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ONTOGENY OF LUTEINIZING HORMONE AND TESTOSTERONE SECRETION

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SUMMARY

Plasma luteinizing hormone (LH) and testosterone (T) were measured by radioimmunoassay in 9 pubertal boys and 9 sexually mature adult men at 20 min intervals for 24 h. Polygraphic monitoring of sleep was also carried out to precisely identify sleep onset, wakefulness and specific sleep stages. In all 9 pubertal boys, plasma LH showed the characteristic augmentation of secretion synchronous with sleep. This increased LH secretory activity was effective in stimulating increased T secretion during sleep that resulted in uniformly higher mean T concentrations during sleep compared with waking. Plasma LH and T were also measured in 3 of these pubertal boys during acute inversion of the sleep wake cycle. The results showed that plasma LH and T were now augmented during the reversed daytime sleep period; the mean LH and T concentrations were significantly higher than during nocturnal waking. Measurement of LH and T in the 9 adult men showed episodic secretion of both hormones during waking and sleep periods with no consistent augmentation of either hormone during sleep.

INTRODUCTION

During the past few years, important observations regarding the secretion of pituitary and steroid hormones has necessitated a re-examination of the traditional concepts of hormonal regulation. In 1970, Hellman and colleagues [1] showed that cortisol was secreted episodically in normal man. The "diurnal variation" in plasma cortisol resulted from the temporal clustering of secretory episodes during the early morning hours. The importance of sampling plasma at frequent intervals throughout the complete sleep wake cycle allowed the recognition of the episodic secretion of LH [2–4] and follicle-stimulating hormone (FSH) [5–7] in adult men and women. The important role of sleep was convincingly shown for growth hormone [8–10], prolactin [11–13], LH and FSH in pubertal girls [14–17] and LH in pubertal boys. Since augmented LH secretion synchronous with sleep is found consistently in "late" prepubertal and pubertal boys, an attempt was made to determine the importance of this phenomenon in stimulating T secretion that results in the development of secondary sexual characteristics during puberty. Toward this end, plasma LH and T were measured in nine pubertal boys at 20-min intervals throughout a control 24 h sleep wake cycle, and in three of these boys during acute inversion of the sleep wake cycle. Plasma LH and T were also measured in nine sexually mature men following a protocol identical to the control studies in the pubertal boys.

EXPERIMENTAL

Subjects and design

Nine healthy pubertal boys (12–15 yr), with no significant medical history, volunteered and were admitted to the sleep laboratory for a control study. Nine normal adult men (19–45 yr) were also studied (two subjects were studied on two occasions). All subjects had been previously adapted to the sleep laboratory. The sleep polygraphic tracings were recorded in an adjacent room and were scored according to standardized criteria [18]. The details of the catheterization technique for withdrawing the 20-min interval blood samples has previously been reported from this laboratory [19]. In three of the pubertal subjects, plasma LH and T were measured during acute sleep wake cycle reversal. One of these subjects was also studied after 3 days of sleep wake cycle reversal. Pubertal stage was assigned according to the criteria of Tanner[20] from prepuberty (P1) to almost complete sexual maturity (P5). The intermediate stages (P2, P3, P4) are based on progressive enlargement of the scrotum, testes and penis.

Methods

Plasma LH was measured by radioimmunoassay described by Midgley[21]. Results are expressed in terms of the 2nd International Reference Preparation of human menopausal gonadotropin (2nd IRP-HMG). The intra-assay coefficient of variation for duplicate samples for the range of values reported in this study was 6–8%. All samples from each 24-h study were assayed in the same run to negate inter-assay variability. Plasma testosterone was measured by radioimmunoassay after plasma extracts were chromatographed on Celite micro-columns. The details of the radioimmunoassay have recently been published [22]. The least detectable dose in 400 μl–1 ml of plasma varied from 9–20 ng/100 ml, but could be increased to 3–5 ng/100 ml by assaying 100 μl of the 300 μl redissolved plasma extract instead of

the usual 100 μl of the 1 ml redissolved plasma extract. The intra-assay and interassay coefficient of variation for T was 7·2% and 13·1%, respectively.

RESULTS

Simultaneous augmented LH and T secretion during sleep

Figure 1 shows the 24-h LH and T secretory patterns derived from 20-min interval plasma sampling in one of the early pubertal subjects (subject 9). The sleep stage sequence is depicted above the period of nocturnal sleep (2300–0700). The augmented LH secretory activity associated with sleep resulted in a mean LH concentration of 7·9 mIU/ml compared with 4·9 mIU/ml during waking. The onset of increased LH secretory activity at 0020 occurred shortly after the first sustained period of stage 4 sleep at 2340. The LH concentration rose from 5 to 13 mIU/ml. Plasma T began to rise at 0040 from 190 to 350 ng/100 ml. The next significant LH secretory episode began at 0140 with LH rising from 8·0 to 10·2 mIU/ml followed 20 min later by an increase in the plasma T concentration. The third LH secretory episode occurred at 0300 with plasma LH rising from 6·3 to 10·1 mIU/ml. An increase in plasma T followed at 0420 rising from 330 to 455 ng/100 ml. The fourth LH sleep associated secretory episode began at 0500 with plasma LH rising from 6·9 to 12·1 mIU/ml. Plasma T did not show any significant change after this LH secretory peak. The last sleep associated LH secretory episode was initiated at 0640 with plasma LH rising from 5·7 to 8·3 mIU/ml. Plasma T rose from 385 to 450 ng/100 ml beginning at 0700. During the awake period there were three significant LH secretory episodes at 1400, 1810 and 2000 which resulted in temporally related rises in plasma T of 130 to 200 ng/100 ml, 100 to 130 ng/100 ml, and 60 to 130 ng/100 ml. The time of onset of T secretion after preceding peak LH concentration ranged from 20 to 40 min with a mean of 31 min. It should

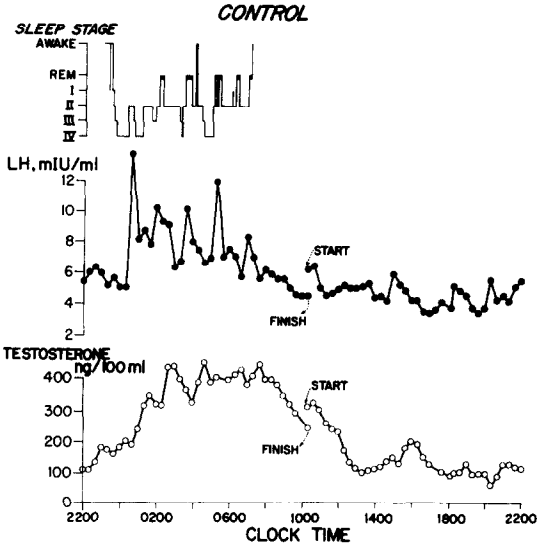

Fig. 1. Plasma LH and testosterone every 20 min during a control 24 h study in Subject 9. The histogram displaying sleep stage sequence is depicted above the period of nocturnal sleep. Sleep stages are REM (rapid eye movement) = ——. Stages I–IV by depth of line graph. Plasma LH (●—●—●) is expressed as mIU/ml 2nd IRP-HMG. Plasma T (○—○—○) is expressed as ng/100 ml.

be noted that after the study was started (1020), plasma T gradually fell from 325 to 100 ng/100 ml. This decrease in plasma T is frequently observed after insertion of the catheter at the beginning of a frequent interval sampling study. It is also of interest that the fall in plasma T concentration did not result in the initiation of an LH secretory episode. Also to be noted is the finding that at 0320 when plasma T was 410 ng/100 ml, a major LH secretory episode was initiated. During sleep the mean plasma T concentration was 333·4 ng/100 ml compared with 185·8 ng/100 ml during waking ($P < 0·001$). The other eight pubertal boys also showed simultaneous augmented secretion of LH and T associated with sleep resulting in significantly increased mean LH and T con-

Table 1. Mean plasma LH and T in pubertal boys asleep and awake*

Subject control	Age yr	Pubertal stage	LH† Asleep	LH† Awake	T Asleep	T Awake
			mIU/ml		ng/100 ml	
1	15	P1	§ 3·7 ± 0·9	2·3 ± 0·4	26·5 ± 8·8	‖ <19·0
2	13	P2	§ 14·4 ± 6·0	6·1 ± 1·8	§ 70·8 ± 20·0	33·0 ± 14·7
3	14	P2	§ 11·0 ± 2·3	6·3 ± 1·2	§ 187 ± 71·0	43·1 ± 46·9
4	12	P3	§ 6·2 ± 2·2	2·5 ± 0·7	§ 278 ± 92·7	180 ± 72·9
5	15	P4	§ 6·0 ± 1·7	2·8 ± 1·1	§ 427 ± 137	272 ± 128
6	15	P4	§ 12·3 ± 3·9	7·7 ± 1·3	§ 371 ± 122	288 ± 122
7	14	P4	†† 6·5 ± 2·8	5·2 ± 1·6	** 336 ± 27·6	290 ± 78
8	14	P4	§ 6·1 ± 1·6	4·3 ± 0·6	¶ 575 ± 65·7	536 ± 91
9	14	P3	§ 7·9 ± 2·1	4·9 ± 0·8	§ 333 ± 99·8	186 ± 106

* Mean values ± S.D.
† 2nd IRP-HMG.
§ Mean LH or T significantly greater than mean LH or T awake ($P < 0·001$).
‖ All T values less than 19 ng/100 ml during waking. Insufficient plasma to repeat with sensitized method.
¶ Mean LH or T asleep significantly greater than mean LH or T awake ($P < 0·05$).
** Mean LH or T asleep significantly greater than mean LH or T awake ($P < 0·01$).
†† Mean LH or T asleep significantly greater than mean LH or T awake ($P < 0·02$).

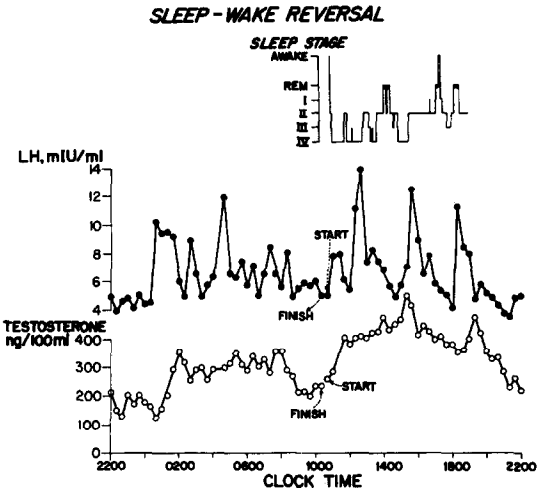

Fig. 2. Plasma LH (●–●–●) and T (O–O–O) concentrations sampled every 20 min in Subject 9, in early puberty during acute inversion of the sleep wake cycle. The sleep histogram shows that diurnal sleep occurred at 1025 and awakening at 1750 h.

centrations during sleep compared with waking (Table 1).

The effect of acute sleep–wake reversal on LH and T secretion

In subjects 7, 8 and 9 acute sleep–wake reversal studies were performed to further elucidate the interrelationship of T secretion to LH secretion and sleep. The results of one such study (subject 9) are shown in Fig. 2. The augmentation of LH secretion occurring during daytime sleep [14] is similar to what has previously been reported. However, during nocturnal waking in the 1 day reversal studies there is a persistence of increased LH secretory activity that is characteristic of the control study. The inability to completely obliterate augmented LH secretion during nocturnal waking with only 1 day sleep wake reversal has been a consistent finding in the pubertal boys we have studied [17]. This study (Fig. 2) was started at 1040 and plasma LH rose from 5·0 to 7·8 mIU/ml while plasma T increased from 260 to 405 ng/100 ml. The first sustained period of daytime sleep occurred at 1100 and a large LH secretory episode followed at noon with the plasma LH concentration rising from 5·4 to 13·9 mIU/ml. This was not associated with a corresponding T secretory episode although plasma T gradually rose to 480 ng/100 ml at 1400. Another augmented LH secretory episode occurred at 1440 with plasma LH rising from 4·9 to 12·5 mIU/ml. This was associated with an increase in plasma T from 470 to 550 ng/100 ml, the highest concentration during the entire 24-h period. The last LH secretory episode associated with sleep occurred at 1800 with plasma LH rising from 4·0 to 11·2 mIU/ml. Plasma T rose 40 min later from 360 to 475 ng/100 ml. With the onset of waking the plasma T concentration declined to its lowest level of the 24-h period (130 ng/100 ml) at 2240. During nocturnal waking, augmented LH secretory episodes occurred at 0020, 0220, 0320 and 0640. The initial LH secretory episode (4·5 to 10·2 mIU/ml) was associated with a large increase in plasma T from 125 to 355 ng/100 ml. Although the other LH secretory episodes were almost equivalent, they were associated with lesser increments in plasma T. The mean plasma T concentration during daytime sleep was 416·3 ng/100 ml compared with 270·1 ng/100 ml during daytime waking. This finding of a significantly increased LH and T concentration during daytime sleep was also found in the other two acute sleep–wake reversal studies (Table 2).

In an attempt to obtain more complete reversal of the LH and T secretory patterns, Subject 9 was restudied after 3 days of sleep wake cycle reversal. The results of this study are shown in Fig. 3. After insertion of the catheter, the plasma T concentration fell from 310 to 180 ng/100 ml. Between 0940 and 1300 the plasma T concentration varied from 140 to 220 ng/100 ml. The first major LH secretory episode occurred at 1200 with plasma LH rising from 6·5 to 20·5 mIU/ml. Twenty minutes after this peak LH concentration, plasma T rose from 190 to 290 ng/100 ml. A further rise in plasma T occurred at 1400 (260 to 360 ng/100 ml) associated with only a change in the rate of decline of the plasma LH concentration. Twenty minutes after the peak LH concentration of the second major LH secretory episode at 1440 (8·2 to 13·6 mIU/ml), plasma T rose from 250 to 480 ng/100 ml. The third and most dra-

Table 2. Mean plasma LH and T asleep and awake during 1-day and 3-day reversal*

Subject 1-day reversal	Age	Pubertal stage	Reversal LH Asleep	Reversal LH Awake	Reversal T Asleep	Reversal T Awake
			mIU/ml		ng/100 ml	
7	14	P4	§ 8·4 ± 2·0	6·7 ± 1·8	§ 322 ± 55·8	264 ± 44·4
8	14	P4	§ 6·0 ± 1·8	4·6 ± 0·7	§ 590 ± 36·1	355 ± 109
9	14	P3	† 7·5 ± 2·4	6·1 ± 1·8	§ 416 ± 55·3	270 ± 76·5
3-day reversal						
9	14	P3	§ 15·8 ± 8·3	9·4 ± 1·9	§ 314 ± 121	178 ± 98·1

* Mean values ± S.D.
† Mean LH and T asleep significantly greater than mean LH and T awake ($P < 0·01$).
§ Mean LH and T asleep significantly greater than mean LH and T awake ($P < 0·001$).

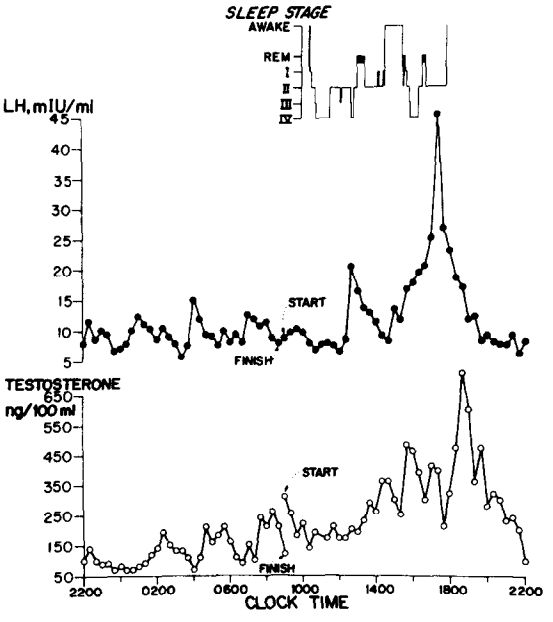

Fig. 3. Plasma LH (●–●–●) and T (○–○–○) sampled every 20 min in Subject 9 after 3 day sleep wake cycle reversal. The sleep histogram shows that diurnal sleep onset occurred at 1025 h and awakening at 1750 h. (Reproduced from *J. Clin. Invest.* **54** (1974) 609–615 with permission from publisher.)

matic LH secretory episode resulted in a rise of plasma LH from 11·7 to 45·6 mIU/ml. The plasma T secretory episodes that followed this LH secretory episode were evident at 1640 (300 to 410 ng/100 ml) and 1740 (210 to 720 ng/100 ml). After the subject's awakening, the plasma T concentration fell from 720 ng/100 ml at 1840 to 70 ng/100 ml at 0040. During nocturnal waking, LH secretory episodes were evident at 2340 (6·9 to 12·5 mIU/ml), 0320 (5·8 to 15·2 mIU/ml), and 0640 (8·2 to 12·8 mIU/ml), and were followed by T secretory episodes at 0040 (70 to 190 ng/100 ml) 0400 (70 to 210 ng/100 ml), 0500 (160 to 210 ng/100 ml), 0640 (90 to 150 ng/100 ml and 0720 (100 to 240 ng/100 ml). The mean ± S.D. LH and T concentrations during daytime sleep were 15·8 ± 8·3 mIU/ml and 314 ± 121 ng/100 ml compared with 9·4 mIU/ml ± 1·9 and 178 ± 98·1 ng/100 ml during nocturnal waking. Comparison of these mean T concentrations with this subject's control study showed complete reversal of the mean T concentration.

LH and T secretion in sexually mature men

Plasma LH and T were also measured at 20 min intervals in nine adult men by a protocol identical to the control studies carried out in the pubertal subjects. Plasma LH was secreted episodically throughout both waking and sleep periods. Only three of the eight subjects in whom LH was measured showed significantly higher mean LH concentrations during sleep compared with waking. Four of the nine subjects showed significantly higher mean T concentrations during sleep compared with waking. Two of the adult men, subjects 1 and 4 had two 24 h studies for LH and T. The results of these four studies in these two subjects showed that the plasma LH and T concentrations were different from day to day in the same subject and that there was no relationship between the LH and resultant T concentration (Table 3). An example of one of the adult men's 24-h LH and T secretory pattern is shown in Fig. 4.

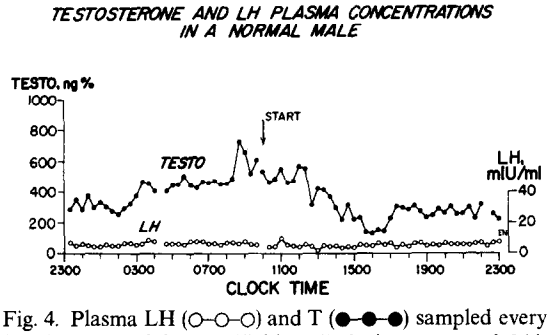

Fig. 4. Plasma LH (○–○–○) and T (●–●–●) sampled every 20 min in an adult man (Subject 1) during a control 24 h study.

Table 3. Mean plasma LH and T in sexually mature men asleep and awake*

Subject	Age	LH†		T	
		Asleep	Awake	Asleep	Awake
		mIU/ml		ng/100 ml	
1	19	6·1 ± 1·2	7·1 ± 1·7	399 ± 80	413 ± 153
	19	‖ 6·3 ± 1·2	5·3 ± 1·5	291 ± 58·3	277 ± 126
2	24	8·6 ± 2·0	8·4 ± 1·7	376 ± 59·6	346 ± 57·3
3	23			‖ 502 ± 50·5	452 ± 72·0
4	23	¶ 13·1 ± 3·4	11·1 ± 3·8	§ 571 ± 45	450 ± 148
		§ 7·0 ± 2·7	4·9 ± 1·2	§ 518 ± 109	321 ± 130
5	24	** 13·4 ± 1·8	12·0 ± 2·1	§ 891 ± 180	709 ± 130
6	21	9·8 ± 2·9	9·1 ± 3·5	378 ± 91	418 ± 130
7	45	20·0 ± 2·4	21·2 ± 3·5	¶ 710 ± 61	664 ± 101
8	45	5·4 ± 0·95	7·4 ± 1·9	371 ± 150	335 ± 51
9	45	7·4 ± 1·1	8·5 ± 1·4	490 ± 48	534 ± 106

* Mean values ± S.D.
† 2nd IRP-HMG.
§ Mean LH or T asleep significantly greater than mean LH or T awake ($P < 0·001$).
‖ Mean LH or T asleep significantly greater than mean LH or T awake ($P < 0·02$).
¶ Mean LH or T asleep significantly greater than mean LH or T awake ($P < 0·05$).
** Mean LH or T asleep significantly greater than mean LH or T awake ($P < 0·01$).

DISCUSSION

The results of this study clearly show that the sleep associated augmentation of LH secretion reported in pubertal boys [14] stimulates testosterone secretion. This simultaneous augmentation of LH and T secretion during sleep is not consistently found in prepubertal boys or adult men. The importance of sleep in driving this pubertal LH-T secretory "program" was clearly demonstrated by the finding of higher mean LH and T concentrations during reversed daytime sleep compared with nocturnal waking. These results show the importance of the central nervous system (CNS) in the regulation of hormonal factors governing the initiation and progression of the normal pubertal process.

The pubertal LH-T secretory "program" described in this study is difficult to explain on the basis of negative feedback control between testosterone and LH. A similar dilemma presented itself when the 24-h cortisol secretory pattern was reported. This difficulty results from the findings in both systems that ACTH and LH secretory episodes are initiated independent of the existing plasma cortisol and testosterone concentration. These observations are more compatible with a CNS-regulated secretory "program" that operates independently of negative feedback influences under normal spontaneous conditions. It should be stated however that under certain conditions feedback control may override the CNS regulated secretory "program" and therefore be the predominant endocrine secretory mechanism. This is clearly the case in patients with Addison's disease and gonadal insufficiency.

The absence of a consistent increase in the mean testosterone concentration during sleep in the adult men is in striking contrast to the findings in the pubertal boys. Although Evans *et al.*[23] reported peaks of testosterone concentration during or adjacent to REM sleep periods, no samples were obtained during waking. These authors also noted a "stepwise" increase in the fluctuating testosterone concentrations as the night progressed following a fall during the first sleep cycle of the sleep period. Judd *et al.*[24] reported a nocturnal rise in plasma testosterone in four young adults, but there were few waking samples to adequately compare to the mean testosterone concentrations during sleep. In our 11 studies in nine subjects, a nocturnal rise in testosterone was found in some subjects, but the maximum testosterone concentration across subjects did not occur until the early morning (Fig. 5). It should also be noted that the lowest level of the 24-h testosterone curve across subjects occurred in the late afternoon and early evening.

Boon *et al.*[25] measured plasma testosterone in 10 men at 6 h intervals and concluded that variation in concentration occurred, but there was no consistency in terms of when the maxima and minima occurred amongst subjects. Some investigators have reported the existence of a circadian rhythm for testosterone in men [26–28], however most of these

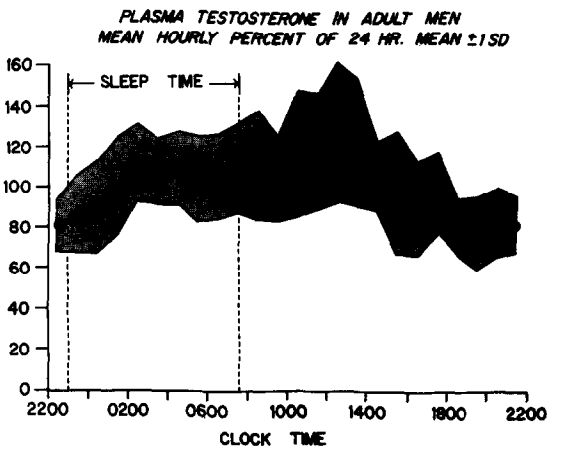

Fig. 5. The hourly mean plasma testosterone concentration across the 9 adult subjects expressed as the percentage of each subject's 24 h mean testosterone concentration. The shaded area represents ±1 standard deviation.

studies sampled plasma at infrequent intervals and were performed before the rapid episodic fluctuations of plasma testosterone were known. Recently, Leymarie *et al.*[29] measured plasma testosterone in four young men at 30 min intervals for 25 h and showed the existence of a circadian rhythm upon which frequent rapid variations were superimposed. Analysis of their four patterns showed that two subjects clearly had a nocturnal rise in plasma T in close proximity to sleep onset, while the two other subjects showed less variation with the maximal testosterone concentration achieved in the early morning after awakening. The findings in this study also suggest that the 24-h pattern of T secretion is not consistent from subject to subject and that not all subjects show the nocturnal rise in plasma T in close proximity to sleep onset. Analysis of the hourly means across all nine subjects based on their individual 24 h means showed a gradual rise with sleep onset and a stepwise rise throughout the night reaching a maximum in the late morning. The curve then falls off so that the minimum concentration occurs in the late afternoon and early evening (Fig. 5). This pattern is in marked contrast to the findings in the pubertal boys who show a consistent rise of plasma T at sleep onset, persisting until awakening and then falling off during the waking period (Fig. 6).

The finding of day to day variability in the mean sleep and wake testosterone concentrations in the two subjects that had two 24-h studies suggests caution in attempting to relate changes in plasma testosterone in response to experimental or therapeutic manipulations. Further studies are required to clarify the degree of variability of the 24 h mean testosterone concentration within a single individual from day to day.

The results of this study clearly show a developmental change in the secretory pattern of LH and T during sexual maturation and into adulthood. Clearly, LH stimulates T secretion in pubertal boys

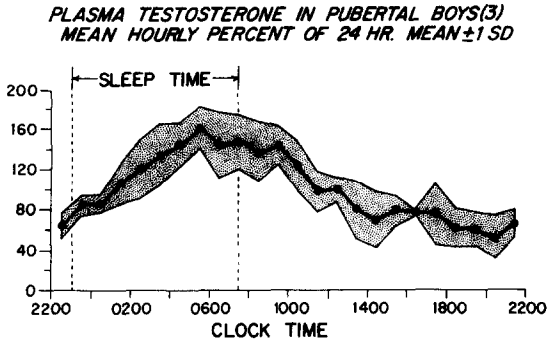

Fig. 6. The hourly mean plasma testosterone concentration across 3 of the pubertal subjects expressed as the percentage of each subject's 24-h mean plasma testosterone concentration. The shaded area represents ± 1 standard deviation.

and is controlled by a sleep associated CNS "program". Since this sleep dependent pubertal LH-T secretory "program" occurs consistently in "late" prepubertal and pubertal boys and not in early prepubertal or adult men it provides an additional biologic index for the identification of puberty. Longitudinal studies are currently underway in an effort to describe the sequential changes in this unique sleep related pubertal "program" in order to understand the findings in adult men where a clear, consistent, relationship between sleep, LH and testosterone secretion is difficult to discern.

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