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Examining multiple sleep behaviors and diurnal salivary cortisol and alpha-amylase: Within- and between-person associations

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Abstract

Sleep has been linked to the daily patterns of stress-responsive physiological systems, specifically the hypothalamic–pituitary–adrenal (HPA) axis and autonomic nervous system (ANS). However, extant research examining sleep and diurnal patterns of cortisol, the primary end product of the HPA axis, has primarily focused on sleep duration with limited attention on other facets of sleep. For example, it is not clear how specific aspects of sleep (e.g., sleep quality, sleep duration variability) are related to specific components of diurnal cortisol rhythms. Salivary alpha-amylase (sAA) has been recognized as a surrogate marker of ANS activity, but limited research has explored relations between sleep and sAA diurnal rhythms. The current study utilized an ecological momentary assessment protocol to examine *within-* and *between-person* relations between several facets of sleep behavior using multiple methods (e.g., subjective report, actigraphy) and salivary cortisol and sAA. Older adolescents ($N = 76$) provided saliva samples and diary entries five times per day over the course of three days. Sleep was assessed via questionnaire, through daily diaries, and monitored objectively using actigraphy over a four day period. *Between-person* results revealed that shorter average objective sleep duration and greater sleep duration variability were related to lower levels of waking cortisol and flatter diurnal slopes across the day. *Within-person* results revealed that on nights when individuals slept for shorter durations than usual they also had lower levels of waking cortisol the next day. Sleep was not related to the cortisol awakening response (CAR) or diurnal patterns of sAA, in either between-person or within-person analyses. However, typical sleep behaviors measured via questionnaire were related to waking levels of sAA. Overall, this study provides a greater understanding of how multiple components of sleep, measured in naturalistic environments, are related to cortisol and sAA diurnal rhythms, and how day-to-day, within-person changes in sleep duration contribute to daily variations in cortisol.

Keywords

Hypothalamic–pituitary–adrenal axis; Cortisol; Salivary alpha-amylase; Sleep; Actigraphy

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Conflicts of interest

None of the authors (S.A.V., L.D.D.) have any conflicts of interest to declare with respect to this manuscript.

Contributors

S.A.V. conducted statistical analyses, completed literature searches and drafted the manuscript. L.D.D. designed the study, wrote the protocol, and contributed to the final manuscript.

1. Introduction

Changes in sleep have been linked with alterations in critical stress-responsive biological systems (Meerlo et al., 2008 for review; Leproult and Van Cauter, 2010). The hypothalamic–pituitary–adrenal (HPA) axis and autonomic nervous system (ANS) have evolved to guide adaptive responses to environmental threats, but also maintain distinct 24-h biological rhythms controlled by the hypothalamic suprachiasmatic nuclei (SCN), the body's principal endogenous circadian pacemaker (Hastings et al., 2003). The SCN, in part, coordinates the diurnal rhythms of these systems, which mobilize energy resources, regulate metabolism, and are closely related to oscillations of the sleep-wake cycle (Reppert and Weaver, 2002). Both animal and human studies indicate the sleep-wake cycle and the diurnal rhythms of these stress-responsive biological systems are closely aligned temporally and functionally, such that sleep at night may be related to alterations in the patterns of these systems during the day (Irwin et al., 1999; Edwards et al., 2001). Thus, the current study examined whether sleep (e.g., sleep duration, sleep quality) moderates the diurnal patterns of cortisol and sAA. This is an important empirical question, as diurnal rhythms of cortisol and sAA are theorized to have important implications for both physical and psychological health (Gunnar and Vazquez, 2001; Miller et al., 2009).

Although there is a well-defined physiological link between sleep and HPA regulation, research examining associations between sleep duration and diurnal cortisol patterns is inconsistent (Elder et al., 2014). For example, some studies utilizing experimental and naturalistic designs have demonstrated associations between sleep duration and various components of the cortisol diurnal rhythm including the cortisol awakening response (CAR; e.g., Kumari et al., 2009; Vargas and Lopez-Duran, 2014) and the linear decline across the waking day (e.g., Zeiders et al., 2011; Kumari et al., 2009; Castro-Diehl et al., 2015), yet other studies have identified no associations between sleep duration and diurnal cortisol (e.g., Pruessner et al., 1997; Federenko et al., 2004). Although prior empirical studies have primarily focused on sleep duration, recent calls in the literature have recognized the need to examine other facets of sleep (Dijk, 2012; Bei et al., 2016), including sleep quality and day-to-day fluctuations in sleep patterns (i.e., sleep variability). However, few studies have explicitly examined associations between sleep quality and variability and the diurnal patterns of cortisol. In one recent study, researchers experimentally manipulated the sleep schedules of a sample of young adults and found that depriving sleep resulted in higher morning cortisol levels, whereas sleep misalignment (i.e., wakefulness at times the SCN is promoting sleep and sleep at times when wakefulness is being reinforced by the SCN) resulted in lower morning cortisol levels (Wright et al., 2015). Other studies among shift workers, who routinely manipulate their sleep schedules, have shown that working later shift times is associated with a reduced CAR compared to individuals on regular sleep (e.g., Williams et al., 2005; Bostock and Steptoe, 2013). Less is known about associations between sleep variability and diurnal cortisol among individuals that experience normative fluctuations in their sleep schedules. The current study seeks to fill this gap in knowledge by testing the associations between several facets of sleep and diurnal cortisol, including sleep variability and quality.

The circadian mechanisms responsible for controlling sleep are also directly involved in modulating the ANS (Leproult and Van Cauter, 2010). Sleep loss or disrupted sleep can activate autonomic activity, with corresponding increases in heart rate and blood pressure and decreases in PNS activity (Zhong et al., 2005). Such increases in ANS activation may be related to ANS activity following a night of shortened or disrupted sleep. For example, in a study examining sleep in children, lower sleep efficiency was related to higher sAA levels during a laboratory stress task (Raikkonen et al., 2010). It remains unclear whether sleep is related to *diurnal* patterns of ANS activity the following day. Recently, research has focused on the diurnal patterns of sAA, which has been identified as a surrogate marker of ANS activity, and a possible indicator of sympathetic nervous system activation (Nater and Rohleder, 2009). However, few studies have examined whether sleep is related to sAA diurnal patterns (for exception see Nater et al., 2007).

Although prior studies have examined sleep and diurnal rhythms of stress-responsive systems, there are several key limitations in the extant literature. The dynamic nature of the human stress response and sleep-wake cycle requires specific measures of multiple components of these systems to accurately examine the relations among these processes. In order to accurately model the diurnal rhythm of cortisol and sAA (e.g., awakening response, linear change across the day) it is necessary to obtain multiple samples a day and measure participant compliance with study protocols, including sample timing (Kudielka et al., 2003; Rotenberg and McGrath, 2014). Further, sleep measurement varies considerably across studies. Depending on the instrument used to measure sleep (e.g., polysomnography, actigraphy, self-report), researchers can derive a variety of sleep indices (e.g., sleep duration, sleep efficiency, sleep duration variability). Previous studies have primarily focused on sleep duration, and the influence of various components of sleep on stress-responsive diurnal patterns has not been explicitly examined.

Responding to recent calls in the literature to examine multiple indicators of sleep and stress physiology (Granger et al., 2012; Gregory and Sadeh, 2012), this study used a multi-method approach (assessing subjective and objective sleep measures) to test relations with multiple components of the diurnal rhythms of both cortisol and sAA. Further, this study tested between- and within-person associations by examining whether typical sleep (i.e., averaged across the study protocol) was associated with average diurnal cortisol and sAA patterns, as well as whether day-to-day changes in sleep predicted daily variation in diurnal cortisol and sAA. For between- and within-person analyses it was hypothesized that shorter sleep duration, poorer sleep quality, and greater sleep duration variability would be related to lower waking levels, a greater CAR, and a flatter linear decline in cortisol across the day (Zeiders et al., 2011; Kumari et al., 2009). Due to limited empirical research examining sleep and diurnal sAA patterns, no specific hypotheses were made regarding direction of effects due to the exploratory nature of these analyses.

2. Method

2.1. Participants

Data for the current study was drawn from the second time point of a longitudinal study examining adjustment during the transition from high school into college (see Doane et al.,

2015). Seventy-six older adolescents (M_{age} = 18.53, SD = .37; 24% male) were assessed during the fall of their first semester of college. Participants were recruited through orientation activities for the psychology department at a large southwestern university or through email. Participants were required to live within 35 miles of the university and be a senior in a local high school during the first assessment. The sample was ethnically diverse, with a race/ethnic makeup of 54% Non-Hispanic White, 16% Latino/Hispanic descent, 4% African–American and 26% multiple race/other. Individuals came from varying socioeconomic backgrounds as measured by their parents' mean levels of education, 3.7% of parents completed some high school, 26.8% had a high school diploma or GED, 23.2% had some college, 11% had an associate's degree, 18.3% had a bachelor's degree, and 17.1% had a graduate degree. Participants were excluded from these analyses if they were non-compliant (see procedure section for full compliance description) with the saliva sampling (n = 6) or actigraphy protocol (n = 1).

2.2. Procedure

Adolescents who agreed to enter the study were asked to select three typical consecutive weekdays to participate. All materials needed for the study were brought to the participant's residence directly by project staff, who explained all procedures and provided the participant with an email address and phone number where they could reach study personnel with questions. Participants signed consent forms upon delivery of project materials. Participants who consented to the study provided saliva samples and diary reports (five times a day) for three days, and wore watch-like devices (that signaled them for sampling times) for four nights. The watches, or actigraphs (wrist-based accelerometer), capture a record of activity across the day from which valid objective measures of waking, bed times, sleep duration and quality can be determined (Sadeh, 2011). Project personnel picked up completed study materials and paid participants $50 for completion of the protocol.

Study materials included three daily diaries, an actigraph, a MEMS 6 (Aardax; Aardex Group, Richmond, VA) track cap compliance device with 16 straws, 16 vials for saliva sampling, and several questionnaires. During the explanation of study procedures, participants were instructed to avoid eating, drinking or brushing their teeth at least 30 min before providing a saliva sample. Participants provided a salivary sample immediately after waking, 30 min later, approximately 3 and 8 h after waking, and at bedtime for 3 consecutive days. In conjunction with saliva samples, participants also completed diary entries documenting their mood, stressful events, caffeine, alcohol, medication and nicotine use, food intake, exercise, and sleeping behavior in the prior hour. In total, participants were required to fill out fifteen diary entries (M = 14.47, SD = 1.04).

2.3. Measures

2.3.1. Salivary cortisol and sAA—Cortisol and sAA were collected by passive drool according to recommendations for best practice (Granger et al., 2012). Participants labeled vials with the time and date of sampling. Completed samples were collected from each participant's home where they had been refrigerated. They were then stored at −20 °C until sent by courier on dry ice over three days to Biochemisches Labor at the University of Trier (Trier, Germany) to be assayed. Precautions were consistent with recommendations for

handling and transporting salivary biomarkers (Granger et al., 2012). sAA samples were assayed in duplicate using a kinetic reaction utilizing a chromagenic substrate, 2-chloro-4-nitrophenyl-*D* Maltrotriosid (CNP-G3; Winn-Deen et al., 1988; Lorentz et al., 1999). For sAA, the intra-assay coefficients of variation ranged from 3.5% to 6.3% and the inter-assay coefficients of variation ranged from 5.5% to 7.6%. sAA values were log transformed and outliers (>3 SD from the mean) were winsorized. Cortisol samples were assayed in duplicate using a solid phase time-resolved fluorescence immunoassay with fluorometric endpoint detection (DELFIA; Dressendörfer et al., 1992). Outlier values were winsorized (cortisol >1.81 μg/dl) and transformed using the natural log transformation to account for positively skewed distributions (Adam and Kumari, 2009). The intra-assay coefficient of variation ranged between 4.0% and 6.7%, and the inter-assay coefficients of variation ranged between 7.1% and 9.0%. Multiple parameters of cortisol and sAA can be assessed by sampling across the day. These include waking levels, the magnitude of the awakening response (i.e., difference in levels between waking and 30 min after waking), and elevation and slope of the diurnal curve (e.g., Adam et al., 2006). In the current study, waking levels, the cortisol and sAA awakening response (CAR; AAR), as well as diurnal slopes were modeled to test both between and with-person associations (see analytic plan for further explanation).

Strict compliance parameters were used to ensure accurate modeling of cortisol and sAA diurnal patterns. Track caps and actigraph watches were utilized to monitor participants' daily compliance, as compliance with sampling timing has been shown to influence the estimation for the measurement of salivary biomarkers (Kudielka et al., 2003; Rotenberg and McGrath, 2014). Participants were considered compliant if (1) their waking sample was within 15 min of their wake time, and (2) their second sample was between 23 and 37 min after their first sample (DeSantis et al., 2010). Individuals were excluded from the analysis if they failed to use track compliance devices during sampling ($n = 6$). See Table 1 for complete information regarding the full and analytic samples.

2.3.2. Subjective sleep—The *Pittsburgh Sleep Quality Index* (PSQI) was used to measure subjective sleep (Buysse et al., 1989). The PSQI is a 19-item self-report instrument that measures sleep quality and sleep disturbance over the past month. The PSQI includes seven scales: sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medication, and daytime dysfunction (Buysse et al., 1989). Participants were asked to answer questions such as, "During the past month, what time have you usually gone to bed at night?" to determine sleep duration. Participants also answered four-point Likert scale questions such as, "During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activities?" to assess sleep quality (i.e., daytime dysfunction). Participant responses were coded from 0 (not during the past month) to 3 (three or more times in a week). Scores on the subscales were summed for an overall global score. The maximum score on the PSQI is 21 and higher overall scores indicate poorer sleep quality and more sleep disturbance. PSQI scores above 5 are considered to be indicative of poor sleep quality (Buysse et al., 1989; Buysse et al., 2008).

2.3.3. Objective sleep—For the duration of the study, participants wore an *Actiwatch Score* (Phillips Respironics, Inc.) on their non-dominant wrist. Participants pressed a button on the watch upon waking and when they got into bed at night. Study staff cross-checked actigraphy-recorded sleep periods with self-reported (i.e., daily diary) bedtime and wake time as an additional sleep-period compliance measure. Sleep data was scored using the Phillips, Actiware (version 6) program, which includes a validated algorithm to measure sleep (Oakley, 1997). Activity counts within each epoch were calculated based on activity levels during the adjacent 2 min period.[1] The threshold was set to 40, with a range of 20–80. Utilizing 1 min epochs and based on significant movement after at least 10 min of inactivity, this algorithm calculates a variety of sleep parameters. The Actiwatch Score and similar sleep algorithms have been used in several other studies examining salivary cortisol (e.g., Smyth et al., 2013).

Sleep duration was calculated by subtracting the total amount of sleep measured in minutes from total time spent in bed. This parameter of sleep also excludes wake periods during the night. Average sleep duration was calculated for each person by averaging sleep duration across the four days sleep was measured via actigraphy. From nightly estimates of sleep duration, sleep duration variability was calculated by assessing the variation in sleep duration (i.e., standard deviation estimate from the person-level average) across four nights of sleep protocol. Last, sleep efficiency was estimated by assessing the percentage of time in bed that an individual is actually sleeping.

Objective indicators of sleep were validated with diary self-reports of bed and wake times to identify significant outliers and equipment malfunction. Days when there was equipment malfunction or days in which there was significant discordance between self-reports and objective measurement were not included in analyses (n = 8 days). In total, 93.4% of participants had actigraphy data for all 4 nights, 5.2% had data for 3 nights of sleep, and 1.4% had 2 or fewer nights of sleep. Fewer than 3 nights of actigraphy may provide a poor estimation of regular sleep (Acebo et al., 1999), and, as such, participants with fewer than three nights of sleep data were excluded from analyses (n = 1).

2.3.4. Covariates—All analyses included person-level covariates that have been shown in previous research to be associated with either cortisol/sAA secretion and/or sleep (Kudielka and Kirschbaum, 2003; Rohleder and Nater, 2009). These included the following: gender (1 = male, 0 = female), race/ethnicity (1 = White, 0 = non-White), parent's average educational status (ranging from 1 = *some high school* to 6 = *graduate school*), and oral contraceptive use. In addition, several momentary covariates were tested, including caffeine use, smoking, eating, or exercising within an hour of each saliva sample. Non-significant covariates were not included in final models.

[1]The following algorithm was used where A denotes activity counts and E denotes epoch: $A = E - 2(1/25) + E - 1(1/5) + E + E + 1(1/5) + E + 2(1/25)$.

3. Data analytic plan

Three-level hierarchical linear growth models were used to examine associations among sleep, cortisol and sAA, and to account for the nested nature of the data (Singer and Willett, 2003). Independent variables were all centered according to recommendations by Enders and Tofighi (2007). Time (level-1 predictor) was centered as hours since waking on each day (e.g., waking = 0) in order to allow for the interpretation of the intercept as the "starting point" of the growth curve, which in this case is cortisol/sAA levels at time of waking. A dummy-coded variable (i.e., 0 or 1) was used to represent the CAR/AAR sample. Level-2 predictors (e.g., sleep duration, sleep efficiency) were within-person centered to represent deviations from an individual's average sleep across the four day sampling protocol. Finally, level-3 sleep parameters (e.g., average sleep duration, sleep duration variability) and covariates were grand-mean centered to represent deviations from an individual's average score relative to the sample average.

The intercepts in all models were estimated to vary across both days and across individuals. All slopes were set as fixed parameters, except for the level-1 time-varying predictor *time since waking* (estimate of the diurnal slope) in cortisol models, which varied significantly across people and was therefore allowed to vary randomly at Level 3 in all subsequent cortisol models. This was determined by testing model fit using likelihood ratio tests to examine the fit of each model with and without the random slope term compared to the unconditional growth model initially estimated.[2] Each model presented below was examined separately for outcome variables, cortisol and sAA.

Initially, a baseline model was constructed to estimate each individual's cortisol and sAA diurnal rhythm. Following model 1, between-person differences were tested by examining how individuals' average sleep duration, sleep duration variability and subjective sleep quality (PSQI score) were related to average cortisol and sAA diurnal patterns. This was accomplished by grand mean centering each sleep parameter and entering them as predictors in the model at level 3. The interaction between the sleep parameters and growth parameters were then tested to examine the relation between sleep and change cortisol or sAA.

To assess within-person associations, sleep duration and sleep efficiency were added in the model at level-2 to assess the effect of day-to-day changes in sleep duration and efficiency on diurnal cortisol and sAA patterns. This was done by examining the interaction between within-person centered sleep duration and efficiency scores and each growth parameter (i.e., intercept or waking, awakening response, linear slope). Below is an example of the estimated model predicting cortisol.

Level 1:

$$\mathrm{LogCort}_{tij}=\pi_{0ij}+\pi_{1ij}(\mathrm{CAR}_{tij})+\pi_{2ij}(\mathrm{Time}_{tij})+\pi_{3ij}(\mathrm{Time}^2{}_{tij})+\mathrm{e}_{tij}$$

Level 2:

[2] χ^2 (2) = 96.35, $p < .001$.

$$\pi_{0ij}=\beta_{00j}+\beta_{01j}(\text{Sleep duration}_j)+\beta_{02j}(\text{sleep efficiency}_j)+r_{0ij}$$
$$\pi_{1ij}=\beta_{10j}+\beta_{11j}(\text{Sleep duration}_j)+\beta_{12j}(\text{sleep efficiency}_j)$$
$$\pi_{2ij}=\beta_{20j}+\beta_{21j}(\text{Sleep duration}_j)+\beta_{22j}(\text{sleep efficiency}_j)$$
$$\pi_{3ij}=\beta_{30j}$$

Level 3:

$$\beta_{00j}=\gamma_{000}+\gamma_{001}(\text{Male})+\gamma_{002}(\text{White})+\gamma_{003}(\text{ParentEd})+\gamma_{004}(\text{Oral Contraceptive})+\gamma_{005}(\text{average sleep duration})+\gamma_{006}(\text{sleep variability})+\gamma_{007}(\text{Sleep qualityPSQI})+u_{00j}$$
$$\beta_{01j}=\gamma_{010}$$
$$\beta_{02j}=\gamma_{020}$$
$$\beta_{10j}=\gamma_{100}+\gamma_{101}(\text{Male})+\gamma_{102}(\text{White})+\gamma_{103}(\text{ParentEd})+\gamma_{104}(\text{Oral Contraceptive})+\gamma_{105}(\text{average sleep duration})+\gamma_{106}(\text{sleep variability})+\gamma_{107}(\text{Sleep qualityPSQI})+u_{10j}$$
$$\beta_{11j}=\gamma_{110}$$
$$\beta_{12j}=\gamma_{120}$$
$$\beta_{20j}=\gamma_{200}+\gamma_{201}(\text{Male})+\gamma_{202}(\text{White})+\gamma_{203}(\text{ParentEd})+\gamma_{204}(\text{Oral Contraceptive})+\gamma_{205}(\text{average sleep duration})+\gamma_{206}(\text{sleep variability})+\gamma_{207}(\text{sleep qualityPSQI})$$
$$\beta_{21j}=\gamma_{210}$$
$$\beta_{22j}=\gamma_{220}$$
$$\beta_{30j}=\gamma_{300}$$

4. Results

All results and sample statistics reflect the analytic sample (see Table 1). Descriptive statistics (means, standard deviations, percentages) and bivariate correlations were assessed for all variables and can be found in Table 2. On average, participants slept 6.24 (SD = .98) h per night and the average sleep efficiency was 83.78 (SD = 5.20). Average subjective sleep quality in the sample, measured via the PSQI, was 5.76 (SD = 2.73). The average person-level variability in sleep duration was 1.06 h, which can be interpreted as a measure of sleep duration consistency (larger values indicate less consistency). Among independent, dependent and control variables there were several statistically significant bivariate correlations (see Table 2).

4.1. Multilevel growth models predicting diurnal cortisol and sAA

Table 3 contains results from multilevel growth models with cortisol as the dependent variable and Table 4 contains results with sAA as the dependent variable. Individuals, on average, demonstrated typical diurnal patterns of cortisol and sAA (see Fig. 1). Overall (Table 3, Model 1), waking levels of cortisol (γ_{000}, intercept = −1.61, $p < .001$, equal to .20 μg/dl) were consistent with prior literature (e.g., Adam et al., 2010), as were individuals' cortisol awakening responses, which demonstrated a 75%[3] increase (γ_{100} = .56, $p < .001$)

[3]Because cortisol/sAA values have been log transformed, these values can be interpreted as percent change per unit change in cortisol/sAA through the calculation of $\beta\%\text{change} = (e^{(\beta\text{raw})})^{-1}$.

within thirty minutes of waking. As expected, cortisol levels declined across the day at a rate of 7.0% per hour at waking (γ_{200} = −.07, p < .001). Further, the quadratic term included to model possible curvilinear effects was also negative and significant (γ_{300} = −.002, p < .001). For sAA (Table 4, Model 1), participants showed waking levels (γ_{000}, intercept = −.97, p < .001, equal to .38 U/ml) consistent with prior studies (e.g., Nater et al., 2007), a significant decline (γ_{100} = −.87, p < .001) within the first thirty minutes after waking, and an increase across the day (γ_{200} = .14, p < .001) at a rate of 15.8% per hour at waking. The quadratic term was negative and significant (γ_{300} = −.007, p < .001) suggesting that the linear rate of change per hour was reduced across the waking day.

4.2. Sleep and diurnal cortisol

Between person results (Table 3, Model 2) revealed that average sleep duration was positively associated with average waking levels of cortisol (γ_{005} = .14, p < .01), such that individuals who slept longer, on average, had higher waking levels of cortisol. Further, average sleep duration was negatively associated with the average diurnal cortisol slope (γ_{205} = −.03, p < .05), such that individuals who slept longer, on average, had a corresponding 2.8% steeper decline in cortisol *per hour* at waking. Average sleep duration was not significantly associated with the CAR (γ_{105} = −.096, *ns*).

Sleep duration variability was negatively associated with average waking levels of cortisol (γ_{006} = −.20, p < .001), indicating that individuals who experienced more day-to-day variability in sleep duration had lower waking levels of cortisol. In addition, average sleep duration variability was positively associated with the average diurnal cortisol slope (γ_{206} = .04, p < .01), such that individuals with higher day-to-day variability in sleep durations also had flatter diurnal slopes, on average. This result suggests that individuals with more daily fluctuation in sleep duration exhibited a 4.1% flatter rate of change in cortisol *per hour* at waking. Subjective sleep quality, as measured by the PSQI, was not associated with waking levels of cortisol (γ_{007} = −.02, *ns*), the CAR (γ_{107} = −.02, *ns*), or the diurnal slope (γ_{207} = .001, *ns*).

Regarding within-person associations (Table 3, Model 2), prior night sleep duration was positively associated with waking levels of cortisol. On days where individuals slept more than their typical amount, they demonstrated higher waking levels of cortisol (γ_{010} = .10, p < .05). Prior night sleep duration was not associated with the CAR (γ_{110} = −.08, *ns*) or diurnal slope (γ_{210} = −.002, *ns*). Prior night sleep efficiency was not associated with waking levels of cortisol (γ_{020} = .002, *ns*), the CAR (γ_{120} = −.10, *ns*), or the diurnal slope (γ_{220} = −.001, n*s*).

4.3. Sleep and diurnal sAA

Similar to cortisol models, sleep parameters were entered into the model (Table 4, Model 2) to examine associations with diurnal sAA. Subjective sleep quality was associated with higher waking values of sAA (γ_{007} = .12, p < .01), indicating that individuals who endorsed worse overall sleep quality also had higher waking levels of sAA, on average. Subjective sleep quality was not associated with the AAR (γ_{107} = −.04, *ns*) or diurnal slope (γ_{207} = −.003, *ns*). Average sleep duration was not associated with waking levels of sAA (γ_{005} = −.

003, *ns*), the AAR (γ_{105} = −.113, *ns*) or diurnal slope (γ_{205} = .001, *ns*). Further, no statistically significant associations were found for average sleep duration variability and waking levels of sAA (γ_{006} = −.008, *ns*), the AAR (γ_{106} = .23, *ns*) or diurnal slope (γ_{206} = −.003, *ns*).

There were no statistically significant associations between prior night sleep duration or efficiency and sAA. Sleep duration was not associated with sAA waking levels (γ_{010} = .112, *ns*), the AAR (γ_{110} = −.061, *ns*), or diurnal slope (γ_{210} = −.01, *ns*). Sleep efficiency was not associated with sAA waking levels (γ_{020} = −.008, *ns*), the AAR (γ_{120} = .02, n*s*), or diurnal slope (γ_{220} = .001, n*s*).

5. Discussion

The sleep-wake cycle and stress-responsive physiological system represent two bio-regulatory processes necessary for everyday functioning. Sleep behavior is an important factor related to individual differences and day-to-day fluctuations of diurnal cortisol (Vgontzas and Chrousos, 2002; Elder et al., 2014). The primary aim of this study was to use multiple methods to examine both *between*-and *within-person* associations between various aspects of sleep (e.g., sleep duration, sleep quality, sleep duration variability) and diurnal patterns of cortisol. Our results support prior theoretical and empirical research that has found individuals who sleep longer tend to have higher waking levels and steeper diurnal slopes (e.g, Zeiders et al., 2011; Kumari et al., 2009). However, contrary to some prior research (e.g., Castro-Diehl et al., 2015; Vargas and Lopez-Duran, 2014), sleep duration and quality were not related to the CAR. *Variability* in the amount of sleep one receives from night to night was associated with higher waking levels and a slower rate of decline in cortisol across the day. Given limited empirical evaluation, a secondary aim of this study was to test associations between the same measures of sleep and sAA diurnal rhythms. Although objective sleep indicators were not associated with diurnal sAA, subjective sleep quality was positively associated with waking levels of sAA. In sum, our results provide evidence that diurnal sAA patterns are not sensitive to sleep in the short-term, but that diurnal cortisol rhythms vary, in part, as a function of both typical sleep durations and variability in sleep durations.

On average, individuals with more variable sleep durations had lower waking cortisol levels, and individuals with greater sleep duration had higher waking levels of cortisol. Within-person results revealed that on nights when an individual slept more than their typical amount they also had higher waking cortisol levels. These differential results regarding sleep duration and variability may be related to the interconnection between cortisol secretion and the sleep-wake cycle. Cortisol levels are lowest during the first half of the sleep-wake cycle, when sleep is deepest (i.e., slow wave sleep). Cortisol, and its secretagogue corticotrophin releasing hormone (CRH), begin to rise during the later phase of sleep, which is more likely to include higher levels of rapid eye movement sleep (REM) sleep and less slow wave sleep (Buckley and Schatzberg, 2005). Given prior findings demonstrating an association between sleep stage and the rise in cortisol before waking (Born et al., 1986), individuals who sleep longer may be more likely to experience greater REM sleep and to wake with higher levels of cortisol by having more time to establish increases in both CRH and cortisol before

waking (Buckley and Schatzberg, 2005). In contrast, individuals who sleep less or who exhibit greater variability (i.e., less consistency) may have less time to establish an increase in cortisol before awakening or likely wake during an earlier phase of the cortisol rise that occurs prior to waking. These findings are consistent with results from other studies examining sleep duration and waking cortisol using both self-reports of sleep (e.g., Kumari et al., 2009) and objective measures (e.g., Zeiders et al., 2011).

Importantly, no associations were found between average sleep duration, sleep duration variability, sleep quality or day-to-day changes in sleep and the CAR. These results stand in contrast to recent studies that have demonstrated a link between reduced sleep duration and an increase in the magnitude of the CAR (e.g., Kumari et al., 2009; Vargas and Lopez-Duran, 2014), but are consistent with other studies that have reported null findings (e.g., Pruessner et al., 1997). Our findings support prior theoretical work positing that the magnitude of the CAR, although subject to external psychosocial and environmental influences (Adam et al., 2006), may not necessarily be associated with *length* of sleep or time of waking (Wilhelm et al., 2007; Clow et al., 2010). That is, individuals who sleep longer may simply shift forward the timing of the start of the CAR (i.e., waking values), without necessarily affecting the magnitude of the increase. Prior studies reporting relations between sleep duration and the CAR have not used electronic compliance devices (e.g., Kumari et al., 2009; Vargas and Lopez-Duran, 2014) to ensure accuracy in the timing of samples needed to model the awakening response, which can skew results (Kudielka et al., 2003; Smyth et al., 2013). Further, in studies that do not use strict compliance protocols, it may be that poor sleep leads to worse morning compliance needed to accurately measure the CAR. The length of ones' sleep may not influence the dynamic increase in cortisol often seen post-awakening, but instead is potentially shifting forward the timing of the start of the response (Clow et al., 2010; Wilhelm et al., 2007). Contrary to what was expected, sleep quality and sleep duration variability were not related to the CAR. However, it may be that there was not sufficient sleep duration variability or poor quality sleep in the current sample to influence a change in the CAR. More extreme levels of sleep duration variability (e.g., shift workers) and poor sleep (e.g., sleep apnea, insomnia) may be necessary to influence consistent changes in the CAR. Further, given our small sample size we may have been under powered to detect these associations.

Between-person results revealed that individuals who slept longer tended to exhibit steeper cortisol slopes (i.e., greater rate of decline per hour estimated at waking), whereas those who had greater variability in sleep duration tended to exhibit flatter cortisol slopes (i.e., less rate of decline per hour estimated at waking). Sleep quality was not related to cortisol slopes across the waking day. Within-person results also revealed that prior-night sleep duration and quality were not related to the diurnal cortisol slope. That is, individual changes in sleep duration and quality were not associated with the diurnal cortisol slope. It could be that four days of sleep may not be sufficient to detect significant within-person variability (Ross et al., 2014). Future studies should aim to examine within-person associations using more days of sleep measurement.

Regarding between-person findings, results are partially in line with prior studies that have demonstrated flatter cortisol slopes in individuals with shortened sleep durations (Zeiders et

al., 2011; Kumari et al., 2009). Flattened diurnal profiles are theorized to be indicators of dysregulation in the HPA axis (Stone et al., 2001). The current findings suggest that individuals who typically sleep longer may demonstrate more adaptive diurnal patterns of cortisol, whereas individuals who exhibit greater fluctuations in their sleep durations demonstrate an inability to reduce cortisol levels across the day resulting in a flattening of the diurnal slope. Shortened sleep or high levels of sleep duration variability may be acting as a stressor influencing cortisol output or may be representative of higher levels of stress influencing sleep behavior. This is consistent with prior studies that have demonstrated changes to 24-h patterns of cortisol in shift workers who naturally vary sleep schedules and sleep durations, as well as with studies that have experimentally manipulated sleep schedules (Rehman et al., 2010).

Both day-to-day changes and average sleep duration were not related to sAA diurnal rhythms, suggesting sleep duration and sleep duration variability, at least in the short term, had little or no association with daily patterns of sAA in this sample. These findings are consistent with past research that demonstrated no association between sleep duration and diurnal sAA among similar populations (e.g., Nater et al., 2007). Results did reveal that individuals who endorsed worse global sleep quality on a subjective report had higher waking levels of sAA, on average. It may be that the PSQI, which assesses sleep behaviors over the previous month, is capturing more chronic sleep problems not detected by four nights of actigraphy. For these individuals, worse sleep quality may be an indicator of more restless sleep or a greater amount of night awakenings, which could result in more active ANS activity throughout the night and upon awakening in the morning (Zhong et al., 2005). Given the lack of empirical examination of sleep and sAA, we speculate that greater changes in sleep behaviors (e.g., chronic sleep deprivation, insomnia) over greater periods of time (e.g., weeks, months) may be required to change diurnal patterns of sAA, given the relative stability of sAA diurnal patterns (Out et al., 2013).

The findings from this study have several implications. First, this study provides a greater understanding of the relations between *multiple* components of sleep and *multiple* indicators of diurnal stress physiology. The majority of the literature examining sleep and stress physiology has focused primarily on cortisol. However, investigators in the field have highlighted the need to go beyond a single system approach that only focuses on the HPA axis (e.g., Granger, 2012). Further, contemporary psychophysiological theorists have acknowledged that measuring, modeling and interpreting *multiple stress-systems* (e.g., HPA axis and ANS) is essential to advancing current knowledge in the field and accounting for individual differences in psychological adaptation across the lifespan (Bauer et al., 2002). Next, although there are studies that have examined both sleep duration and sleep quality, this is the first study to test relations between variability in sleep durations and diurnal patterns of cortisol and sAA. This study also utilized a multi-method approach to measuring sleep, allowing for delineation between relations of both subjective and objective measures. Third, electronic monitoring devices were used to ensure compliance with morning saliva sampling procedures and to reduce bias in estimating the waking and awakening response. Fourth, by testing both within- and between-person associations this study was able to examine whether typical sleep is related to diurnal cortisol and sAA at the aggregate level, but also if day-to-day changes in sleep are associated with day-to-day changes in diurnal

stress physiology. Last, this study provides evidence in support of theoretical work hypothesizing that the CAR may be independent of diurnal variations in HPA axis activity and sleep behaviors, and instead represents the response to awakening (Clow et al., 2010). Although the CAR may be sensitive to external inputs (e.g., sleep timing), including psychological and environmental experiences, we did not find any evidence that the CAR was associated with sleep behavior.

This study was not without limitations. First, future studies should aim to replicate these results with a larger, more heterogeneous sample. For example, the average score on the PSQI in this sample is greater than 5 suggesting that more than half of our sample experienced significant sleep disturbances. While increased sleep disturbances may be typical for college-going youth (e.g., Lund et al., 2010), these sleep patterns may not be representative of the general adult population. Further, the small sample size may have limited our ability to detect some of the tested associations, particularly for analysis examining between-person effects. For example, there was a non-significant association between sleep duration variability and the CAR/AAR, but we may have been limited by a small sample size to detect these effects. Future studies should examine associations between sleep variability and the awakening responses of cortisol and sAA in larger samples and over a greater number of days. Indeed, although this study had multiple nights of sleep and salivary collection, measuring only four nights of sleep may have obscured findings due to limited within-person variability in sleep behaviors. Future studies should collect more nights of sleep to further examine within-person changes in sleep and associations with stress physiology. Last, sleep was only examined during weekdays making comparisons to weekend sleep behaviors not possible. Sleep behavior may be even more variable and disrupted on weekends (e.g., social jetlag), particularly in this population. However, despite only collecting sleep during a more stable time (e.g., during the week), results still revealed significant associations.

Despite the noted limitations, this study provides further evidence of relations between typical durations and patterns of sleep, measured using multiple methods, and cortisol and sAA diurnal rhythms. Further, this study is one of the few to examine associations between day-to-day changes in sleep and variations in cortisol and sAA. Our study is also one of the first to test relations between sleep and sAA diurnal patterns, which represents an advance in understanding relations between sleep and the diurnal pattern of the ANS, an important component of the physiological stress response. Although prior research has identified a link between sleep and the HPA axis, this study takes an important first step in determining if sleep is related to the diurnal patterns of the ANS. We did not find evidence for a link between objective measures of sleep and sAA in the current study, but future studies should test these relations in larger samples. Overall, our findings support prior theoretical and empirical research examining relations between sleep duration and diurnal patterns of cortisol, including associations with waking levels of cortisol and the diurnal slope. However, contrary to some prior research, sleep was not related to the CAR. The current study also demonstrated that *variability* in the amount of sleep one receives may have important implications for daily functioning of the HPA axis. In conclusion, this study provides evidence that sleep behaviors, specifically fluctuations in sleep durations, could have implications for bio-regulatory mechanisms important for responding to stress.

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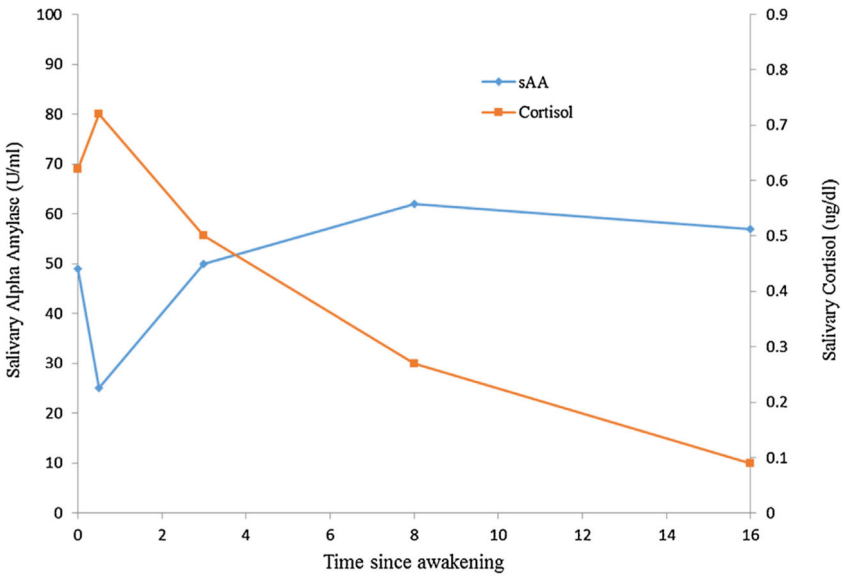

Fig. 1.

Average salivary cortisol and alpha-amylase rhythms across the waking day. Values are aggregated across days (n = 295) and individuals (n = 69). The cortisol and sAA values represent the average level at the following time points across the day: waking, +30 min after waking, approximately 3 and 8 h after waking, and immediately before bedtime.

Table 1

Full and analytic samples.

	Full sample	Analytic sample
Level-1 (moments)	1106	958
Salivary cortisol		950
Salivary alpha-amylase		893
Level-2 (days)	295	205
Sleep duration		205
Sleep efficiency		205
Level-3 (individuals)	76	69

Notes: Individuals, day, and/or moments were excluded from analyses based on compliance and/or insufficient saliva.

Table 2

Descriptive statistics and intercorrelation Table of cortisol, salivary alpha-amylase, sleep and covariates ($N = 69$).

	Mean (SD)	Min	Max	Percentage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. Cortisol waking	.24 (.13)	.01	.59	–	1																		
2. Cortisol 30 min post waking	.42 (.20)	.01	1.02	–	**.584****	1																	
3. Cortisol 3 h post waking[a]	.22 (.09)	.03	.52	–	**.282***	.204	1																
4. Cortisol 8 h post waking[a]	.11 (.07)	.01	.45	–	**.430****	.195	**.456****	1															
5. Cortisol bedtime	.06 (.05)	.01	.36	–	−.183	−.138	.133	.175	1														
6. sAA waking	49.88 (51.15)	1.31	300	–	−.069	−.002	.089	.086	.179	1													
7. sAA 30 min post waking	18.67 (18.34)	1.31	97.31	–	.150	.089	−.095	.213	.005	.236	1												
8. sAA 3 h post waking[a]	53.82 (39.60)	3.28	185.43	–	.205	.078	**−.240***	−.054	.008	**.245***	**.469****	1											
9. sAA 8 h post waking[a]	70.11 (53.51)	3.28	300	–	.077	−.054	−.014	.023	−.066	**.363****	**.297***	**.547****	1										
10. sAA bedtime	54.70 (48.92)	2.41	300	–	.055	−.005	.027	.136	.059	**.690****	**.350****	**.477****	**.584****	1									
11. Sleep duration (hours)[a]	6.24 (.98)	3.38	8.63	–	.225	−.004	.045	−.079	−.130	−.099	−.185	.005	.136	−.032	1								
12. Sleep duration variability (hours)[a,b]	1.06 (.56)	.07	2.49	–	−.170	−.205	−.218	−.094	.237	−.168	.104	−.035	−.039	−.160	.127	1							
13. Sleep efficiency[a]	83.78 (5.20)	64.66	92.52	–	.037	.114	.121	.215	.056	.039	.072	−.030	−.087	.025	**.304***	.235	1						
14. Sleep quality (PSQI)	5.76 (2.73)	1.00	13	–	−.115	−.074	.089	−.088	.051	**.251***	.011	−.012	.173	.131	.021	.192	.198	1					
15. Age	18.53 (.37)	17.18	19.12	–	.069	.018	.131	.073	−.048	.120	−.041	−.142	.066	.079	.136	−.123	.056	−.151	1				
16. Parent education level	3.35 (1.44)	1.00	6.00	–	.049	−.004	.049	.150	.001	.192	.067	−.011	.099	.120	.198	−.068	.109	−.056	.005	1			
17. Non-Hispanic White (white = 1)	–	–	–	54%	.007	−.068	.150	−.058	−.035	.232	.018	−.052	−.057	.095	.129	−.156	−.007	−.173	.193	**.242***	1		
18. Gender (Male = 1)	–	–	–	24%	.086	.025	−.148	.168	.018	.081	**.316****	**.250***	.048	**.265***	−.172	.071	.003	−.006	−.036	.027	−.047	1	
19. Oral contraceptive use	–	–	–	28%	−.025	−.156	.026	−.044	.191	−.005	−.135	.006	.140	−.036	.236	.097	.020	−.024	−.099	.020	.073	**−.344****	1

Notes: Cortisol and sAA values represents the average for all individuals across three days for each sample time;

$^{*}p < .05$

$^{**}p < .01$.

[c]Average sleep duration variability for all individuals across 4 nights of sleep; *$p < .05$ **$p < .01$. Cortisol values are in ug/dl; sAA values are in U/mL; PSQI = Pittsburgh Sleep Quality Index.

[a]3 and 8 h post waking are approximate and ranged by a half an hour depending on the day.

[b] Aggregate between-person actigraph measures of sleep.

Table 3

Multilevel model regression estimates predicting cortisol.

	Salivary cortisol			
	Model 1		Model 2	
	Coefficient	SE	Coefficient	SE
Intercept: waking levels, γ_{000}	**.199*****	.077	**.197*****	.681
Male, γ_{001}			.084	.147
White, γ_{002}			.049	.119
Parent education, γ_{003}			.002	.034
Oral contraceptive use, γ_{004}			.014	.133
Level 2: prior night sleep duration, γ_{010}			**.102****	.042
Level 2: prior night sleep efficiency, γ_{020}			.002	.008
Level 3: average sleep duration, γ_{005}			**.141****	.045
Level 3: sleep duration variability, γ_{006}			**−.199****	.138
Level 3: sleep quality (PSQI), γ_{007}			−.021	.024
Awakening response (CAR), γ_{100}	**.557*****	.057	**.573*****	.055
Male, γ_{101}			−.001	.169
White, γ_{102}			−.111	.138
Parent education, γ_{103}			−.023	.039
Oral contraceptive use, γ_{104}			−.155	.131
Level 2: prior night sleep duration, γ_{110}			−.078	.046
Level 2: prior night sleep efficiency, γ_{120}			−.096	.069
Level 3: average sleep duration, γ_{105}			−.096	.066
Level 3: sleep duration variability, γ_{106}			.128	.117
Level 3: sleep quality (PSQI), γ_{107}			−.017	.019
Time since waking: slope, γ_{200}	**−.068*****	.015	**−.061*****	.015
Male, γ_{201}			.002	.011
White, γ_{202}			−.001	.011
Parent education, γ_{203}			.001	.003
Oral contraceptive use, γ_{204}			**.032***	.015
Level 2: prior night sleep duration, γ_{210}			−.002	.004
Level 2: prior night sleep efficiency, γ_{220}			−.001	.001
Level 3: average sleep duration, γ_{205}			**−.028*****	.005
Level 3: sleep duration variability, γ_{206}			**.038****	.010
Level 3: sleep quality (PSQI), γ_{207}			.001	.002
Time since waking squared, γ_{300}	**−.002****	.001	**−.002*****	.001
Within-person pseudo R^2	–		.07	
Slope variance, *time since waking*	–		.35	

	Salivary cortisol			
	Model 1		Model 2	
	Coefficient	SE	Coefficient	SE
Between-person pseudo R^2	–		.06	

Notes: $N = 69$; individuals, days and moments were excluded from analyses based on compliance issues (see Table 1).

All fixed effects are with robust standard errors.

* $p < .05$,

** $p < .01$,

*** $p < .001$.

All cortisol levels reflect log10 ug/dl.

PSQI—Pittsburgh Sleep Quality Index.

Table 4

Multilevel model regression estimates predicting alpha-amylase.

	Salivary alpha-amylase			
	Model 1		Model 2	
	Coefficient	SE	Coefficient	SE
Intercept: waking levels, γ_{000}	**.379** ***	.141	**.386** ***	.139
Male, γ_{001}			−.066	.351
White, γ_{002}			−.025	.249
Parent education, γ_{003}			.002	.086
Oral contraceptive use, γ_{004}			.425	.238
Level 2: prior night sleep duration, γ_{010}			.112	.082
Level 2: prior night sleep efficiency, γ_{020}			−.008	.016
Level 3: average sleep duration, γ_{005}			−.003	.130
Level 3: sleep duration variability, γ_{006}			−.008	.189
Level 3: sleep quality (PSQI), γ_{007}			**.122** **	.037
Awakening response, γ_{100}	**−.874** **	.113	**−.864** ***	.113
Male, γ_{101}			.239	.288
White, γ_{102}			−.180	.207
Parent education, γ_{103}			.096	.071
Oral contraceptive use, γ_{104}			−.224	.231
Level 2: prior night sleep duration, γ_{110}			−.061	.095
Level 2: prior night sleep efficiency, γ_{120}			.016	.013
Level 3: average sleep duration, γ_{105}			−.113	.109
Level 3: sleep duration variability, γ_{106}			.234	.200
Level 3: sleep quality (PSQI), γ_{107}			−.045	.036
Time since waking: slope, γ_{200}	**.138** ***	.031	**.144** ***	.031
Male, γ_{201}			.025	,017
White, γ_{202}			−.024	.013
Parent education, γ_{203}			−.001	.004
Oral contraceptive use, γ_{204}			−.004	.014
Level 2: prior night sleep duration, γ_{210}			−.010	.008
Level 2: prior night sleep efficiency, γ_{220}			.001	.001
Level 3: average sleep duration, γ_{205}			.001	.001
Level 3: sleep duration variability, γ_{206}			−.003	.013
Level 3: sleep quality (PSQI), γ_{207}			−.003	.002
Time since waking squared, γ_{300}	**−.007** ***	.001	**−.007** **	.001
Within-person pseudo R^2	–		.06	
Between-person pseudo R^2	–		.04	

Notes: N = 69; individuals, days and moments were excluded from analyses based on compliance issues (Table 1).

All fixed effects are with robust standard errors.

*$p < .05$,

**$p < .01$,

***$p < .001$.

sAA levels reflect log10 U/mL.

PSQI—Pittsburgh Sleep Quality Index.