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Effects of Acutely Displaced Sleep on Testosterone

John Axelsson, Michael Ingre, Torbjörn Åkerstedt, and Ulf Holmbäck

National Institute for Psychosocial Medicine (J.A., M.I., T.Å.) and Karolinska Institute (J.A., T.Å.), 171 77 Stockholm, Sweden; and Department of Medical Sciences, Nutrition (U.H.), Uppsala University, 751 85 Uppsala, Sweden

Context: It is not yet clear whether the diurnal variation in testosterone is regulated by circadian or homeostatic (sleep) influences.

Objective: The present study tested whether testosterone is driven by a circadian-independent sleep effect by shifting sleep acutely to daytime in a 24-h sampling regiment.

Design, Setting, and Participants: In the sleep laboratory, seven healthy young men (age, 22–32 yr) participated in three conditions: habituation (sleep between 2300–0700 h), night sleep (2300–0700 h), and day sleep (0700–1500 h), the latter two in a balanced order.

Intervention and Main Outcome Measure: Serum testosterone was, in all conditions, sampled by hourly blood drawing for 24 h during constant bed rest.

Results: Mean testosterone levels increased as a log-linear function of time (hours) across both sleep periods ($b = 4.88$; $P < 0.001$), from 15.3 ± 2.1 to 25.3 ± 2.2 nmol/liter during night sleep and from 17.3 ± 2.1 to 26.4 ± 2.9 nmol/liter during day sleep. Similarly, mean testosterone levels decreased with time (log-linear) awake ($b = -1.80$; $P < 0.001$). There was also evidence of a weak circadian component (acrophase ranging between 0651–0924 h) and an increase with time in the laboratory. Moreover, all these effects, except for the increase during sleep, differed significantly between individuals.

Conclusion: In conclusion, testosterone increased during sleep and fell during waking, whereas circadian effects seemed marginal. Individual differences were pronounced. **(*J Clin Endocrinol Metab* 90: 4530–4535, 2005)**

A BETTER UNDERSTANDING of the relationship between sleep and testosterone is not only of theoretical interest. It may also contribute to understanding of the mechanisms behind certain health problems associated with sleep disturbances, aging, and shift work. The former two are, for example, related to metabolic changes, lower testosterone levels, and fatigue (1–4). Shift work is also associated with a number of health effects (5), and recently it was found that shift workers with sleep and fatigue problems had lower testosterone levels than workers without these problems (6).

In healthy adult men, circulating levels of testosterone have a distinct pattern, with increasing levels during sleep toward a maximum around the time of awakening and a decrease during the day (7–9). This pattern is often referred to as a circadian rhythm, despite the fact that disturbed sleep reduces or blunts the nocturnal rise of testosterone (3, 10). Furthermore, although diurnal sleep increases testosterone in prepubertal boys (9), this has not been seen in adult men (11). However, both studies included only a few subjects.

Most research has focused on how testosterone, or, rather, the nocturnal rise in testosterone, is related to LH peaks, rapid eye movement sleep (REM)-sleep/latency, and, to some degree, prolactin levels (4, 10, 12–14). However, these provide no information on the relative influence of sleep and circadian regulation. Most study protocols have been restricted in time; few cover more than 12 h. To determine whether testosterone is driven by a circadian rhythm-independent sleep effect, one would need to shift sleep within a 24-h sampling regimen. This was the purpose of the present experiment. Apart from the night-day sleep comparison, we explored the mechanisms behind circulating testosterone levels by simultaneously estimating the effects of sleep, wakefulness, and circadian factors by means of mixed effects regression analysis.

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Abbreviation: REM, Rapid eye movement sleep.

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Subjects and Methods

Participants

Seven healthy males (mean ± SE age, 25 ± 1 yr; range, 22–32 yr) participated in the study. All were in good health, nonsmokers, nonobese (body mass index range, 21–25 kg/m^2), moderate alcohol consumers with a body fat composition of 16 ± 4%, and were taking no medication. The study was approved by the local ethical committee at Karolinska Institute, applying the Helsinki committee rules. All participants gave their informed written consent after the procedures had been fully explained.

Study protocol

One week before entering the sleep laboratory and throughout the study period, the subjects adhered to a sleep protocol with bedtime at 2300 h ± 30 min and rise time at 0700 h ± 30 min. The participants came to the sleep laboratory on three occasions. Each time they arrived at 1730 h and stayed until 2100 h the following evening for habituation sleep (2300–0700 h), nocturnal sleep (2300–0700 h), and diurnal sleep (0700–1500 h), with the latter two in a balanced order. At 1800 h, an iv catheter was inserted into a forearm vein and was kept patent by a slow infusion of 0.9% NaCl. Blood samples (10 ml) were drawn from an adjacent room every hour for 24 h starting at 2100 h. Subjects remained in a reclining position from 1930–2100 h the following evening. Polysomnography was recorded continuously during the same time. The light at bedside was approximately 70 lux. To ensure 24-h energy balance, standardized meals (45% fat and 40% carbohydrates) were given at 1800, 2200, and 1600 h during both conditions, at 0200 and 0600 before diurnal sleep, and at 0800 and 1200 h after nocturnal sleep.

Analysis of sleep stages

Electrodes were attached for recording two electroencephalograms (C3-A2 and C4-A1), two electroocculograms, and one electromyogram (submental). Sleep stages were scored in 30-sec epochs using standardized methods (15). The parameters used were time in bed (lights out to awakening), sleep latency (time from lights out to first stage 2 sleep), wake time after sleep onset, total sleep time (real sleep time), sleep efficiency [after sleep onset = total sleep time/(time in bed − sleep latency)], REM latency (time to first REM), and sleep stages 1, 2, 3, and 4 (stages 3 and 4 are presented as slow-wave sleep) and REM.

Hormone analysis

Serum samples were centrifuged, separated, frozen at −20 C, and stored at −73 C directly after each laboratory day until analyzed. Serum testosterone levels were determined by specific immunofluorometric assay (Autodelphia, Wallac Oy, Turku, Finland) with intra- and interassay coefficients of variation of 2.6% and 6.1%, respectively. Other blood substances were analyzed, but will be published later.

ANOVA

A repeated measures ANOVA was used to analyze testosterone for the entire 24-h sampling period. Contrasts were calculated between corresponding time points. Separate ANOVAs compared nocturnal sleep with nocturnal waking (both 2300–0700 h) and nocturnal sleep with diurnal sleep (2300–0700 *vs.* 0700–1500 h).

Mixed effects regression analysis

A linear mixed model approach was used to explore the mechanisms behind circulating testosterone levels. The approach has several advantages over traditional analyses (*e.g.* ANOVA/analysis of covariance). It allows for more flexible model specifications and for fixed as well as random effects to be estimated. The random effects describe individual differences that are explicitly estimated and included in the model as latent variables. The fixed effects are conditional on the random effects, and the estimates describe an average subject, similar to the group mean in the case of a truly linear model [see Raudenbush and Bryk (16) for a discussion of mixed effects models within a hierarchical linear modeling framework and Skrondal and Rabe-Hesketh (17) for a more general review within a generalized latent variable modeling framework].

The aim was to model testosterone levels as a function of sleep, wakefulness, and circadian rhythm. However, adaptation to the laboratory experiment may also influence the data. This may occur, for example, when an active subject is put into a supine position and there is a reorganization of blood and plasma volumes as well as of protein levels between blood and vascular compartments (18, 19). Thus, an experimental effect had to be included in the model. The experimental effect should account for effects on testosterone levels as a function of time in the experiment, *i.e.* the time the subjects have spent in a reclining position during that condition.

Two different functions were considered for modeling the effect of time asleep, time awake, and time in the experiment. In the first model, only linear effects were considered. However, many effects of time on physiological processes show nonlinear relations, especially in the context of sleep (20, 21). To capture a nonlinear effect, log-transformations of all time variables were also considered. All combinations of log-linear and/or linear effects of time asleep, time awake, and time in experiment were tested (all models had the same number of parameters), and the model with the best fit, indicated in a higher log likelihood, was chosen as the final model.

To model the circadian rhythm, a sine and a cosine function of time of day were also added to the model (22). Two different constants were included in the model to ease interpretation, one that accounted for the intercept in the regression equation during sleep and one during wakefulness. All parameters were modeled as both fixed and random to explore individual differences.

ANOVA models were estimated with SuperAnova version 1.11 with the Hyunh-Feldt ϵ correction applied to adjust for violations against the assumption of sphericity. The mixed effect models were estimated by means of restricted maximum likelihood estimation using the software hierarchical linear modeling version 6.0 (23). An α level of 0.05 was used to test for significance; however, trends less than 0.10 are also discussed when appropriate.

Results

Sleep

The only significant difference between conditions, except for bed times and rise times, was seen in a shorter sleep latency before diurnal sleep (Table 1). None of the sleep periods was particularly disturbed (the lowest sleep efficiency after sleep onset was 85%).

Twenty-four-hour levels of testosterone

The 24-h mean testosterone level did not differ between the night sleep and day sleep conditions (19.9 ± 0.4 *vs.* 19.2 ± 0.5 nmol/liter; $F_{1,6} = 1.1$; $P = 0.343$; see Fig 1). Testosterone varied substantially across time ($F_{24,144} = 13.3$; $P < 0.001$), and there was a significant interaction between condition and time ($F_{24,144} = 8.1$; $P < 0.001$). Predetermined contrasts between corresponding time points showed that these effects were largely due to the timing of sleep. Significant differences were found between the latter part of sleep and for the first hour after awakening. Maximum testosterone levels always occurred during sleep for all individuals and both conditions.

Analysis of nighttime levels

The analysis of data between 2300 and 0700 h showed that testosterone was higher during night sleep than during night waking (21.1 ± 0.8 *vs.* 16.8 ± 0.8 nmol/liter; $F_{1,6} = 7.6$; $P = 0.033$). The main effect across time indicated that testosterone increased across both conditions between 2300 and 0700 h ($F_{8,48} = 12.1$; $P < 0.001$). The significant interaction showed that testosterone increased more during sleep (from 15.3 ± 2.1 to 25.3 ± 2.2 nmol/liter) than during the time awake (from 13.4 ± 2.2 to 17.3 ± 2.4 nmol/liter; $F_{8,48} = 2.9$; $P = 0.018$).

Analysis of sleep levels

Figure 1 illustrates that testosterone increased across both sleep periods combined ($F_{8,48} = 25.5$; $P < 0.001$), from 15.3 ± 2.1 to 25.3 ± 2.2 nmol/liter during night sleep and from 17.3 ± 2.1 to 26.4 ± 2.9 nmol/liter during day sleep. There was a trend for testosterone to be higher during day sleep

TABLE 1. Mean and SE of polysomnographic data for night sleep and day sleep

Parameters	Night sleep	Day sleep
TST (min)	401 ± 15	422 ± 5
Sleep latency (min)	22 ± 6	5 ± 1[a]
WTASO (min)	39 ± 6	51 ± 6
Sleep efficiency (%)	92 ± 1	89 ± 1
% Stage 1	2 ± 0	3 ± 0
% Stage 2	47 ± 5	48 ± 3
% Slow-wave sleep	26 ± 4	27 ± 4
% REM	25 ± 2	23 ± 2
REM latency (min)	94 ± 17	75 ± 10

P values were determined using repeated measures ANOVA. TST, Total sleep time; WTASO, wake time after sleep onset.

[a] $P < 0.05$.

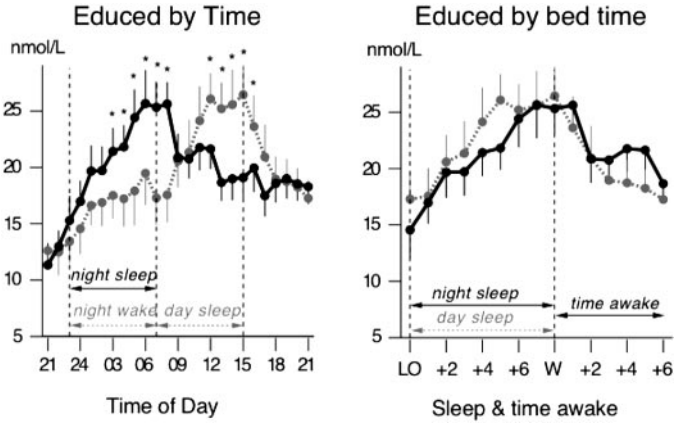

FIG. 1. Shown are the mean and SE for circulating testosterone. *Left*, Night sleep and day sleep conditions presented across 24 h. *Right*, Day sleep *vs.* night sleep, with conditions educed by bed times (lights out). *Black lines*, Night sleep condition; *gray dotted lines*, day sleep condition. LO, Lights out; W, wake; +numbers represent hours asleep or awake. *, $P < 0.05$ or $P < 0.01$.

(22.7 ± 0.9 *vs.* 21.1 ± 0.8 nmol/liter; $F_{1,6} = 5.3$; $P = 0.061$), and the interaction between condition and time was nonsignificant ($F_{8,48} = 1.1$; $P = 0.394$). The latter indicates an essentially similar development during sleep in the two conditions.

Mixed effect regression analysis

A set of mixed effect regression models was fitted to estimate the effect of sleep, wakefulness, circadian factors, and time in experiment. The model with all time effects log-transformed showed the best fit to data and was selected as the final model. The estimates are presented in Table 2.

TABLE 2. Mixed effect regression analysis

Parameters	Estimates		
Fixed effects	*b*	SE	*P*
Constant for being asleep	9.39	1.74	0.000
Constant for being awake	17.61	1.57	0.000
Log of time asleep	4.88	0.71	0.000
Log of time awake	−1.80	0.52	0.016
Sine of time of day	1.14	0.36	0.023
Cosine of time of day	−0.60	0.32	0.108
Log of time in experiment	2.00	0.51	0.010
Wald test	χ^2	df	*P*
Sine + cosine of time of day	14.32	2	0.001
Random effects	Var	χ^2	*P*
Constant for being asleep	12.62	11.79	0.066
Constant for being awake	9.78	11.94	0.063
Log of time asleep	1.64	8.40	0.209
Log of time awake	1.42	28.72	0.000
Sine of time of day	0.55	13.86	0.031
Cosine of time of day	0.19	3.37	>0.500
Log of time in experiment	1.16	13.54	0.035
Log likelihood	−831.41		
df	29		

The table shows the estimated coefficients (b), SE, and *P* values for the fixed effects and estimated variance (Var), χ^2 statistics, and *P* value for the random effects. A Wald test is used to test the total effect of the circadian rhythm by combining the estimate of the sine and cosine of time of day. Estimated covariances of the random effects are omitted.

The results indicate significant fixed effects for all parameters included in the model. The cosine component alone was not significant; however, the total effect of the circadian factors (sine plus cosine of time of day) was highly significant. There were also significant individual differences observed for time awake, circadian factors, and time in experiment, as indicated by the significant random effects variances. The random effects for the two constants (sleep and wake) only showed a trend toward significance. The estimated individual differences illustrated in Fig. 2 were subject-specific (empirical Bayes) predictions, which are plotted as a function of the parameters in the model. The differences in slope are clearly visible for time awake and time in experiment. Also, the circadian rhythm shows apparent individual differences in both phase and amplitude, with the acrophase ranging from 0651–0924 h. One subject showed almost no circadian rhythm.

The estimates of the combined fixed effects are also plotted together with the observed mean and SE in Fig. 3, indicating a relatively high degree of fit between the predicted and observed levels of testosterone. The explained variance in the observed group mean from the predicted mean is estimated to 90%.

Discussion

This study is the first to show that testosterone increases during day sleep in the same way as it does during night sleep in healthy young men. The reverse pattern is seen after waking: testosterone falls. The increase in testosterone during day sleep is in line with earlier findings in adult boys (9), but contrasts with findings in men (11). However, the latter study only included two men and should be interpreted with caution. Our study also confirms that sleep, rather than circadian influences, is critical for testosterone regulation (24). Previous work has shown that both low sleep efficiency and sleep apnea are related to reduced nocturnal testosterone levels (3, 24). In contrast, this was not found in a study with an ultra short sleep protocol (10). That study found no main difference between fragmented and undisturbed sleep, but included no condition without sleep. On the whole, our data support the idea that testosterone is under strong influence of sleep, and to a lesser extent, under circadian influence, resembling the regulation of prolactin (25, 26). Thus, healthy young men can expect comparable amounts of testosterone during sleep independently of time of day if they manage similar sleep durations.

The design of the study was aimed at evaluating how an acute change in sleep from night to daytime would affect testosterone levels. Our main results are reasonable with respect to both study design and the literature. We had good control of previous sleep episodes, and the conditions were presented in a balanced order. Blood was withdrawn from an adjacent room to minimize sleep disturbances. No sleep episode was considered particularly disturbed. The choice of a homogenous group of fit, nonobese, sexually active, healthy young men increased the power of finding effects, but also carried some limitations. Generalizations to other groups should be made cautiously. For example, healthy middle-aged men differ from young men with respect to nocturnal testosterone regulation (4).

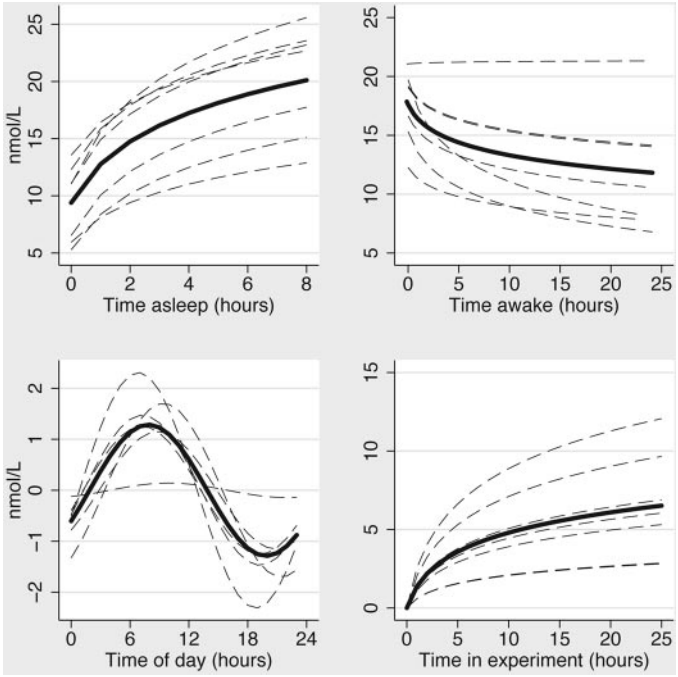

FIG. 2. Predicted testosterone levels as a function of time asleep (*top left*), time awake (*top right*), the circadian rhythm (*lower left*), and the experiment effect (*lower left*). *Thick lines* indicate the fixed effect estimate of an average subject, and *dashed lines* indicate model based (empirical Bayes) predictions for individual subjects.

Testosterone levels were higher at the end of the 24-h protocol than at the start. The exact reason for this laboratory effect is unknown, but several explanations exist. For example, it is well known that on lying down there are changes in both blood and plasma levels as well as in protein concentrations (18, 19). To control for such effects, subjects stayed in a reclining position from 1.5 h before the first blood sample and throughout the protocol. In addition, other laboratory effects may also exist, for example, time with an iv catheter and problems drawing blood (27). The subject's anticipation of finishing/leaving a 25.5-h protocol may also have contributed to this effect; a positive anticipation of an outcome seems related to increased testosterone levels (28, 29). This effect was also added to the mixed effects regression analysis, and it was estimated as a logarithmic function of time being in the experiment (*i.e.* within each condition). Similar effects may be present in other studies, even though this is not explicit, because few studies have run 24-h protocols. Without being able to address the exact reason for this laboratory effect, we will have to await results from experiments specifically focused on this issue.

The mixed effects approach used in the present study had several advantages over traditional statistical models (*e.g.* ANOVA/analysis of covariance). This made it possible to estimate the circadian factor by means of a cosinor analysis (22) and to test for a linear *vs.* log-linear effect of all time variables in the model. The best-fit model consisted of all time effects log-linearly transformed, which agrees well with the homeostatic drive for sleep/wakefulness (30). The approach also made it possible to explicitly estimate individual differences and include them as latent variables in the model. Thus, the reported fixed effects are controlled for individual differences.

The individual differences apparent in the plots represent empirical Bayes estimates that have very desirable properties. They are estimated with a shrinkage factor that biases the subject-specific estimates toward the group mean based on the reliability of the subject-specific *vs.* group estimate. The more uncertainty in the subject-specific estimate and the less uncertainty in the group estimate, the more the subject-specific estimate is shrunken toward the group mean. This means that the individual differences apparent in the plots are more than just plain residuals; they represent a systematic deviation from the fixed effect model as a function of the random parameters included in the model (16).

The mixed model approach supported the ANOVA results and added information about the relation between sleep/wake and circadian factors as well as estimates of individual differences. The results showed significant fixed (group average) effects of time asleep and time awake, but could also support the existence of a circadian component, although weak, and an effect of time in the experiment.

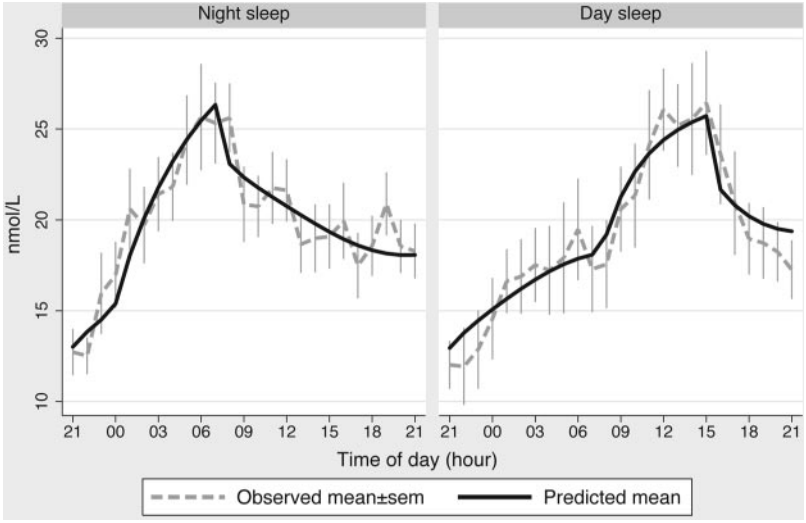

FIG. 3. Observed and predicted testosterone levels for the night sleep (*left*) and day sleep (*right*) conditions.

There were significant differences between individuals with respect to time awake, circadian factors, and time in experiment, but not for sleep. Thus, it seems that the increasing effect of sleep was reasonably stable for all participants. In addition, there were significant fixed effects for the intercepts (adjusting constants for differences in testosterone levels) for being asleep and being awake, but only trends of a difference between individuals. The latter may have been due to the homogeneity (healthy young adult males) of the group, resulting in low interindividual variation and low power to find different effects between individuals. Hence, we might expect differences between individuals for time asleep in a more heterogeneous sample of individuals. Indeed, other studies have shown that it is the increase in testosterone during sleep that is disturbed with increasing age, sleep apnea, and other sleep disturbances (3, 4, 31). However, these studies have found differences between groups. Our study is the first to test whether testosterone regulation differs on an individual level. Moreover, a mixed model approach may be of even greater benefit when evaluating risk groups and patient groups, because it is possible to evaluate how they differ from normal on an individual level.

With respect to how these functions affect testosterone, the log-linear increase across sleep suggests that sleep length is crucial for testosterone levels; short sleep would reduce testosterone and long sleep would do the opposite. This would be in line with the homeostatic sleep component and the fact that increasing age is related to a blunting of the expected increase during sleep (4, 31). However, studies manipulating sleep length are needed before such effects can be confirmed. Another interesting aspect is whether reduced testosterone levels, due to short or disturbed sleep, are compensated for in the same manner as the release of GH (32).

The reduction of testosterone after waking suggests that time awake is as important a factor to consider as time of day. For example, this may explain why testosterone is lower in the summer than in the winter, a phenomenon reported by Svartberg *et al.* (33) in Tromsø, a town with the latitude of 69.65°N. The interpretation is that sleep is shorter in summer than in winter at this latitude (34), often resulting in a longer wake span before samples are taken, which agrees with the researchers' own suggestions in a later study (35). To monitor these effects, we suggest that sleep duration, sleep quality, and time between waking and sampling should be measured to determine testosterone levels.

The circadian component was highly significant, (about ±1 nmol/liter). The subject-specific acrophases were estimated between 0651 and 0924 h, which supports earlier findings in studies without manipulations of sleep (7, 8, 36). On a mechanistic level, several possible factors may explain a circadian variation, such as LH, Leydig sensitivity to LH, gonadal factors (such as inhibin B), the circadian clock, and processes related to testicular blood flow. For instance, the testicular glycoprotein inhibin B has a similar rhythm, although the circadian component was not independent of possible sleep effects (37).

Even though the circadian factor was highly significant in the present study, the design was not ideal for separating the circadian effect from sleep and wakefulness. The main weakness is that the sleep periods were not balanced across the entire 24-h window; no sleep occurred between 1500 h and 2300 h, a time of day with a small likelihood of permitting 7 h of sleep (38). We could hence only partially support the existence of a circadian rhythm. Additional research with a completely balanced design would be necessary to confirm the circadian effect found in the present study. Moreover, circadian and sleep influences should preferably be determined from studies covering several circadian cycles, and the results are only tentative.

In the present study we have modeled effects over long periods of time while testosterone is released in bursts. Hence, there are fluctuations that are unaccounted for. Additional research should include the biological mechanisms of the regulation of testosterone, *i.e.* LH bursts, REM latency, REM sleep, erections, and prolactin levels. These variables have the possibility of adding to the model and explaining more of the variance. For example, the small burst of testosterone a few hours after awakening from night sleep that was observed in the present study may be due to the prolactin burst shortly after waking.

The ecological interpretation of our findings is speculative, but one might expect disturbed sleep, such as that in sleep apnea and shift work, to result in an acute reduction of testosterone. Likewise, low morning testosterone levels may be indicative of disturbed sleep. There is a need for experiments on the effects of sleep restriction and sleep fragmentation on testosterone levels.

In conclusion, the present study has shown that sleep increases, and wakefulness decreases, testosterone levels. Some evidence was seen for a weak circadian influence. There were, however, considerable individual differences in temporal patterns. Our findings suggest that sleep is a more potent regulator of testosterone than circadian factors.

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Address all correspondence and requests for reprints to: Dr. John Axelsson, National Institute for Psychosocial Medicine and Karolinska Institute, Box 230, 171 77 Stockholm, Sweden. E-mail: john.axelsson@ipm.ki.se.

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