The impact of prior day sleep and physical activity on the cortisol awakening response

By: Travis Anderson, Gail Corneau, Laurie Wideman, Kari Eddington, Suzanne Vrshek-Schallhorn

Anderson, T., Corneau, G., Wideman, L., Eddington, K., Vrshek-Schallhorn, S. (2021). The impact of prior day sleep and physical activity on the Cortisol Awakening Response. Psychoneuroendocrinology, 126:105131. https://doi.org/10.1016/j.psyneuen.2021.105131

Abstract:

The cortisol awakening response (CAR) describes the increase in cortisol within the first 30–60 min after waking from nocturnal sleep, and is a common biomarker used within psychoneuroendocrinology, but the effect of sleep on the CAR is currently unclear. A previous study suggested that reported discrepancies may be due to other lifestyle behaviors such as physical activity; given the role of the CAR in energy regulation and preparation for the day, it is theoretically plausible that activity level would influence the CAR. However, no study has yet utilized objective monitoring of day-to-day sleep and physical activity to investigate potential effects on the CAR. This study aimed to test the hypotheses that either sleep duration or sleep quality would interact with the prior 24 h′ physical activity to predict the CAR on the following morning. Salivary samples were collected from 85 young adults (mean = 19.1 years, SD = 1.89) immediately after waking from nocturnal sleep and again 30 min after waking; two complete and consecutive days were used. Participants wore accelerometers (ActiGraph, wGT3X-BT) throughout this phase of a larger study, which provided objective measures of sleep duration, number of awakenings, and amount of physical activity. Mixed-effects models with post-hoc regions of significance decompositions tested the hypothesized interaction effects. Results demonstrated a significant interaction between prior day sleep duration and physical activity predicting the next day CAR, wherein short sleep duration and high levels of physical activity resulted in an augmented CAR. Although more sleep clearly predicted a smaller next day CAR in main effect, this study provides additional support that sleep duration effects are also moderated by prior day physical activity. Both behavioral factors should be considered when assessing the CAR and the association between the CAR other psychoneuroendocrine outcomes.

Keywords: actigraphy | exercise | cortisol awakening response

Article:

1. Introduction

The cortisol awakening response (CAR) reflects the increase in cortisol concentrations upon waking from nocturnal sleep and is considered distinct from the circadian regulation of cortisol (Stalder et al., 2016). The CAR is implicated in the pathophysiology of type II diabetes (Bruehl et al., 2009), metabolic syndrome (Kuehl et al., 2015), depression (Vrshek-Schallhorn et al., 2013), and anxiety disorders (Adam et al., 2014). Elucidating factors that influence the CAR, such as sleep, may improve the prevention and intervention strategies to ameliorate these conditions and positively impact public health. Fekedulegn et al. (2018) reported that the effect of sleep on the CAR depends upon physical activity level. However, no prior investigations have studied the association of the CAR with objectively measured sleep and physical activity. The present study therefore aimed to test whether prior day objectively measured physical activity would interact with either objectively measured sleep quantity or sleep quality to predict next day CAR.

1.1. Sleep and the CAR

The association between sleep and the hypothalamic-pituitary-adrenal axis (HPA-axis) is complex and bidirectional (Balbo et al., 2010), as alterations in sleep parameters can disturb circadian cortisol rhythms, whilst hyperactivity of the HPA-axis can also inhibit sleep and increase the frequency of awakening (Tsai et al., 2019). Elevated cortisol concentrations have been observed after both a single acute sleep deprivation event (Wright et al., 2015) and sleep restriction periods (Reynolds et al., 2012). Consistently poor self-reported sleep quality has also been associated with a larger cortisol increase during the waking period, but a flatter overall diurnal cortisol profile (Abell et al., 2016).

Specific to the CAR, research has indicated that patients suffering from insomnia tend to have a lower CAR (Backhaus et al., 2004, Castro-Diehl et al., 2015) and nurses reporting low sleep quality tend to produce flatter CARs (Tsai et al., 2019). In contrast, shorter sleep duration has been associated with an increased CAR (Kumari et al., 2009). Not all studies, however, report significant relations between sleep and the CAR (Eek et al., 2012, Garde et al., 2012). Thus, although the balance of prior research appears to support that acute sleep deprivation is linked to larger CAR while chronic sleep deprivation is linked with smaller CAR, there is also inconsistency in these findings, suggesting between-study moderators.

One difference across studies is the variation in sleep assessment tools used. Some researchers have highlighted the poor agreement between objective and subjective measurement of sleep variables and have suggested the reason for this discrepancy may lie in the subjective sleep questionnaires capturing other traits, such as perceived stress (Girschik et al., 2012, Van Den Berg et al., 2008). Further, self-report sleep tools often provide average subjective sleep quality rather than data specific to the day when the CAR is measured. Since the CAR has high day-to-day variability and is highly dependent on state factors (Hellhammer et al., 2007), capturing day-level sleep values would permit a more temporally proximate assessment of sleep's relationship to the CAR.

1.2. Physical Activity and the CAR

The physical activity or exercise on the previous day is another potential moderating variable not often examined in CAR studies. There are at least four theoretical considerations to suggest that

physical activity ought to impact the CAR, although these postulations are not easily reconcilable and may in fact be contradictory. First, exercise training can reduce basal cortisol levels (Marx et al., 2001), and increased physical fitness has been shown to reduce cortisol reactivity to psychological stressors (Mücke et al., 2018). Hypothetically, this may also be applicable to the response to waking, suggesting that more physical activity would reduce the CAR. Indeed, Martikainen et al. (2014) reported a negative association between the CAR and average levels of vigorous physical activity in adolescents.

Second, trained athletes can demonstrate an augmented cortisol response to maximal exercise, attributable to adrenal hypertrophy (Viru, 1992). If the CAR is reflective of maximal adrenal capacity (Schmidt-Reinwald et al., 1999), exercise training may produce an augmented CAR. This effect was potentially observed in a group of older adults, where a 6-month aerobic exercise program resulted in an increased CAR (Drogos et al., 2019).

Third, acute exercise can increase circulating cortisol concentrations, which may have residual effects on the cortisol rhythm the following day. Indeed, Hackney and Viru (1999) demonstrated reduced cortisol concentrations during the night following a day-time exercise bout. These residual effects may then continue to impact HPA-axis function during the waking period. Fourth, exercise can have a moderate antidepressant effect (Josefsson et al., 2014), and its influence on improved mental health may extend to perception of demands, another known correlate of the CAR.

One model that has been posited to explain the functional role of the CAR is termed the "boost" hypothesis. This model suggests the CAR acts to promote mobilization and serves to support an individual in their response to the demands of the upcoming day (Adam et al., 2006). In this regard, increased physical activity may create a physiological expectation of needing increased resources and thus may provide an increased "boost" to meet upcoming demands. Despite the theoretical basis, the directionality of the effects of physical activity on the CAR are currently speculative and thus investigating the effect of daily physical activity on the CAR is clearly necessary.

Fekedulegn et al. (2018) first hypothesized that moderating lifestyle factors, including leisure time physical activity, could explain the somewhat disparate findings of sleep quality and duration on the CAR. The researchers reported that for law enforcement officers who reported that they did not meet physical activity guidelines, self-reported poor sleep quality was associated with a significantly flatter CAR. Thus, the purpose of the present study was to further elucidate the role of prior day physical activity and sleep on the CAR by objectively assessing physical activity and sleep characteristics (quantity and quality) on the day immediately prior to the CAR measurement. Based on the available evidence, we hypothesized that sleep duration would be negatively associated with the CAR on the following day. We also hypothesized that physical activity would separately interact with sleep quantity and sleep quality to predict the CAR the following day. However, because of limited and conflicting prior evidence, we made no a priori hypotheses with regards to the directionality of the effect of physical activity, or the directionality of interaction effects of physical activity and sleep duration or sleep quality.

2. Materials and methods

2.1. Participants

Research participants were recruited from a midsized southeastern university in the United States to participate in a larger 14-day diary study (total N for larger study = 162), in which they provided samples of saliva for cortisol assay and wore wrist accelerometers for the first 4 days. Participants were initially excluded from cortisol assays if they reported use of psychotropic or steroid-based medications (excluding birth control). Participants in the present secondary analysis of the data (N = 85) were selected from the larger sample. To accurately represent the influence of activity over the entire 24-h period prior to morning cortisol sampling, as well as to capture the cortisol awakening response over multiple days, only participants who had two complete consecutive days of data (i.e., activity one day to predict the next morning's CAR) and saliva samples from the following morning were included in analysis. The two consecutive days were therefore required in order to have multiple days of awakening salivary samples, coupled with an entire 24-hour period of actigraphy prior to saliva collection.

Participants were excluded if there was no actigraphy or low actigraphy wear time (< 80% wear time; excluded 36 participants), they did not have all saliva samples on the required days (excluded 23 participants), they did not have actigraphy data (including sleep) on the appropriate day to align with saliva (excluded 17 participants), or they did not have demographic data (excluded 1 participant). The demographics for the final sample (n = 85) included in the current study were not statistically different from the total sample in age (19.06 vs. 18.96 years), sex (72.9% vs. 75.2% female), or minority racial status (44.7% vs. 46.6% white), all p-values > 0.05. Participants provided signed, written documentation of informed consent; all procedures were approved by the University's Institutional Review Board.

2.2. Procedure

Participants wrist-wore accelerometers (ActiGraph, wGT3X-BT) for 4 consecutive weekdays and nights (Monday to Thursday, or Tuesday to Friday), beginning at 5 p.m. on the first day of the study (either a Monday or Tuesday), and ending between 10 a.m. and 5 p.m. on the final day of the study (corresponding to either a Thursday or Friday). On each morning of the study, participants self-collected saliva into sterile cryogenic vials by passive drool immediately after waking (S1) and again 30 min after waking (S2); additional samples were collected at bedtime but were not included in the present statistical models. Participants completed a brief tutorial at study enrollment on the importance of sampling at the designated times, i.e., immediately upon waking, and were provided with a digital timer set to 30 min to aid in obtaining the second morning sample each day. To objectively monitor sampling times, Medication Event Monitoring System (MEMS) TrackCap (Aardex) collection bottles stored straws for use during sampling, and experimenters informed participants that the TrackCaps would record timing. Participants were instructed to refrigerate samples until their return to the University, where they were stored at − 20 °C until they were shipped to Trier, Germany in one batch on dry ice for assay. Samples were analyzed for cortisol in duplicate via dissociation enhanced lanthanide fluoroimmunoassay by an independent laboratory (Dressendörfer et al., 1992). The cortisol awakening response was calculated as the change in cortisol concentration between the waking sample and the sample taken 30 min after waking (Boehringer et al., 2015).

Accelerometer data were analyzed in ActiLife software (v6.13.3). Physical activity was calculated and determined via the ActiLife implementation of the Freedson (1998) algorithm. Since hip-based actigraphy algorithms do not generalize well to wrist-worn accelerometers, we defined physical activity as time spent in what would be considered moderate to vigorous physical activity. However, due to the anatomical difference in placement of the accelerometer, this was conceptualized only as physical activity and we refrain from interpreting the intensity or level of exertion of this physical activity. Therefore, the total duration of physical activity is expected to be greater than a typical range of moderate to vigorous physical activity for this population. Sleep periods were analyzed via the ActiLife implementation of the Sadeh algorithm (Sadeh et al., 1995). Total sleep time and the number of awakenings metrics were used to represent sleep duration and sleep quality, respectively.

Additional data were collected at study enrollment and included in analysis as a priori covariates, in keeping with recent recommendations (Stalder et al., 2016). Participants' age, body mass index, and average parental education (indicator of socioeconomic status) were included as continuous time-invariant covariates, while self-reported wake time and time between samples (as determined by recorded TrackCap timestamps) were included as continuous time-varying covariates. Gender (0 = female, 1 = male), minority racial status (0 = white, 1 = non-white), nicotine use (0 = no, 1 = yes), hormonal contraceptive use among females (0 = no, 1 = yes), and menstrual cycle phase (self-reported within 8 days since last menses: 0 = follicular phase; otherwise: 1 = not follicular phase) were entered as binary time-invariant covariates.

2.3. Statistical analytic plan

Multilevel linear models assessed the extent to which sleep duration, physical activity, and their interaction predicted CAR (Model 1), and the extent to which sleep quality, physical activity, and their interaction predicted CAR (Model 2). We first fit models using maximal likelihood estimation, which permits comparison of models through − 2 log likelihood testing (Zuur et al., 2009). Unconditional models assessed appropriateness of mixed model structure via intraclass correlation coefficients (ICC). During the model selection process, both random slope and random slope-intercept models failed to converge and thus we chose random intercept only models. Following model selection, final model parameters were estimated using restricted maximum likelihood estimation to avoid downwardly biased estimates produced by models fit with maximum likelihood estimation (Zuur et al., 2009). Observations with missing person-level covariates were omitted from the models. In the event of a significant interaction effect, the interaction was decomposed using a regions of significance analysis with an online tool (Preacher et al., 2006). The code generated by this tool was subsequently imported into R and figures were reproduced using the ggplot2 package (Wickham, 2016).

All continuous predictor variables and time-varying continuous covariates were grand-mean centered to test the average effect of predictors across participants on the CAR. We chose grand-mean centering of day-level variables to permit inclusion of interaction effects, to appropriately interpret parameter estimates, and because this focuses the interpretation on the total or marginal effects of the predictors rather than decomposing the within- and between-person effects (Curran and Bauer, 2011). The ability to make meaningful interpretations of within-person effects is limited by including only two observations per person, decreasing the reasonableness of the person-mean centering approach. Moreover, decomposing the within-person and between-person effects by including both person-mean centering and person means in the model resulted in

models failing to converge (due to low numbers of observations within each person). Thus, results for sleep and activity should be interpreted as comparisons across the sample including both day and person level effects, and not strictly comparisons within the person.

In addition to these analyses, for any significant effect on CAR for physical activity, sleep duration, or sleep quality, follow-up analyses predicted S1 and S2 cortisol concentrations separately to probe whether observed relationships were specific and independent to the CAR. All mixed-effect models were computed via the lmer package (Bates et al., 2015) using R statistical software (R Core Team, 2018; version 3.5.2). Significance was set at $p < 0.05$ for all analyses.

Table 1. Participant demographics and mean (SD) values of predictor variables.

Variable	Proportion of Sample or Mean	Standard Deviation
Gender (% Female)	73	–
Contraceptive Use (% using; females only)	37.5	–
Menstrual Cycle Phase (% in follicular)	61.0	–
Nicotine Use (% using)	13	–
Minority Racial Status		
White (% of sample)	44.7	–
Black/African American (% of sample)	38.8	–
Asian (% of sample)	12.9	–
Non-white Hispanic (% of sample)	9.4	–
Middle-Eastern (% of sample)	2.4	–
Multi-Racial/Other (% of sample)	9.4	–
Age (years)	19.1	1.9
BMI (kg/m^2)	26.1	6.2
TrackCap Time Between Samples (mins)	31.10	6.95[a]
Wake Time (HH:MM)	8:07	1:22
Cortisol Wake (nmol/L)	8.11	5.16
Cortisol Wake + 30 min (nmol/L)	13.46	7.01
CAR (nmol/L)	5.35	6.65
Activity (h)	4.29	1.45
Sleep (h)	6.19	1.64
Number of Awakenings (#)	17.00	7.41

a The standard deviation reported here is not reflective of the TrackCap values that were included in the final model. The average time between S1 and S2 samples of the data used in the reported modelswas mean = 36.0 min, SD = 36.7 min. This relatively large standard deviation is a result of three participants with TrackCap values of 204, 190, and 385 min. To assess whether these values affected our models, we reanalyzed the dataset following the removal of these apparent outlier values. The reanalyzed models align with the results reported here, with the exception of the parameter estimate and 95% confidence interval for the TrackCap estimate being slightly altered (new TrackCap parameter estimate = 0.01; 95% CI: -0.16 – 0.18). Parameter estimates for predictor variables of interest remained significant and the directionality of effects remained the same. Without adequate justification for removing these participants from the analysis, and because the reanalyzed models were almost equivalent, we chose to retain all data in the analysis.

3. Results

3.1. Preliminary analyses

Participants demographics and values for predictor variables are detailed in Table 1. Cortisol concentrations demonstrated the expected increase in the awakening period, increasing on average 105% from waking for an average CAR of mean= 5.35, SD= 6.65 nmol/L.

3.2. Sleep duration, physical activity, and the cortisol awakening response (model 1)

Participants engaged in, on average, 4.29 h of physical activity (SD = 1.45) and had 6.19 h of total sleep duration (SD = 1.64) prior to each CAR assessment. The ICC of the unconditional model was 13%, which supports a nested structure with a modest contribution of within-subject variance. The fully conditional model with random intercept structure and including interaction effects is presented in Table 2.

Table 2. Model estimates for the association of total sleep time and physical activity on the cortisol awakening response the following morning.

	Model 1		
Predictors	***β* Estimates**	**CI**	**p**
(Intercept)	-1.92	-12.90 – 16.74	0.800
Wake Time	-0.28	-1.18 – 0.63	0.552
BMI	0.02	-0.17 – 0.20	0.864
Contraceptive Use	2.02	-1.00 – 5.05	0.190
Menstrual Cycle Phase	-3.76	-6.62 – -0.89	**0.010**
TrackCap mins	-0.01	-0.04 – 0.02	0.532
Nicotine Use	-0.64	-4.05 – 2.77	0.714
Minority Racial Status	-1.97	-4.40 – 0.46	0.112
Gender	2.55	-1.12 – 6.22	0.173
Age	0.22	-0.37 – 0.81	0.468
Average Parental Education	0.42	-0.34 – 1.18	0.279
Physical Activity	-0.05	-0.89 – 0.80	0.915
Total Sleep Time	-1.19	-1.95 – -0.44	**0.002**
Physical Activity x Total Sleep Time	-0.96	-1.55 – -0.38	**0.001**
N	73		
Observations	132		
Marginal R^2 / Conditional R^2	0.171 / 0.200		

Note: Wake Time = self-reported wake time on the morning of sample collection, in metric time; BMI = body mass index (height (m)/mass (kg)2); TrackCap Mins = objectively recorded time between the two CAR sample collections (mins); SES = socioeconomic status. Statistically significant effects are bolded.

Model results demonstrated a significant interaction between prior day physical activity and total sleep time (Fig. 1; $\beta = -0.96$, standard error (SE) = 0.30, $p = 0.001$).1 Decomposition demonstrated a negative effect of physical activity on the next day CAR when total sleep time was 1 SD above the average, and a positive effect of physical activity on the next day CAR when total sleep time was 1 SD below the average (Fig. 1A). In the converse decomposition, results demonstrated

increasing total sleep time had a negative effect on the next day CAR with average and above average physical activity, but total sleep time had no effect on the next day CAR with below average physical activity (Fig. 2A).2

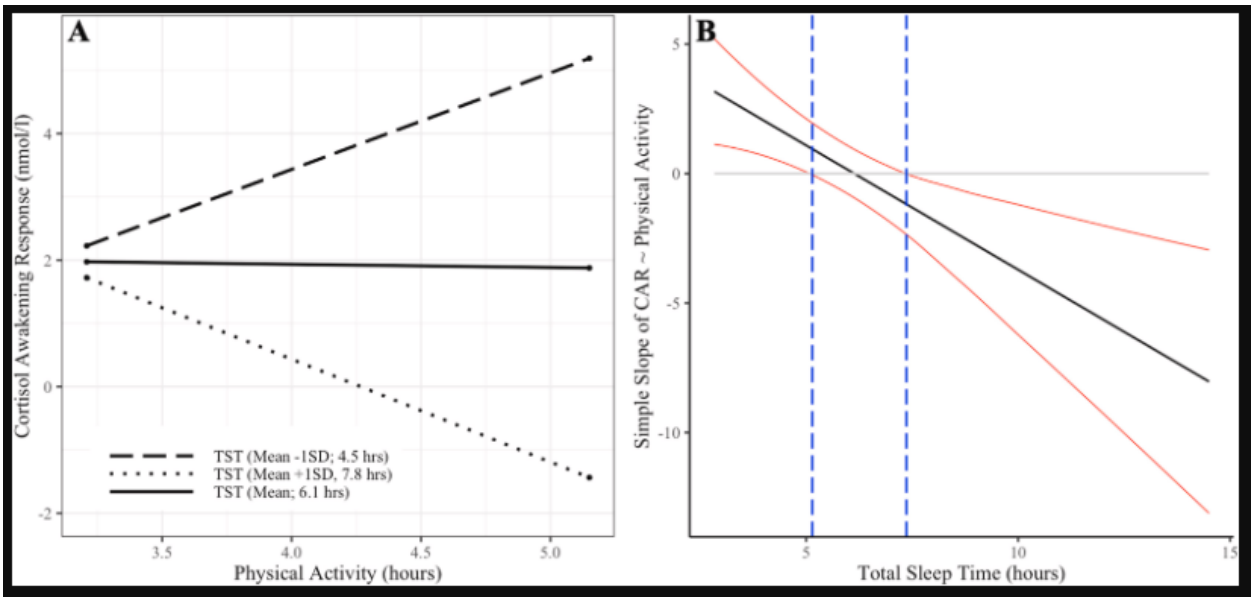

Fig. 1. Effect of prior day physical activity on the CAR at varying levels of total sleep time (A). Effect of prior day physical activity on the CAR is significant when TST < 5.14 h or TST > 7.37 h (B). TST = total sleep time.

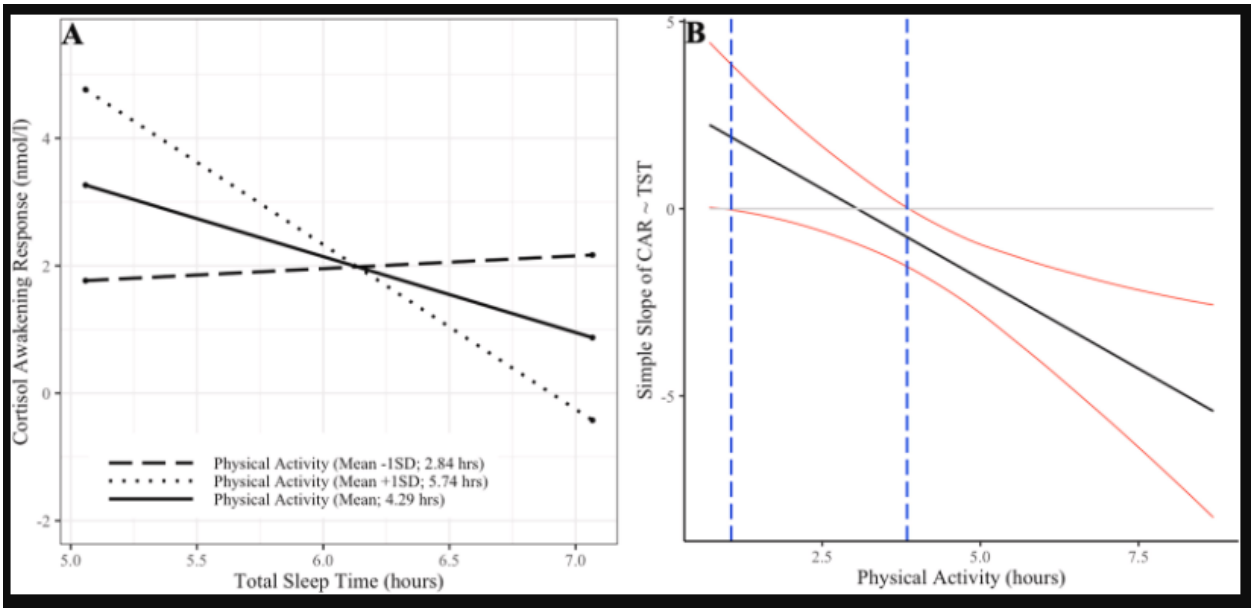

Fig. 2. Effect of prior night total sleep time on the CAR at varying levels of physical activity (A). Effect of prior night total sleep time on the CAR is significant when physical activity < 1.06 h or physical activity > 3.84 h (B). TST = total sleep time.

Regions of significance analysis on this interaction effect demonstrated the effect of physical activity on the next day CAR was significant when total sleep time was less than 5.14 h (47.1% of participants on at least one day), or greater than 7.37 h (32.9% of participants on at least one day; Fig. 1B). Conversely, the effect of total sleep time on the next day CAR (Fig. 2B) was significant when physical activity was less than 1.06 h (1.2% of participants on at least one day), or greater than 3.84 h (72.9% of participants, on at least one day).

3.3. Number of awakenings, physical activity, and the cortisol awakening response (Model 2)

Models examining sleep quality as number of nighttime awakenings demonstrated lack of improved model fit with the inclusion of predictor variables over the unconditional model ($X2(13) = 11.21$, $p = 0.59$). Results of the fixed effect parameter estimates, with non-significant interaction effect for awakenings and physical activity predicting next day CAR, are presented in Table 3.

Table 3. Model estimates for the association of number of awakenings and physical activity on the cortisol awakening response the following morning.

	Model 2		
Predictors	***β* Estimates**	**CI**	**p**
(Intercept)	9.17	-7.42 – 25.77	0.279
Wake Time	-0.49	-1.47 – 0.49	0.328
BMI	-0.02	-0.23 – 0.20	0.874
Contraceptive Use	0.57	-2.74 – 3.89	0.734
Menstrual Cycle Phase	-3.30	-6.47 – -0.13	**0.041**
TrackCap mins	-0.01	-0.05 – 0.02	0.502
Nicotine Use	-0.59	-4.36 – 3.19	0.761
Minority Racial Status	-1.58	-4.25 – 1.09	0.247
Gender	2.76	-1.29 – 6.81	0.181
Age	-0.08	-0.74 – 0.58	0.813
Average Parental Education	0.24	-0.60 – 1.09	0.572
Physical Activity	0.08	-0.83 – 0.99	0.866
Number of Awakenings	-0.14	-0.32 – 0.05	0.143
Physical Activity x Number of Awakenings	-0.04	-0.18 – 0.09	0.505
N	73		
Observations	132		
Marginal R^2 / Conditional R^2	0.077 / 0.203		

Note: Wake Time = self-reported wake time on the morning of sample collection, in metric time; BMI = body mass index (height (m)/mass (kg)2); TrackCap Mins = objectively recorded time between the two CAR sample collections (mins); SES = socioeconomic status. Statistically significant effects are bolded.

3.4. Post-hoc analysis of Individual Sample Levels Comprising CAR for Model 1

In order to rule out whether the significant effects of CAR were being driven by either of its constituent samples S1 or S2 alone, and whether a single saliva sample might actually provide more utility than the CAR, the significant interaction of sleep duration and physical activity on the next day CAR was probed further. Models were recomputed with the individual sample cortisol

levels used to calculate the CAR (the waking sample (S1), or the waking + 30 min sample (S2), as the dependent variable) and also assessed these relations while controlling for the CAR. Results for S1 demonstrated that total sleep time and physical activity did not significantly interact or independently predict S1. However, similar to findings in Model 1, when regressing on S2, total sleep time and physical activity demonstrated a significant interaction effect ($\beta = -0.90$, SE = 0.30, $p = 0.007$), and total sleep time had a significant independent effect ($\beta = -1.04$, SE = 0.39 $p = 0.008$). However, results do not support that S2 predicts beyond the CAR: when assessing the effect of prior day total sleep time and physical activity on S1 and S2 (in separate models) after controlling for the CAR, no significant interaction or main effects of S1 and S2 were observed.

4. Discussion

For the first time, and in agreement with our hypothesis regarding an interaction between physical activity and sleep duration, we demonstrate that when assessed using objective actigraphy measures, the effects of prior-night sleep quantity on next-morning CAR significantly depend upon prior-day physical activity level in a sample of emerging adults. Specifically, these results demonstrate that when total sleep time on the previous night was less than the sample average, increased physical activity levels on the previous day had a positive effect on the CAR, while above average total sleep time resulted in a negative effect on the CAR with increasing previous day physical activity. Alternatively, for average and above average physical activity, longer sleep duration on the previous night had a negative effect on the CAR, while sleep duration on the previous night had little to no effect on the CAR when physical activity was below average. However, in contrast to our hypothesis, there was no significant interactive effect for physical activity and sleep quality, as assessed by the number of actigraphy-identified awakenings, on the CAR.

4.1. Contextualizing the role of sleep on the CAR

The direct effect of sleep variables on the CAR is still under investigation, although several studies suggest that shortened sleep duration can result in a compensatory increase in the CAR. For example, Wüst et al. (2000) first reported a small increase in the CAR when sleep duration was shorter. Others have found similar effects, with a steeper CAR observed when sleep duration was shortened (Kumari et al., 2009, Vargas and Lopez-Duran, 2014). Although not an effect on the CAR per se, Serpell et al. (2019) demonstrated a negative relation between sleep duration and the salivary cortisol concentration 45 min after waking.

Our results further support that increasing sleep duration has a negative effect on the next day CAR, which likely explains the exaggerated CAR responses observed in participants who had shorter than average sleep. Within the context of the present study, the negative effect of increasing sleep duration was observed both for average and above average physical activity levels, while physical activity levels below the group average seemed to mask or decouple the effect of sleep duration on the CAR. Within the framework of the "boost" hypothesis, our results suggest that shortened nocturnal sleep, especially on nights preceded by moderate to high levels of physical activity, requires a greater activation of the HPA-axis and mobilization of resources the next morning to meet the demands of the upcoming day. Although likely an appropriate and necessary (i.e., healthy) response to these conditions, if the high CAR level we observed was suggestive of cortisol levels that are too high, persist too long, or represent frequent elevations, that this increased

glucocorticoid exposure may likely to be detrimental, as chronically high cortisol concentrations have been linked to a variety of health disorders (Stalder et al., 2013).

It is also possible that the effect of sleep observed in the present study is the result of sleep directly affecting those physiological mechanisms that regulate the CAR. Indeed, it has been suggested that the CAR is altered directly by sleep architecture and that the associations between the CAR and other health conditions are actually facilitated by disruptions to sleep patterns (Elder et al., 2016). Although the shortened sleep time in this study does not necessarily indicate an absolute or relative reduction in any specific stage of sleep, it is possible that some stages of sleep also become particularly disrupted when total sleep time is reduced. Recent evidence suggests that the CAR is positively associated with time spent in Stage 2 sleep specifically (Devine and Wolf, 2016, Elder et al., 2016, Lemola et al., 2015); however more work is needed to identify these physiological mechanisms and to establish whether physical activity interacts with disruptions to specific stages of sleep.

4.2. Role of physical activity

Prior day physical activity appears to buffer the effect of short sleep duration on the CAR, such that having average or above average physical activity can produce an augmented next day CAR even when sleep durations decrease, and increasing prior day physical activity levels can augment the CAR when sleep is below average. This is perhaps because greater physical activity compensates for the physiological stressfulness of limited opportunity to sleep. It is important to note that physical activity engagement and sedentary time are zero-sum and therefore these reported associations may actually be due to reduced sedentary behaviors. Sedentary behavior is known to negatively affect health outcomes (Wilmot et al., 2012) and the CAR has been reported to be positively associated with the average amount of sedentary time in adolescents (Martikainen et al., 2014). Further, because we did not delineate specific physical activity intensities in the current study, the directionality of the relation between physical activity and health, and consequently the CAR, may have been altered. However, previous work has suggested that both overall and vigorous physical activity are negatively associated with the CAR in adolescent girls (Martikainen et al., 2014). Results from this same study also provide further support for the results reported in the present study. For example, Martikainen et al. (2014) reported a negative association between physical activity and the CAR and although an interaction between sleep and physical activity was not specifically tested by the researchers, the average sleep duration for girls (8.1 h) exceeded the duration of sleep we reported in our interaction decomposition (7.37 h) that resulted in a negative effect of physical activity on the CAR. It should be noted however that in contrast to the present study, Martikainen and colleagues used an average of vigorous activity levels over the study period (> 4 days), studied a younger population (~12.4 years old), recorded 71% of the CARs on a weekend compared to the weekday CAR assessment in the present study, and the relations were only observed in adolescent girls despite total and vigorous activity being greater in the adolescent boys (Martikainen et al., 2014).

Conceptually, high levels of physical activity may indicate a generally good health status and thus result in more typical (or “healthy”) CARs—defined as a 50–156% increase in cortisol concentrations within the first hour after waking (Clow et al., 2004). Reduced levels of physical activity have been previously linked to poor cardiometabolic health (Camhi et al., 2011, Chastin et al., 2015, Henson et al., 2013). This effect may be influencing the present results, as the CAR is reported to have a negative relation with some metabolic syndrome symptoms such as elevated

blood pressure (Kuehl et al. 2015). However, it is important to consider that the effect of physical activity may be moderated by the context in which the physical activity occurs. For example, physical activity accumulated as a function of one's occupation may be detrimental to health, whereas leisure-time physical activity is protective (Holtermann et al., 2012, Holtermann et al., 2018). Though this distinction cannot be formally made in the present analysis, because participants in this study represented students within an academic institution it is likely that much of the physical activity occurred outside of an occupational context.

Alternatively, high physical activity, as recorded in this study, may represent an acute exercise bout that results in elevated cortisol concentrations, which may remain elevated into the morning waking period. The increase in cortisol over the waking period is likely to be lessened when initial cortisol concentration is higher, and therefore we may expect high activity to be associated with a low CAR. This is, however, a separate consideration from whether there is an interaction between chronic or habitually high physical activity levels and single bouts of acute activity that deviate from the levels an individual is accustomed to. It is possible that excessively high quantities of high-intensity physical activity would produce an excessively high CAR, given that exercise intensity is related to momentary cortisol response (Hill et al., 2008) and thus the intensity of physical activity engagement should be considered in future studies. Because of this study's focus on acute assessment of daily behaviors, habitual physical activity measures are regrettably not available for the present analyses and therefore no conclusions are made about whether the two days assessed here represent participants' typical activity levels.

Although the present study did not directly investigate exercise engagement, comparisons to the effect of exercise on the CAR are pertinent. Few studies have directly investigated this relation, but Anderson et al. (2018) demonstrated a positive correlation between the physiological load within normal exercise training in endurance runners and the CAR on the next day. Since exercise can affect blood glucose levels, and cortisol concentrations may be affected into the sleep period and into the following day, negative feedback to the hypothalamus may become disturbed and have an indirect effect on the CAR. The present results suggest that this may only occur in the context of longer sleep durations, although the increased sleep durations of the current sample only approach what is considered to be an optimal duration of sleep (> 7 h).

4.3. Factors influencing the interaction of sleep and physical activity

The present results partially replicate the association reported by Fekedulegn et al. (2018), as we also demonstrated an interaction of physical activity and sleep predicting the CAR. In contrast however, we did not observe an independent effect of objective sleep quality, but rather an interaction between physical activity and sleep duration. Moreover, we demonstrated that with increasing previous day physical activity, lower sleep duration resulted in an augmented CAR. These findings do however support those reported by Fekedulegn et al. in that combined low physical activity and sleep duration predicted a lower CAR. Our findings may differ from these, however, because the self-reported sleep and physical activity data in Fekedulegn and colleagues' study was reflective of the previous 30 and 7 days, respectively, whereas our data is temporally coupled to the CAR assessment in a day-to-day fashion. As such, it may be true that the CAR will respond differentially to acute disruptions as opposed to changes in global sleep quality when aggregated over many days or weeks. Importantly, there are a number of distinctions between the results reported by Fekedulegn et al. and the present study. For example, the current study recruited young adults primarily from the college environment, which is a notably distinct population from

the adult emergency services personnel studied by Fekedulegn et al. who may be more likely than the present sample to engage in shift work or have varying sleep schedules throughout the day. Additionally, the metric used in the present study as an estimate of "sleep quality" was the number of awakenings recorded. Fekedulegn et al. utilized a composite measure of seven sleep variables, where both sleep duration and the number of nightly awakenings were sub-components. The lack of interaction observed in Model 2 may be reflective of the metric chosen rather than sleep quality per se.

Our results suggest a relation between physical activity, sleep duration, and the next day CAR; however, it is unknown whether these behaviors are directly causative. Low physical activity is related to a range of health concerns such as cancer, cardiovascular disease, and all-cause mortality (Powell et al., 2018), and shortened sleep duration is associated with hypertension (Gottlieb et al., 2006) and diabetes mellitus (Gottlieb et al., 2005). It is therefore possible that an unobserved health-related construct such as cardiometabolic risk is affecting the CAR, and sleep duration and physical activity are merely representing this latent variable. Despite the relatively young of age of this sample, there is evidence that cardiometabolic risk factors can already be present in young adults (Camhi and Katzmarzyk, 2010, Dalleck and Kjelland, 2012), including in those that are physically active (Hruby et al., 2017) and not obese (Otelea et al., 2019). Moreover, physical activity and sleep are unlikely to be merely passive indicators of underlying health as they both interact dynamically with these health constructs. Improvements in physical activity and sleep behaviors can result in a multitude of physiological and psychological benefits which may also have secondary effects on the CAR. However, in the absence of additional data, the impact of these health-related constructs remains speculative. We also acknowledge that little is known about the effect of lifestyle behaviors on health outcomes during adolescence and emerging adulthood, and thus more research on this specific developmental window is required.

The interaction of physical activity and sleep duration is admittedly not easily interpretable, as these two variables have bidirectional effects on one another. Adequate levels of physical activity are known to improve both objective and subjective sleep quality (Kredlow et al., 2015). However, poor sleep quality or duration can often result in lethargy and worsening physical activity behaviors. Nonetheless, our results suggest that if researchers are attempting to characterize the relation between physical activity and the CAR, then the duration of sleep must also be considered in order to appropriately identify physical activity's effects. The present study was observational in nature and thus the effect of systematically manipulating physical activity and sleep on the CAR should be considered in future work.

4.4. Limitations

Despite several strengths including objective actigraphy measurement of sleep variables and activity level, objective measurement of the time between the two CAR samples, and measurement of many recommended covariates (Stalder et al., 2016), this study is not without limitations. First, actigraphy data were collected via wrist-worn accelerometers. Although commonly used, algorithms for the detection and quantification of activity at specifically identified intensities have not been reported and validated for wrist-worn actigraphy in the extant literature. This is an active area of research (Montoye et al., 2020) and despite the option to specify the accelerometers as wrist-worn within the software (which scales the counts per minute values to new cutoffs), this may not be an appropriate method for determining intensity of activity. We therefore opted to report these results as physical activity only and make no inference to the intensity of the activity.

Second, given the inclusion criteria used to ensure validity of the model findings, the analytic sample was limited to 85 participants. Although a total of 170 separate observations were captured in analyses, replication of these effects in an independent sample is recommended. Third, saliva samples were collected at only two timepoints for the calculation of the CAR. It has been recommended to collect multiple samples at 15 min intervals in the post-waking period (Stalder et al., 2016). This recommendation allows the observation of the true peak value (the peak may occur at 45 min after waking in females; Schlotz et al., 2004, Wust et al., 2000), as well as to allow the calculation of other CAR indices such as the area under the curve, which may be differentially associated with the sleep and physical activity metrics analyzed in the present study. These results should therefore be replicated using a more frequent sampling procedure, whilst remaining mindful of the increasing participant burden. Fourth, we did not measure participants' chronotypes. It has recently been demonstrated that self-reported evening-ness or morning-ness will affect the CAR (Petrowski et al., 2020). This intrinsic difference in the timing of sleeping and waking may also interact with physical activity patterns and should be studied further.

5. Conclusions

We demonstrated that the interaction of objectively measured sleep duration and physical activity is associated with the CAR on the following morning. Given the interaction between these variables, these models suggest that if the effect of either prior night sleep or prior day physical activity on the CAR is to be investigated, they should be studied together. Moreover, these data support the notion that prior day physical activity and sleep can acutely affect the CAR and thus should be considered as covariates in future studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The research was supported by internal institutional funds awarded to SVS.

References

Abell, J.G., Shipley, M.J., Ferrie, J.E., Kivim¨aki, M., Kumari, M., 2016. Recurrent short sleep, chronic insomnia symptoms and salivary cortisol: a 10-year follow-up in the Whitehall II study. Psychoneuroendocrinology 68,, 91–99. https://doi.org/10.1016/j.psyneuen.2016.02.021.

Adam, E.K., Hawkley, L.C., Kudielka, B.M., Cacioppo, J.T., 2006. Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults. Proc. Natl. Acad. Sci. USA 103 (45), 17058–17063. https://doi.org/10.1073/pnas.0605053103.

Adam, E.K., Vrshek-Schallhorn, S., Kendall, A.D., Mineka, S., Zinbarg, R.E., Craske, M.G., 2014. Prospective associations between the cortisol awakening response and first onsets of anxiety disorders over a six-year follow-up–2013 Curt Richter Award Winner. Psychoneuroendocrinology 44, 47–59. https://doi.org/10.1016/j.psyneuen.2014.02.014.

Anderson, T., Lane, A.R., Hackney, A.C., 2018. The cortisol awakening response is associated with training load in endurance runners. Int. J. Sports Physiol. Perform. 1–21. https://doi.org/10.1123/ijspp.2017-0740.

Backhaus, J., Junghanns, K., Hohagen, F., 2004. Sleep disturbances are correlated with decreased morning awakening salivary cortisol. Psychoneuroendocrinology 29 (9), 1184–1191. https://doi.org/10.1016/j.psyneuen.2004.01.010.

Balbo, M., Leproult, R., Van Cauter, E., 2010. Impact of sleep and its disturbances on hypothalamo-pituitary-adrenal axis activity. Int. J. Endocrinol. 2010, 1–16. https://doi.org/10.1155/2010/759234.

Bates, D., M¨achler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Van Den Berg, J.F., Van Rooij, F.J., Vos, H., Tulen, J.H., Hofman, A., Miedema, H.M., Neven, A.K., Tiemeier, H., 2008. Disagreement between subjective and actigraphic measures of sleep duration in a population-based study of elderly persons. J. Sleep Res. 17 (3), 295–302. https://doi.org/10.1111/j.1365-2869.2008.00638.x.

Boehringer, A., Tost, H., Haddad, L., Lederbogen, F., Wüst, S., Schwarz, E., Meyer- Lindenberg, A., 2015. Neural correlates of the cortisol awakening response in humans. Neuropsychopharmacology 40 (9), 2278–2285. https://doi.org/10.1038/npp.2015.77.

Bruehl, H., Wolf, O.T., Convit, A., 2009. A blunted cortisol awakening response and hippocampal atrophy in type 2 diabetes mellitus. Psychoneuroendocrinology 34 (6), 815–821. https://doi.org/10.1016/j.psyneuen.2008.12.010.

Camhi, S.M., Katzmarzyk, P.T., 2010. Tracking of cardiometabolic risk factor clustering from childhood to adulthood. Int. J. Pediatr. Obes. 5 (2), 122–129.

Camhi, S.M., Sisson, S.B., Johnson, W.D., Katzmarzyk, P.T., Tudor-Locke, C., 2011. Accelerometer-determined moderate intensity lifestyle activity and cardiometabolic health. Prev. Med. 52 (5), 358–360. https://doi.org/10.1016/j.ypmed.2011.01.030.

Castro-Diehl, C., Diez Roux, A.V., Redline, S., Seeman, T., Shrager, S.E., Shea, S., 2015. Association of sleep duration and quality with alterations in the hypothalamic- pituitary adrenocortical axis: the Multi-Ethnic Study of Atherosclerosis (MESA). J. Clin. Endocrinol. Metab. 100 (8), 3149–3158. https://doi.org/10.1210/jc.2015-1198.

Chastin, S.F., Palarea-Albaladejo, J., Dontje, M.L., Skelton, D.A., 2015. Combined effects of time spent in physical activity, sedentary behaviors and sleep on obesity and cardio-metabolic health markers: a novel compositional data analysis approach. PLoS One 10 (10), e0139984. https://doi.org/10.1371/journal.pone.0139984.

Clow, A., Thorn, L., Evans, P., Hucklebridge, F., 2004. The awakening cortisol response: methodological issues and significance. Stress 7 (1), 29–37.

Curran, P.J., Bauer, D.J., 2011. The disaggregation of within-person and between-person effects in longitudinal models of change. Annu. Rev. Psychol. 62, 583–619. https://doi.org/10.1146/annurev.psych.093008.100356.

Dalleck, L.C., Kjelland, E.M., 2012. The prevalence of metabolic syndrome and metabolic syndrome risk factors in college-aged students. Am. J. Health Promot. 27 (1), 37–42.

Devine, J.K., Wolf, J.M., 2016. Determinants of cortisol awakening responses to naps and nighttime sleep. Psychoneuroendocrinology 63, 128–134. https://doi.org/10.1016/j.psyneuen.2015.09.016.

Dressend¨orfer, R., Kirschbaum, C., Rohde, W., Stahl, F., Strasburger, C., 1992. Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. J. Steroid Biochem. Mol. Biol. 43 (7), 683–692. https://doi.org/10.1016/0960-0760(92)90294-S.

Drogos, L.L., Wynne-Edwards, K., Zhou, R., Hall, S.E., Tyndall, A.V., Longman, R.S., Eskes, G., Poulin, M.J., 2019. Aerobic exercise increases cortisol awakening response in older adults. Psychoneuroendocrinology 103, 241–248. https://doi.org/10.1016/j.psyneuen.2019.01.012.

Eek, F., Karlson, B., Garde, A.H., Hansen, Å.M., Ørbæk, P., 2012. Cortisol, sleep, and recovery–Some gender differences but no straight associations. Psychoneuroendocrinology 37 (1), 56–64. https://doi.org/10.1016/j.psyneuen.2011.05.003.

Elder, G.J., Ellis, J.G., Barclay, N.L., Wetherell, M.A., 2016. Assessing the daily stability of the cortisol awakening response in a controlled environment. BMC Psychol. 4 (1), 3. https://doi.org/10.1186/s40359-016-0107-6.

Fekedulegn, D., Innes, K., Andrew, M.E., Tinney-Zara, C., Charles, L.E., Allison, P., Violanti, J.M., Knox, S.S., 2018. Sleep quality and the cortisol awakening response (CAR) among law enforcement officers: the moderating role of leisure time physical activity. Psychoneuroendocrinology 95, 158–169. https://doi.org/10.1016/j. psyneuen.2018.05.034.

Garde, A.H., Albertsen, K., Persson, R., Hansen, Å.M., Rugulies, R., 2012. Bi-directional associations between psychological arousal, cortisol, and sleep. Behav. Sleep Med. 10 (1), 28–40. https://doi.org/10.1080/15402002.2012.636272.

Girschik, J., Fritschi, L., Heyworth, J., Waters, F., 2012. Validation of self-reported sleep against actigraphy. J. Epidemiol. 22 (5), 462–468. https://doi.org/10.2188/jea.JE20120012.

Gottlieb, D.J., Punjabi, N.M., Newman, A.B., Resnick, H.E., Redline, S., Baldwin, C.M., Nieto, F.J., 2005. Association of sleep time with diabetes mellitus and impaired glucose tolerance. Arch. Intern. Med. 165 (8), 863–867. https://doi.org/10.1001/archinte.165.8.863.

Gottlieb, D.J., Redline, S., Nieto, F.J., Baldwin, C.M., Newman, A.B., Resnick, H.E., Punjabi, N.M., 2006. Association of usual sleep duration with hypertension: the Sleep Heart Health Study. Sleep 29 (8), 1009–1014. https://doi.org/10.1093/sleep/29.8.1009.

Hackney, A., Viru, A., 1999. Twenty-four-hour cortisol response to multiple daily exercise sessions of moderate and high intensity. Clin. Physiol. 19 (2), 178–182. https://doi.org/10.1046/j.13652281.1999.00157.x.

Hellhammer, J., Fries, E., Schweisthal, O., Schlotz, W., Stone, A., Hagemann, D., 2007. Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. Psychoneuroendocrinology 32 (1), 80–86.

Henson, J., Yates, T., Biddle, S.J., Edwardson, C.L., Khunti, K., Wilmot, E.G., Gray, L.J., Gorely, T., Nimmo, M.A., Davies, M.J., 2013. Associations of objectively measured sedentary

behaviour and physical activity with markers of cardiometabolic health. Diabetologia 56 (5), 1012–1020. https://doi.org/10.1007/s00125-013-2845-9.

Hill, E., Zack, E., Battaglini, C., Viru, M., Viru, A., Hackney, A., 2008. Exercise and circulating cortisol levels: the intensity threshold effect. J. Endocrinol. Invest 31 (7), 587–591.

Holtermann, A., Hansen, J., Burr, H., Søgaard, K., Sjøgaard, G., 2012. The health paradox of occupational and leisure-time physical activity. Br. J. Sports Med. 46 (4), 291–295.

Holtermann, A., Krause, N., Van Der Beek, A.J., Straker, L., 2018. The physical activity paradox: Six reasons why occupational physical activity (OPA) does not confer the cardiovascular health benefits that leisure time physical activity does.

Hruby, A., Bulathsinhala, L., McKinnon, C.J., Hill, O.T., Montain, S.J., Young, A.J., Smith, T.J., 2017. Body mass index at accession and incident cardiometabolic risk factors in US army soldiers, 2001–2011. PLoS One 12 (1), e0170144.

Josefsson, T., Lindwall, M., Archer, T., 2014. Physical exercise intervention in depressive disorders: meta-analysis and systematic review. Scand. J. Med. Sci. Sports 24 (2), 259–272. https://doi.org/10.1111/sms.12050.

Kredlow, M.A., Capozzoli, M.C., Hearon, B.A., Calkins, A.W., Otto, M.W., 2015. The effects of physical activity on sleep: a meta-analytic review. J. Behav. Med. 38 (3), 427–449. https://doi.org/10.1007/s10865-015-9617-6.

Kuehl, L.K., Hinkelmann, K., Muhtz, C., Dettenborn, L., Wingenfeld, K., Spitzer, C., Kirschbaum, C., Wiedemann, K., Otte, C., 2015. Hair cortisol and cortisol awakening response are associated with criteria of the metabolic syndrome in opposite directions. Psychoneuroendocrinology 51, 365–370. https://doi.org/10.1016/j.psyneuen.2014.09.012.

Kumari, M., Badrick, E., Ferrie, J., Perski, A., Marmot, M., Chandola, T., 2009. Self- reported sleep duration and sleep disturbance are independently associated with cortisol secretion in the Whitehall II study. J. Clin. Endocrinol. Metab. 94 (12), 4801–4809. https://doi.org/10.1210/jc.2009-0555.

Lemola, S., Perkinson-Gloor, N., Hagmann-von Arx, P., Brand, S., Holsboer-Trachsler, E., Grob, A., Weber, P., 2015. Morning cortisol secretion in school-age children is related to the sleep pattern of the preceding night. Psychoneuroendocrinology 52, 297–301. https://doi.org/10.1016/j.psyneuen.2014.12.007.

Martikainen, S., Pesonen, A.-K., Lahti, J., Heinonen, K., Pyh¨al¨a, R., Tammelin, T., Kajantie, E., Strandberg, T.E., Reynolds, R.M., R¨aikk¨onen, K., 2014. Physical activity and hypothalamic–pituitary–adrenocortical axis function in adolescents. Psychoneuroendocrinology 49, 96–105.

Marx, J.O., Ratamess, N.A., Nindl, B.C., Gotshalk, L.A., Volek, J.S., Dohi, K., Bush, J.A., G´omez, A.L., Mazzetti, S.A., Fleck, S.J., 2001. Low-volume circuit versus high- volume periodized resistance training in women. Med. Sci. Sports Exerc. 33 (4), 635–643.

Montoye, A.H., Clevenger, K.A., Pfeiffer, K.A., Nelson, M.B., Bock, J.M., Imboden, M.T., Kaminsky, L.A., 2020. Development of cut-points for determining activity intensity from a wrist-worn ActiGraph accelerometer in free-living adults. J. Sports Sci. 38, 2569–2578.

Mücke, M., Ludyga, S., Colledge, F., Gerber, M., 2018. Influence of regular physical activity and fitness on stress reactivity as measured with the trier social stress test protocol: a systematic review. Sports Med. 48 (11), 2607–2622. https://doi.org/10.1007/s40279-018-0979-0.

Otelea, M.R., Streinu-Cercel, A., B˘aicus, C., Nitescu, M., 2019. The adipokine profile and the cardiometabolic risk in non-obese young adults. Balk. Med. J. 36 (3), 155–161.

Petrowski, K., Schmalbach, B., Stalder, T., 2020. Morning and evening type: the cortisol awakening response in a sleep laboratory. Psychoneuroendocrinology 112,, 104519. https://doi.org/10.1016/j.psyneuen.2019.104519.

Powell, K.E., King, A.C., Buchner, D.M., Campbell, W.W., DiPietro, L., Erickson, K.I., Hillman, C.H., Jakicic, J.M., Janz, K.F., Katzmarzyk, P.T., 2018. The scientific foundation for the physical activity guidelines for Americans. J. Phys. Act. Health 16 (1), 1–11. https://doi.org/10.1123/jpah.2018-0618.

Preacher, K.J., Curran, P.J., Bauer, D.J., 2006. Computational tools for probing interactions in multiple linear regression, multilevel modeling, and latent curve analysis. J. Educ. Behav. Stat. 31 (4), 437–448. https://doi.org/10.3102/10769986031004437.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 〈https://www.R-project.org〉. Reynolds, A.C., Dorrian, J., Liu, P.Y., Van Dongen, H.P., Wittert, G.A., Harmer, L.J., Banks, S., 2012. Impact of five nights of sleep restriction on glucose metabolism, leptin and testosterone in young adult men. PLoS One 7 (7), e41218. https://doi.org/10.1371/journal.pone.0041218.

Sadeh, A., Hauri, P.J., Kripke, D.F., Lavie, P., 1995. The role of actigraphy in the evaluation of sleep disorders. Sleep 18 (4), 288–302. https://doi.org/10.1093/sleep/18.4.288.

Schlotz, W., Hellhammer, J., Schulz, P., Stone, A.A., 2004. Perceived work overload and chronic worrying predict weekend–weekday differences in the cortisol awakening response. Psychosom. Med. 66 (2), 207–214.

Schmidt-Reinwald, A., Pruessner, J., Hellhammer, D., Federenko, I., Rohleder, N., Schürmeyer, T., Kirschbaum, C., 1999. The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. Life Sci. 64 (18), 1653–1660.

Serpell, B.G., Horgan, B.G., Colomer, C.M., Field, B., Halson, S.L., Cook, C.J., 2019. Sleep and salivary testosterone and cortisol during a short preseason camp: a study in professional Rugby Union. Int. J. Sports Physiol. Perform. 00, 1–9. https://doi.org/ 10.1123/ijspp.2018-0600.

Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S.R., Gao, W., Miller, R., Stark, S., Bosch, J.A., Fischer, J.E., 2013. Cortisol in hair and the metabolic syndrome. J. Clin. Endocrinol. Metab. 98 (6), 2573–2580. https://doi.org/10.1210/jc.2013-1056.

Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D.H., 2016. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology 63, 414–432.

Tsai, J.-C., Chou, K.-R., Tsai, H.-T., Yen, Y.-C., Niu, S.-F., 2019. Effects of nocturnal sleep quality on diurnal cortisol profiles and attention in nurses: a cross-sectional study. Biol. Res. Nurs. 21 (5), 510–518. https://doi.org/10.1177/1099800419861695.

Vargas, I., Lopez-Duran, N., 2014. Dissecting the impact of sleep and stress on the cortisol awakening response in young adults. Psychoneuroendocrinology 40, 10–16. https://doi.org/10.1016/j.psyneuen.2013.10.009.

Viru, A., 1992. Plasma hormones and physical exercise. Int. J. Sports Med. 13 (03), 201–209. https://doi.org/10.1055/s-2007-1021254.

Vrshek-Schallhorn, S., Doane, L., Mineka, S., Zinbarg, R.E., Craske, M., Adam, E.K., 2013. The cortisol awakening response predicts major depression: Predictive stability over a 4-year follow-up and effect of depression history. Psychol. Med. 43 (3), 483–493. https://doi.org/10.1017/S0033291712001213.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer.

Wilmot, E.G., Edwardson, C.L., Achana, F.A., Davies, M.J., Gorely, T., Gray, L.J., Khunti, K., Yates, T., Biddle, S.J., 2012. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta- analysis. Diabetologia 55, 2895–2905. https://doi.org/10.1007/s00125-013-2842-z.

Wright, K.P., Drake, A.L., Frey, D.J., Fleshner, M., Desouza, C.A., Gronfier, C., Czeisler, C. A., 2015. Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. Brain Behav. Immun. 47, 24–34. https://doi.org/10.1016/j.bbi.2015.01.004.

Wüst, S., Wolf, J., Hellhammer, D.H., Federenko, I., Schommer, N., Kirschbaum, C., 2000. The cortisol awakening response-normal values and confounds. Noise Health 2 (7), 79.

Wust, S., Wolf, J., Hellhammer, D.H., Federenko, I., Schommer, N., Kirschbaum, C., 2000. The cortisol awakening response-normal values and confounds. Noise Health 2 (7), 79.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed effects modelling for nested data. Mixed Effects Models and Extensions in Ecology with R. Springer, pp. 101–142.