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Diurnal Rhythm of Testosterone and Luteinizing Hormone in Hypogonadal Men

STEPHEN J. WINTERS

From the Department of Medicine, Montefiore Hospital and University of Pittsburgh, Pittsburgh, Pennsylvania.

ABSTRACT: To determine the relationship between increasing luteinizing hormone (LH) production and the diurnal secretion of LH and testosterone (T) in adult men, studies were performed on five men with gonadotropin insufficiency associated with prolactinoma, five eugonadal men, and five men with primary testicular failure. Blood samples were drawn every 10 to 20 minutes for 24 hours beginning at 8:00 to 8:30 AM to evaluate diurnal periodicity. Mean (± SEM) LH levels in the three groups were 7.67 ± 1.46 mIU/ml, 13.9 ± 3.2 mIU/ml, and 62.3 ± 14.4 mIU/ml, respectively, and mean serum T levels were 8.05 ± 1.49 nmol/L, 13.9 ± 3.5 nmol/L, and 9.15 ± 1.3 nmol/L, respectively. Cosinor analysis revealed that each hyperprolactinemic man had a T rhythm with a significant 24-hour periodicity; the mean acrophase was at 5:00 AM. Testosterone levels were 35.0 ± 10.6% less at 4:00 PM than at 8:00 AM. Eugonadal men also demonstrated a significant diurnal T rhythm with an acrophase at 6:00 AM, and T levels were 15.8 ± 5.3% less at 4:00 PM than at 8:00 AM. By contrast, there was no significant diurnal rhythm in T secretion among the men with testicular failure, although serum T levels were 11.5 ± 3.7% less at 4:00 PM than at 8:00 AM. For LH, hyperprolactinemic men demonstrated a significant 24-hour rhythm with an acrophase at 1:30 AM, whereas no significant 24-hour periodicity was identified among either eugonadal men or men with testicular failure. The diurnal LH rhythm characteristic of pubertal boys is a property of the gonadotropin-receptor hormone (GnRH) pulse generator that is expressed in adult men with hyperprolactinemia and gonadotropin insufficiency. These data are consistent with the possibility that the expression of this rhythm reflects an enhanced gonadotropin-suppressive action of T in these men. As gonadotropin secretion increases to and beyond normal levels, there is a progressive dampening of the diurnal patterns of T and LH secretion.

Key words: Circadian rhythms.

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There is a prominent sleep-associated increase in luteinizing hormone (LH) secretion in adolescent boys that is followed by an early morning rise in serum testosterone (T) concentrations and a subsequent decrease in LH and T levels (Boyar et al, 1974b). With sexual maturity, the diurnal difference in circulating T levels persists but is less pronounced (Resko and Eik-Nes, 1966). The mechanism for diurnal secretion of T in adult men remains controversial since most (Krieger et al, 1972; Miyatake et al, 1980; Spratt et al, 1988; Tenover et al, 1988), but not all, studies (Nankin and Troen, 1972; Veldhuis et al, 1987) have concluded that there is no consistent diurnal rhythm in LH secretion in adult men to explain the T changes.

One proposed explanation for these findings is a day-night difference in the sensitivity of the hypothalamic-pituitary unit to the negative feedback actions of androgens during puberty (Hale et al, 1988; Foster et al, 1990) that declines with sexual maturity. This change may reflect aging or increasing gonadotropin secretion. According to this construct, the diurnal LH rhythm attenuates in adult men because the morning T increase is less effective in reducing daytime LH secretion. Consequently, LH levels are nearly similar throughout the day and night, and the nocturnal T rise is much less pronounced than during puberty. To probe this hypothesis, 24-hour LH and T secretion in two groups of hypogonadal men were studied. Group 1 was composed of men with gonadotropin deficiency and hyperprolactinemia since studies in rats have indicated that hyperprolactinemia increases the negative feedback effects of gonadal steroids (McNeilly et al, 1983). Group 2 was composed of men with gonadotropin hypersecretion and primary testicular failure in whom there is a partial resistance to the negative feedback effect of androgens (Winters et al, 1979). I reasoned that an enhanced negative feedback effect of T in adult hyperprolactinemic men might permit the reexpression of the pubertal diurnal rhythm in LH secretion. Studies were also performed on eugonadal men.

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Correspondence to: Stephen J. Winters, MD, Montefiore Hospital, Department of Medicine, 3459 Fifth Avenue, Pittsburgh, Pennsylvania 15213.

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Materials and Methods

Subjects

The clinical characteristics of the study subjects are found in Table 1. Group 1 was composed of five hypogonadal subjects (mean age, 33 ± 2 years) with previously untreated prolactin-producing pituitary adenomas. Each subject had an intrasellar adenoma that ranged in size from 6 to 20 mm, as determined by a computerized tomographic head scan. Serum prolactin levels ranged from 183 to 734 ng/ml. All subjects were euthyroid with thyroxine levels greater than 95 nmol/L (7.4 μg/dl), and all responded normally with an increment in serum cortisol of greater than 193 nmol/L (7 μg/dl) to insulin-provoked hypoglycemia. Normal growth hormone responses, with peak levels greater than 0.372 pmol/L (8 ng/ml), were observed in four of the five subjects. Group 2 consisted of five subjects (mean age, 27 ± 1 years) with primary testicular failure. Two subjects had Klinefelter's syndrome, two had bilateral cryptorchidism, and one had mixed gonadal dysgenesis. Group 3 consisted of five men (mean age, 33 ± 3 years) who sought medical attention as partners in infertile couples. All subjects had normal morning serum levels of LH, follicle-stimulating hormone (FSH), and testosterone. Their sperm output ranged from 7.0 to 94 million cells/ml.

Protocol

Subjects were admitted to the Clinical Research Unit of the University of Pittsburgh after providing informed consent. The following morning, a needle was inserted into a forearm vein and blood samples were drawn beginning at 8:00 to 8:30 AM every 10 or 20 minutes for 24 hours. Samples were frozen at −20°C for radioimmunoassay (RIA).

Assays and Analysis

Luteinizing hormone was measured using an established double antibody RIA with anti-human LH RD21 (Lot 280477, Wellcome Reagents Unlimited, London, UK). Follicle-stimulating hormone was measured by RIA in the first three samples using NIH human FSH (hFSH) antiserum #5. The FSH and LH standard used was the Second International Reference Preparation of Human Menopausal Gonadotropins (Second IRP-HMG). The NIH human chorionic gonadotropin α (hCGα; CR 119), at a dose of 10 ng/ml, displaced the antibody binding of the radioiodinated ligand equivalent to 1 mIU/ml (Second IRP-HMG) in the LH assay and less than 0.5 mIU/ml (Second IRP-HMG) in the FSH assay. Hourly serum pools were assayed for testosterone. All specimens for a given individual were analyzed in one RIA. Radioimmunoassay potency estimates were determined using the computer program of Rodbard (1972). The intra-assay coefficient of variation for LH ranged from 8.9% to 13%, and the interassay coefficient of variation was 11.8%. The within-assay and between-assay coefficients of variation were 6.9% and 11% for FSH, and 10% and 5.8% for testosterone, respectively.

Differences among group means were determined by a one-way analysis of variance, and post-hoc differences were tested by a t-statistic. The 24-hour profiles were tested for diurnal variation by a computer program for cosinor analysis (Vagnucci, 1979). This program assumes that the data points outline a 24-hour sinusoidal pattern and describes the series of observations in terms of its component sinusoidal forms. A significant ($P < 0.05$) fit was defined as one in which the possibility that the data represented a horizontal line rather than a cosine curve was less than 5%. Missing values were interpolated linearly from bracketing results. The acrophases represent the times of occurrence of maximal levels of the best fit pattern; they are not necessarily the observed maximal values. The amplitude was defined as 50% of the difference between the acrophase and the nadir values. Mean values for the five subjects in each group also were analyzed for diurnal periodicity.

Due to differences in mean hormone concentrations among subjects, the data were also transformed for graphic illustration. For each subject, the arithmetic mean of the series was calculated, and individual values were contrasted with the mean. The relationship between LH and T secretion was investigated by calcu-

TABLE 1. *Clinical characteristics and hormone profiles of men with testicular failure and hyperprolactinemia*

Subject (age, years)	Testosterone (nmol/L)	LH (mIU/ml)	FSH (mIU/ml)	Prolactin (ng/ml)
Hyperprolactinemia				
A (38)	11.0	12.8	11.0	734
B (32)	6.00	4.34	3.2	186
C (34)	7.08	8.40	5.7	195
D (33)	4.19	5.54	7.2	183
E (26)	12.0	7.28	8.4	617
Mean ± SEM	8.05 ± 1.49	7.67 ± 1.46	7.1 ± 1.3	383 ± 121
Testicular failure				
F (29)	13.2	42.1	60.0	10.1
G (25)	9.54	32.9	36.5	14.9
H (31)	9.65	57.1	39.8	9.0
I (26)	8.24	63.7	62.7	2.0
J (24)	5.10	116	48.3	6.0
Mean ± SEM	9.15 ± 1.30	62.3 ± 14.4	49.5 ± 4.7	8.4 ± 2.1
Eugonadal men (33 ± 6)	13.9 ± 3.5	13.9 ± 3.2	7.2 ± 31	4.9 ± 1.0

Clinical diagnoses are: E = MEN 1; F, J = Klinefelter's syndrome; G, I = 46,XY bilateral cryptorchidism with previous unilateral orchiectomy; and H = mixed gonadal dysgenesis.

lating the coefficient of variation of coincident values and for various time lags of the T series. Data are presented as the mean ± SEM.

Results

The hormone profiles of the study subjects are listed in Table 1. The mean 24-hour serum T level in men with hypogonadism and hyperprolactinemia was 8.05 ± 1.49 nmol/L (2.28 ± 0.40 ng/ml) and was similar to the level in men with testicular failure of 9.15 ± 1.30 nmol/L (2.61 ± 0.37 ng/dl). Mean T levels in each subject group were less ($P < 0.05$) than those of the eugonadal men.

Figure 1 illustrates the 24-hour T profiles for the three groups of subjects. A pronounced diurnal T rhythm was observed in hyperprolactinemic men with an acrophase at 5:00 AM. The amplitude of this rhythm was 2.15 nmol/L (0.61 ng/ml). Testosterone levels at 4:00 PM were 35.0 ± 10.6% less ($P < 0.05$) than those at 8:00 AM. When the individual series were analyzed independently, each hyperprolactinemic man had a significant 24-hour periodicity (Table 2). Cosinor analysis of the group mean serum T levels among eugonadal men also revealed a significant ($P < 0.05$) diurnal rhythm, which is in agreement with previous studies. The mean amplitude of this rhythm was 1.4 nmol/L (0.41 ng/ml). Testosterone levels at 4:00 PM were 15.8 ± 5.3% less ($P < 0.05$) than those at 8:00 AM. The mean acrophase occurred at 6:00 AM. Significant 24-hour periodicities were observed in two of the five eugonadal men. By contrast, there was no significant ($P > 0.25$) diurnal rhythm in T secretion among the men with testicular failure and no individual's hourly values expressed a 24-hour periodicity. Serum T levels in these men, however, were 11.5 ± 3.7% less at 4:00 PM than at 8:00 AM. This difference was significant at the $P = 0.10$ level by sign test.

Group mean 24-hour LH levels are plotted in Figure 2. The LH levels in men with hyperprolactinemia increased abruptly at approximately 11:00 PM and remained elevated for 4 hours. Cosinor analysis identified a significant ($P < 0.05$) fit and indicated that peak LH concentrations occurred at 1:30 AM. Analysis of individual profiles revealed that four of the five hyperprolactinemic men had significant diurnal rhythms of LH secretion. The timing of acrophases ranged from 11:00 PM to 7:20 AM (Table 2). By contrast, there was no diurnal LH rhythm among men with testicular failure or eugonadal men. Cosinor analysis detected a significant 24-hour LH rhythm in one of the five men with testicular failure but failed to detect a significant 24-hour LH periodicity in any of the five eugonadal men.

Figure 3 illustrates serum LH concentrations measured every 20 minutes and T levels in hourly serum pools in a subject with hyperprolactinemia. Testosterone secretion declined during the morning from 12.8 to 4.0 nmol/L over 7 hours. After a lag of several hours, LH secretion increased during the evening followed by a rise in serum T levels. Thereafter, LH concentrations declined as the study ended, presumably initiating another 24-hour cycle. Figure 4 summarizes the 24-hour LH and T profiles of men with hyperprolactinemia after the data were adjusted for the series means. These data reveal an abrupt rise in LH secretion at 11:00 PM. Changes in LH secretion preceded changes in T by 2 hours, with a maximum cross-correlation coefficient of 0.82 ($P < 0.01$). Testosterone secretion declined gradually from 8:00 AM to 4:00 PM.

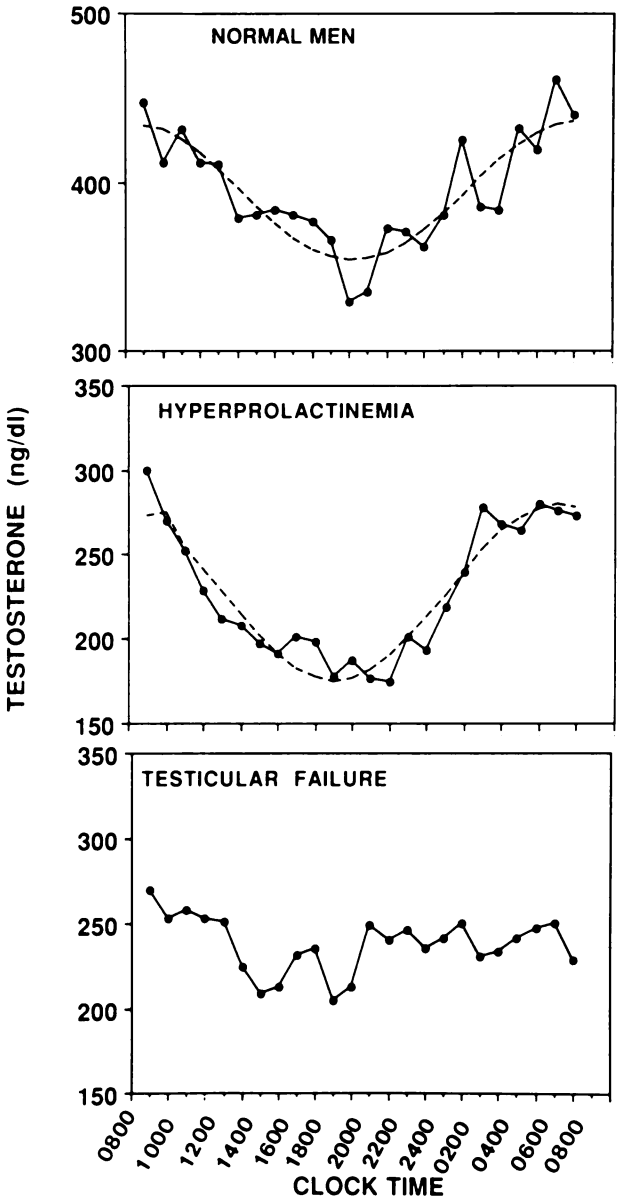

FIG. 1. Twenty-four-hour mean serum testosterone profiles of men with hyperprolactinemia, testicular failure, and of normal men. The interrupted lines represent the 24-hour "best-fit" cosine regression plots of the data.

TABLE 2. *Cosinor analysis of luteinizing hormone and testosterone secretory patterns in men with hyperprolactinemia*

	Luteinizing Hormone*		Testosterone	
Subject	Amplitude (mIU/ml)	Acrophase (hours)	Amplitude (nmol/L)	Acrophase (hours)
A	5.10	2306	2.24	0147
B	6.78	0716	1.64	0745
C	2.97	0157	4.24	0555
D		NS	1.09	0334
E	3.17	0022	1.55	0726
Mean ± SE	4.50 ± 0.90	0210 ± 0174	2.15 ± 0.56	0500 ± 0115

NS = the diurnal change in luteinizing hormone secretion in this subject was not significant.

Discussion

This study demonstrates pubertal patterns of LH and T secretion in adult men with hypogonadism and hyperprolactinemia. Twenty-four-hour periodicities for LH and T were revealed, with peak levels at 1:30 AM and 5:00 AM, respectively. The timing of these acrophases is similar to those previously reported for pubertal boys (Boyer et al, 1974b; Parker et al, 1975; Lee et al, 1976; Hale et al, 1988). These data indicate that the mechanism regulating the diurnal secretion of LH and T in puberty persists into adulthood and is modified by factors other than age, including increasing gonadotropin secretion. Pubertal patterns of LH secretion have previously been reported in young adult women with anorexia nervosa (Boyar et al, 1974a).

Murray et al (1984) identified a diurnal difference in T secretion in men with large prolactin-producing pituitary tumors; most of the men had undergone pituitary surgery and radiation treatment. In that study, the pattern and amplitude of T secretion were similar in normal and hyperprolactinemic men. The striking diurnal change in T levels in untreated men with hyperprolactinemia identified in the current study offers an explanation for the occasional finding of normal serum T levels in men with hyperprolactinemia and clinical hypogonadism (Ambrosi et al, 1981).

The contribution of testicular negative feedback factors to the expression of the diurnal pattern of LH (and, presumably, gonadotropin-receptor hormone [GnRH]) secretion in men with hypogonadism and hyperprolactinemia cannot be assessed directly from this study. Testosterone, however, was more effective in suppressing the postcastration rise in LH secretion in male rats with hyperprolactinemia from ectopic pituitary grafts than in control animals (McNeilly et al, 1983). Thus, the observed 24-hour LH and T profiles are consistent with enhanced T negative feedback during waking in hyperprolactinemic men and support the possibility that the limited expression of a diurnal LH rhythm in normal adult men relates to a reduced negative feedback response to T during the daytime. Nevertheless, this study has not addressed the role of other factors influencing the GnRH pulse

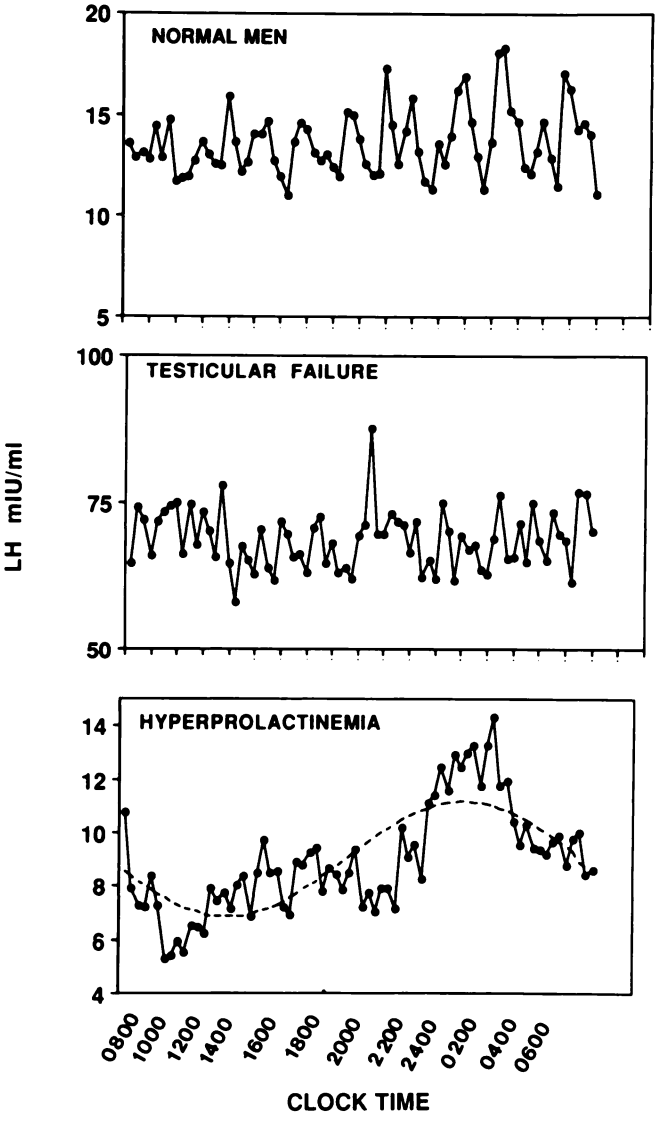

FIG. 2. Twenty-four-hour mean serum luteinizing hormone profiles of men with hyperprolactinemia, testicular failure, and of normal men. The interrupted line represents the 24-hour "best-fit" cosine regression plot for men with hyperprolactinemia. There was no statistically significant 24-hour profile for normal men or men with testicular failure.

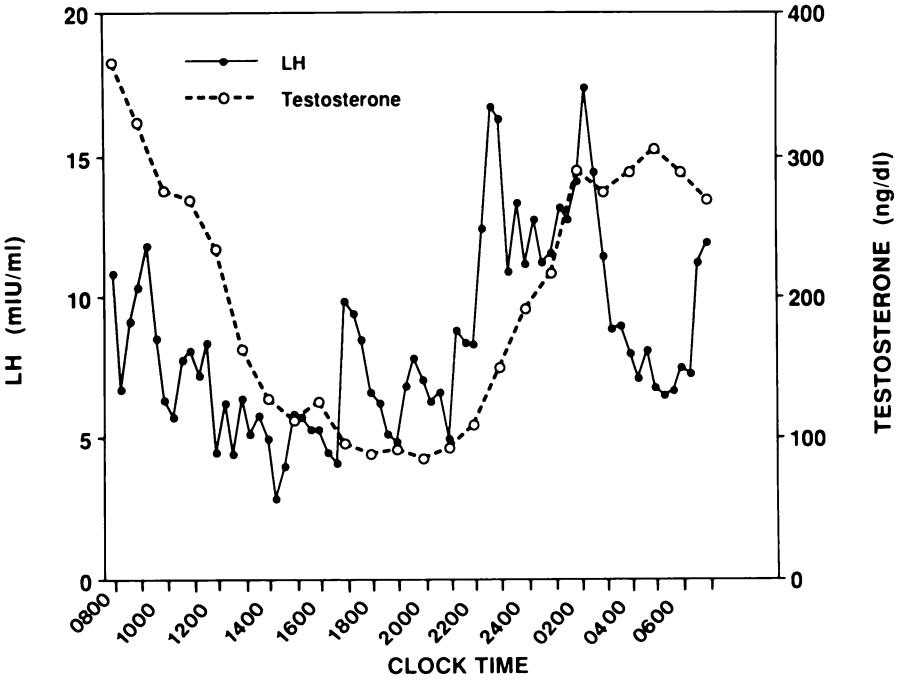

FIG. 3. Luteinizing hormone levels in samples drawn every 20 minutes and testosterone levels in hourly pooled samples from subject C with a prolactinoma.

generator or the various intratesticular regulators of LH-stimulated T secretion.

In repeated studies of two normal men, the metabolic clearance of T was found to be similar during the night and day, indicating that the early morning increase in circulating T levels results from an increase in T production (Southren and Gordon, 1975). There has been one sleep-wake reversal study performed in normal adult men in which the morning increase in T levels was reported to be independent of sleep (Miyatake et al, 1980), implying a fundamental difference in the mechanism for the diurnal secretion of T in adult men compared with pubertal boys. This conclusion, however, followed from studies in only two men, and reexamination of the data reveals several peaks of T secretion during sleep and waking that suggest other interpretations of those results.

The current results also reveal that the diurnal rhythm of T secretion is absent, or markedly attenuated, in men with primary testicular failure. A much larger study population is needed to distinguish between these possibilities. Our re-

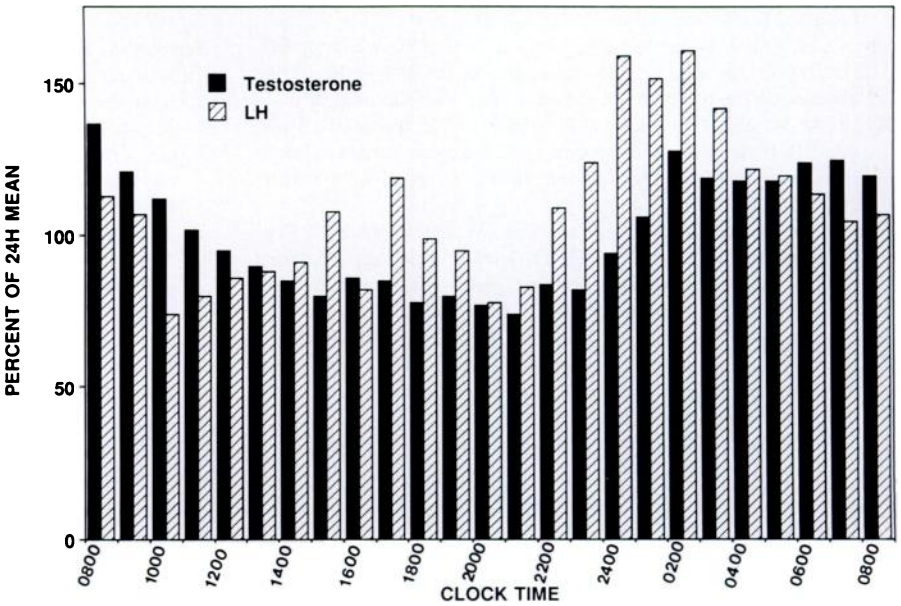

FIG. 4. Mean 24-hour luteinizing hormone and testosterone levels in men with hyperprolactinemia. Results were pooled from individuals by calculating the 24-hour mean of each series and comparing each individual value with the series mean.

sults extend the previous finding that plasma T levels were 17% less at 4:00 PM than at 8:00 AM in men with Klinefelter's syndrome (Smals et al, 1975). Serum T levels were 11.5% less at 4:00 PM than at 8:00 AM among our subjects with various etiologies for primary testicular failure in whom there was no significant circadian rhythmicity for T secretion based on cosinor analysis. Among eugonadal men, by comparison, serum T levels were 15.8% less at 4:00 PM than at 8:00 AM, and among hypogonadal hyperprolactinemic men, this difference was 35.0%. The results in the control group of eugonadal men who presented as partners in infertile couples were similar to those previously reported for normal young men (Resko and Eik-Nes, 1966; Miyatake et al, 1980; Spratt et al, 1988; Tenover et al, 1988). Diurnal T rhythm has been found to be absent or attenuated in healthy elderly men (Tenover et al, 1988). Our results suggest that this change could reflect the Leydig cell dysfunction associated with aging (Takahashi et al, 1983; Neaves et al, 1984).

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