the rhythmic variation is statistically significant.

the percentage of the total daily variation that is attributable to the rhythm.

A statistically significant rhythm with a 24-h period was detected for the concentrations of sex-hormone-binding globulin in serum ($P < 0.01$), testosterone in serum ($P < 0.01$) and testosterone in saliva ($P < 0.00001$), and for the ratio between the serum concentrations of testosterone and sex-hormone-binding globulin ($P < 0.001$), and a statistically significant rhythm with a 12-h period was detected for the serum concentrations of sex-hormone-binding globulin ($P < 0.05$) and testosterone ($P < 0.01$).

The cosinusoidal function that best fits all the 78 experimental data for each quantity, and mean values ($n = 13$) for six mean times of day, are shown in figure 1.

Discussion

Daily within-subject variation

Although usually the within-subject biological variation obtained is higher when the study covers a longer time span (39, 40), the daily within-subject biological variation in men obtained in the present study for the concentrations of lutropin and testosterone in serum, and testosterone in saliva, are higher than those observed in a study performed in one year (41). This is probably due to the daily rhythmic variation of these quantities (42). As far as we know, no previous data have been published on daily within-subject biological variation of the quantities studied. Nevertheless, data have been published on the biological variation of the quantities considered here obtained from month-to-month measurements during one year (41), and on the biological variation of serum concentrations of follitropin, lutropin and testosterone, obtained from day-to-day measurements during one week (43).

Daily rhythmic variations

Follitropin and lutropin

No significant daily rhythmic 24-h or 12-h components were detected for serum concentrations of follitropin and lutropin. Some groups have reported a 24-h rhythmic variation in the serum concentration of follitropin in men (8, 10, 11, 13, 30), and others have not (5, 6, 9, 25–29). Likewise, a review of the studies on circadian variations of lutropin shows a large disagreement on the results; some authors have found significant 24-h rhythmic variations (9–11, 13, 25, 26, 28–30), whereas others have not (5, 6, 12–14, 27, 29).

In the publications we reviewed on the circadian rhythmic variation in serum concentrations of follitropin and lutropin, we found no relationship between significant detection of rhythmic variation and other variables, such as the interval between specimen collections, the geographical area, the size of the group of subjects under study or the mathematical analysis.

The 12-h rhythmic component of follitropin and lutropin concentrations in serum was not studied in the articles reviewed.

Episodic release of lutropin in men has been demonstrated, and that of follitropin has been more difficult to define (8–11, 28, 44, 45). The pulsatile release can produce an important daily within-subject biological variation of serum concentrations of these hormones, although as our results reveal, these pulses can hinder the detection of circadian rhythmic variations in these quantities. The median CV_{Bw} of serum concentrations of follitropin and lutropin are 5.5% and 31.9%, respectively. The observed difference may be the result of the great amplitude of lutropin episodic release.

Sex-hormone-binding globulin

Our results show rhythmic variations with periodicities of 24 h and 12 h for the serum concentration of sex-hormone-binding globulin in men. The daily profile is very similar to that for the concentrations of protein and albumin in serum (46), and, as for these quantities, the circadian variation would be related to postural changes (24).

These results are particularly interesting in relation to our geographical area, since few studies on this biochemical quantity have been carried out in it. We have found no published reports about a 12-h rhythmic component. Our finding of a 24-h rhythmic variations in serum concentrations of sex-hormone-binding globulin in men and its acrophase, late morning/early afternoon hours, are consistent with previously published studies (12, 21, 22, 24). In contrast, *Montanini* et al. (6) did not find a 24-h variation in this quantity in men, using population mean consinor analysis, probably due to the smaller number of subjects studied.

Plymate et al. (21) suggest that, since the half-life of sex-hormone-binding globulin is 6 days, the 24-h rhythmic variation of its concentration would be unlikely to be due to variations in production. However, according to *Yie* et al. (22), sex-hormone-binding globulin acts as a buffer to stabilise the serum concentration of unbound testosterone in the presence of fluctuating testosterone production patterns.

According to *Plymate* et al. (21), the 24-h rhythmic variations in the serum concentration of protein in men are greater than those for sex-hormone-binding globulin; possible explanations include a change in plasma water and distribution of proteins between the plasma and extravascular system. In this situation, lower molecular mass proteins would be expected to show a greater variation than higher molecular mass proteins; since the majority of serum proteins have molecular masses less than sex-hormone-binding globulin, a greater 24-h variation in the concentration of protein than in the concentration of sex-hormone-binding globulin would be expected. However, considering our results for the 24-h rhythm of sex-hormone-binding globulin, and for the 24-h rhythm of protein obtained in a previous study which used the same study design, subjects and statistical analysis (46) as the present one, the changes in serum concentrations of sex-hormone-binding globulin and protein during 24-h were not significantly different. Therefore, the conclusion of *Plymate* is not in accordance with our data.

Testosterone in serum

Rhythmic components of 24 h and 12 h have been detected for the concentration of testosterone in serum. Several studies have demonstrated 24-h circadian rhythmic changes of serum testosterone concentration in healthy men (5–25), although this rhythm seems to decrease with age (6, 16, 21, 27, 47). The acrophases obtained by most of the studies performed in France and Italy (6, 15, 20, 25) are similar to our finding of 10:24, while the highest concentration reported by most of the studies performed in distant geographical locations (USA, Brazil, China ...) (5, 7, 8, 14, 16, 17) occurs at about 08:00. The geographical location is very important because of the different influences of synchronisers on biological rhythms (e. g. behavioural habits, sleeping/waking patterns, meal times, hours of light and darkness) in different places.

The significant daily 24-h and 12-h rhythmic components for the concentration of testosterone in serum were also detected by *Levi* et al. (20), but with higher amplitudes than those observed in the present study.

Testosterone in saliva

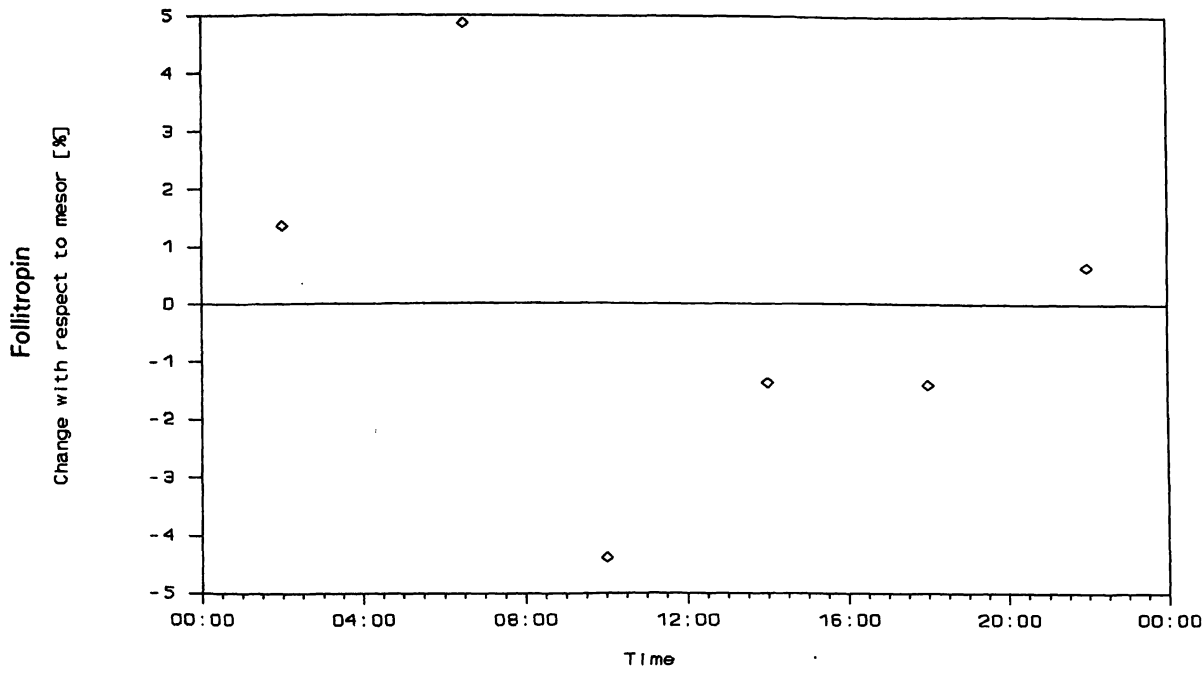
Some authors (7, 31–35) have found a significant 24-h rhythm in the salivary testosterone concentration of men, with acrophases similar to those observed for the circadian rhythm of serum testosterone concentration. These results are consistent with ours.

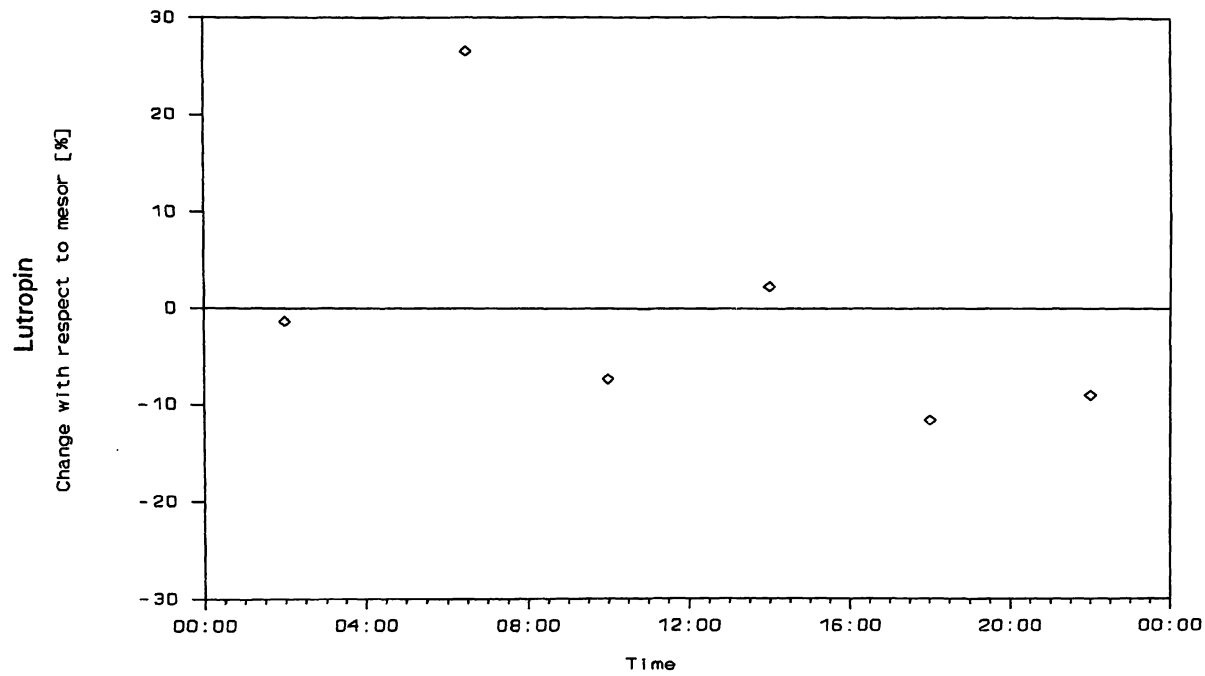
Considering that salivary testosterone concentration represents the circulating serum unbound testosterone (7, 48), our data are in agreement with those reported by other authors (6, 7, 22, 24) who have found significant 24-h variations in serum unbound testosterone, with similar acrophases. Our results show that the daily rhythmic variations of serum testosterone concentration are less than those of salivary testosterone concentration. This finding is in accordance with a previous report by *Kahn-Dawood* et al. (7), who demonstrated that salivary testosterone concentrations show wider variations; likewise, our results are indirectly in agreement with those reported by other authors who observed that serum unbound testosterone concentrations have higher rhythmic daily variations than serum bound and unbound testosterone concentrations (6, 24). In contrast to our study, *Yie* et al. (22) found that daily variations of the serum unbound testosterone concentration in men are much less than those of serum bound and unbound testosterone concentrations.

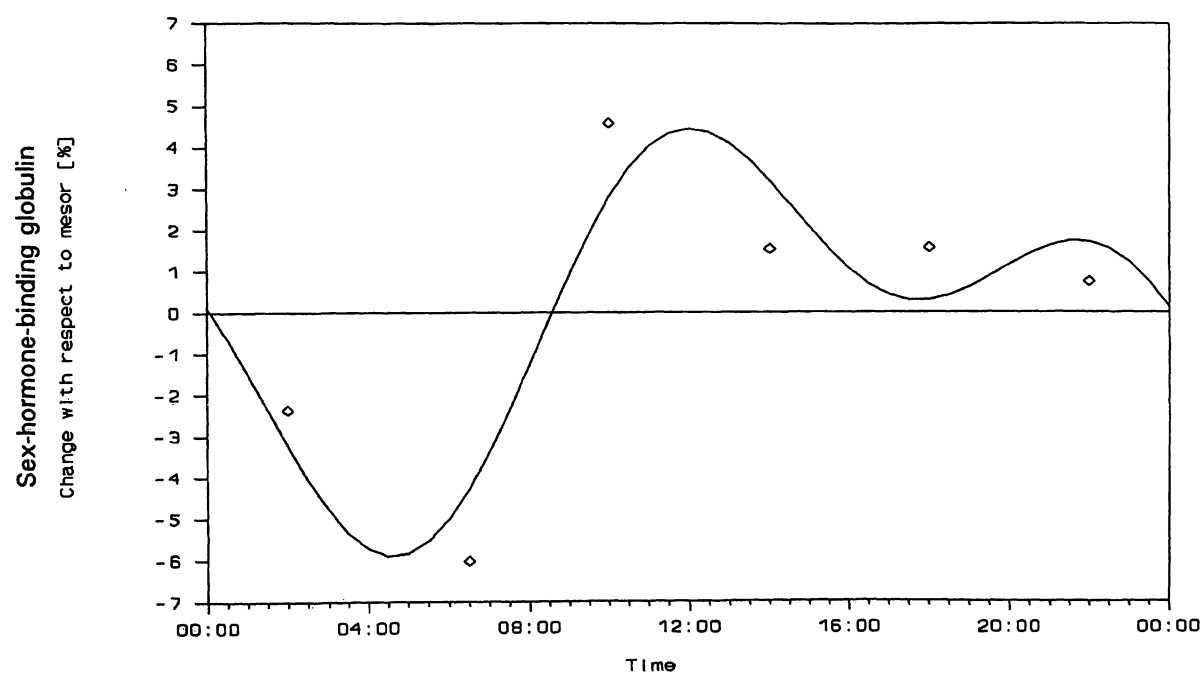
No rhythmic 12-h component was detected for the concentrations of testosterone in saliva. We found no published reports about this rhythmic component.

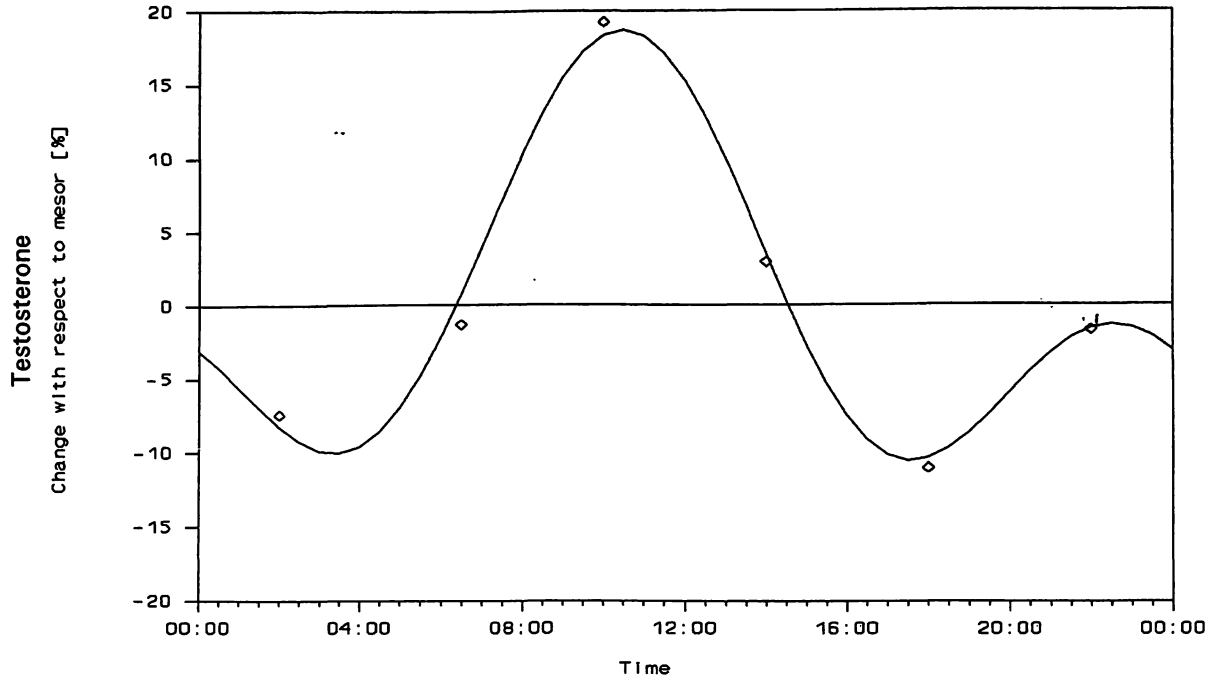
The salivary testosterone concentration exhibits relatively high total and rhythmic daily variations. This quantity reflects the serum concentration of unbound testosterone (7, 48); likewise, episodic release of testos-

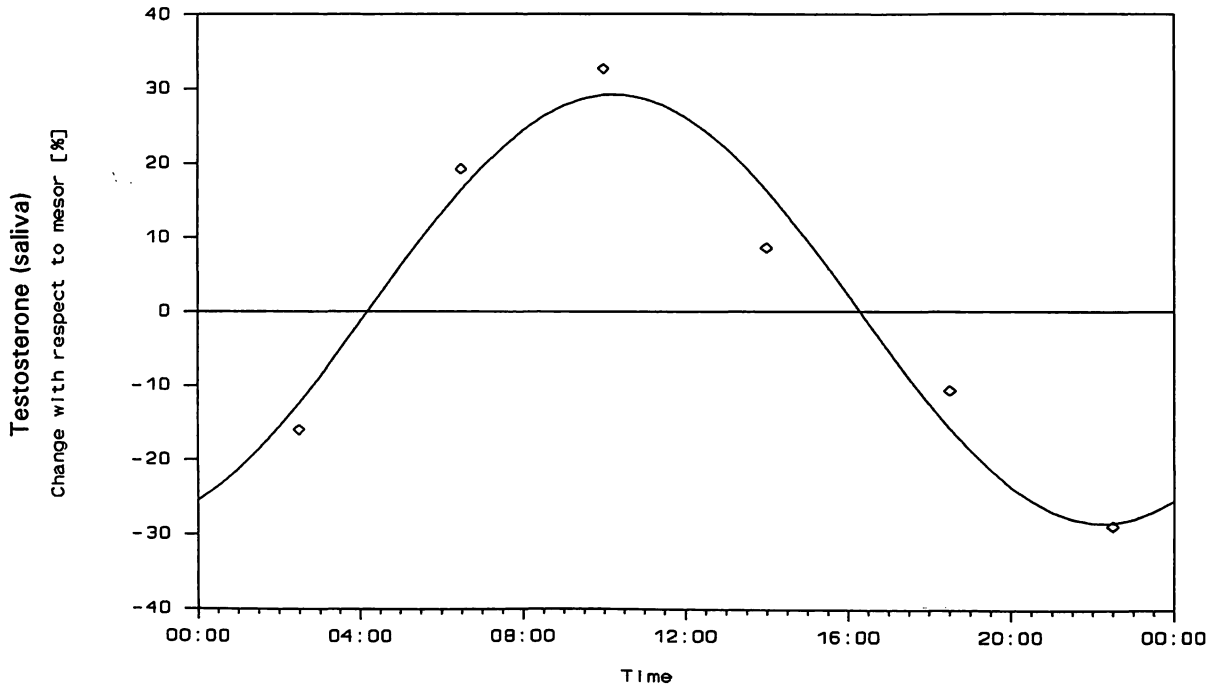
Fig. 1 The curves represent the rhythmic functions resulting from cosine functions that best fit all the 78 experimental data, expressed as a percentage of the mesor. The curves are shown only when the rhythmic variation is statistically significant. The points represent mean values ($n = 13$) for six sampling times during a 24-h period.

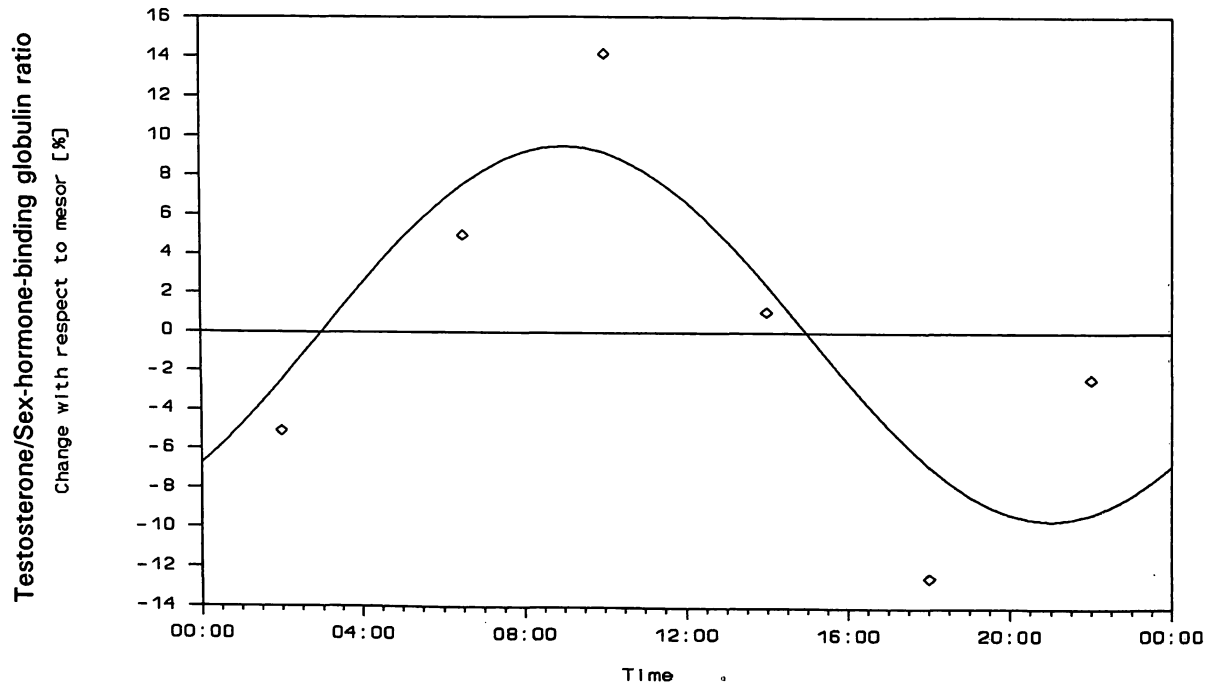

Follitropin
Change with respect to mesor [%]
5
4
3
2
1
0
-1
-2
-3
-4
-5
00:00
04:00
08:00
12:00
16:00
20:00
00:00
Time


Lutropin
Change with respect to mesor [%]
30
20
10
0
-10
-20
-30
00:00
04:00
08:00
12:00
16:00
20:00
00:00
Time


Sex-hormone-binding globulin
Change with respect to mesor [%]
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
00:00
04:00
08:00
12:00
16:00
20:00
00:00
Time


Testosterone
Change with respect to mesor [%]
20
15
10
5
0
-5
-10
-15
-20
00:00
04:00
08:00
12:00
16:00
20:00
00:00
Time


Testosterone (saliva)
Change with respect to mesor [%]
40
30
20
10
0
-10
-20
-30
-40
00:00
04:00
08:00
12:00
16:00
20:00
00:00
Time


Testosterone/Sex-hormone-binding globulin ratio
Change with respect to mesor [%]
16
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
00:00
04:00
08:00
12:00
16:00
20:00
00:00
Time

terone follows the pulsatile release of lutropin (8–11). The daily within-subject biological variation of the concentrations of lutropin in serum and testosterone in saliva are similar; since the daily rhythmic variations of these quantities are different, the changes in the salivary testosterone concentrations cannot be causally linked to variations of lutropin.

Ratio between the serum concentrations of testosterone and sex-hormone-binding globulin

For this quantity, only the 24-h component has been detected, with an acrophase similar to those observed for the rhythms of serum and salivary concentrations of testosterone. From the reviewed publications, only *Yie* et al. (22) studied and detected a significant 24-h rhythmic variations for this quantity.

No significant 12-h rhythmic component has been detected in our study, and, to our knowledge, no other studies on this rhythmic component have been previously reported.

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