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# CIRCADIAN VARIATIONS OF SERUM SEX HORMONE BINDING GLOBULIN BINDING CAPACITY IN NORMAL ADULT MEN AND WOMEN

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**Summary**—Diurnal variations of serum sex hormone binding globulin (SHBG), testosterone (T) and estradiol (E2) in five normal adult men and five normal adult women were investigated. SHBG binding capacity was measured by both polyacrylamide gel electrophoresis and dextran-coated charcoal technique (DCC); T and E2 were assayed by RIA and free T and free E2 were determined by means of equilibrium dialysis. In male subjects the variations of SHBG binding capacity was associated with the changes of total T, free T and T/SHBG index, which had the highest concentrations in the morning and the lowest levels in the evening during the 24 h test period, but percentage free T remained unchanged. Serum protein concentrations did not change significantly during 24 h. No significant diurnal changes of SHBG binding capacity, total E2, free E2, percentage free E2 and percentage free T were found in female subjects in the mid-luteal phase of the menstrual cycle, although significant fluctuations of total T, free T and T/SHBG index were observed throughout the day. The results suggested that SHBG may play a buffer role in the presence of fluctuations of testosterone production during 24 h period, allowing stabilization of a bioactive fraction of the hormone both in normal adult male and female. However, the concentrations of T in normal adult women may be too low to drive any change of SHBG levels while there were no significant variations of E2 throughout a day in the mid-luteal phase of the menstrual cycle.

## INTRODUCTION

Sex hormone binding globulin (SHBG) plays an important role in the determination of the biologically available testosterone (T) and estradiol (E2) in human plasma [1, 2]. The levels of SHBG are predominantly influenced by estrogens and androgens in certain physiological states and disease processes [3–12].

Many studies have confirmed a diurnal rhythm of plasma T in normal adult men with the highest level in the morning and lowest concentration in the evening [13]. In normal adult women, Lachelin *et al.*[14] and Rosenfield and Helke[15] have also determined the existence of a circadian rhythm of androgens. Yen's study on two normal women demonstrated that E2 is secreted in a rhythmic, pulsatile fashion during 24 h in the early follicular phase of the menstrual cycle [14].

At present it is not known whether fluctuations of plasma T and E2 during 24 h period in normal adult men and women have any effect on SHBG binding capacity. Clair *et al.*[16] reported circadian changes of plasma SHBG with a peak at the beginning of the afternoon and a nadir at midnight in 7 adult men. This variation was in agreement with the circadian rhythm of the total plasma proteins other than sex hormones. The present study was undertaken to determine the diurnal variation in serum SHBG binding capacity and the relationship of this variation to changes in serum T concentration in normal adult men and of serum E2 and T levels in normal adult women.

## MATERIALS AND METHODS

### *Subjects*

Five men and five women volunteered for this study. The range of age, height and weight of male subjects were 30–37 yr, 168–175 cm and 51–65 kg, respectively. The range of age, height and weight of female subjects were 25–35 yr, 158–165 cm and 50–55 kg, respectively. Each subject had a normal medical history and physical examination. Each woman had a regular and spontaneous menstrual cycle. Blood samples of men were drawn hourly during a 24 h test period. The sleep period was 23:00–07:00 h. All women were studied for a day in the mid-luteal phase of the menstrual cycle. Progesterone levels were approximately 40 nmol/l. Blood

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samples were also taken hourly and the sleep period was the same as for men. All samples from each individual were assayed for each hormone and SHBG binding capacity in a single assay.

*Assay of SHBG binding capacity and hormones*

Serum samples of men were assayed for SHBG binding capacity both by a polyacrylamide gel electrophoresis described by Rezen *et al.*[17] and a simplified method for measuring SHBG reported by Fattah and Chard[18]. Briefly, the method consisted of a multiple ligand dose and serial dilutions of a pool of pregnancy serum as standard. The method was modified by using dextran-coated charcoal (DCC) for separation of bound and free labelled steroids instead of saturated ammonium sulphate. Since a good correlation between the two methods (male sample assay: $r = 0.80$, d.f. $= 236$, $P < 0.001$) (Fig. 1) was obtained, SHBG binding capacity in female samples were only measured by DCC. The intra- and interassay coefficients of variation were 4.33–7.87% and 7.26–14.90% respectively.

Serum T and E2 were measured by radioimmunoassay according to the WHO manual [19] using the matched reagents provided by WHO. The intra- and interassay coefficients of variation were, respectively, 5.40–11.40% and 7.40–14.70%. Percentage free T and percentage free E2 in serum were determined by means of equilibrium dialysis described by Bammann *et al.*[20].

Serum proteins were determined by the Lowry method [21] using the equivalent of 1.25 $\mu$l of serum.

*Statistical analysis*

The concentrations of free T and free E2 were calculated according to the equation of Bammann *et al.*[20]. Ratio of T to SHBG both in male and female subjects were all calculated from levels of SHBG assayed by DCC. The daily variations were analyzed by one-way analysis of variation. A $P$ value of 0.05 was considered significant. The linear regression analysis was used to test the relationship between sex hormones and SHBG binding capacity.

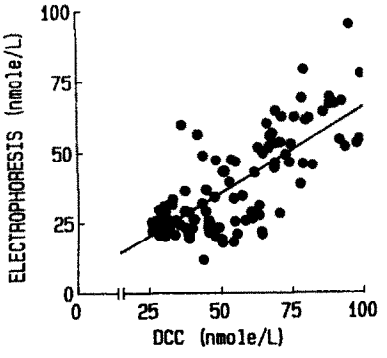


Fig. 1. Correlation between serum SHBG binding capacity as assayed by polyacrylamide gel electrophoresis and dextran-coated charcoal (DCC) technique. $r = 0.80$, d.f. $= 236$, $y = 14.644 + 1.044x$.

## RESULTS

Mean levels of serum T, free T, SHBG binding capacity and T/SHBG index in male subjects and mean levels of serum T, E2, free T, free E2, SHBG binding capacity and T/SHBG index in female subjects in each individual during the 24 h test period are presented in Table 1 and 2, respectively. The $F$ values of one-way analysis of variation for daily fluctuations of all indices including percentage free T and percentage free E2 in both male and female are shown in Table 3.

In male subjects differences in free T and T/SHBG index were less than those in T and SHBG binding capacity during the 24 h study period and percentage free T remained unchanged, although a morning rise and an afternoon fall of all indices were clearly evident (Fig. 2). The T pattern closely paralleled the diurnal variations in free T and T/SHBG index. The pattern of SHBG binding capacity had almost an hour lag behind those of T, free T and T/SHBG index. Regression analysis demonstrated a significant correlation between T and SHBG, free T and SHBG as well as T/SHBG and SHBG, with a higher $r$ value between T and SHBG during the 24 h test period (Table 4).

In female subjects, higher deviations from 08:00–14:00 and lower levels at night were observed for T, free T and T/SHBG index (Fig. 3). There were no significant differences in levels of E2, free E2, SHBG, percentage free E2 and percentage free T (Table 3). However, the relationship between SHBG binding capacity and all other indices except free E2 were significant with higher $r$ values between SHBG and testosterone indices (Table 4).

## DISCUSSION

Changes in SHBG have been described for a variety of physiological and pathological states including fetal life, puberty, menstrual cycle, pregnancy and advancing age in men and women [2]. As far as we are aware, there is only one recent report on the diurnal variation of SHBG in normal men where a peak was found in the afternoon and nadir at midnight [16]. The present report confirms and extends these findings. However, our peak in SHBG binding capacity was observed in the late morning and our nadir occurred around midnight for men. Although

Table 1. Mean values of serum T, free T, SHBG binding capacity and T/SHBG index in normal adult male subjects during the 24 h test period

| Subjects | Age | T (nmol/l)[a] | free T (nmol/l)[a] | SHBG bound T (nmol/l)[a] | T/SHBG[a] |
|---|---|---|---|---|---|
| M.T. | 31 | 9.8 ± 2.2 | 0.28 ± 0.08 | 33.3 ± 6.44 | 0.30 ± 0.07 |
| Y.S. | 37 | 23.4 ± 7.3 | 0.43 ± 0.12 | 77.4 ± 22.5 | 0.32 ± 0.09 |
| W.R. | 34 | 16.3 ± 6.5 | 0.32 ± 0.13 | 59.8 ± 15.2 | 0.27 ± 0.08 |
| W.D. | 32 | 13.7 ± 5.5 | 0.34 ± 0.17 | 35.5 ± 8.7 | 0.40 ± 0.16 |
| Z.F. | 31 | 29.2 ± 9.9 | 0.41 ± 0.16 | 71.1 ± 17.1 | 0.43 ± 0.17 |

[a]Mean ± SD.

Table 2. Mean values of serum E2, free E2, T, free T, SHBG binding capacity and T/SHBG index in normal adult women during the 24 h test period

| Subject | Age | E2 (pmol/l)[a] | Free E2 (pmol/l)[a] | T (nmol/l)[a] | Free T (pmol/l)[a] | SHBG bound T (nmol/l)[a] | T/SHBG[a] |
|---|---|---|---|---|---|---|---|
| L.X. | 35 | 549.4 ± 302.1 | 18.3 ± 11.7 | 0.86 ± 0.38 | 24.9 ± 11.9 | 56.4 ± 9.1 | 0.24 ± 0.12 |
| L.G. | 32 | 564.3 ± 221.2 | 9.4 ± 3.0 | 1.30 ± 0.41 | 15.7 ± 7.7 | 107.2 ± 18.4 | 0.010 ± 0.003 |
| L.M. | 29 | 597.1 ± 161.2 | 25.5 ± 8.5 | 0.99 ± 0.44 | 27.2 ± 12.7 | 59.2 ± 10.3 | 0.022 ± 0.008 |
| S.C. | 28 | 403.2 ± 154.6 | 8.9 ± 5.6 | 1.26 ± 0.3 | 26.8 ± 8.0 | 69.8 ± 13.7 | 0.023 ± 0.007 |
| T.X. | 25 | 664.3 ± 223.2 | 25.2 ± 8.3 | 1.16 ± 0.25 | 20.9 ± 8.7 | 98.1 ± 37.6 | 0.009 ± 0.003 |

[a]Mean ± SD.

fluctuations occurred in women in the mid-luteal phase no definite diurnal rhythm was detectable. Our results in men show that the daily variations of free T are much less than those of total T although it also has a circadian rhythm similar to that of total T. Percent free T remained unchanged during the 24 h study period. In addition the T/SHBG index that is recently thought to reflect the plasma level of the active fraction of T, e.g. free T and albumin bound T [22, 23] also remained very stable compared with the fluctuations of total T and SHBG binding capacity. Changes in total serum proteins during 24 h were not significantly different. All these results suggest that the SHBG is acting as a buffer to stabilize free hormone levels in the presence of fluctuating testosterone production patterns [24].

Many studies have demonstrated that androgens tend to drive down SHBG levels [1]. However, the finding is based upon the changes of SHBG levels with age, sex and administration of androgens [25]. The long-term effect of T on SHBG production differs probably from the short-term influence of diurnal variations in T on SHBG levels. *In vitro* cell cultures show that both estrogens and androgens stimulate SHBG synthesis and secretion when evaluated with the use of antibodies to SHBG [26]. Our data shown in Fig. 2 suggest that this may also occur *in vivo*.

Contradictory results about the effect of cyclic variations in estrogen secretion during the menstrual cycle on the changes of SHBG levels have been reported [27–30]. Odlind *et al.*[28] proposed that a threshold level of E2 (>400 pg/ml) is needed to increase SHBG production. Plymate *et al.*[29] and Solomon *et al.*[30] reported a significant rise in SHBG levels in the luteal phase of the cycle, due to the increase in serum E2 concentrations. We therefore thought that any diurnal changes in SHBG levels might be more detectable in the luteal phase than in the follicular phase. Female subjects were all in the mid-luteal phase of the menstrual cycle. However, no significant changes in E2 and SHBG binding capacity were found despite a significant circadian rhythm of T. Even if a rise of E2 concentrations in the luteal

Table 3. Summary of one-way analysis of variation of serum SHBG binding capacity, T and E2 assayed hourly during the 24 h study period in normal adult men and women

| Measurements | *F* value | *P* |
|---|---|---|
| Male | | |
| SHBG | 4.602 | <0.01 |
| T | 4.056 | <0.01 |
| T/SHBG | 2.892 | <0.01 |
| Free T | 1.833 | <0.05 |
| Percentage free T | 0.631 | NS |
| Protein | 1.127 | NS |
| Female | | |
| SHBG | 0.906 | NS |
| E2 | 0.715 | NS |
| Free E2 | 0.446 | NS |
| Percentage free E2 | 0.230 | NS |
| T | 2.127 | <0.01 |
| Free T | 1.776 | <0.05 |
| Percentage free T | 0.737 | NS |
| T/SHBG | 1.650 | <0.05 |

NS = not significant.

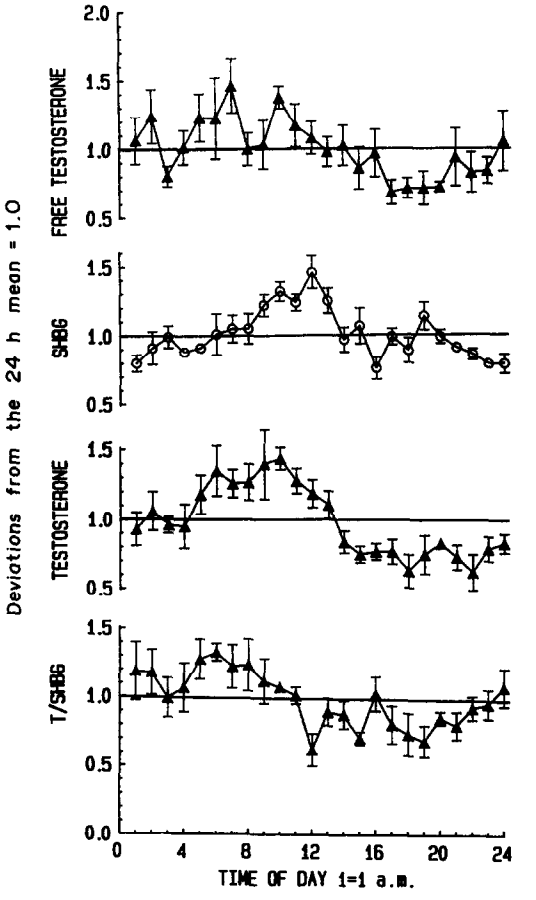


Fig. 2. Daily variations of circulating SHBG binding capacity, T, free T and T/SHBG index. Hormones and SHBG levels were determined in hourly samples obtained from 5 normal adult men during a 24 h period. The data expressed as percent deviations from the 24 h mean concentrations, with 100% = 1.0 = mean.

Table 4. Correlation between changes in serum T, E2 and changes in serum SHBG binding capacity during the 24 h study period in adult men and women

| Measurements | r | d.f. | P |
|---|---|---|---|
| Male | | | |
| T:SHBG | 0.665 | 236 | <0.001 |
| Free T:SHBG | 0.374 | 236 | <0.01 |
| T/SHBG:SHBG | −0.155 | 236 | <0.05 |
| Female | | | |
| E2:SHBG | 0.236 | 218 | <0.01 |
| Free E2:SHBG | −0.067 | 214 | NS |
| T:SHBG | 0.302 | 212 | <0.01 |
| Free T:SHBG | −0.335 | 212 | <0.01 |
| T/SHBG:SHBG | 0.660 | 212 | <0.001 |

NS = not significant.

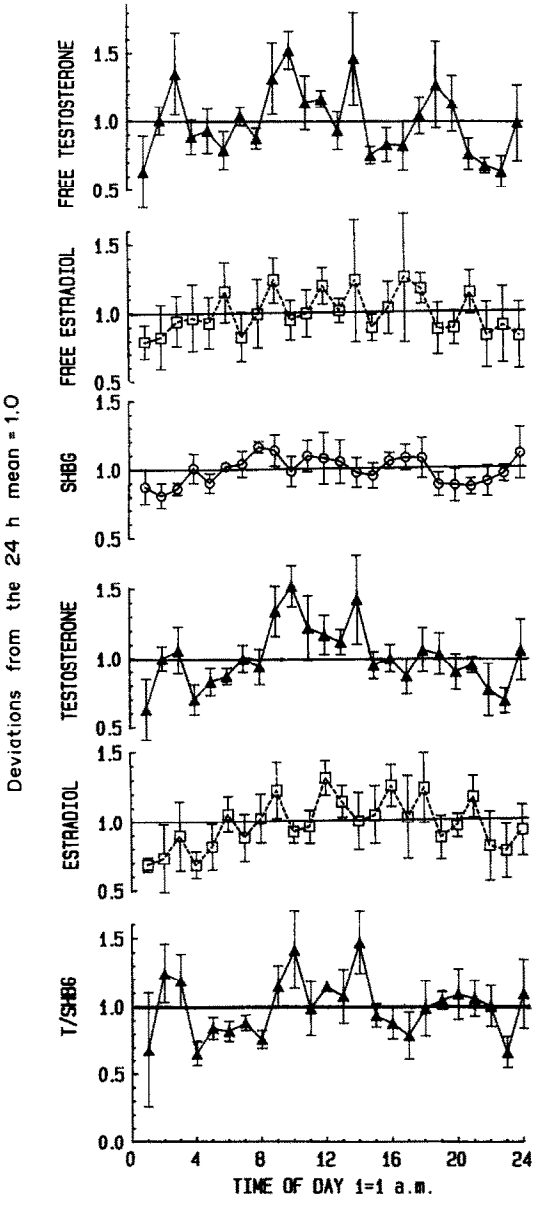


Fig. 3. Daily variations of circulating SHBG binding capacity, E2, free E2, T, free T and T/SHBG index. Hormones and SHBG were determined in hourly samples obtained from 5 normal women throughout the day in the mid-luteal phase of the menstrual cycle. The data expressed as percent deviations from the 24 h mean concentrations as in Fig. 2.

phase has an effect on SHBG production non-significant diurnal changes in serum E2 secretion may not influence SHBG levels. On the other hand, the concentrations of testosterone in normal adult females are too low to drive any change in SHBG production (T concentration in female is almost 1/20 of T level in male). Nevertheless, the stability of percentage free T observed in this study indicates that SHBG may also play a buffer role in normal adult female during 24 h period. In addition, the relationship between SHBG binding capacity and T is closer than that between SHBG and E2. Thus it appears that T modulates SHBG production to a greater extent than E2 in this situation.

In summary, the results suggest that the diurnal variations in testosterone secretion has a promoting effect on SHBG production. The latter in turn play a buffer role to prevent acute effects of rapid increases in hormone levels and maintain the levels of bioactive fraction both in normal adult male and female.

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