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Chronotype, social jetlag and sleep loss in relation to sex steroids

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ABSTRACT

Chronotype describes preferences for functioning at different times of the day. At the onset of puberty, a sharp shift towards eveningness starts, reaching its peak at the end of adolescence, followed by a steady shift towards morningness as the ageing process occurs. Puberty is also the time when sex differences appear, with men being more inclined to eveningness than women, which diminishes around menopause; the described pattern of changes in chronotype leads to the hypothesis that reproductive hormones may be the driving factor behind this conversion. In the present study, we aimed to verify this hypothesis by analysing participants' testosterone, progesterone and dehydroepiandrosterone (DHEA) levels in the three months, as indicated by assays in 3-cm hair strands from the scalp. Participants ($n = 239$) of both sexes also completed the Munich Chronotype Questionnaire. The results showed that in men higher testosterone levels were related to eveningness and less sleep loss, whereas greater sleep loss was associated with lower levels of DHEA. In women, no associations between chronotype and levels of the analysed hormones were found. The results support the hypothesis that testosterone levels play a role in shaping eveningness. We further hypothesised that a possible cause of the higher secretion level of testosterone in men with the evening chronotype is a mechanism to offset the negative consequences of sleep loss.

1. Introduction

Chronotype, also called morningness-eveningness or circadian typology (Adan et al., 2012), is an individual characteristic describing preferences for functioning at different times of the day and sleep-wake timing. Although it is a dimensional variable, researchers often use categories (e.g. evening types, morning types) to facilitate communication. Evening types, in contrast to morning types, exhibit a shift towards later hours in circadian rhythms, such as sleep-wake rhythms or the secretion of hormones such as cortisol (Bailey and Heitkemper, 2001) and melatonin (Burgess and Fogg, 2008).

The evening chronotype has been related to a number of adverse health outcomes, including both mental (Jankowski and Dmitrzak-Węglarz, 2017; Taylor and Hasler, 2018) and physical health problems (Gariépy et al., 2018). These adverse outcomes are often claimed to be a side effect of the conflict between biologically driven preferences for late sleep timing and morning activities imposed by society (work, school, family care, etc.), a phenomenon called social jetlag (Wittmann et al., 2006). Aside from circadian misalignment (misalignment of sleep to the biological night; Baron and Reid, 2014), the evening chronotype can also lead to sleep loss if an individual attempts to sleep according to their biological night but needs to wake up early to fulfil morning social obligations, like the start of work or school (Jankowski, 2017; Vollmer et al., 2017).

Amongst the biological factors related to chronotypes, age demonstrates a robust effect, which is further moderated by sex (Jankowski, 2015; Roenneberg et al., 2004). In general, morningness dominates during childhood, and sex differences are not apparent (Randler et al., 2017, 2019). At the onset of puberty, a sharp shift towards eveningness begins and reaches its peak at around 18–21 years of age (Jankowski, 2015; Roenneberg et al., 2004). Afterwards, a mild shift to morningness continues with ageing. Puberty is also the time when sex differences appear, with men being more inclined to eveningness than women, which diminishes around menopause (Roenneberg et al., 2004).

The described pattern of changes in chronotype, as well as its existence in animals, has led researchers to hypothesise that reproductive hormones may be the driving factor behind chronotype (Hagenauer and Lee, 2012; Mong et al., 2011). Particular interest was paid to androgen hormones, based on the observation that gonadectomies in mice diminished evening activity in males, but not in females, and that androgen supplementation restored the sex difference in daily rhythmicity (Iwahana et al., 2008). Increasing testosterone was postulated to lead to

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later bedtimes in humans (Wittert, 2014). This hypothesis, linking higher testosterone with eveningness, has not been extensively tested. Randler et al. (2012b) illustrated that eveningness is related to salivary testosterone levels sampled during the morning hours (8:00–9:00). This method, however, can confound the results because different chronotypes may be tested at different time intervals from their morning testosterone peak (Brambilla et al., 2009; Lacerda et al., 1973). On the other hand, Maestripieri (2014) found no association between salivary testosterone and chronotype tested in the afternoon in men or women (13:30 – 17:00).

Moreover, social jetlag and sleep loss are salient variables that should also be considered in the context of an association between chronotypes and sex hormones. It has been suggested that social jetlag has adverse endocrine effects (Rutters et al., 2014), but no associations with sex hormones have been reported so far. On the other hand, associations between sleep loss in humans and the levels of numerous hormones have been documented (Kim et al., 2015; Van Cauter et al., 2005). Sleep loss has been related to diminished morning testosterone levels in men (Penev, 2007), and this effect was particularly prominent if sleep loss occurred in the second half of the night (Schmid et al., 2012). When both sexes were analysed, the effect of sleep loss on testosterone levels appeared in men but not in women (Cote et al., 2013). Therefore, these two sleep variables can potentially confound links between chronotype and hormone levels.

In the present study, we aimed to clarify and extend previous findings by testing long-term testosterone levels for the first time, taking advantage of testing hair (as opposed to testing saliva), according to chronotype. Furthermore, we also aimed to analyse two other sex hormones for the first time – dehydroepiandrosterone (DHEA) and progesterone in the context of chronotype. Finally, we aimed to test two salient chronotype-related sleep variables concurrently – social jetlag and sleep loss – in the context of the abovementioned hormones and to consider the associations not only in men but also in women.

2. Methods

2.1. Participants and procedures

In total, 239 subjects were analysed. There were 124 males aged 19–41 (24.73 ± 3.98 years) and 115 females aged 19–38 (23.14 ± 3.17 years). Using self-reporting, they were assessed as healthy and were not taking any medications or substances affecting hormone levels (including anabolic steroids and contraceptive pills) during the sampling period or in the three months prior to it. Additional exclusion criteria were pregnancy, lactation, hair inadequate for sampling (≤3 cm, dreadlocks, hair extensions) working night shifts and crossing time zones within the past 2 weeks. The majority of the sample were students (62%), followed by working students (19%), workers (16%) and the unemployed (3%). Participants were recruited through an online questionnaire asking for health conditions and medication use; those willing to participate in the study were asked to leave an email address or phone number at which they could be contacted. Individuals who were willing to participate and met the study requirements (health and hair condition) were contacted by an experimenter. Upon arrival, subjects were briefed comprehensively on the study procedures, gave written informed consent and underwent measurements. Subjects received a shopping voucher as remuneration for participation. The study was approved by the Committee for Ethics in Scientific Research of the Institute of Psychology, Polish Academy of Sciences.

2.2. Questionnaires

Assessment of chronotype, social jetlag and sleep loss was performed using the Polish version of the Munich Chronotype Questionnaire (MCTQ; Jankowski, 2015; Roenneberg et al., 2003). The MCTQ asks participants for the timing of numerous sleep behaviours on working days and free days separately. Mid sleep on free days sleep corrected (MSFsc) was used as an indicator of chronotype (Roenneberg et al., 2004). MSFsc is the halfway point between sleep onset and wake-up time shifted back proportionally to the time people sleep off the sleep loss accumulated during workdays; MSFsc is expressed in local time, and later values indicate the presence of more evening chronotypes. Social jetlag sleep corrected (SJLsc; Jankowski, 2017) was used to distinguish between circadian misalignment and sleep loss. SJLsc is expressed in time units (hh:mm) and shows the absolute difference in sleep timing between days off and workdays. Greater values indicate more pronounced social jetlag (greater discrepancy in sleep timing). Weekly workday sleep loss was calculated as the number of workdays *x* (mean weakly sleep duration – mean workday sleep duration). The resultant sleep loss is also expressed in time format (hh:mm), with greater values indicating more sleep loss across working days. The descriptive statistics of sleep variables are illustrated in Table 1.

Table 1
Descriptive statistics of the sample.

	N	Median	Mean	*SD*	Minimum	Maximum
Men						
Testosterone*	114	1.14	1.22	.65	.10	4.14
DHEA*	124	20.12	42.49	88.67	7.80	645.30
Progesterone*	122	.75	1.03	1.00	.31	8.87
Chronotype*	123	5:07	5:15	1:15	1:45	9:17
Social jetlag	123	0:45	0:55	0:44	0:00	3:15
Sleep loss	124	1:42	2:20	2:10	0:00	13:25
Women						
Testosterone	47	.28	.48	.54	.09	2.90
DHEA	115	11.45	20.44	27.24	6.03	195.75
Progesterone	114	2.19	2.56	1.81	.44	11.70
Chronotype	115	4:35	4:36	1:13	1:17	8:49
Social jetlag	115	0:35	0:43	0:34	0:00	3:00
Sleep loss	115	1:59	2:15	1:50	0:00	12:36

Note. DHEA – dehydroepiandrosterone; *statistically significant differences (p = .00) between men and women as indicated by U-Mann Whitney test.

2.3. Hair sampling

Hair sampling was conducted according to the instructions of the Laboratory of Biological Psychology at the Technical University of Dresden (TUD). Trained study personnel collected two strands of hair from the posterior vertex region of participants' heads. Strands were cut with scissors; the cut was made as close as possible to the scalp before the strand was wrapped in aluminium foil and shipped to the TUD laboratory. The analyses were conducted on the 3 cm of hair closest to the scalp, which corresponds to the 3 months prior to sampling, assuming an average hair growth of 1 cm per month. Hormone levels were assayed using the liquid chromatography tandem mass spectrometry protocol (Gao et al., 2013). Previous research supports the validity of hormone measurement in hair (Wang et al., 2019).

2.4. Statistical analyses

At first descriptive statistics were calculated, which showed salient missing testosterone data in women. Because the distribution of hormone levels is known to be positively skewed, which was also true for our data, we used Spearman's rank correlations as an initial analysis to test associations between study variables. These zero-order correlations between study variables were calculated separately for men and women. The associations found in zero-order analyses were further investigated using Spearman's rank partial correlations, controlling for age, assumed workload (0 = unemployed and students; 1 = working students and workers), and sleep variables (e.g. control of SJLsc and sleep loss if the chronotype was under investigation). In supplementary

analyses regression models were tested with ln(hormone) as dependent variable, sex, sleep loss and chronotype as predictors entered in the first block; sex*chronotype, sex*sleep loss and chronotype*sleep loss interactions entered in the second block and sex*chronotype*sleep loss interaction entered in the third block. Regression models for ln(testosterone) were tested separately in men and women due to the small number of women with measurable testosterone levels. To rule out effects of age on the results, regression models were re-run with age as a predictor entered in the first block. Because distributions of ln(hormone) were also skewed, regression coefficients were computed using bootstrapping (1000 samples). All analyses were conducted using IBM SPSS Statistics version 25.

3. Results

3.1. Descriptive statistics

The descriptive statistics of the main study variables are displayed in Table 1. One can observe that there were only 47 female participants with testosterone data; in the remainder, testosterone levels were below the lower limit of sensitivity. The same was true for only 10 males. DHEA and progesterone data were available for almost all men and women (Table 1). The distribution of hormone levels was heavily right-skewed (skewness between 2.0 and 5.7) and using natural log transformation did not make the distribution of DHEA and progesterone in men and testosterone in women symmetrical (skewness between 1.1 and 1.6). We used the natural log transformation because it made the data symmetrical to a greater (or similar) extent compared to other common transformations for positively skewed data (log10, square root, inverse) and it has advantages in terms of interpretability (Gelman and Hill, 2007).

3.2. Zero-order correlations

Spearman's rank correlations proved that higher testosterone levels in men were related to the evening chronotype and less sleep loss but unrelated to social jetlag (Table 2). Higher DHEA levels were associated with lower levels of sleep loss but unrelated to chronotypes or social jetlag. Progesterone was unrelated to sleep variables. In women, the levels of the three sex steroids were unrelated to sleep variables. Furthermore, in both men and women, the evening chronotype was associated with greater social jetlag, but was unrelated to sleep loss. Sleep loss and social jetlag were not interrelated in either sex.

3.3. Partial correlations

To confirm that the revealed association of chronotype with testosterone as well as the associations of sleep loss with testosterone and DHEA were not cofounded, we conducted partial Spearman's rank correlations (Table 3). After controlling for sleep loss, SJLsc, age, and workload, the chronotype remained positively correlated with testosterone in men, which means that men with evening chronotypes had higher levels of testosterone than men with morning chronotypes. Furthermore, after controlling for chronotype, SJLsc, age, and workload, sleep loss remained negatively correlated with testosterone in men, which means that men with greater sleep loss had lower levels of testosterone than men with lower sleep loss. Finally, after controlling for chronotype, SJLsc, age, and workload, sleep loss remained negatively correlated with DHEA in men, which means that men with greater sleep loss had lower levels of DHEA than men with lower sleep loss.

Table 2
Spearman's rank correlations (p two-tailed) between the studied variables.

	Chronotype	Social jetlag	Sleep loss
Men			
Testosterone	.25* (.01)	.09 (.36)	−.31* (.00)
DHEA	−.07 (.42)	.08 (.38)	−.22* (.02)
Progesterone	.01 (.94)	.16 (.08)	−.07 (.46)
Chronotype		.30* (.00)	.08 (.40)
Social jetlag			−.10 (.28)
Women			
Testosterone	.08 (.60)	.04 (.82)	−.04 (.81)
DHEA	.04 (.65)	.16 (.08)	.04 (.68)
Progesterone	.10 (.31)	.05 (.59)	−.03 (.77)
Chronotype		.35* (.00)	−.13 (.16)
Social jetlag			−.05 (.62)

Note. *Statistically significant associations.

Table 3
Spearman's rank partial correlations (p two-tailed) between sleep variables and hormone levels controlling for age, assumed workload and sleep variables (e.g. control of social jetlag and sleep loss if the chronotype was under investigation).

	Chronotype	Social jetlag	Sleep loss
Men			
Testosterone	.27* (.00)	−.02 (.85)	−.32* (.00)
DHEA	−.09 (.35)	.09 (.35)	−.20* (.03)
Progesterone	−.06 (.51)	.17 (.07)	−.06 (.52)
Women			
Testosterone	−.02 (.92)	.07 (.64)	−.03 (.84)
DHEA	−.03 (.73)	.17 (.07)	.05 (.59)
Progesterone	.07 (.49)	.03 (.72)	.01 (.92)

Note. *Statistically significant associations.

3.4. Regression models

Regression prediction of ln(DHEA) showed that the model with main effects was statistically significant and explained 11% of the variance in ln(DHEA), while interaction terms did not explain additional variance (Table 4). Higher levels of ln(DHEA) were predicted by male sex and lower sleep loss. Levels of ln(DHEA) according to sleep loss and chronotype is illustrated in Fig. 1 (men) and Fig. 2 (women). From the B coefficients (Table 4) the shift in sleep loss in hours that occurs in relation to DHEA levels can be computed by substituting the characteristics of a subject of interest. For instance, in the case of a male with a mean chronotype of 6:00 and no sleep loss, the estimated ln (DHEA) value is 3.81, translating to 45.15 pg/mg DHEA. In his case, an increase in sleep loss by 1 h leads to a decrease in ln(DHEA) to 3.76, translating to 42.95 pg/mg DHEA – a decrease in DHEA of 2.20 pg/mg. Overall, in men with the typical chronotype (4:00 – 7:00) and typical sleep loss (0:00 – 5:00), a 1 -h shift in sleep loss is related to an ˜1.9 pg/mg difference in DHEA.

Regression prediction of progesterone showed that the model with main effects was statistically significant and explained 36% of the variance in progesterone, while interaction terms did not explain additional variance (Table 4). Higher levels of progesterone were predicted by female sex.

Regression prediction of ln(testosterone) in men showed that the model with main effects was statistically significant and explained 16% of the variance in ln(testosterone), whereas interaction terms did not explain the additional variance (Table 5). Higher levels of ln(testosterone) were predicted by the evening chronotype and lower sleep loss. Fig. 3 illustrates levels of ln(testosterone) according to sleep loss and chronotype in men. From the B coefficients (Table 5) it can be estimated that in men with typical chronotype (4:00 – 7:00) and typical sleep loss (0:00 – 5:00), a 1 -h shift in chronotype is related to an ˜0.2 pg/mg difference in testosterone, and a 1 -h shift in sleep loss is related to an ˜0.1 difference in testosterone. In women, regression prediction of ln (testosterone) showed that all considered predictors were statistically non-significant (Table 5). Including age as a control variable did not

Table 4
Results of regression analyses with ln (natural logarithm) of hormone levels as the outcome predicted by variables entered in subsequent blocks (model).

	Ln(DHEA)					Ln(Progesterone)				
Model	*B*	*p*	95% *CI*	R^2	*p*	*B*	*p*	95% *CI*	R^2	*p*
constant	3.21	.00	2.67; 3.74	.11	.00	−.18	.36	−.59; .21	.36	.00
sex	−.50	.00	−.71; -.31			.91	.00	.72; 1.09		
chronotype	.01	.80	−.09; .12			.01	.81	−.06; .07		
sleep loss	−.05	.01	−.09; -.01			−.02	.37	−.05; .02		
sex*chronotype	−.05	.62	−.23; .16	.01	.54	.08	.24	−.04; .21	.01	.45
sex*sleep loss	.03	.48	−.05; .12			.02	.63	−.07; .11		
chronotype*sleep loss	−.02	.26	−.06; .02			.02	.14	−.01; .05		
sex* chronotype*sleep loss	.01	.89	−.09; .09	.00	.92	−.02	.43	−.09; .05	.00	.54

Note. Sex coded 0 = men, 1 = women; chronotype and sleep loss expressed in hours; *CI* confidence interval for coefficients.

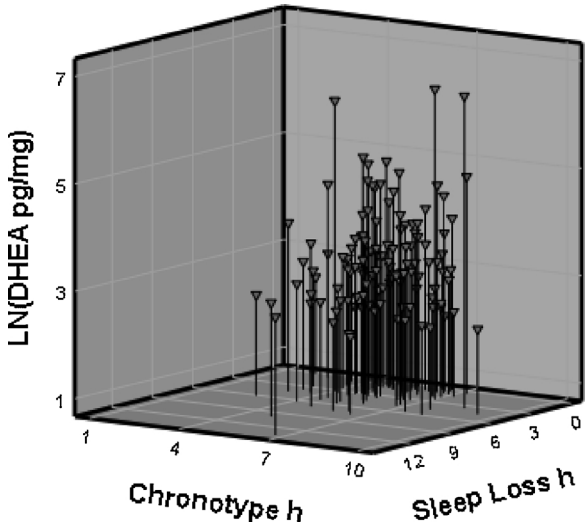

Fig. 1. Scatterplot showing natural logarithm (LN) of DHEA levels across range of chronotype (in hours) and sleep loss (in hours) in men.

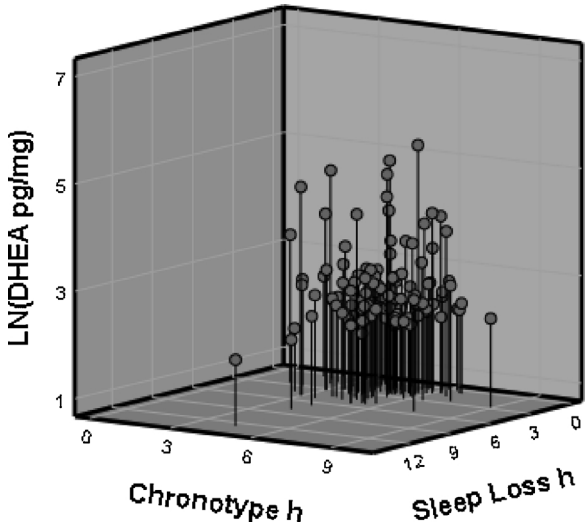

Fig. 2. Scatterplot showing natural logarithm (LN) of DHEA levels across range of chronotype (in hours) and sleep loss (in hours) in women.

change the outcomes of the above analyses (Appendix A).

4. Discussion

The main study finding is that testosterone levels are higher in men with a later chronotype and lower levels of sleep loss, while higher levels of DHEA are associated with lower levels of sleep loss. In contrast, the analysed hormones in women appeared to be unrelated to sleep variables, but conclusively demonstrating this sex difference in the relationship between hormone levels and sleep variables would require a larger sample size.

The finding of higher testosterone levels in men with an evening chronotype is in line with the observations reported by Randler et al. (2012b) but not with the results of Maestripieri (2014), who reported a lack of association. These results suggest that testosterone levels assayed from saliva collected in the morning (Randler et al., 2012b) but not in the afternoon (Maestripieri, 2014) are a better proxy of participants' 3-month testosterone levels. Taking the results of the three studies together, the hypothesis that chronotype is dependent on testosterone levels gains further support. Nevertheless, it still has the status of a hypothesis, given the cross-sectional nature of the current and previous studies. Interventional studies, which are scarce, focusing on older men undergoing androgen therapy report impacts of testosterone on sleep duration but not necessarily on sleep timing (Liu et al., 2003). It is also plausible that there is a third factor that drives both increased testosterone production and eveningness and, hence, association between testosterone levels and chronotype is parallelism. Interventional studies (i.e. exogenous testosterone administration) with adolescent/young healthy males could possibly provide a stronger argument but would be ethically questionable. Another possibility to test causality between testosterone and chronotype could be a study of young anabolic-androgenic steroid users (Westerman et al., 2016) or transgender men undergoing testosterone therapy (Irwig, 2017).

The result showing lower testosterone levels in men experiencing sleep loss is in line with previous reports (Cote et al., 2013; Penev, 2007; Schmid et al., 2012). Our study clarifies that chronic sleep loss translates to long-lasting effects on testosterone levels, with a duration of at least 3 months.

The observation of higher testosterone in evening chronotypes and men with less sleep loss triggers a novel hypothesis that higher secretion of testosterone in evening chronotypes could be a mechanism to offset the negative consequences of sleep loss on hormone levels. Because evening chronotypes are at greater risk of sleep loss, especially in the testosterone-sensitive second half of the night (Schmid et al., 2012), due to the morning organisation of social life (early school/work starts), individuals could have developed a mechanism to protect themselves from insufficient testosterone levels. The hypothesis is based on the assumption that sleep loss due to the evening chronotype is not a modern phenomenon, supported by observations that in pre-industrial societies, in localizations of human origin (around the equator), sleep-wake patterns are similar to those observed today (Yetish et al., 2015). Some support for this hypothesis comes from a recent study, which showed that men who delayed their sleep timing by 45 min and lost half an hour of sleep per night for a 1-week period did not experience a decrease in testosterone levels (Zander et al., 2019). This hypothesis could be tested further in future research in a sample of individuals with restricted morning sleep (e.g. high school students or workers with

Table 5
Results of regression analyses with ln (natural logarithm) of testosterone levels as the outcome predicted by variables entered in subsequent blocks (model), separately for men and women.

	Ln(Testosterone) in Men					Ln(Testosterone) in Women				
Model	*B*	*p*	95% *CI*	R^2	*p*	*B*	*p*	95% *CI*	R^2	*p*
constant	−.56	.04	−1.12; -.04	.16	.00	−1.31	.00	−2.20; -.64	.05	.35
chronotype	.15	.01	.05; .25			.06	.53	−.09; .25		
sleep loss	−.06	.03	−.13; -.02			−.07	.10	−.13; .07		
chronotype*sleep loss	.018	.49	−.04; .07	.01	.30	−.06	.38	−.19; .07	.01	.44

Note. Sex is coded 1 = women, 0 = men; chronotype and sleep loss expressed in hours; *CI* confidence interval for coefficients.

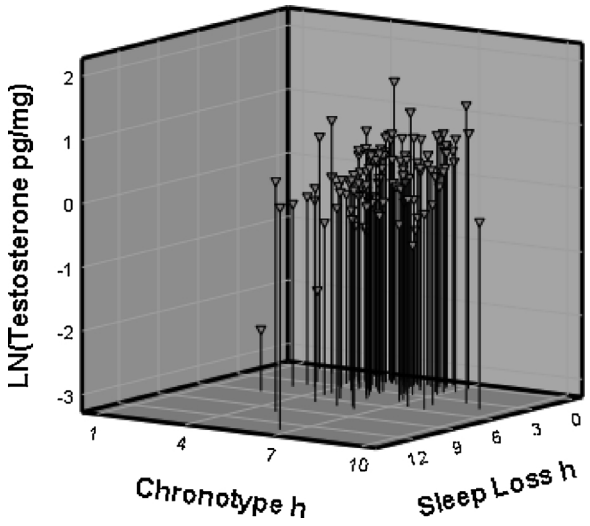

Fig. 3. Scatterplot showing natural logarithm (LN) of testosterone levels across the range of chronotypes (in hours) and sleep loss (in hours) in men.

a regular schedule); if it holds true, then no association between eveningness and higher testosterone levels should be visible in zero-order correlations, but it should emerge after controlling for sleep loss (suppression effect).

Another conclusion from the abovementioned results is that evening-oriented males who do not experience sleep loss have the highest levels of testosterone. This conclusion seems to be further supported indirectly by some behavioural data. Specifically, men with higher testosterone levels are more interested in multiple sexual partners (van Anders, 2013). This interest is related to eveningness in samples dominated by university students, who presumably experience lower sleep loss due to having greater freedom to live according to their biological clocks (Matchock, 2018; Randler et al., 2012a; 2016), but is unrelated to chronotype in worker-dominated samples, who presumably have more sleep restrictions (Jankowski et al., 2014).

The lack of association between chronotype and DHEA in men suggests that chronotype may not depend on steroids nonspecific for sex. On the other hand, the result showing that sleep loss is related to lower DHEA levels is a novel finding requiring further replication, given that previous reports on DHEA levels in the context of sleep are scarce. For instance, Schüle et al. (2003) showed that DHEA does not change as a reaction to sleep loss in a small sample of patients of both sexes suffering from major depression.

Our study showed that none of the analysed hormones in women were related to sleep variables. Maestripieri (2014) demonstrated a similar result – in women, there were no associations between testosterone and chronotype. One possible explanation is that testosterone levels in the vast majority of women are too low to affect sleep-wake behaviour. Furthermore, testosterone levels in women did not react to sleep loss (Cote et al., 2013).

Our regression model for DHEA showed that associations between sleep variables and DHEA do not differ between men and women (interactions with sex were statistically non-significant), but a correlation analysis conducted on the women subsample showed no relationship. This conflicting result is likely due to an insufficient sample size to prove a statistically significant difference between men and women in the strength of the correlations between DHEA and sleep loss. Specifically, considering the abovementioned correlations in both sexes, a sample of at least 165 participants per sex would be needed to show a statistically significant difference between the coefficients (−.22 vs. − .04; Lenhard and Lenhard, 2014). Furthermore, the coefficient of correlation between DHEA and sleep loss in women (-.04) is below the accepted level for a meaningful correlation; thus, we conclude that there is no such association in women (though it exists in men). Previous studies have devoted little attention to DHEA levels in relation to sleep in women; the aforementioned study by Schüle et al. (2003) can be considered to be in line with our results showing no association between levels of DHEA and sleep in women. Finally, we found no links between progesterone and the analysed sleep variables in women. This result does not rule out the possibility that progesterone plays a role in sleep parameters other than chronotype, social jetlag and sleep loss. For instance, Deurveilher and colleagues (2009) showed that in female rats, progesterone impacts sleep architecture following sleep loss.

In the current study, we did not analyse oestrogen, which is the main limitation of the conclusions. Specifically, if one hypothesises that major sex hormones impact chronotypes, then one can expect that higher levels of oestrogen would be related to the evening chronotype in women. Though oestrogen is known to play a role in some aspects of women's sleep, such as sleep quality (Mong et al., 2011), the proposed hypothesis regarding oestrogen's role in chronotype requires direct testing in future research. To provide a deeper insight, future research could also record other variables that we have not recorded, such as body mass index, smoking, marital status or having children, as these variables can eventually relate to hormone levels and sleep variables. For instance, a higher body mass index was observed in men with lower testosterone (Osuna et al., 2006) and those with an evening chronotype (Lucassen et al., 2013). It should also be considered that sleep deprivation increases stress (Minkel et al., 2012), which can eventually inhibit hair growth (Peters et al., 2006) and, consequently, affect hormone concentrations in hair.

Conflict of interest

The authors report no conflicts of interest.

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Appendix A

Regression models controlling for age
See Tables A1 and A2.

Table A1
Results of regression analyses with ln (natural logarithm) of hormone levels as the outcome predicted by variables entered in subsequent blocks (model) controlling for age.

	Ln(DHEA)					Ln(Progesterone)				
Model	*B*	*p*	95% *CI*	R^2	*p*	*B*	*p*	95% *CI*	R^2	*p*
constant	2.91	.00	1.65; 4.27	.11	.00	.00	1.00	−.83; .79	.36	.00
age	.01	.66	−.03; .05			−.01	.58	−.03; .02		
sex	−.48	.00	−.69; -.27			.90	.00	.74; 1.07		
chronotype	.02	.70	−.08; .13			.00	.94	−.07; .07		
sleep loss	−.05	.03	−.09; -.00			−.02	.31	−.05; .02		
sex*chronotype	−.05	.59	−.25; .13	.01	.47	.08	.20	−.04; .22	.01	.41
sex*sleep loss	.04	.43	−.05; .14			.02	.67	−.06; .11		
chronotype*sleep loss	−.02	.24	−.07; .01			.02	.11	−.01; .05		
sex* chronotype*sleep loss	.00	.93	−.08; .08	.00	.95	−.02	.48	−.09; .04	.00	.56

Note. Sex coded 0 = men, 1 = women; chronotype and sleep loss expressed in hours; *CI* confidence interval for coefficients.

Table A2
Results of regression analyses with ln (natural logarithm) of testosterone levels as the outcome predicted by variables entered in subsequent blocks (model), separately for men and women, controlling for age.

	Ln(Testosterone) in Men					Ln(Testosterone) in Women				
Model	*B*	*p*	95% *CI*	R^2	*p*	*B*	*p*	95% *CI*	R^2	*p*
constant	−1.10	.07	−2.28; .02	.17	.00	−.40	.70	−2.33; 1.54	.07	.39
age	.02	.23	−.01; .05			−.04	.31	−.10; .03		
chronotype	.16	.01	.06; .28			.05	.59	−.11; .23		
sleep loss	−.06	.04	−.13; -.02			−.09	.08	−.17; .05		
chronotype*sleep loss	.02	.54	−.04; .06	.01	.34	−.05	.45	−.20; .11	.01	.50

Note. Sex is coded 1 = women, 0 = men; chronotype and sleep loss expressed in hours; *CI* confidence interval for coefficients.

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