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Circadian Variation in Testosterone, Sex Hormone-Binding Globulin, and Calculated Non-Sex Hormone-Binding Globulin Bound Testosterone in Healthy Young and Elderly Men

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The circadian pattern in levels of serum total testosterone (T) in men becomes blunted with normal aging. However, because T not bound to sex hormone-binding globulin (non-SHBG-T) is felt to be a better representative of biologically available T than is total T, the possibility of a 24-h variation in non-SHBG-T in young men and the possibility that aging is associated with a blunting of that rhythm were investigated. Hourly blood samples were drawn on 10 normal young men (mean age 27.3 years) and 10 normal elderly men (mean age 70.7 years) over a 24-h period and the serum was assayed for total T, sex hormone-binding globulin (SHBG), and total protein; non-SHBG-T was calculated. SHBG was determined by radioimmunoassay as well as by a steroid-binding assay. Young men had a significantly higher ($p < 0.05$) mean 24-h level of non-SHBG-T (1.91 ± 0.62 nm/l) than did the elderly men (0.86 ± 0.01 nM/l). Also, each young man showed a significant circadian rhythm in non-SHBG-T, with a group mean daily variation of 1.42 ± 0.38 nM/l. In contrast, only 60% of the elderly men demonstrated a significant circadian rhythm in non-SHBG-T, and the group mean rhythm was blunted (maximum excursion 0.38 ± 0.07 nM/l) compared with that of the young men. SHBG and total protein levels demonstrated similar 24-h variations in the two age groups. It was concluded that non-SHBG-T serum levels, similar to serum total T levels, demonstrate a circadian pattern in young men and this circadian rhythmicity becomes blunted with normal aging.

Key words: testosterone, SHBG, circadian rhythm, aging.

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A circadian variation in serum total testosterone (T) has been well documented in young men and this circadian variation has been shown to be blunted in older men (Bremner et al, 1983; Moroz and Verkhratsky, 1985; Montanini et al, 1988). This age-associated decline in circadian variation in serum T levels is accompanied by a significant decrease in

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mean 24-h serum total T levels (Bremner, et al 1983; Moroz and Verkhratsky, 1985; Montanini et al, 1988). In addition, when free or non-sex-hormone-binding globulin bound T (non-SHBG-T) has been measured or calculated in single daily blood samples, a more marked-difference has been demonstrated between young and elderly men than has been seen when only total T is measured (Nankin and Calkins, 1986; Tenover et al, 1987). Non-sex hormone-binding globulin bound T has been suggested to be a better reflection of the actual portion of circulating T that is available for a metabolic effect than is the measurement of total T (Lasnitski and Franklin, 1972; Pardridge et al, 1980; Garden et al 1988), and since non-SHBG-T is determined not only by total T, but also by the quantity of steroid binding proteins, especially SHBG in the circulation, a fluctuation in the level of either T or SHBG could change non-SHBG-T.

Recently, a circadian pattern in SHBG levels has been described in young men (Clair et al, 1985). The reported SHBG pattern had its zenith at about 1400 hours which is the same general time of day that has been reported for the nadir of the daily total T cycle. If this SHBG cycle were absent or the same in older men compared with younger men, the circadian differences in non-SHBG-T might be exaggerated between these two groups, especially since older men generally have higher SHBG levels than younger men. Conversely, if the SHBG cycle were reversed in the older men, there might be little or no difference in the 24-h pattern of non-SHBG-T between the two age groups. The following study was undertaken to determine and compare the 24-h pattern of non-SHBG-T levels in healthy young and older men.

Materials and Methods

Subjects

Ten healthy young men, mean age 27.3 years, and 10 healthy older men, mean age 70.7 years were studied. All men were recruited by advertising. They were within 10% of their ideal body weight, night sleepers, non-smokers, non-abusers of alcohol, and were not elite athletes. They took no medications. All of the men were healthy as determined by physical examination, complete blood count, urinalysis, and blood chemistry screen. In addition, all men had testis volumes > 15 ml and were eugonadal, as defined by T > 9.7 nmol/l, LH < 80 μg/l, and FSH < 240 μg/L. A more complete description of these gonadotropin and sex steroid profiles has been previously presented (Tenover et al, 1987; 1988).

Experimental Protocol

The men were admitted to the Clinical Research Center of the University of Washington Hospital for acclimatization the night prior to the beginning of the blood sampling. Each group was then divided equally in order to begin blood sampling between either 0800 and 1000 h or between 2000 and 2100 h. Blood was drawn every 10 min for 24 h from an indwelling cannula placed in an arm vein and kept open with heparinized lactated Ringer's solution. During the study, the men were on an unrestricted diet with meals at fixed times (0800, 1200, 1730 h). Lights were turned out some time between 2300 and 0100 h and during the night the person performing the sampling was in an adjacent room so that the subjects were not disturbed. All subjects awakened between 0630 and 0730. The first blood sample from each hour was used in this study. The remaining samples were used in previously reported studies of gonadotropin pulse frequency (Tenover et al, 1988).

Testosterone (T)

The RIA for T employed reagents provided by the World Health Organization (WHO) Matched Reagent Program. The methodologies have been previously described (Matsumoto et al, 1983). The T assay was preceded by ether extraction, and separation of bound from free hormone was accomplished with dextran-coated charcoal. The assay sensitivity was 0.1 ng/ml (0.35 nM/l SI). The intra- and interassay variabilities were 5.1% and 9.8%, respectively.

Sex Hormone Binding Globulin (SHBG) and Total Protein

SHBG was measured by radioimmunometric assay (IRMA) and by a tritiated dihydrotestosterone (DHT) saturation analysis binding assay. The SHBG IRMA assay was performed with reagents provided by Farmos Diagnostica (Oulunsalo, Finland). This assay has intra- and interassay coefficients of variation of 3.5% and 5.6%, respectively. The specifics of the binding assay have been previously described (Plymate et al, 1981). The intra- and interassay coefficients of variation for the binding assay are 7.4% and 9.6%, respectively. The correlation between the IRMA and binding assays for SHBG was $r = 0.96$. Total protein was measured by a Coomassie blue G-250 affinity dye technique with reagents obtained from Bio-Rad Laboratories (Los Angeles, CA).

Non-SHBG Bound T

The non-SHBG-T was calculated from the total molar concentration of T and SHBG according to a modification of the mass action equation as previously described (Plymate et al, 1987). The IRMA measurement of SHBG was used in this calculation. We have previously demonstrated that this method has a good correlation with the brain uptake of T, salivary T measurements and the Sodergard equation for non-SHBG-T although absolute values for each type of measurement may differ (Garden et al, 1989).

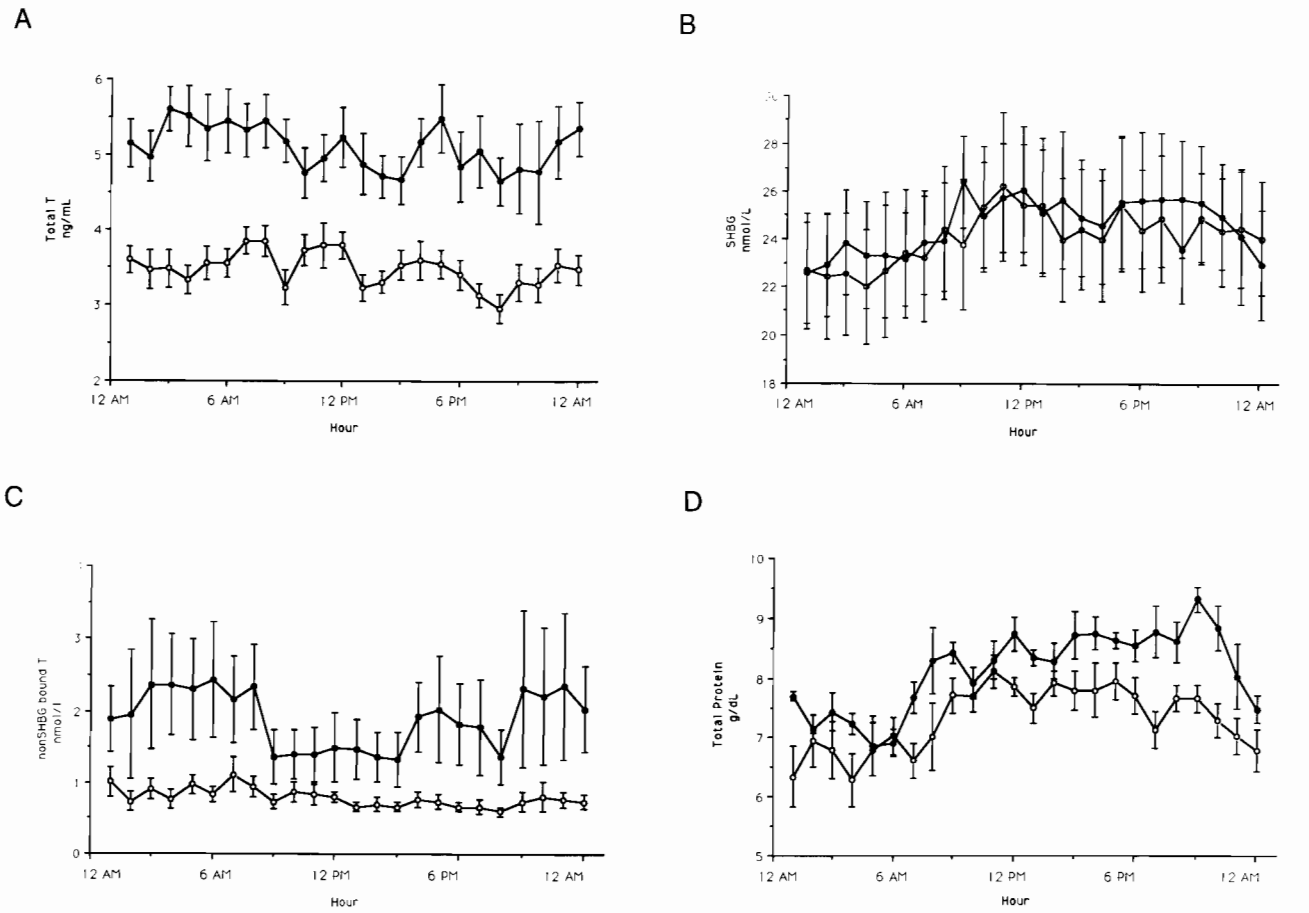

Fig. 1. Serum total testosterone (A), SHBG (B), non-SHBG-bound T (C), and total protein (D) measurements in normal young (closed circles) and elderly (open circles) men measured hourly for a 24-h period.

Statistics

Nonpaired Student's t-test with Bonferoni modification to correct for number of comparisons was used to compare individual 24-h mean data between the two age groups. To identify and characterize any circadian rhythm that might be present in an individual's serum T levels, SHBG, non-SHBG-T, or total protein, 24-h cosine regression analysis of individual hourly data was performed (Nelson et al, 1979). This analysis provides estimates of the amount of fluctuation (amplitude) and the time of maximum fluctuation (phase) of the cosine curve that best fits the data. (Doubling the cosine amplitude gives the maximum excursion of the "best fit" cosine curve). Using this method, a data set is felt to demonstrate a signficant circadian rhythm if the 24-h cosine regression was significant at $p < 0.05$.

Results

Mean hourly total T levels over a 24-h period in young and elderly men are shown in Fig. 1A. As has been reported previously (Tenover et al, 1987), the young men had significantly higher ($p < 0.05$) 24-h mean total T levels than the elderly men (Table 1). Cosine regression analysis found that seven of the 10 young men and four of the 10 older men had significant individual circadian variations in total T and that the average daily change in total T in the young men was more than twice (4.9 ± 0.7 nMol/l) that of the elderly men (2.1 ± 0.3 nMol/l). Both young and elderly men as a group had their highest serum total T levels in the early morning hours.

Hourly SHBG levels, as measured by IRMA, as shown in Fig. 1B for both young and elderly men. Using the DHT binding assay to determine SHBG levels produced similar data (results not shown). The two age groups had similar mean 24-h SHBG levels (Table l) and similar SHBG patterns over 24-h (Fig. 1B). Maximum SHBG levels occurred in the late

morning/early afternoon hours in both age groups. Cosine regression analysis demonstrated a significant circadian variation in SHBG in eight of the 10 young men and five of the 10 elderly men, with a mean daily maximum variation in SHBG level that was similar in the two groups (6.4 ± 1.3 nMol/l and 5.2 ± 0.9 nMol/l, young men and elderly men, respectively).

Hourly non-SHBG-T levels in the two age groups were calculated using hourly total T and SHBG values (Fig. 1C). As described previously (Tenover et al, 1987), the young men had significantly ($p < 0.05$) higher mean 24-h non-SHBG-T levels than did the elderly men (Table 1). Cosine regression analysis found that all 10 young men had significant circadian rhythms in non-SHBG-T, with a mean daily change of 1.42 ± 0.38 nMol/l. In contrast, six of 10 elderly men had a significant circadian rhythm in non-SHBG-T and this rhythm, like the rhythm for total serum T levels, was blunted (maximum excursion 0.38 ± 0.07 nMol/l) relative to that found in the young men. The cosine regression analysis reported was determined on the basis of the IRMA measurements, as previously stated. In addition, since the binding assay gave similar quantitative results for each time point, the circadian variations seen are for a functional binding protein in addition to protein mass.

To evaluate if the 24-h changes in SHBG paralleled those of other serum proteins in both age groups, hourly total serum protein levels also were determined over the 24-h period. As shown in Fig. 1D and Table 1, there was no difference in pattern or maximal 24-h variation in serum total protein or mean 24-h total protein levels between the two age groups. In addition, comparison of Fig. 1D to 1B shows that maximal daily changes in total protein are a greater percentage (22%) of the daily total protein level than is the maximal daily change in SHBG when compared to its daily mean (14%).

Discussion

This study demonstrates that the 24-h patterns of non-SHBG-T in healthy young and elderly men are similar to the 24-h patterns of total T and that, like the total T circadian pattern, the 24-h variation in non-SHBG-T in elderly men is not as marked as in the young men. The patterns of non-SHBG-T are similar to those for total T in the two age groups because the 24-h variations in SHBG levels are similar.

The finding of a blunted 24-h pattern in total T variation in normal elderly men is consistent with other studies (Bremner et al, 1983; Moroz and Verkhratsky, 1985; Montanini et al, 1988). In addition, our finding of a circadian variation in serum levels of SHBG in a majority of young and elderly men is consistent with previous work demonstrating a circadian variation of SHBG in young men (Clair et al, 1985). We have determined non-SHGB-T by calculations, using the relationship between T and SHBG. A recent report of bioavailable T, estimated by ammonium sulfate precipitation of SHBG-bound T, has shown similar results for mean non-SHBG-T levels (Kaiser et al, 1988).

TABLE 1. Average 24-h Serum Levels and Maximum 24-h Excursions of Total Testosterone, Sex Hormone Binding Globulin, Non-Sex Hormone Binding Globulin-Bound Testosterone, and Total Protein in Normal Young and Elderly Men

	Young (n = 10)	Elderly (n = 10)	
Total T (ng/ml)	5.1 ± 0.3	3.5 ± 0.2	$p < 0.05$
(nMol/l)	17.7 ± 1.0	12.1 ± 0.7	
Mean excursion of cosinor (ng/ml) (2 × amplitude)	1.4 ± 0.2	0.6 ± 0.1	
(mMol/l)	4.9 ± 0.7	2.1 ± 0.3	
SHBG (nMol/l)	30.1 ± 2.7	28.1 ± 2.1	NS
Mean excursion	6.4 ± 1.3	5.2 ± 0.90	
non-SHBG-T (ng/ml)	0.55 ± 0.18	0.25 ± 0.03	$p < 0.05$
non-SHBG-T (nMol/l)	1.91 ± 0.62	0.86 ± 0.10	
Mean excursion (ng/ml)	0.41 ± 0.11	0.11 ± 0.02	
(nMol/l)	1.42 ± 0.38	0.38 ± 0.07	
Total protein (g/dl)	8.1 ± 0.5	7.3 ± 0.3	NS

Another investigation also has demonstrated a circadian rhythm in T and free T in young men, as measured by ultrafiltration dialysis (Montanini et al, 1988). Similar to our findings, this study also detected a loss of the T rhythm in the older men; although the rhythm in free T persisted. In contrast to our study, Montanini et al (1988) did not find circadian variations in SHBG in either the young or elderly men. This may have been due to the smaller number of subjects or the longer 4-h sampling interval as opposed to the 1-h interval in the current study.

In this study we have demonstrated the endogenous variation of two substances, one of which (SHBG) has a major effect on the availability of the other (T) for its cellular action and metabolic clearance. Since the circadian variations of T and SHBG tend to have opposing zeniths and nadirs, the differences between the two groups are accentuated when non-SHBG-T is calculated. This accentuation is seen not only in the larger difference between the groups in non-SHBG-T but also in the increased number of young men with evident non-SHBG-T circadian rhythms and the flattening of circadian rhythm amplitude in the older men.

Figures 1D and B show that the variations in total protein were greater than those for SHBG over the 24-h period in both groups of men. The rhythms for both SHBG and total protein parallel each other. Possible explanations for these circadian variations include a change in plasma water and distribution of proteins between the plasma and extravascular system. In this situation, lower molecular weight proteins would be expected to show a greater variation than higher molecular weight proteins. Since the majority of serum proteins have molecular weights less than SHBG (100,000 Da), a greater circadian variation in total protein than SHBG would be expected (Hyltoft-Peterson et al, 1981). Since the half-life of SHBG is 5–7 days, the circadian variation would be unlikely to be due to variations in production. Also, because the fluctuations are present for both the binding assay and IRMA, the variation is not due to a change in binding.

Circadian rhythnms for steroid hormones have been described for a number of years, the most well-known of these being cortisol (Montanini et al, 1988). Circadian rhythms for T have also been described by several investigators (Bremner, et al, 1983; Moroz and Verkhratsky, 1985; Montanini, et al, 1988). As with most circadian rhythms, however, the causes of the T rhythm are not known. Among the candidates for generation of the T rhythm are the pineal gland, with its secretion of melatonin and subsequent control of gonadotropin secretion (Moore, 1978; Martin, et al, 1980), and hypothalamic catecholamines, specifically norepinephrine, which may control GnRH secretion (Moore, 1983). More recently, a relationship between CRF and GnRH has been described (Olster and Ferin, 1987). It also may be that the circadian total T and non-SHBG-T rhythms are the result of some mechanism of regulation, not yet described, occurring directly at the level of the testis.

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