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Loss of Circadian Rhythmicity in Blood Testosterone Levels with Aging in Normal Men*

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ABSTRACT. Previous studies concerning the relationship of serum testosterone levels to aging in normal men have yielded apparently inconsistent results. Studies performing blood sampling in the morning have often shown an age-related decrease in testosterone levels, while those using afternoon samples have failed to show such a decrease. These results suggested to us the possibility that the circadian rhythm in serum testosterone levels might be altered with normal aging in men.

Hourly blood samples were obtained for 24 h from 17 young (mean age, 25.2 yr) and 12 old (mean age, 71 yr) healthy men. Total testosterone levels were measured by RIA. The circadian rhythm in serum testosterone levels found in normal young men was markedly attenuated or absent in healthy elderly men; the early morning rise in testosterone levels characteristic of young men was not present in old age. Mean testosterone levels for the entire 24-h day were lower in healthy old men than in young men. These results demonstrate a clear decrease in serum testosterone levels in healthy old men compared to those in young men and provide an explanation for the inability to demonstrate an age-related decline in testosterone levels in earlier studies using serum samples obtained in the afternoon. (*J Clin Endocrinol Metab* **56:** 1278, 1983)

THE EXISTENCE of a male climacteric analogous to menopause in the female has been debated for many years (1, 2). It is generally recognized that libido and potency decrease with age in normal men (3). Many other changes in aging men (*e.g.* loss of musculature and of body hair) could also be aspects of a male climacteric (1).

In an effort to understand the physiological reasons for these age-related changes, many workers have measured the hormones of the male reproductive system (4–6). Testosterone levels have been studied because of the central role this hormone plays in maintaining sexual characteristics in man. Several studies using single blood samples obtained at 0800–1000 h have shown a decrease in plasma testosterone levels with age in men (4–6).

In contrast, a recent, carefully performed study found no decrease in serum testosterone levels with aging in normal men (7). An important difference between this study (7) and earlier ones was that the blood samples were obtained between 1400 and 1500 h, rather than the more usual time of 0800–1000 h. Normal young men exhibit a circadian rhythm in serum testosterone, with highest levels around 0800 h and lowest levels in the late afernoon and early evening (8). We reasoned that a possible explanation for the discrepancy between the recent study (7) and older ones (4–6) was that the amplitude of the circadian rhythm in testosterone could decrease with age. This would make the ability to detect a change in testosterone level with increasing age dependent upon the time of day that blood sampling was performed. We resolved to study the effect of normal aging upon the circadian rhythmicity of serum testosterone levels in man.

Materials and Methods

We studied 29 normal men, 17 young men (age range, 23–28 yr; mean ± SD, 25.2 ± 1.8 yr) and 12 old men (age range, 58–82 yr; mean ± SD, 71.0 ± 7.8 yr). The subjects were paid volunteers, recruited by advertising for normal men. The men were all of normal weight (within 15% of ideal body weight). Each was a nonsmoker, a nonabuser of alcohol, and receiving no medication. Normality was confirmed by medical history, physical examination, electrocardiogram, complete blood count, and urinalysis.

Each man was admitted to the Clinical Research Center and allowed to adjust for 24 h. On the second morning, hourly blood sampling through an indwelling peripheral venous cannula was begun at 0800 h and continued until 0700 h the next morning. The person performing the sampling was in an adjacent room so that subjects were not bothered by the procedure of blood withdrawal. Electroencephalographic monitoring was per-

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formed to confirm normal sleep patterns. All men had normal percent wakefulness, stages 3 plus 4, and rapid eye movement sleep for their age groups (9).

Total testosterone levels were measured in each serum sample by RIA, as described previously (10). Assay sensitivity was less than 10 pg/tube (<0.1 ng/ml). Intraassay and interassay variabilities were 51% and 9.8%, respectively. All specimens from each subject were measured in a single assay run. Analysis of variance and Duncan's multiple range test were used to assess differences between the groups at each time. Student's t test and cosinor analysis (11) were used for other comparisons, as noted in *Results*.

Results

Young men demonstrated a clear circadian rhythm in serum testosterone levels (Fig. 1), as has been reported in many previous studies (8). Maximal levels occurred at approximately 0800 h, while minimal levels were found between 1900 and 2100 h. In old men, however, the circadian rhythm was much less apparent (Fig. 1). Mean testosterone levels in young men were significantly higher than those in old men at each time point studied between 0200 and 1300 h. During the remainder of the day (1400–0100 h), testosterone levels did not differ significantly between the two age groups, although younger men tended to have higher levels. The circadian excursion of serum testosterone levels (highest point minus lowest point) was greater in young men (mean ± SEM, 3.54 ± 0.3 ng/ml) than in old men (2.05 ± 0.2 ng/ml; $P < 0.001$, by unpaired t test). Similarly, the amplitude of the circadian rhythms determined by cosinor analysis was significantly greater in the young men (mean ± SEM, 1.64 ± 0.18 ng/ml) than in the old men (0.68 ± 0.09 ng/ml; $P < 0.001$). Although the amplitudes were markedly lower, a slight circadian rhythm was detectable ($P < 0.05$, by cosinor analysis) in six of the elderly men.

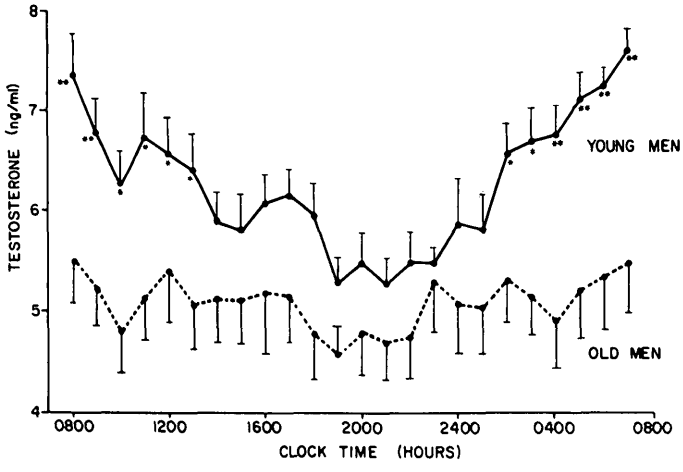

FIG. 1. Hourly serum testosterone levels (mean ± SEM) in normal young (n = 17) and old (n = 12) men. Blood samples were obtained using an indwelling peripheral venous cannula, which allowed free movement and normal sleep. *, $P < 0.05$; **, $P < 0.01$ (significance levels of the differences between young and old men at each time point). The *absence of an asterisk* denotes that there was no significant difference at that time point.

Although mean testosterone levels did not differ significantly in the two groups of men between midafternoon (1400 h) and late evening (0100 h), testosterone values averaged throughout the 24-h period were significantly higher in young men (mean ± SEM, 6.17 ± 0.26 ng/ml) than in old men (5.08 ± 0.41 ng/ml; $P < 0.05$, by unpaired t test).

Discussion

These data demonstrate clear differences between circulating testosterone levels in normal young and old men. Since the old men we recruited were physically and mentally active and had no coexistent disease, their lower testosterone levels cannot be attributed to inanition or illness. It seems likely that the differences in mean testosterone levels and circadian patterns that we observed are consequences of normal aging in man.

Studies that have found lower testosterone levels in old men have used blood samples obtained between 0800 and 1000 h (4–6), while a major study not finding an age-related decline sampled blood between 1400 and 1500 h (7). Our results imply that the ability to demonstrate an age-related decrease in testosterone levels in man depends in part upon the time of day that blood sampling is performed. Samples obtained in the morning are most likely to allow demonstration of an age effect, while those obtained in the afternoon or evening are least likely to differ between young and old men.

Two very recent studies (12, 13), despite using samples obtained in the morning, have been unable to demonstrate a difference in testosterone levels between young and old men. However, even in these studies, serum LH and FSH levels were higher in elderly men than in younger ones. Furthermore, the testosterone responsiveness to hCG stimulation was decreased in older men (13). Those findings appear to be consistent with a decrease in testicular endocrine function with age, despite the fact that these two studies were unable to demonstrate lower basal testosterone levels in older men. It may be that frequent blood sampling over longer periods of time in each man would have allowed demonstration of age-related changes in testosterone levels. It is also possible that the stress of being asked to present themselves for experimental studies may have partially suppressed the circadian increase in testosterone levels in the normal young men in these two studies. The mean testosterone levels reported were lower than those found in other studies of normal young men (4–6, 8).

Our finding of a loss in circadian testosterone rhyth-

micity with aging raises the question of whether this loss is due to age-related changes in gonadotropin production or to decreased testicular responsiveness to gonadotropins. Careful studies of circadian patterns of gonadotropin secretion and testicular responses to gonadotropins with normal aging will be required to answer this question.

The demonstration of a loss of circadian rhythmicity in testosterone levels raises the possibility that decreases in other circadian rhythms may be a normal concomitant of aging. Recent studies have demonstrated a reduction with normal aging in the circadian rhythm of pineal melatonin content in animals (14) and serum melatonin levels in humans (15). The pineal plays an important role in the neuroendocrine control of gonadotropin secretion in many species and often calcifies with aging in human beings. Whether there is a causal relationship between the loss of the circadian rhythm in melatonin acting through gonadotropins to affect testosterone is unknown. In contrast to these results for melatonin and testosterone, the circadian rhythmicity of cortisol in man appears to be unaffected by age (16, 17).

Another possible mechanism for loss of circadian rhythmicity in T relates to hypothalamic neurotransmitter control of gonadotropin secretion. Hypothalamic catecholamines are known to exert an important influence on the production of LHRH into the hypothalamic-hypophyseal portal system and, thereby, upon gonadotropin production from the pituitary (18, 19). Important changes in hypothalamic catecholamine content with aging have been described in both experimental animals and humans (18). Decreases in norepinephrine levels and turnover rates have been demonstrated in old rats; furthermore, the administration of L-dopa or iproniazid, which increase hypothalamic catecholamines, can reinitiate estrous cycles in old, constant estrous female rats (20). It is possible that an age-associated alteration in hypothalamic catecholamine production in men could alter a circadian pattern of gonadotropin secretion and, thereby, decrease the circadian rhythm in serum testosterone levels.

Our results have demonstrated that, in addition to a loss of the circadian rhythmicity of serum testosterone levels, normal older men have an overall decrease in mean serum testosterone levels when averaged over 24 h. Zumoff *et al.* (21) have also recently reported that the 24-h mean testosterone level is lower in healthy elderly men than in young men. The effect of these changes in testosterone secretion on male sexual behavior is presently unknown. While it is well known that testosterone administration to hypogonadal men will stimulate sexual behavior (22), little critical work has been performed on the ability of testosterone administration to affect sexual behavior in normal elderly men. Similarly, it may be that the decreases in testosterone that we have found in older men may be important in the loss of body hair and musculature that are well known concomitants of aging in man.

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