Sleep Medicine 48 (2018) 117–126

Contents lists available at ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

Slow-wave sleep and androgens: selective slow-wave sleep suppression affects testosterone and 17α-hydroxyprogesterone secretion

Yu.V. Ukraintseva [a, f, *], K.M. Liaukovich [a, e], A.A. Polishchuk [a, b], O.V. Martynova [a, e], D.A. Belov [d], E.S. Simenel [d], M. Meira e Cruz [c], A.N. Nizhnik [d]

[a] Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Butlerova St. 5A, 117485, Moscow, Russia
[b] Department of Biology, Lomonosov Moscow State University, Leninskiye Gory, 1-12, 119234, Moscow, Russia
[c] Sleep Unit of Cardiovascular Center of University of Lisbon, Faculty of Medicine, Alameda da Universidade 1649-004 Lisbon, Portugal
[d] ArhiMed Clinique for New Medical Technologies, Vavilova St. 68/2, 119261, Moscow, Russia
[e] Centre for Cognition and Decision Making, National Research University Higher School of Economics, Myasnickaya St. 20, 101000, Moscow, Russia
[f] ITMO University, Kronverksky Pr. 49, 197101, Saint Petersburg, Russia

ARTICLE INFO

Article history:
Received 6 March 2018
Received in revised form
22 April 2018
Accepted 25 April 2018
Available online 12 May 2018

Keywords:
Slow-wave sleep
Testosterone
Androstenedione
Dehydroepiandrosterone
17α-hydroxyprogesterone
Cortisol

ABSTRACT

Objectives: Levels of steroid hormones such as androgens and cortisol exhibit circadian variation, and their fluctuations are related to the sleep-wake cycle. Currently, the functional role of different stages of sleep in steroid hormone secretion remains unclear. The present study aims to explore the effect of slow-wave sleep (SWS) suppression on morning levels of cortisol and androgens.
Methods: Twelve healthy male volunteers participated in two experimental sessions: a session with selective SWS suppression during night sleep and a session with regular night sleep (control). SWS suppression was achieved by stimulation using an acoustic tone. Salivary samples were collected in the morning immediately after awakening and again 40 min later. The samples were analysed by liquid chromatography-tandem mass spectrometry for testosterone, androstenedione (Ad), dehydroepiandrosterone (DHEA), 17α-hydroxyprogesterone (17-OHP), and cortisol.
Results: SWS suppression reduced overall SWS duration by 54.2% without significant changes in total sleep time and sleep efficiency. In the session with selective SWS suppression, the average level of morning testosterone was lower than in the control session ($p = 0.017$). Likewise, 17-OHP was lower in the SWS suppression condition ($p = 0.011$) whereas the ratio of DHEA/Ad was higher ($p = 0.025$). There were no significant differences between sessions in cortisol, Ad, or DHEA concentrations.
Conclusions: The effect of selective SWS suppression on morning levels of testosterone and 17-OHP points to the importance of SWS for the synthesis and secretion of androgens. These results suggest that chronic sleep problems, which lead to reduced SWS, increase the risk for the development of androgen deficiency in the long term.

1. Introduction

Steroid hormone secretion exhibits daily oscillations which are closely connected to the sleep-wake cycle (Ghiciuc et al., [1]; Mezzullo et al., [2]). The highest concentrations of cortisol and androgens [such as testosterone, androstenedione (Ad), dehydroepiandrosterone (DHEA), and 17α-hydroxyprogesterone (17-OHP)] are observed in serum and saliva in the morning immediately after awakening for androgens and within 30–45 min of awakening for cortisol [1,2]. Fluctuations in cortisol concentrations are driven by circadian oscillators as well as metabolic factors. A major function

List of Abbreviations: Ad, androstenedione; BMI, body mass index; DHEA, dehydroepiandrosterone; EEG, electroencephalography; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17-OHP, 17α-hydroxyprogesterone; nREM, non-rapid eye movement sleep; PVT, psychomotor vigilance task; PSG, polysomnography; REM, rapid eye movement sleep; SSS, Stanford Sleepiness Scale; SWS, slow wave sleep; TST, total sleep time; VASS, Visual Analogue Sleepiness Scale; WASO, wake after sleep onset.

* Corresponding author. Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Butlerova St. 5A, 117485, Moscow, Russia. Fax: +7 499 7430056.
E-mail address: ukraintseva@yandex.ru (Yu.V. Ukraintseva).

https://doi.org/10.1016/j.sleep.2018.04.012
1389-9457/

of cortisol is mobilizing energy resources in the presence of insufficient reserves (eg, during hypoglycaemia). Therefore, immediately after awakening in the morning, the brain's increased energy needs invoke the release of cortisol (Benedict et al., [3]). Androgen secretion fluctuations also seem to be temporally associated with the sleep period [2]. In this regard, the most androgen secretion fluctuation studies are sleep-related changes in testosterone release. Testosterone concentrations begin to increase shortly after falling asleep, exhibit a distinct rise until the beginning of the first rapid eye movement (REM) sleep episode, and then remain at that level until awakening (Luboshitzky et al., [4]); or continue to increase as a log-linear function of sleep duration (Axelsson et al., [5]). Luboshitzky et al., found a positive correlation between the rise of testosterone in the beginning of the night sleep and REM latency [4]. Furthermore, a longer latency period is associated with a slower increase in testosterone levels [4]. After awakening, testosterone decreases dramatically. In a study by Axelsson et al., in which the sleep period was shifted from night to day, testosterone levels increased during both night- and day-time sleep [5]. Of note, a peak in testosterone secretion during day-time sleep was almost equal to the maximal secretion of testosterone during night-time sleep [5]. Consequently, the circadian rhythm does not seem to have much impact on fluctuations in testosterone levels.

Although the sleep-wake cycle plays a crucial role in regulating steroid hormone secretion, data on the association between the secretion of these hormones and sleep duration are contradictory (Arnal et al., [6]; Jauch-Chara et al., [7]; Leproult and Van Cauter, [8]; Rabat et al., [9]; Reynolds et al., [10]; Schmid et al., [11]; Voderholzer et al., [12]; Wu et al., [13]). In several studies, morning testosterone levels decreased significantly after a night of total sleep deprivation [6,7]. Studies on the effect of sleep restriction on testosterone levels reported a similar effect (Leproult and Van Cauter, [8,9]) whereas others did not [10,11]. Moreover, data on the association between cortisol and sleep duration is inconsistent. In one study, cortisol concentrations declined after total sleep deprivation as well as after 3 h of night-time sleep [6,13]. In another study, cortisol did not decrease after sleep was restricted for up to 5 h [12]. Collecting data under conditions of partial or total sleep deprivation could explain these inconsistent findings. Furthermore, indirect factors such as a disrupted circadian rhythm or an aroused state due to being awake at a time when one usually sleeps might influence hormone production. Thus, it remains unclear whether hormone secretion is impaired due to insufficient sleep by itself or a deficit in some specific sleep stages critical for steroid release.

The functional role of different sleep stages in steroid secretion is unknown. Slow-wave sleep (SWS), the deepest stage of sleep, predominates during the non-rapid eye movement (nREM) portion of the first sleep cycle. Considering the evident rise of testosterone levels during the first period of nREM, we assume that SWS plays an important role in hormone secretion. This assumption is indirectly supported by the well-known influence of SWS on different neuroendocrine systems: hypothalamic-pituitary-adrenal axis (Born and Fehm [14]), sympatho-adrenal-medullary system (Brandenberger et al., [15]), carbohydrate metabolism (Maquet, [16]; Tasali et al., [17]; Herzog et al., [18]). Unfortunately, SWS's role in androgen secretion has not been systematically studied. Previous studies have examined the relationship between cortisol secretion and time spent in SWS, however the results were inconsistent (Born et al., [19]; Van Liempt et al., [17,20]).

Studies on the role of sleep in pituitary-gonadal axis activity and male health are often based solely on testosterone measurements [6,7,10,11]. However, additional measurements of its precursors such as Ad, DHEA, and 17-OHP may improve diagnostics. DHEA and 17-OHP are converted into Ad, and Ad is transformed into testosterone. Therefore, concentration ratios of these metabolites may serve as an index of enzyme activity underlying steroidogenesis (Damgaard-Olesen et al., [21]).

The aim of the present study was to estimate the influence of time spent in SWS on cortisol and androgen (ie, testosterone, Ad, DHEA, and 17-OHP) secretion. For the experimental manipulation of SWS duration, we applied a paradigm of selective SWS suppression that did not affect the circadian rhythm or total sleep time (TST) [18]. Hence, the latter can be considered an adequate model to study the functional role of SWS in regulating endocrine processes.

2. Research design and methods

2.1. Participants

Sixteen male volunteers participated in a crossover balanced study. All participants were undergraduates from Lomonosov Moscow State University. They completed an Epworth Sleepiness Scale (ESS) and a written questionnaire about sleep quality, habitual sleep time, physical and mental health, use of medications, and health behaviours (eg, smoking, alcohol consumption, and work and study schedule) 1–2 weeks before the study began (Johns [22]). The exclusion criteria were as follows: a history of head injury; chronic or acute illness; current medication of any kind; alcohol or drug abuse; smoking; shift work; excessive daytime somnolence (ie, ESS>11); sleep complaints; and the presence of any oral inflammatory processes with or without evident bleeding.

In the week before the experimental sessions commenced, the volunteers were instructed to maintain a regular sleep-wake cycle with bedtimes between 23:00 and 23:30 h and wake-up times between 07:00 and 07:30 h as well as refrain from taking naps during the day. Compliance with these instructions was confirmed by accelerometric recordings (Xiaomi Mi band 2). Volunteers were asked to complete a daily sleep diary and wear a wrist actigraph during the week before each experimental session. Two volunteers failed to keep the prescribed sleep-wake cycle; and were excluded from the study. Saliva samples of one volunteer showed signs of coloration possibly due to contamination with blood, and he was also excluded. One volunteer was not included in the analysis due to symptoms of sleep disorder identified during the first experimental session. Therefore, the final sample included twelve participants.

All participants had a regular sleep-wake cycle without jetlag in the 4 weeks prior to taking part in the experimental sessions, a habitual sleep duration, mean ± SEM, 7.9 ± 0.16 h (range: 7–9 h), bedtimes of 23:20 ± 0:12 (range: 22:00–0:00), and wake times of 07:14 ± 0:10 (range: 06:00–08:00). Their average age was 23.4 ± 0.6 years (range: 20–25 years) and their average body mass index (BMI) was 22.3 ± 1.1 kg/m^2.

Our study was performed according to the Declaration of Helsinki on research involving human participants. The ethics committee of the Institute of Higher Nervous Activity and Neurophysiology of Russian Academy of Sciences approved the study protocol. All participants were informed in detail of the methods, study procedure, possible side effects, and handling measures. Written informed consent was obtained from each participant before the study procedures began. Moreover, each participant received financial compensation of 2000 RUB (34.3 USD).

2.2. Procedure

Due to the study's randomized, balanced crossover design, each volunteer participated in two experimental sessions: a session with selective SWS suppression during night sleep and a session with regular night sleep as a control condition (Fig. 1). Except for SWS

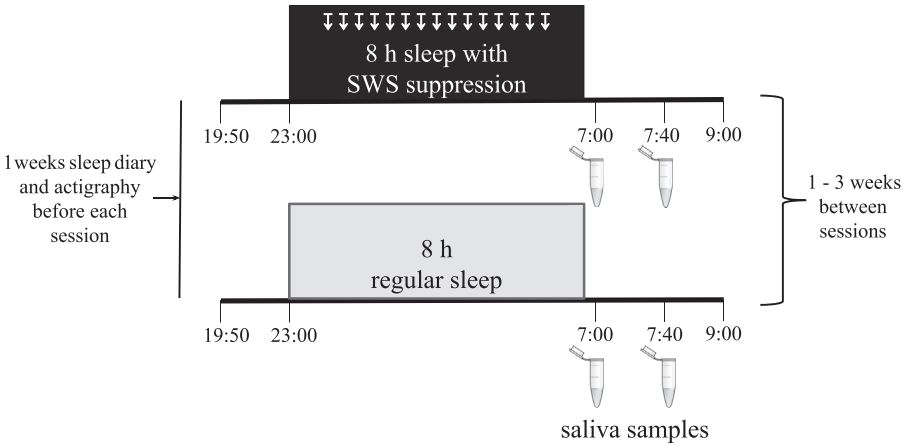

Fig. 1. Study Design. The schema illustrates the two experimental conditions: one session with slow-wave sleep (SWS) suppression during 8 h of night-time sleep and one session with 8 h of regular night-time sleep (control).

suppression, the design of both sessions was identical, and the participants received the same instructions. We did not tell them about subsequent SWS suppression, however in each session before sleep, we informed them that sounds would be presented sometime during the night. There was an interval of 1–3 weeks between the sessions.

One to two weeks before the first session, the participants visited the laboratory to familiarize themselves with the experimental conditions and equipment as well as undergo daytime nap polysomnography (PSG) for 1.5 h to adapt to the PSG recording procedure. None of the participants had difficulty falling asleep or with sleep maintenance.

On the day prior to the first experimental session, the participants were asked to avoid drinks that could induce insomnia, such as coffee, tea, or cola.

The participants arrived at the research unit at 19:45 h for each experimental session in a fasting state (4 h of fasting). After the participants consumed a meal (20:00 h) standardized for all participants, they completed the psychomotor vigilance task (PVT), Visual Analogue Sleepiness Scale (VASS), and Stanford Sleepiness Scale (SSS). All tests were completed by 22:20 h. At 22:40 h, participants were prepared for nocturnal polysomnography. Electrodes were attached for registration of electroencephalography (EEG), electrooculography, electromyography and electrocardiography. At 23:00 h, the participants went to bed, and the lights were turned off. The next day, at 07:00 h, the participants were woken up. Immediately after waking, while still lying in bed, a saliva sample was obtained, and another sample was taken 40 min later (07:40 h). During the 40 min period between collecting the first and second saliva samples, intense physical activity was prohibited. After removing the electrodes, the participants spent time sitting (only reading and writing were allowed during this time). At 07:50 h, each participant's vigilance level were measured using the PVT, SSS, and VASS. Afterward, at 08:00 h, participants ate breakfast.

2.3. Measures

2.3.1. Test instruments for vigilance and sleepiness assessment

The PVT was used to examine the level of vigilance before and after the night-sleep session. The PVT is a simple reaction time test used to assess subjects' sustained attention capacity (Dinges and Powell, [23]). A fixation cross appeared on a black screen for 400 ms, followed by a target stimulus (red circle, 300 ms) at randomized (2–9 s) intervals. The test lasted approximately 10 min and consisted of 85 target stimulus presentations. Participants were required to press the space button on a keyboard as quickly as possible after the target stimulus appeared. The number of lapses (ie, response latency exceeding 500 ms) and anticipated responses (ie, responses faster than 150 ms) as well as average response latencies were calculated.

Subjective sleepiness was assessed using the SSS and VASS. The SSS (Maclean et al., [24]) is a single-item seven-point self-report questionnaire that measures levels of sleepiness. This scale is a subjective assessment of the subject's level of alertness. The VASS consists of two statements with opposite meanings (sleepy and alert) located at the ends of a 100-mm line. Participants were asked to put a vertical mark on the line between these statements at a point that best reflected their perceived alertness.

2.3.2. Polysomnographic data acquisition and scoring

PSG recordings were performed using a digital EEG amplifier Encephalan-EEGR-19/26 (Medicom MTD, Taganrog, Russia) with a sampling rate of 250 Hz. PSG recordings included an EEG (F3, F4, C3, C4, O1, and O2, placed in accordance with the International 10–20 System), electrooculogram, electromyogram, and an electrocardiogram in lead II.

Polysomnograms were scored offline by two scorers who were blinded to the experimental conditions. Visual scoring of each 30 s epoch of PSG recording as awake, nREM sleep stage 1, 2, or 3 (SWS) and REM sleep was performed according to standard AASM criteria (Iber et al., [25]). Inter-scorer reliability was >92%. The PSG variables analysed included sleep onset latency, TST, wakefulness after sleep onset (WASO), sleep period time (SPT) and sleep efficiency. SPT was measured as the period beginning when the participant fell asleep and ending at the last wake-up, including the duration of awakenings if they occurred. Sleep efficiency was calculated as a percent value of TST referred to SPT.

2.3.3. SWS suppression

During the session with SWS suppression, PSG recordings were scored online by an expert (P.A.A. or L.K.M.) according to standard AASM criteria. SWS suppression was conducted using the method described by Herzog et al., with some modifications [18]. When subjects reached SWS, a standardized acoustic tone of 532 Hz was presented, starting at level 35. If necessary, the expert increased the sound intensity in 5 dB increments to a maximum of 95 dB using a specifically developed script for E-Prime software (E_Prime 1.2,

Psychology Software Tools, Pittsburgh, PA, USA). The sounds were emitted by two loudspeakers located about 30 cm behind the participant's head. If the maximum volume tone did not control delta activity, the researcher knocked on the bedroom door. EEG criteria for starting the acoustic stimulation were defined as follows: ≥6 delta waves with an amplitude of ≥75 mV and a frequency of ≤2 Hz within a 30 s period of sleep. The sound presentation was immediately stopped by the expert when less than six delta waves per 30 s occurred or when EEG criteria indicating lighter sleep stages such as k-complexes, α-waves, or arousals were detected. Extreme care was taken to avoid waking the participant.

2.3.4. Salivary samples

Saliva samples were used to evaluate the secretion of cortisol and androgens because saliva sampling is non-invasive and has been previously validated for measuring bioactive steroid hormones [2]. Salivary samples were collected twice: immediately upon awakening when androgen levels are at their highest and again 40 min after wakening when cortisol levels peak. By collecting repeated samples, we aimed to improve the accuracy of the estimated levels of androgens due to the previously-described cyclic changes in levels of these hormones (Brambilla et al., [26]). Saliva sampling was performed using special sampling devices: SaliCap equipped with a special straw. Saliva was collected by passive drool; it usually took approximately 2 min to obtain the required 1–1.5 ml of saliva. Samples were collected during a fasting state more than 30 min after drinking and teeth brushing and stored at −60 °C. Samples with even minimal coloration due to blood contamination were excluded from the analysis. In all samples concentrations of steroid hormones: testosterone, cortisol, Ad, DHEA, and 17-OHP were measured by liquid chromatography-tandem mass spectrometry.

2.3.5. LC–MS/MS analysis of steroids

The detection and quantitation of steroids were performed with the AB SCIEX QTRAP 6500 tandem mass spectrometer (AB SCIEX, Concord, ON, Canada) using the Waters® ACQUITY UPLC® H-Class System for the chromatographic separation.

17-hydroxyprogesterone, androstenedione, DHEA, cortisol and testosterone were purchased from Steraloids, Inc (Newport, RI, USA). The deuterated internal standards (ISs): 17-hydroxyprogesterone-d8, cortisol-d4, DHEA-d2, and c3-testosterone were obtained from Cambridge Isotope Laboratories (MA, USA). MS-grade methanol, and water, HPLC-grade acetone, MTBE, and formic acid were purchased from PanReac AppliChem.

Stock solutions of steroid hormones and ISs were prepared in ethanol at concentrations of 1 mg/ml and were stored at −20 °C until use. The substock solution contained all steroid hormones at a concentration 100 times higher than the highest working calibrator. Working calibrators were prepared before the analysis by serially diluting the standard substock in methanol and then by adding 10 μl of each dilution to 190 μl of deionized water. The working calibrators covered the ranges 0.1–100 ng/ml for all steroids. Seven levels of calibrators were used for all the analytes. The working IS solution was prepared by diluting the each steroid IS stock solution with methanol. Quality controls (QCs) were prepared in deionized water at three levels (low, medium, and high) by spiking with the substock solution to the concentrations corresponding to 1%, 10%, and 50% of the highest working standard. All the solutions of standards, calibrators, and QCs were stored in glass 2.0 ml autosampler sample vials with screw caps at −20 °C.

For the current study, 10 μl of the working IS solution and 0.2 ml of acetone were added to 0.2 ml of patient saliva/calibrator solution in 10 ml borosilicate glass vials with disposable caps and the mixture was vortexed for 1 min. After that, the solution was extracted with 2 ml MTBE for 1 min with vigorous stirring. The vials were allowed to freeze for 30 min at −20 °C, and after that the organic layers were transferred to 10-ml borosilicate glass test tubes and evaporated to dryness in a stream of nitrogen at 35 °C. The residues were reconstituted in 0.2 μl of methanol–water mixture (1:1), and after centrifugation were transferred to 2.0 ml autosampler vials with 0.25 ml glass inserts.

Following extraction, 10 μl of the reconstituted sample were injected into a reverse phase column (Acquity UPLC BEH C18, 1.7 μ, 2.1 × 100 mm, and 0.2 μ in-line precolumn filter). LC separation was performed using a gradient mobile phase: phase A (water+0.1% formic acid) and phase B (methanol +0.1% formic acid) at a flow rate of 0.25 ml/min. The column temperature was maintained at 45 °C throughout the separation. The detection and quantitation were achieved by MS/MS using an AB SCIEX QTRAP 6500 tandem mass spectrometer fitted with an atmospheric pressure chemical ionization (APCI) source. The nebulizer current was set at 3 mA with a source temperature of 400 °C. Nitrogen and dry air were produced by a PEAK Scientific generator (Parker Balston, Haverhill, MA, USA) GENIUS 3031. The steroids were monitored in positive-ion mode using multiple-reaction monitoring (MRM).

2.3.6. BMI estimation

BMI was computed using objectively measured height and body weight using the following formula: $\text{BMI} = \frac{\text{weight in kilograms}}{(\text{height in meters})^2}$.

2.4. Statistical analysis

The data analysis was performed using Statistica 10 software (Stat Soft. Inc., Tulsa, OK, USA). Normal distribution of the sleep and hormone data was evaluated using the Kolmogorov-Smirnov & Lilliefors test. Paired *t*-tests were performed to compare sleep variables in the two experimental conditions. Ratios of androgen concentrations (ie, 17-OHP/Ad, DHEA/Ad, and Ad/T) were calculated. Since androgen ratios were not normally distributed, they were log-transformed before analysis. A repeated-measures analysis of variance (ANOVA) was conducted to examine the differences in androgen and cortisol concentrations as well as in androgen ratios; a series of 2 × 2 [time (Immediately after awakening versus 40 min later] x condition [regular sleep versus SWS suppression)] comparisons assessed between- and within-conditions differences. For post hoc analyses, the Newman-Keuls test was performed. Pearson correlation analysis was used to assess the correlations between steroid concentrations and sleep variables. Statistical significance was taken at $p < 0.05$.

3. Results

3.1. Sleep architecture

Sleep data recorded by PSG are summarized in Table 1. There were significant differences in the amount of SWS, stage 1 and stage 2 in the two experimental sessions. As expected, according to the experimental protocol, SWS suppression reduced the time spent in SWS by 56.21 min ($p < 0.0001$), which was 54.2% of the amount of SWS in the control condition. Additionally, SWS suppression led to a 15 min increase in the duration of stage 1 ($p = 0.004$) and a 25.9 min increase in the duration of stage 2 ($p = 0.044$). Notably, selective SWS suppression did not significantly affect TST, WASO, or sleep efficiency.

3.2. Hormone data

The results related to morning hormone concentrations are shown in Fig. 2. In both sessions (ie, with selective SWS suppression

Table 1
Mean values of polysomnographic data.

	Regular Sleep	SWS suppression	*p*
Total sleep time (min)	422.50 (11.61)	402.13 (14.77)	0.135
Sleep period time (min)	463.83 (6.29)	452.92 (7.73)	0.296
WASO (min)	40.96 (8.13)	49.67 (8.60)	0.199
Sleep efficiency %	91.00 (1.78)	88.56 (2.15)	0.126
Sleep onset latency (min)	19.13 (2.86)	24.83 (6.57)	0.424
Latency of SWS (min)	10.13 (0.92)	11.71 (2.47)	0.558
Latency of REM (min)	86.04 (9.44)	112.96 (16.17)	0.133
Stage 1 (min)	15.63 (1.97)	30.67 (4.58)	**0.004**
Stage 2 (min)	202.42 (9.77)	228.33 (6.89)	**0.044**
SWS (min)	103.79 (7.09)	47.58 (5.63)	**<0.001**
REM (min)	95.71 (1.43)	90.42 (10.67)	0.438

Note. Data are mean values and standard error of mean. WASO, wakefulness after sleep onset; SWS, slow-wave sleep; REM, rapid eye movement sleep. *p*-values are derived from a paired Student's *t*-test. Significant differences are highlighted in bold.

and with regular sleep), the dynamics of morning salivary testosterone concentrations were similar (Fig. 2A). Immediately after awakening, testosterone concentrations were high and decreased 40 min thereafter. The ANOVA revealed a significant time effect: $F(1, 11) = 58.130$, $p < 0.001$. The post hoc analyses found significant differences between two morning measurements in the same conditions ($p = 0.007$ for control and $p = 0.011$ for SWS suppression). The average morning testosterone level (averaged over two morning measurements collected immediately after wakening and 40 min later) was significantly lower in the session with SWS suppression compared to the control session with regular sleep: $F(1, 11) = 7.8188$, $p = 0.017$ for the ANOVA condition effect (Fig. 2B).

The morning dynamics of salivary 17-OHP concentrations differed in the control and SWS suppression conditions; the ANOVA showed a significant time x condition effect: $F(1, 11) = 7.6486$, $p = 0.018$. Levels of 17-OHP were high after awakening and then decreased after 40 min in the control condition (Fig. 2A). Conversely, in the SWS suppression condition, they were significantly lower than those of the control immediately after wakening (post hoc: $p = 0.017$) and remained at the same level 40 min later. The average morning 17-OHP level was lower in the session with SWS suppression than in the control condition; a significant condition effect was observed: $F(1, 11) = 9.2145$, $p = 0.011$ (Fig. 2B).

There were no significant differences in cortisol, Ad, or DHEA levels in the two conditions.

Concerning the ratios of androgens, ANOVA revealed a significant time x condition effect for the DHEA/Ad ratio: $F(1, 11) = 6.2040$, $p = 0.030$. Also there was a significant difference in the DHEA/Ad ratio immediately after wakening (Fig. 3A). The ratio was higher in the SWS suppression condition than in the control condition ($p = 0.025$). The difference between the average DHEA/Ad ratios was not significant (Fig. 3B).

In order to more clearly identify the links between morning steroid concentrations and sleep quality Pearson correlation analysis was performed. Results of the correlation analysis are summarized in Table 2. Positive correlations were found between SWS duration and testosterone concentrations in both morning saliva samples (0.408, $p = 0.048$ and 0.405, $p = 0.049$), as well as with average morning level of testosterone (0.455, $p = 0.026$). Similarly, SWS was positively correlated with 17-OHP immediately after wakening (0.469, $p = 0.021$) and with the average 17-OHP level (0.408, $p = 0.048$). Time spent in stage 1 was negatively associated with testosterone and 17-OHP in the moment of awakening (−0.466, $p = 0.022$ and −0.501, $p = 0.013$), with average testosterone level (−0.408, $p = 0.048$) and with Ad concentrations (−0.417, $p = 0.042$ with Ad on awakening; −0.407, $p = 0.048$ with Ad 40 min after wakening and −0.454, $p = 0.026$ with average Ad). Sleep efficiency was positively correlated with Ad in the moment of awakening (0.503, $p = 0.012$), as well as with its average level (0.470, $p = 0.021$), while WASO was negatively correlated with Ad on awakening (−0.490, $p = 0.015$) and with average Ad (−0.472, $p = 0.020$). TST was positively associated with Ad in the moment of awakening (0.502, $p = 0.012$), and with its average level (0.475, $p = 0.019$), as well as with average DHEA (0.416, $p = 0.043$).

3.3. Vigilance and sleepiness data

Selective SWS suppression had no significant effects on vigilance and sleepiness according to data from the PVT, VASS, and SSS. The data are shown in Table 3.

4. Discussion

To our knowledge, this is the first study to show that selective SWS suppression affects the secretion of androgen hormones in male human subjects, reducing SWS duration by 54.2%. Moreover, SWS suppression was followed by a significant decrease in morning testosterone levels. There were significant differences between conditions in testosterone levels averaged over two morning measurements: immediately after awakening and 40 min later. The lack of statistical significance for each measurement might be explained by the episodic release of this hormone in response to a pulsatile gonadotropin stimulus (Foresta et al., [27]). As reported earlier, since the level of testosterone varies markedly over intervals of approximately 60 min, collecting repeated samples is recommended to control for this variation [26].

Many studies have consistently demonstrated that total sleep deprivation lowered testosterone levels [6,7,11]. Yet, findings concerning the effect of sleep restriction on testosterone secretion are contradictory. For example, one study found a significant decrease in testosterone concentrations after sleep time was reduced to 5 h per night for eight nights [8], and another study found similar results when it was reduced to 4 h per night for six nights [9]. Conversely, other studies found that neither two days [11] nor five days [10] of sleep restriction led to significant changes in testosterone levels. Thus, sleep restriction for more than six nights seems to be necessary to affect testosterone secretion.

However, the previous studies did not examine whether the observed changes in testosterone levels were sleep-stage specific or due to the detrimental effects of sleep debt. Usually, chronic sleep restriction does not lead to a substantial reduction in SWS; previous research demonstrated that subjects exposed to a chronic lack of sleep (ie, 4 or 5 h per night instead of 8 h) spent less time in light sleep stages (ie, stages 1 and 2) and REM and proportionally more time in SWS [8,9]. The present study's findings highlight that the amount of SWS is more important in testosterone production than total sleep duration. Our results are in line with the findings of a cohort study which showed that total testosterone levels were unrelated to habitual sleep duration, but men with decreased testosterone levels had low sleep efficiency and increased nocturnal awakenings and spent less time in SWS [28].

Notably, between the conditions in our study there were no substantial differences in the level of sleepiness and sustained attention. This suggests that compared to partial or total sleep deprivation, the selective SWS suppression does not induce neurobehavioral deficits. Moreover, selective SWS suppression for one night did not affect cortisol concentrations, implying that a decrease in deep sleep did not act as a stressor for the participants. Another important finding of the present study was that despite a major decrease (more than 50%) in the quantity of SWS, the architecture of sleep was not greatly disturbed; both TST and sleep efficiency remaining unchanged at the group level. Thus, we can conclude that the observed alterations in the release of hormones

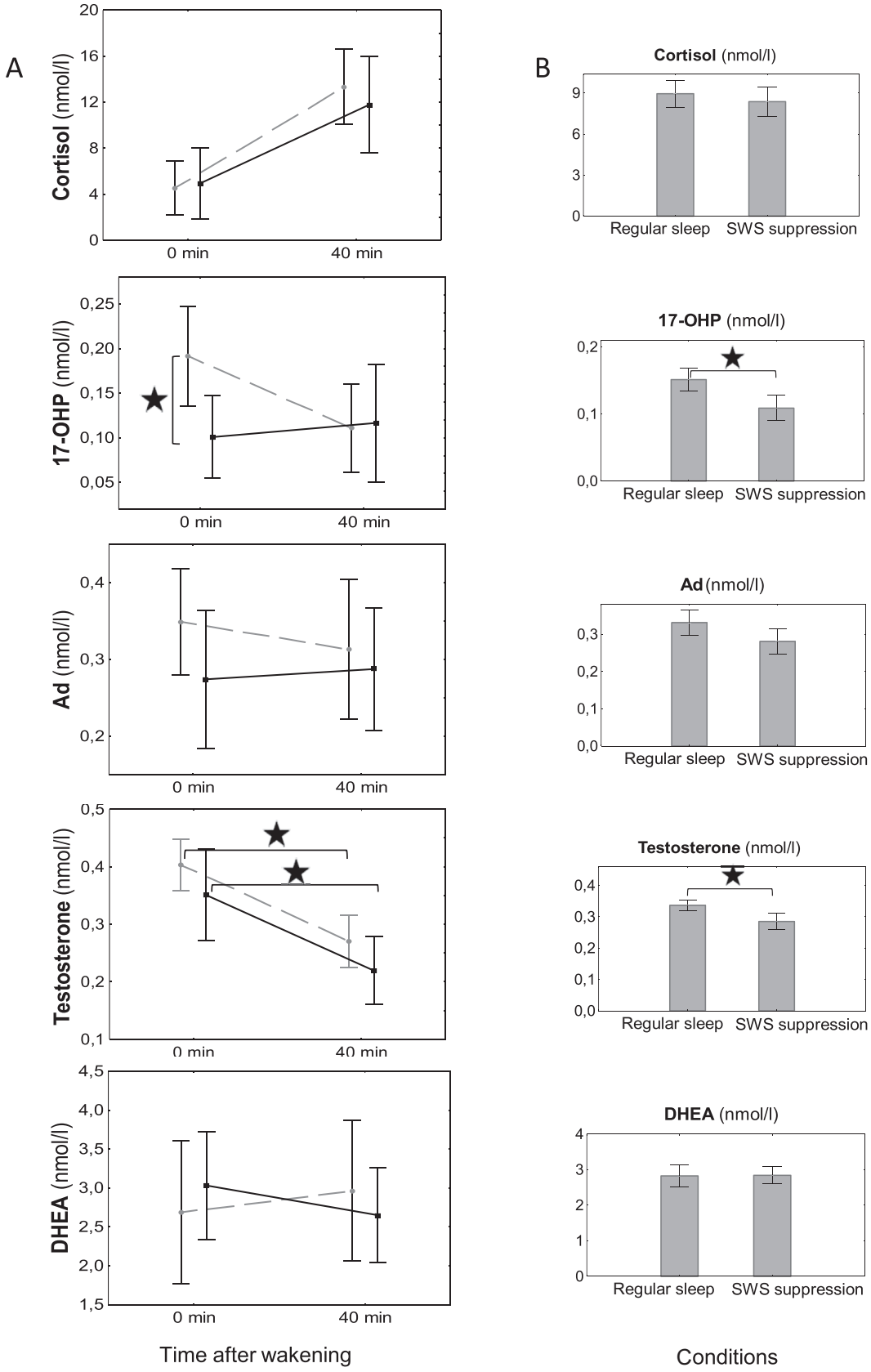

Fig. 2. Morning salivary steroid hormones: Testosterone, 17α-hydroxyprogesterone (17-OHP), dehydroepiandrosterone (DHEA), androstenedione (Ad), and cortisol. A) Dynamic: 0 = moment of awakening, and 40 = 40 min after wakening. The solid black line denotes the session with slow-wave sleep (SWS) suppression, and the grey dashed line denotes the control session with regular sleep. The vertical bars denote 95% confidence intervals. B) Mean values (averaged over two morning measurements) in each condition. The vertical bars denote the standard error of mean. Asterisks represent significance ($p < 0.05$).

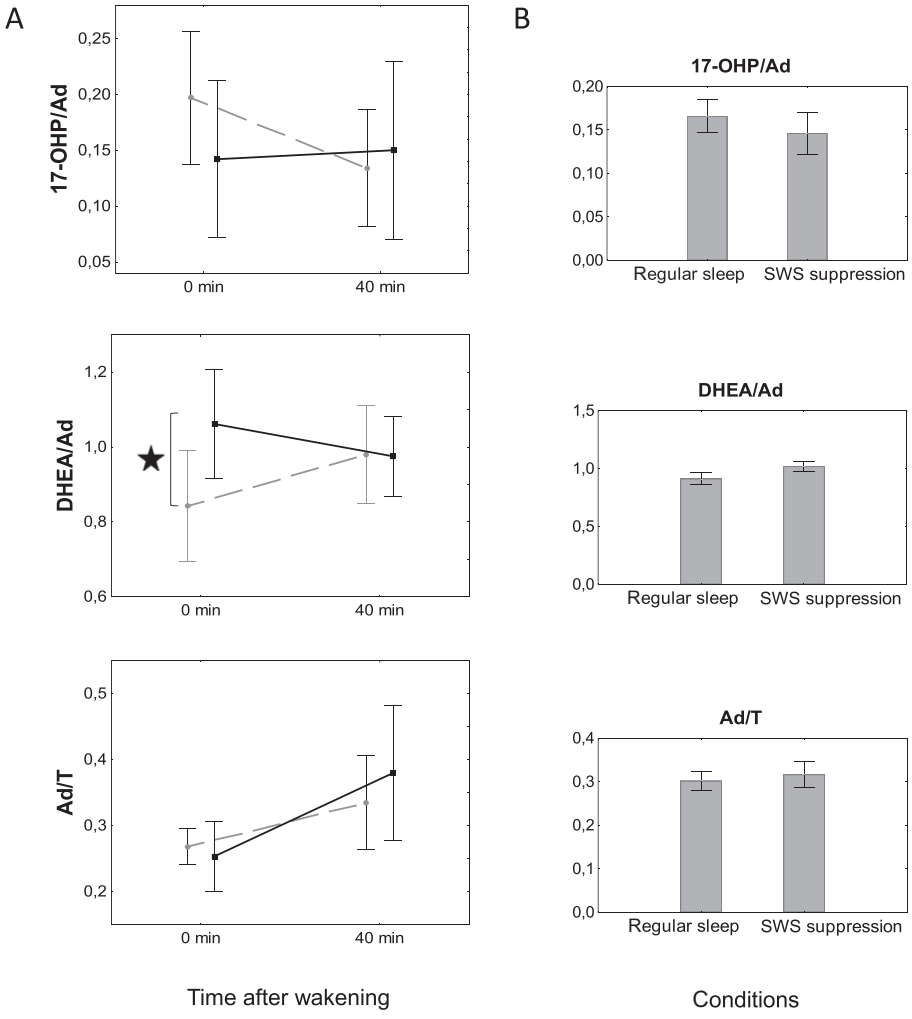

Fig. 3. Log-transformed ratios of morning salivary steroid hormones concentrations: dehydroepiandrosterone and androstenedione (DHEA/Ad), 17α-hydroxyprogesterone and androstenedione (17-OHP/Ad), and androstenedione and testosterone (Ad/T). A) Dynamic: 0 = the moment of awakening, and 40 = 40 min after wakening. The solid black line denotes the session with slow-wave sleep (SWS) suppression, and the grey dashed line denotes the control session with regular sleep. The vertical bars denote 95% confidence intervals. B) Mean values (averaged over two morning measurements) in each condition. The vertical bars denote the standard error of mean. Asterisks represent significance ($p < 0.05$).

were not caused by general sleep disturbance or insufficiency but were associated mainly with the time spent in SWS.

The present study also revealed different dynamics in morning 17-OHP secretion after normal sleep and after disturbed sleep. In the SWS suppression condition, the salivary 17-OHP concentration immediately after awakening was significantly lower than in the control condition, and the concentration remained approximately the same after 40 min. Yet, in the control session the amount of 17-OHP was high after wakening and decreased after 40 min. Furthermore, the average morning level of 17-OHP was lower in the SWS suppression condition than in the control condition. These findings support the assumption that disrupting deep sleep affects not only testosterone but also the secretion of its precursors. Considering that the DHEA/Ad ratio also changed in the SWS suppression condition, we can conclude that SWS plays a significant role in the regulation of steroidogenesis, and a certain amount of undisturbed deep sleep is necessary for normal androgen secretion. The enzyme 17,20-lyase converts 17-OHP to Ad, and 3β-hydroxysteroid dehydrogenase (3β-HSD) is crucial in converting DHEA to Ad. Ad is converted to testosterone by 17β-hydroxysteroid dehydrogenase. Hence, the concentration ratios of androgen hormones may be used as indices of the activity of enzymes underlying steroidogenesis [21,29]. DHEA is mainly produced by the adrenal glands, and smaller amounts are produced by the testes (Melmed et al., [30]). Decreased activity of the 3β-HSD enzyme results in the reduced conversion of DHEA to Ad and an increase in the DHEA/Ad ratio. For example, a previous study reported that the DHEA/Ad ratio was significantly elevated in patients with a 3β-HSD deficiency, which led to incomplete masculinization (Moisan et al., [31]). Correspondingly, the increased DHEA/Ad ratio observed after SWS suppression in the present study may be correlated with decreased 3β-HSD activity. Since Ad is a precursor of testosterone, reducing the amount of DHEA converted to Ad may lead to a decrease in testosterone concentration.

The exact mechanisms involved in SWS suppression's effects on steroidogenesis are unknown. The release of testosterone from the testes occurs in response to a pulsatile luteinizing hormone (LH) stimulus [27]. However, experimental studies failed to demonstrate a suppressive effect of sleep restriction or SWS disruption on LH release (Killick et al., [32]; Shaw et al., [33]). Therefore, in this case, the secretion of androgens seems to be influenced by changes in other hormones. Among the candidates is growth hormone (GH);

Table 2
Results of correlation analysis.

	Total sleep time (min)	WASO (min)	Sleep efficiency (%)	Stage 1 (min)	Stage 2 (min)	SWS (min)
cortisol 0	0.2103	−0.1620	0.1970	0.0529	0.1769	−0.0078
	p = 0.324	p = 0.449	p = 0.356	p = 0.806	p = 0.408	p = 0.971
cortisol 40	−0.1812	0.2190	−0.1987	0.0707	−0.1364	0.1956
	p = 0.397	p = 0.304	p = 0.352	p = 0.743	p = 0.525	p = 0.360
cortisol av	−0.0239	0.0839	−0.0462	0.0899	−0.0069	0.1567
	p = 0.912	p = 0.697	p = 0.830	p = 0.676	p = 0.974	p = 0.465
17-OHP 0	0.3838	−0.3766	0.3831	**−0.5009**	0.0147	**0.4694**
	p = 0.064	p = 0.070	p = 0.065	**p = .013**	p = 0.946	**p = .021**
17-OHP 40	−0.1451	0.1322	−0.1706	0.0831	0.0808	0.0776
	p = 0.499	p = 0.538	p = 0.426	p = 0.700	p = 0.707	p = 0.719
17-OHP av	0.2139	−0.2158	0.1988	−0.3401	0.0577	**0.4077**
	p = 0.316	p = 0.311	p = 0.352	p = 0.104	p = 0.789	**p = .048**
Ad 0	**0.5020**	**−0.4896**	**0.5026**	**−0.4173**	0.2073	0.2842
	p = .012	**p = .015**	**p = .012**	**p = .042**	p = 0.331	p = 0.178
Ad 40	0.3628	−0.3696	0.3525	**−0.4068**	0.1996	0.1672
	p = 0.081	p = 0.075	p = 0.091	**p = .048**	p = 0.350	p = 0.435
Ad av	**0.4750**	**−0.4721**	**0.4696**	**−0.4535**	0.2239	0.2476
	p = .019	**p = .020**	**p = .021**	**p = .026**	p = 0.293	p = 0.243
Testosterone 0	0.1976	−0.2927	0.2584	**−0.4661**	−0.0832	**0.4078**
	p = 0.355	p = 0.165	p = 0.223	**p = .022**	p = 0.699	**p = .048**
Testosterone 40	0.1237	0.0412	−0.0304	−0.2392	−0.1276	**0.4053**
	p = 0.565	p = 0.849	p = 0.888	p = 0.260	p = 0.552	**p = .049**
Testosterone av	0.1842	−0.1609	0.1450	**−0.4081**	−0.1152	**0.4548**
	p = 0.389	p = 0.453	p = 0.499	**p = .048**	p = 0.592	**p = .026**
DHEA 0	0.3425	−0.3931	0.3804	0.0021	0.4007	−0.2254
	p = 0.101	p = 0.057	p = 0.067	p = 0.992	p = 0.052	p = 0.290
DHEA 40	0.3007	−0.2065	0.2347	−0.2509	0.0401	0.1271
	p = 0.153	p = 0.333	p = 0.270	p = 0.237	p = 0.852	p = 0.554
DHEA av	**0.4161**	−0.3906	0.3998	−0.1562	0.2911	−0.0697
	p = .043	p = 0.059	p = 0.053	p = 0.466	p = 0.168	p = 0.746

Notes: Data are Pearson correlation coefficients and p values, n = 24 (data of 12 subjects in two sessions). 17-OHP, 17α-hydroxyprogesterone; DHEA, dehydroepiandrosterone; Ad, androstenedione. WASO, wakefulness after sleep onset; SWS, slow-wave sleep. 0, concentration in the moment of awakening; 40, concentration 40 min after wakening; av, mean concentration (averaged over two morning measurements). Significant correlations are highlighted in bold.

Table 3
Mean values of the PVT, SSS and VAS.

	Regular Sleep		SWS suppression	
	Evening	Morning	Evening	Morning
PVT				
Anticipated responses	0.91 (0.42)	0.73 (0.45)	1.06 (0.53)	0.57 (0.29)
Lapse	6.73 (1.61)	8.82 (2.35)	7.91 (2.15)	7.64 (1.49)
Reaction time (ms)	396.51 (10.01)	399.18 (12.57)	410.16 (12.30)	407.27 (9.44)
SSS	2.73 (0.20)	2.64 (0.31)	3.01 (0.32)	2.77 (0.31)
VAS (mm)	38.45 (5.69)	31.91 (5.07)	42.61 (5.99)	36.83 (5.79)

Note. Data are mean values and standard error of mean. PVT, Psychomotor Vigilance Task; lapse, response with latency exceeding 500 ms; anticipated responses, responses faster than 150 ms; SSS, Stanford Sleepiness Scale; VAS, Visual Analogue Sleepiness Scale.

its temporal and quantitative association with SWS has been repeatedly demonstrated (Gronfier et al., [34,35]). Animal and in vitro studies have also revealed that GH influences steroidogenesis. Specifically, it enhances the responsiveness of Leydig cells to LH stimulation (for review, see Hull and Harvey, [36]). GH also promotes steroidogenesis by increasing the production of enzymes involved in the steroidogenic pathway: steroidogenic acute regulatory protein and 3β-HSD (Kanzaki and Morris, [37]). However, it is not clear whether SWS suppression is sufficient to affect GH release. According to Born et al.'s study, selective suppression of this sleep stage did not cause significant changes in the amplitude of GH secretory peaks, but the authors only suppressed SWS during the first 3 h of night sleep [19]. Therefore, to unravel the mechanisms through which SWS suppression affects steroidogenesis, further investigations are needed.

Testosterone, 17-OHP, Ad, DHEA, their precursors, and their metabolites have neurogenic and neuroprotective properties. These androgens can also be synthesized in the brain by neurons and glial cells, in which case they have been termed neurosteroids (Baulieu [38]). The enzyme 3β-HSD also plays an important role in neurosteroidogenesis (Porcu et al., [39]). Previous studies reported associations between the secretion of androgens and mood (Romero-Martínez et al., [40]) and cognitive functions (Moffat et al., [40,41]). Studies have also reported that impaired neurosteroidogenesis is involved in the development of anxiety disorders [42] (Longone et al., [43]). Besides mental functions, steroid hormones, particularly testosterone (Zitzmann [44]) and DHEA (Kang et al., [45]), which influences carbohydrate metabolism, participate in regulating metabolic processes. In this regard, our data may have some clinical relevance. Based on the findings in the literature and those of the current study, we can assume that chronic sleep disorders such as obstructive sleep apnoea, which is characterized by reduced SWS (Redline et al., [46]), may lead to long-term changes in androgen secretion and have serious consequences for metabolic and cognitive processes. Previous studies have consistently found that sleep disorders increase the risk of

developing various diseases, particularly metabolic disorders such as type 2 diabetes (Shan et al., [47]) as well as anxiety (Roberts and Duong, [48]) and cognitive (Banks and Dinges, [49]) disorders. Considering the influence of androgens on carbohydrate metabolism, cognitive functions, and mood, we can conclude that changes in androgen secretion caused by insufficient or poor sleep may significantly affect the development of metabolic and psychiatric disorders.

Some limitations of the present study should be mentioned. Although the findings underline the importance of SWS in androgen secretion, the data do not provide insight into the possible role of other sleep stages. Additionally, because the study did not include an active control condition involving the presentation of sounds during sleep when SWS was absent, there is a possibility that the acoustic stimulation's arousing effect influenced the synthesis and release of androgens rather than SWS suppression. Furthermore, in our study we reduced overall SWS duration by only 54.2%, whereas Tasali et al., suppressed SWS by more than 80% [17]. The explanation for this is likely to be found in the type of sleep intervention. In the study of Tasali et al., if there was no response to sound, the experimenter entered the bedroom and shook the shoulder of the subject [17]. We considered this procedure as potentially stressful for participants and used only acoustic stimuli for SWS disturbance. However, we should note that in young healthy men it is difficult to suppress SWS by more than 50% using only acoustic stimuli. Other limitations of our study are the relatively small number of subjects tested, which limits the statistical power to detect more discrete effects.

5. Conclusions

Selective SWS suppression by acoustic stimuli resulted in a 54.2% reduction in SWS. Nevertheless, it did not greatly disturb the architecture of night-time sleep and did not induce sleepiness or impair sustained attention. The decline in SWS was sufficient to affect the secretion of testosterone and its precursors. SWS suppression was associated with a decrease in morning testosterone and 17-OHP levels and an increase in the DHEA/Ad ratio in saliva. Moreover, SWS suppression did not significantly influence morning cortisol levels. Thus, the present study's findings indicate that SWS plays an essential role in the regulation of androgen synthesis and secretion.

Financial support

This work was supported by the Russian Foundation for Basic Research (RFBR grant number 18-013-01187 A). M.O.V. was supported within the framework of the Basic Research Program at the National Research University Higher School of Economics (HSE) and subsidy by the Russian Academic Excellence Project '5-100'. The supporting agencies had no role in the design or conduct of the study; the collection, analysis, or interpretation of the data; or the writing or approval of the manuscript.

Authors contributions

Conceived and designed the experiments: U.Yu.V., P.A.A., L.K.M., B.D.A., S.E.S., M.C.M., M.O.V., N.A.N.

Performed the experiments: P.A.A., L.K.M., Analysed the data: U.Yu.V., N.A.N., B.D.A., S.E.S., M.C.M.

Wrote the paper: U.Yu.V., M.O.V., N.A.N., B.D.A.

Revisiting the manuscript: U.Yu.V., P.A.A., L.K.M., N.A.N., B.D.A., S.E.S., M.C.M., M.O.V.

Final approval of the version to be submitted U.Yu.V., P.A.A., L.K.M., B.D.A., S.E.S., M.C.M., M.O.V., N.A.N.

Acknowledgements

We gratefully acknowledge the help of the volunteers who participated in this study. We thank our colleagues, especially Olga Kashevarova, for help in conducting the experiments.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2018.04.012.

References

[1] Ghiciuc CM, Cozma-Dima CL, Pasquali V, et al. Awakening responses and diurnal fluctuations of salivary cortisol, DHEA-S and alpha-amylase in healthy male subjects. Neuroendocrinol Lett 2011;32:475–80.
[2] Mezzullo M, Fazzini A, Gambineri A, et al. Parallel diurnal fluctuation of testosterone, androstenedione, dehydroepiandrosterone and 17OHprogesterone as assessed in serum and saliva: validation of a novel liquid chromatography-tandem mass spectrometry method for salivary steroid profiling. Clin Chem Lab Med 2017;55(9):1315–23. https://doi.org/10.1515/cclm-2016-0805.
[3] Benedict C, Kern W, Schmid SM, et al. Early morning rise in hypothalamic-pituitary-adrenal activity: a role for maintaining the brain's energy balance. Psychoneuroendocrinology 2009;34(3):455–62. https://doi.org/10.1016/j.psyneuen.2008.10.010.
[4] Luboshitzky R, Herer P, Levi M, et al. Relationship between rapid eye movement sleep and testosterone secretion in normal men. J Androl 1999;20(6):731–7. https://doi.org/10.1002/j.1939-4640.1999.tb03378.x.
[5] Axelsson J, Ingre M, Åkerstedt T, et al. Effects of acutely displaced sleep on testosterone. J Clin Endocrinol Metab 2005;90(8):4530–5. https://doi.org/10.1210/jc.2005-0520.
[6] Arnal PJ, Drogou C, Sauvet F, et al. Effect of sleep extension on the subsequent testosterone, cortisol and prolactin responses to total sleep deprivation and recovery. J Neuroendocrinol 2016;28(2):12346. https://doi.org/10.1111/jne.12346.
[7] Jauch-Chara K, Schmid SM, Hallschmid M, et al. Pituitary-gonadal and pituitary-thyroid axis hormone concentrations before and during a hypoglycemic clamp after sleep deprivation in healthy men. PLoS One 2013;8(1):e54209. https://doi.org/10.1371/journal.pone.0054209.
[8] Leproult R, Van Cauter E. Effect of 1 week of sleep restriction on testosterone levels in young healthy men. JAMA 2011;305(21):2173–4. https://doi.org/10.1001/jama.2011.710.
[9] Rabat A, Gomez-Merino D, Roca-Paixao L, et al. Differential kinetics in alteration and recovery of cognitive processes from a chronic sleep restriction in young healthy men. Front Behav Neurosci 2016;10:95. https://doi.org/10.3389/fnbeh.2016.00095.
[10] Reynolds AC, Dorrian J, Liu PY, et al. Impact of five nights of sleep restriction on glucose metabolism, leptin and testosterone in young adult men. PLoS One 2012;7(7):e41218. https://doi.org/10.1371/journal.pone.0041218.
[11] Schmid SM, Hallschmid M, Jauch-Chara K, et al. Sleep timing may modulate the effect of sleep loss on testosterone. Clin Endocrinol (Oxf) 2012;77(5):749–54. https://doi.org/10.1111/j.1365-2265.2012.04419.x.
[12] Voderholzer U, Piosczyk H, Holz J, et al. The impact of increasing sleep restriction on cortisol and daytime sleepiness in adolescents. Neurosci Lett 2012;507(2):161–6. https://doi.org/10.1016/j.neulet.2011.12.014.
[13] Wu H, Zhao Z, Stone WS, et al. Effects of sleep restriction periods on serum cortisol levels in healthy men. Brain Res Bull 2008;77(5):241–5. https://doi.org/10.1016/j.brainresbull.2008.07.013.
[14] Born J, Fehm HL. Hypothalamus-pituitary-adrenal activity during human sleep: a coordinating role for the limbic hippocampal system. Exp Clin Endocrinol Diabetes 1998;106(3):153–63. https://doi.org/10.1055/s-0029-1211969.
[15] Brandenberger G, Ehrhart J, Piquard F, et al. Inverse coupling between ultradian oscillations in delta wave activity and heart rate variability during sleep. Clin Neurophysiol 2001;112(6):992–6. https://doi.org/10.1016/S1388-2457(01)00507-7.
[16] Maquet P. Sleep function(s) and cerebral metabolism. Behav Brain Res 1995;69:75–83. https://doi.org/10.1016/0166-4328(95)00017-N.
[17] Tasali E, Leproult R, Ehrmann DA, et al. Slow-wave sleep and the risk of type 2 diabetes in humans. Proc Natl Acad Sci USA 2008;105(3):1044–9. https://doi.org/10.1073/pnas.0706446105.
[18] Herzog N, Jauch-Chara K, Hyzy F, et al. Selective slow wave sleep but not rapid eye movement sleep suppression impairs morning glucose tolerance in healthy men. Psychoneuroendocrinology 2013;38:2075–82. https://doi.org/10.1016/j.psyneuen.2013.03.018.

[19] Born J, Muth S, Fehm HL. The significance of sleep onset and slow wave sleep for nocturnal release of growth hormone (GH) and cortisol. Psychoneuroendocrinology 1988;13(3):233–43. https://doi.org/10.1016/0306-4530(88)90021-2.
[20] Van Liempt S, Arends J, Cluitmans PJM, et al. Sympathetic activity and hypothalamo-pituitary-adrenal axis activity during sleep in post-traumatic stress disorder: a study assessing polysomnography with simultaneous blood sampling. Psychoneuroendocrinology 2013;38(1):155–65. https://doi.org/10.1016/j.psyneuen.2012.05.015.
[21] Damgaard-Olesen A, Johannsen TH, Holmboe SA, et al. Reference ranges of 17-hydroxyprogesterone, DHEA, DHEAS, androstenedione, total and free testosterone determined by TurboFlow-LC-MS/MS and associations to health markers in 304 men. Clin Chim Acta 2016;454:82–8. https://doi.org/10.1016/j.cca.2015.12.042.
[22] Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14(6):540–5. https://doi.org/10.1093/sleep/14.6.540.
[23] Dinges DF, Powell JW. Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. Behav Res Meth Instrum Comput 1985;17:652–5. https://doi.org/10.3758/BF03200977.
[24] Maclean AW, Fekken GC, Saskin P, et al. Psychometric evaluation of the Stanford sleepiness scale. J Sleep Res 1992;1(1):35–9. https://doi.org/10.1111/j.1365-2869.1992.tb00006.x.
[25] Iber C, Ancoli-Israel S, Chesson A, et al. The AASM manual for the scoring of sleep and associated events: rules. Terminology and technical specifications. 1st ed. Westchester: American Academy of Sleep Medicine; 2007.
[26] Brambilla DJ, McKinlay SM, McKinlay JB, et al. Does collecting repeated blood samples from each subject improve the precision of estimated steroid hormone levels? J Clin Epidemiol 1996;49(3):345–50. https://doi.org/10.1016/0895-4356(95)00569-2.
[27] Foresta C, Bordon P, Rossato M, et al. Specific linkages among luteinizing hormone, follicle-stimulating hormone, and testosterone release in the peripheral blood and human spermatic vein: evidence for both positive (feed-forward) and negative (feedback) within-axis regulation. J Clin Endocrinol Metab 1997;82:3040–6. https://doi.org/10.1210/jc.82.9.3040.
[28] Barrett-Connor E, Dam T-T, Stone K, et al. The association of testosterone levels with overall sleep quality, sleep architecture, and sleep-disordered breathing. J Clin Endocrinol Metab 2008;93(7):2602–9. https://doi.org/10.1210/jc.2007-2622.
[29] Søeborg T, Frederiksen H, Mouritsen A, et al. Sex, age, pubertal development and use of oral contraceptives in relation to serum concentrations of DHEA, DHEAS, 17α-hydroxyprogesterone, Δ4-androstenedione, testosterone and their ratios in children, adolescents and young adults. Clin Chim Acta 2014;437:6–13. https://doi.org/10.1016/j.cca.2014.06.018.
[30] Melmed S, Polonsky KS, Larsen P, et al. Williams textbook of endocrinology 13th edition. Elsevier; 2016. https://doi.org/10.1017/CBO9781107415324.004.
[31] Moisan AM, Ricketts ML, Tardy V, et al. New insight into the molecular basis of 3β-hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the HSD3B2 gene in eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. J Clin Endocrinol Metab 1999;84(12):4410–25. https://doi.org/10.1210/jcem.84.12.6288.
[32] Killick R, Hoyos CM, Melehan KL, et al. Metabolic and hormonal effects of "catch-up" sleep in men with chronic, repetitive, lifestyle-driven sleep restriction. Clin Endocrinol (Oxf) 2015;83(4):498–507. https://doi.org/10.1111/cen.12747.
[33] Shaw ND, Butler JP, Nemati S, et al. Accumulated deep sleep is a powerful predictor of LH pulse onset in pubertal children. J Clin Endocrinol Metab 2015;100:1062–70. https://doi.org/10.1210/jc.2014-3563.
[34] Gronfier C, Luthringer R, Follenius M, et al. A quantitative evaluation of the relationships between growth hormone secretion and delta wave electroencephalographic activity during normal sleep and after enrichment in delta waves. Sleep 1996;19:817–24. https://doi.org/10.1093/sleep/19.10.817.
[35] Van Cauter E, Kerkhofs M, Caufriez A, et al. A quantitative estimation of growth hormone secretion in normal man: reproducibility and relation to sleep and time of day. J Clin Endocrinol Metab 1992;74(6):1441–50. https://doi.org/10.1210/jcem.74.6.1592892.
[36] Hull KL, Harvey S. Growth hormone and reproduction: a review of endocrine and autocrine/paracrine interactions. Int J Endocrinol 2014. https://doi.org/10.1155/2014/234014.
[37] Kanzaki M, Morris PL. Growth hormone regulates steroidogenic acute regulatory protein expression and steroidogenesis in Leydig cell progenitors. Endocrinology 1999;140:1681–6. https://doi.org/10.1210/endo.140.4.6661.
[38] Baulieu EE. Neurosteroids: a novel function of the brain. Psychoneuroendocrinology 1998;23:963–87. https://doi.org/10.1016/S0306-4530(98)00071-7.
[39] Porcu P, Barron AM, Frye CA, et al. Neurosteroidogenesis today: novel targets for neuroactive steroid synthesis and action and their relevance for translational research. J Neuroendocrinol 2016;28(2). https://doi.org/10.1111/jne.12351. 10.1111.
[40] Romero-Martínez Á, Ruiz-Robledillo N, Moya-Albiol L. Depressive mood and testosterone related to declarative verbal memory decline in middle-aged caregivers of children with eating disorders. Int J Environ Res Publ Health 2016;13(3):286. https://doi.org/10.3390/ijerph13030286.
[41] Moffat S, Zonderman A, Metter E, et al. Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. J Clin Endocrinol Metab 2002;87(11):5001–7. https://doi.org/10.1210/jc.2002-020419.
[42] Giltay EJ, Enter D, Zitman FG, et al. Salivary testosterone: associations with depression, anxiety disorders, and antidepressant use in a large cohort study. J Psychosom Res 2012;72(3):205–13. https://doi.org/10.1016/j.jpsychores.2011.11.014.
[43] Longone P, di Michele F, D'Agati E, et al. Neurosteroids as neuromodulators in the treatment of anxiety disorders. Front Endocrinol (Lausanne) 2011;2:55. https://doi.org/10.3389/fendo.2011.00055.
[44] Zitzmann M. Testosterone deficiency, insulin resistance and the metabolic syndrome. Nat Rev Endocrinol 2009;5(12):673–81. https://doi.org/10.1038/nrendo.2009.212.
[45] Kang J, Ge C, Yu L, et al. Long-term administration of dehydroepiandrosterone accelerates glucose catabolism via activation of PI3K/akt-PFK-2 signaling pathway in rats fed a high-fat diet. PLoS One 2016;11(7):e0159077. https://doi.org/10.1371/journal.pone.0159077.
[46] Redline S, Kirchner HL, Quan SF, et al. The effects of age, sex, ethnicity, and sleep-disordered breathing on sleep architecture. Arch Intern Med 2004;164:406–18. https://doi.org/10.1001/archinte.164.4.406.
[47] Shan Z, Ma H, Xie M, et al. Sleep duration and risk of type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care 2015;38(3):529–37. https://doi.org/10.2337/dc14-2073.
[48] Roberts RE, Duong HT. Is there an association between short sleep duration and adolescent anxiety disorders? Sleep Med 2017;30:82–7. https://doi.org/10.1016/j.sleep.2016.02.007.
[49] Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. J Clin Sleep Med 2007;3(5):519–28. https://doi.org/10.1055/s-0029-1237117.Neurocognitive.