RESEARCH ARTICLE

The Cortisol Awakening Response and Depressive Symptomatology: The Moderating Role of Sleep and Gender

Ivan Vargas*†, Stefanie Mayer & Nestor Lopez-Duran

Department of Psychology, University of Michigan, Ann Arbor, MI, USA

Abstract

The association between depression and the cortisol awakening response (CAR) has been widely examined, yet the results are mixed and factors responsible for such inconsistencies are poorly understood. The current study investigated whether the link between depressive symptomatology and CAR varied as a function of two such factors: sleep and gender. The sample included 58 young adults (30 females; $M_{age} = 18.7$; $SD_{age} = 0.91$). Participants completed the Beck Depression Inventory as well as the Consensus Sleep Diary to assess depressive symptomatology and daily sleep patterns, respectively. Participants also provided four salivary cortisol samples (0, 30, 45 and 60 min after awakening) during two consecutive weekdays. Results demonstrated that greater depressive symptoms were associated with a greater CAR but only when depressive symptoms were linked to a shorter sleep time. In addition, gender significantly moderated the association between depressive symptoms and CAR. While greater depressive symptoms were associated with an elevated CAR among females, they were associated with a blunted CAR among males. These findings provide some insight into potential mechanisms linking depressive symptomatology and CAR, and suggest that future studies examining CAR as a biomarker of depression should account for differences in sleep and gender.

Received 11 October 2015; Revised 8 June 2016; Accepted 9 June 2016

Keywords

depressive symptoms; HPA-axis; cortisol awakening response; sleep; gender

***Correspondence**

Ivan Vargas, Department of Psychology, University of Michigan, 530 Church Street, 2245 East Hall, Ann Arbor, MI 48109, USA.

†E-mail: ivargas@umich.edu

Published online 28 July 2016 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/smi.2691

Introduction

Given the established link between stress and depression (Hammen, 2005), efforts to identify biomarkers of depression have focused on the hypothalamic–pituitary–adrenal–axis (HPA-axis), and its end-product, cortisol (Pariante & Lightman, 2008; Lopez-Duran, Kovacs, & George, 2009). Recently, these efforts have targeted the cortisol awakening response (CAR; Chida & Steptoe, 2009; Fries, Dettenborn, & Kirschbaum, 2009), a sudden rise in cortisol that occurs immediately following awakening (Pruessner et al., 1997). CAR is commonly viewed as a potential marker of HPA-axis sensitivity to stress (Schlotz, Hellhammer, Schulz, & Stone, 2004; Schulz, Kirschbaum, Prubner, & Hellhammer, 1998) that can provide investigators with the opportunity to conduct large-scale, epidemiological studies on biomarkers of depression given its relatively low cost and ease of collection in field settings (e.g. home; Adam & Kumari, 2009). This has led to a plethora of studies investigating whether atypical CAR reflects an underlying neuroendocrine sensitivity to stress that can play a role in depression (Chida & Steptoe, 2009). However, variability in CAR may reflect individual and contextual factors beyond basic neuroendocrine functioning, such as sleep (Elder et al., 2014), gender (Fries et al., 2009) and anticipatory stress (Rohleder, Beulen, Chen, Wolf, & Kirschbaum, 2007). Little has been done to dissect whether the link between depression and CAR reflects atypical HPA-axis functioning or any of the multiple factors that modulate the awakening response. Therefore, the current study investigated two such factors (i.e. sleep and gender) previously linked to both depression (Nolen-Hoeksema, 2001; Riemann, Berger, & Voderholzer, 2001) and CAR (Elder, Wetherell, Barclay, & Ellis, 2014; Pruessner et al., 1997; Wüst et al., 2000).

Despite research indicating that CAR may inform our understanding of the pathophysiology of depression (e.g. Bhagwagar, Hafizi, & Cowen, 2005; Stetler & Miller, 2005; Vreeburg et al., 2009), the exact nature

of the CAR–depression link is unclear and findings have been equivocal. For example, several studies have demonstrated an elevated CAR among both depressed and at-risk samples (Adam et al., 2010; Ulrike, Reinhold, & Dirk, 2013; Vrshek-Schallhorn et al., 2013). Depression is associated with an increased sensitivity to stress (Monroe & Harkness, 2005; Spijker & van Rossum, 2012), which may explain the relationship between greater CAR and depression. However, depression has also been associated with a blunted CAR (Dedovic et al., 2010; Mangold, Marino, & Javors, 2011; Stetler & Miller, 2005), which may reflect blunted HPA-axis sensitivity resulting from more chronic stress exposure in depressed populations (Hammen, 2005). Alternatively, other studies have also suggested that particular sub-types of depression (e.g. anhedonic depression) have a non-linear association with CAR (Veen et al., 2011; Wardenaar et al., 2011).

It is possible that CAR variability in depression reflects other correlates of CAR that are also linked to depression. For example, lower sleep quantity (Vargas & Lopez-Duran, 2014) and quality (Kumari et al., 2009) are associated with an elevated CAR. Sleep problems or reduced sleep time may make the process of awakening more stressful, resulting in a greater CAR (Wilhelm, Born, Kudielka, Schlotz, & Wüst, 2007). There is also evidence that morning endocrine activation begins prior to awakening and may reflect circadian activity not associated with stress (Born, Hansen, Marshall, Mölle, & Fehm, 1999). Thus, CAR may depend on the time of awakening in relation to the person's total sleep period, with shorter sleep time producing a greater cortisol response (Vargas & Lopez-Duran, 2014). The link between sleep and depression is more heterogeneous. While diagnostic criteria (e.g. DSM-5; APA, 2013) and relevant research (e.g. Liu et al., 2007) suggest that depression symptom profiles are heterogeneous, little is known about the mechanisms that explain this divergence. Specifically, depression is commonly associated with difficulty sleeping (i.e. insomnia) and reduced sleep time, which may increase CAR. Alternatively, many depressed individuals report increased sleep (i.e. hypersomnia), comorbid insomnia and hypersomnia or no sleep difficulties at all (Breslau, Roth, Rosenthal, & Andreski, 1996; Liu et al., 2007; Tsuno, Besset, & Ritchie, 2005). Thus, the association between depression and sleep may drive variability in the CAR–depression link. That is, because depression can have contrasting effects on sleep, individual differences in the direction of the sleep–depression link likely determine the direction of the CAR–depression link. For example, depressive symptomatology may be associated with an elevated CAR only among those in which greater depressive symptoms are associated with less sleep.

In addition, the CAR–depression link may also vary by gender differences in neuroendocrine functioning, stress sensitivity, and sleep. Most studies that have linked greater depressive symptomatology to an elevated CAR have been oversampled with females (e.g. Adam et al., 2010; Bhagwagar, Hafizi, & Cowen, 2003; Kern et al., 2011; Ulrike et al., 2013; Vreeburg et al., 2009). The greater prevalence of depression among females has also been frequently attributed to an increased psychological and physiological vulnerability to stress (Kuehner, 2003; Maciejewski, Prigerson, & Mazure, 2001; Nolen-Hoeksema, 2001; Weiss, Longhurst, & Mazure, 1999). In addition, depressed females are more likely to report impaired sleep (e.g. insomnia symptoms, reduced sleep) compared to depressed males (Angst et al., 2002; Baji et al., 2009; Kroenke & Spitzer, 1998). There are a number of potential explanations for this gender difference. For example, there are hypothesized differences in the way that men and women cope with stress and depression (Nolen-Hoeksema, 2012). While men have a greater tendency to use externalizing coping behaviours (e.g. physical activity, alcohol), women use internalizing coping behaviours (e.g. rumination) that may be more likely to impair sleep or increase risk for insomnia (Carney, Harris, Falco, & Edinger, 2013; Thomsen, Yung Mehlsen, Christensen, & Zachariae, 2003). Therefore, the link between increased CAR and depression may be attributed to the relative difference in sleep patterns and/or stress sensitivity among depressed females.

In the current study, we were not interested in examining whether the impact of depressive symptoms on CAR was moderated by sleep, as this would assume that the CAR–depression link varies simply as a function of sleep. Instead, we were interested in a unique type of moderation in which the impact of depressive symptoms on CAR is a function of the *association* between depressive symptoms and sleep for each individual. That is, because depressive symptomatology can have opposite effects on sleep (i.e. insomnia, hypersomnia or both), the degree and direction in which depressive symptoms impacts CAR could be a function of how (i.e. in what direction) depressive symptoms impact sleep. We predicted: (H1) greater depressive symptoms would be associated with an elevated CAR among those for whom greater depressive symptoms are associated with shorter sleep, and (H2) that this relationship between sleep and depressive symptoms would be moderated by gender, such that the link would be stronger among females compared to males. In summary, the current study may provide some insight into the previously mixed findings on the association between depressive symptomatology and CAR, and potentially advance our understanding of the contextual and individual factors that explain the variability in the CAR–depression link.

Methods

Participants

Participants included 58 young adults (30 females; $M_{age} = 18.7$, $SD_{age} = 0.91$) from the volunteer

psychology participant pool at a large American research university. Participants were recruited through an online sign-up procedure and were given course credit points for their participation. Participants were initially screened and excluded from the study if they had a history of chronic medical conditions (e.g. cancer), endocrine disorders (e.g. Cushing's disease) or pregnancy. No participant met any of the exclusion criteria. The Institutional Review Board at the university approved the study, and participants completed written informed consent. This study was conducted in the USA.

Protocol

Participants initially came in to the laboratory on a Monday for an intake appointment, in which they met with a trained research assistant to review the collection procedures and completed the questionnaires (i.e. BDI-II). Participants were given eight clearly marked Salivette tubes (Sarstedt AG & Co., Nümbrecht, Germany), a detailed instruction sheet, a sleep diary and a collection log to take home. Salivary cortisol was used to assess the CAR. Saliva was collected via unstimulated drool without the use of cotton swabs. Participants deposited saliva into the base of the Salivette. All cortisol assessments were conducted across two consecutive weekdays. Cortisol assessments began 1–2 days after the intake appointment (Tuesday – Thursday). Participants were instructed to refrain from eating or drinking until all samples were collected. If the participant ate or drank anything, they were asked to indicate this in the collection log. Participants were also asked to refrain from brushing their teeth and any vigorous activity (e.g. exercise) until the end of the morning sample collection phase. A first sample was obtained immediately after awakening and additional samples were then taken at 30, 45 and 60 min after awakening. Participants were instructed to use a saliva collection log to record the actual time (s) they completed each sample. Participants also received two reminder text messages each morning at their expected time of awakening and 30 min later in order to increase consistency in the timing of cortisol collection.

Depressive symptoms

The Beck Depression Inventory (BDI-II; Beck, Steer, & Brown, 1996) was used to assess current depressive symptomatology (i.e. during the past two weeks). The BDI-II is a 21-item, self-report instrument with excellent reliability and validity (Dozois, Dobson, & Ahnberg, 1998), and can be used as a screening tool for depressive symptomatology (Subica, Fowler, & Elhai, 2014). Total scores on the full 21-item scale range from 0 to 63. Many studies have examined the factor analytic structure of the BDI-II, and suggest a two-factor model. While studies have indicated that the BDI-II is not a unidimensional construct, it does have strong support for its use as a single factor (Subica et al., 2014; Ward, 2006). We were not able to test the factor analytic structure of the current data because of our small sample size. This measure demonstrated good internal consistency in the current sample ($\alpha = 0.79$). The BDI-II was administered during the intake visit.

Sleep patterns

Sleep diary

The Core Consensus Sleep Diary (Carney et al., 2012), developed by a committee of sleep research experts at the Pittsburgh Assessment Conference, is a nine-item self-report measure used to collect information about daily sleep patterns. The diary asked participants to report each day the time they attempted to fall asleep, how long it took them to fall asleep, the number and duration of awakenings experienced and their final time of awakening. Participants completed sleep diaries on both days that salivary cortisol was assessed. The current study specifically focused on the association between CAR and total sleep time (TST), given that a previous report of this data suggested variability in CAR was accounted for by differences in TST, but not other sleep parameters (Vargas & Lopez-Duran, 2014). However, the following sleep parameters were controlled for in the current analyses: sleep onset latency (SOL; how long it took them to initiate sleep, in minutes), wake after sleep onset (WASO; sum of their nocturnal awakenings, in minutes) and waketime (time of final awakening). TST was equal to the difference between the final waketime and the attempted sleep time (in minutes), minus SOL and WASO. Sleep diaries are a widely used self-report measure of daily sleep patterns, and have been shown to be significantly correlated with objective reports of sleep, particularly TST (Monk et al., 1994; Wilson, Watson, & Currie, 1998).

Cortisol assessment

Participants were instructed to store all samples in a freezer within 1 h of collection until they were returned to the laboratory. Within two days of completing saliva collection, participants returned all cortisol collection materials to the laboratory. Samples were stored at −20 °C until assayed and were centrifuged at 1500 × *g* (at 3000 rpm) for 15 min prior to assay. Samples were assayed in duplicates and averaged using a commercial Enzyme Linked Immunosorbent Assay (ELISA) kit (Salimetrics LLC, Carlsbad, CA, USA). To avoid inter-assay variability all samples from the same participant were assayed in the same batch. Duplicates varying more than 15% were re-assayed. The inter-assay and intra-assay coefficients of variability were 10.6% (high = 14.0% and low = 7.2%) and 5.1%, respectively.

Statistical analysis

In the current analyses, we were interested in examining the impact of the *association* between depressive symptoms and sleep on CAR, given the heterogeneous relationship between depressive symptomatology and sleep. We operationalized the association between BDI scores and TST by determining the degree to which BDI scores were linked with increased or decreased TST for each individual. To this end, we computed an adjusted ratio score (DeviationRatio) that reflected whether each individual's BDI and TST coordinate score was closer with a line representing a perfect positive correlation between BDI and TST or a line representing a perfect negative correlation between BDI and TST (examples provided in Figure 1). A BDI/TST coordinate that is closer to the perfect negative correlation line would have a DeviationRatio score that reflects a more negative association between BDI and TST.

To compute the DeviationRatio score, we used the range of TST and BDI scores for this sample to estimate two lines representing perfect positive and negative correlations between TST and BDI (Figure 1). Next, at each participant's BDI score level, we calculated two absolute distances between the participant's actual TST value (based on their sleep diary data) and the estimated TST value assuming (1) the perfect positive correlation line (PosDeviation) and (2) the perfect negative correlation line (NegDeviation). Accordingly, the estimated TST value used to calculate PosDeviation and NegDeviation was equal to the TST value that corresponded with the participant's BDI score level, under the condition of a perfect positive and negative correlation between BDI and TST (see Figure 1). We then estimated the ratio (DeviationRatio) of these two distances so that values above 1 indicated that the participant's ratio score reflected that his or her BDI-TST coordinate fell closer to the perfect positive correlation line relative to the negative correlation line. Likewise, as values decreased from one (and approached 0) the participant score got closer to the negative correlation line.

$$\text{DeviationRatio} = \text{NegDeviation} / \text{PosDeviation}$$

Ratio scores are not normally distributed and must be transformed when used in parametric tests (Cohen, Cohen, West, & Aiken, 2013; Osbourne, 2002). Therefore, we log transformed then mean-centered the LogDeviationRatio to create a final BDI-TST Index.

$$\begin{aligned}\text{LogDeviationRatio} &= \text{Log10}(\text{DeviationRatio})\\ \text{BDI-TST Index} &= \text{LogDeviationRatio} - \\ &\quad \text{Mean}(\text{LogDeviationRatio})\end{aligned}$$

Therefore, increasing positive values in this BDI-TST Index score indicated that the individual was closer to the perfect positive BDI-TST trajectory (i.e. TST increases as BDI increases), while decreasing negative values indicated that the individual was closer to the perfect negative BDI-TST trajectory (i.e. TST decreases

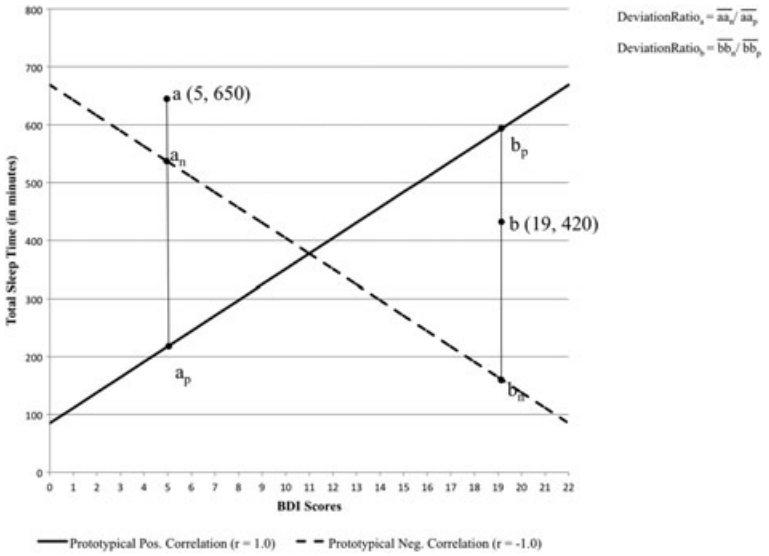

Figure 1. Prototypical trajectories (lines) representing a perfect positive and negative correlation between BDI scores and total sleep time (TST). As ratios increase from one, the BDI-TST coordinate gets closer to the perfect positive correlation line. As ratios decrease from one, the BDI-TST coordinate gets closer to the perfect negative correlation line. In this example, coordinate a (5, 650) receives a Deviation ratio of 0.25, which is close to 0 suggesting the coordinate is close to the negative correlation line. The opposite is true of coordinate b (19, 420) which is greater than one, and therefore, closer to the positive correlation line. Deviation $\text{Ratio}_a = \text{NegDeviation}_a$ $\text{PosDeviation}_a = |650 - 540| |650 - 210| = 110\ 440 = 0.25$. Deviation $\text{Ratio}_b = \text{NegDeviation}_b$ $\text{PosDeviation}_b = |420 - 160| |420 - 600| = 260\ 180 = 1.44$

as BDI increases). Finally, in our sample a zero value reflected no link between BDI and TST for that individual.

We used multiple adjusted random effects growth curve models via SPSS MIXED to examine the impact of our BDI-TST Index, gender, depressive symptoms and their interactions on the cortisol trajectory from awakening (i.e. CAR). Specifically, we examined their effect on awakening cortisol (intercept) and cortisol reactivity (slope) from awakening. We used mixed modelling as opposed to repeated measures ANOVA in order to model the correct covariate structure of the interrelated repeated measures data (Gueorguieva & Krystal, 2004; Hruschka, Kohrt, & Worthman, 2005). We used mixed modelling as opposed to basic examinations of Area Under the Curve (AUC) because it allows for better characterization of patterns of activation and thus can be more sensitive to subtle differences in cortisol reactivity (Lopez-Duran, Mayer, & Abelson, 2014). All models included day of collection, intercept and reactivity slopes as random effects within subjects. Cortisol and other skewed variables were log-transformed to attain normality. However, model estimates and standard errors were back-transformed in our figures to aid interpretability of the results (Jorgensen & Pedersen, 1998). Age, medication use, menstrual cycle, oral contraceptive use, time of awakening and other sleep covariates were first examined via independent unadjusted models to determine their effect on CAR.

Results

Descriptive statistics

Table I presents correlations between all study variables. As expected, the correlation between TST and BDI scores was not significant, $r = -.14$, $p > .20$, which is consistent with the conceptualization of the sleep and depression link as following two opposite patterns. Further, while the correlation between TST and the BDI-TST Index (BTI) was relatively high, $r = -0.59$, $p < .01$, it was not a perfect correlation, confirming that BTI is not simply a proxy for TST. Table II includes the means and standard deviations, as well as gender comparisons, for all predictor and dependent variables. There were no significant gender differences in age, sleep patterns (e.g. TST, time of awakening) or depressive symptoms (i.e. BDI). There were, however, significant mean gender differences in morning cortisol. Specifically, females had significantly greater cortisol compared to males at all four sampling times (Table II). Participants reported a broad range of depressive symptoms (0–22), with 21% reporting mild to moderate levels of depressive symptomatology (BDI $\geq$ 10). Among females, approximately 63% ($n = 19$) were in the follicular phase and 37% in the luteal phase ($n = 11$) of their menstrual cycle. Forty-three percent ($n = 13$) were currently using oral contraceptives.

Unconditional CAR

Modelling CAR alone suggested that cortisol increased linearly after awakening, Time $b = .008$, $t(264) = 8.00$, $p < .001$, and this was followed by a significant deceleration of this increase, Time^2 $b = -0.0001$, $t(231) = -6.93$, $p < .001$, reflecting the expected rise and fall of cortisol levels after awakening. The quadratic model was a better fit to the data (linear model AIC = −19.24 versus quadratic model AIC = −42.63); thus, only quadratic conditional models were tested (i.e. models including both linear and quadratic effects).

Table I. Correlations (Pearson's *r*) between all predictor variables

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
(1) Gender	—												
(2) Age	−.048	—											
(3) BDI	.121	−.037	—										
(4) PSQI	.139	.004	.440**	—									
(5) TST	.165	−.061	−.144	−.149	—								
(6) SOL	−.067	−.009	.006	.165	−.228*	—							
(7) WASO	−.150	.042	−.093	.025	−.057	.026	—						
(8) Waketime	−.053	−.022	.089	.048	.398**	−.089	.150	—					
(9) Cort 0	.232*	.160	.023	−.119	.385**	−.095	−.060	.066	—				
(10) Cort 30	.241**	.180	.052	−.036	.207*	.000	.103	.017	.670**	—			
(11) Cort 45	.296**	.197*	.060	−.022	.057	.000	.085	−.015	.510**	.863**	—		
(12) Cort 60	.292**	.157	−.054	.017	.019	.015	.071	−.056	.431**	.798**	.903**	—	
(13) BTI	−.249**	−.039	.098*	.055	−.589**	.121*	.059	−.095	−.283**	−.221*	−.158	−.172	—

*$p < .05$;
**$p < .01$
Table includes correlations between all study variables for the enitre sample.
BDI: Beck Depression Inventory; PSQI: Pittsburgh Sleep Quality Index; TST: Total Sleep Time; SOL: Sleep Onset Latency; WASO: Wake After Sleep Onset; BTI: BDI-TST index.

Table II. Group means and standard deviations, as well as gender comparisons, for all predictor and dependent variables

	Males ($N = 28$)		Females ($N = 30$)		
	Mean	SD	Mean	SD	p-value
Age	18.79	0.17	18.70	0.17	0.72
BDI	5.82	1.03	7.13	1.00	0.36
PSQI	5.71	0.52	6.47	0.50	0.30
TST (min)	385.51	16.53	421.78	16.20	0.12
SOL (min)	30.15	6.00	24.30	5.74	0.48
WASO (min)	14.21	4.19	4.97	4.11	0.12
Waketime (h:min)	8:46	0:18	8:33	0:17	0.59
Cortisol (μg/dL)					
Wake	0.27	0.03	0.35	0.03	0.04
Wake +30	0.38	0.05	0.55	0.05	0.03
Wake +45	0.37	0.06	0.58	0.05	0.01
Wake +60	0.34	0.06	0.55	0.06	0.01

Accordingly, p-value's less than 0.05 indicate a significant difference between males and females.

BDI: Beck Depression Inventory; PSQI: Pittsburgh Sleep Quality Index; TST: Total Sleep Time; SOL: Sleep Onset Latency; WASO: Wake After Sleep Onset.

Main effects of covariates on CAR

We conducted a series of separate (unadjusted) models to examine potential covariates that impact cortisol at awakening (intercept) and trajectories from awakening. Our results indicated a significant main effect of SOL on CAR. Specifically, SOL was associated with a steeper post-awakening cortisol rise (slope), however, only in females, SOL × Time $b = .007$, $t(311) = 1.97$, $p = .05$. No other covariates (age, medication use, menstrual cycle, oral contraceptive use, waketime and WASO) were significantly associated with individual differences in cortisol at awakening or slope after awakening (data reported elsewhere, Vargas et al., 2014), and were therefore not included in the subsequent analyses. SOL was also not included in subsequent models, given its relatively small effect in females and statistically significant correlation with TST (but results did not change if included).

Main effects of TST, BDI-TST index, gender and depressive symptoms on CAR

The main effect of TST on CAR in the current sample is reported in detail elsewhere (Vargas & Lopez-Duran, 2014); however, shorter TST was associated with lower cortisol at awakening, TST $b = .001$, $t(104) = 5.70$, $p < .001$, and a greater post-awakening linear cortisol activation, TST × Time $b = -.00003$, $t(308) = -2.54$, $p = .01$. BDI-TST index (BTI) was associated with cortisol at awakening in that greater cortisol was linked to those participants for whom greater depressive symptoms were associated with shorter sleep, BTI $b = -.242$, $t(106) = -4.72$, $p < .001$. In contrast, the effects of BTI on the linear or quadratic slopes were not significant. Results also revealed that males had lower cortisol at awakening relative to females, Gender $b = -.141$, $t(59) = -2.21$, $p = .03$. Yet, there were no significant gender differences on post-awakening cortisol trajectories. In contrast, depressive symptoms among the entire sample were not associated with cortisol at awakening or post-awakening cortisol trajectories. See Table III under *Main Effects Models* for all estimates.

Moderators of the link between depressive symptoms and CAR

BTI as a moderator

There was a significant two-way interaction between depressive symptoms and BTI predicting awakening cortisol, BTI × Depressive Symptoms $b = .041$, $t(106) = 3.42$, $p < .001$, and the post-awakening linear slope, BTI × Depressive Symptoms × Time $b = -.002$, $t(297) = -3.61$, $p < .001$. Specifically, among those participants in which greater depressive symptoms were associated with a shorter TST, greater depressive symptoms were associated with lower cortisol at awakening, and a steeper post-awakening cortisol rise (Figure 2a). In contrast, among those participants for whom greater depressive symptoms were associated with a longer TST, greater depressive symptoms were associated with greater cortisol at awakening, and a flatter post-awakening cortisol rise (Figure 2b). See Table III under *BDI-TST Index (BTI) Model* for all model estimates. We repeated these analyses while controlling for TST in order to assess the specificity of the finding to the BTI effect. The interaction between BTI and depressive symptoms predicting the post-awakening linear slope remained significant, BTI × Depressive Symptoms × Time $b = -.002$, $t(282) = -2.36$, $p = .02$, suggesting that this effect is not because of any variance accounted for by TST.

Gender as a moderator

Results from our two-way interaction indicated that the impact of depressive symptoms on the post-awakening cortisol rise varied by gender, Gender × Depressive Symptoms × Time $b = -.001$, $t(267) = -3.27$, $p = .001$. For females, greater depressive symptoms were associated with a steeper post-awakening rise, Depressive Symptoms × Time $b = .0007$, $t(267) = 2.80$, $p < .01$, and greater deceleration over time, Depressive Symptoms × Time^2 $b = -0.000009$, $t(228) = -2.62$, $p < .01$ (Figure 3a). Yet, for males, greater depressive symptoms were associated with a flatter post-awakening rise, Depressive Symptoms × Time $b = -.0006$, $t(267) = -1.94$, $p = .05$ (Figure 3b). In contrast, the link between depressive symptoms and cortisol at awakening did not differ by gender, Gender × Depressive Symptoms $b = .016$, $t(57) = 1.34$, $p = .19$. See Table III under *Gender Model* for all model estimates.

Table III. Unadjusted random effects models for our BDI-TST index, depressive symptomatology and gender predicting morning cortisol

	Cortisol at awakening			Linear slope			Quadratic slope		
	b	SE	*t*-value	*b*	SE	*t*-value	*b*	SE	*t*-value
Main Effects Models									
BTI	−0.242	0.051	−4.72***	0.003	0.002	1.47	1.60E − 05	3.19E − 05	−0.50
Gender[a]	−0.141	0.064	−2.21*	0.001	.002	0.40	−1.62E − 05	3.01E − 05	−0.54
BDI	0.005	0.006	0.82	1.67E − 04	1.94E − 04	0.86	−5.25E − 06	2.76E − 06	−1.90
TST	0.001	2.37E − 04	5.70***	−2.67E − 05	1.05E − 05	−2.54*	7.61−E − 08	1.55E − 07	0.49
Total Sleep Time (TST) Model									
TST	0.001	2.28E − 04	6.17***	−2.67E − 05	1.05E − 05	−2.55*	4.53E − 08	1.56E − 07	0.29
BDI	0.008	0.006	1.41	6.17E − 05	2.05E − 04	0.30	−5.45E − 06	3.01E − 06	−1.81
TST × BDI	−1.42E − 04	4.70E − 05	−3.03**	3.19E − 06	2.15E − 06	1.49	−4.21E − 08	3.19E − 08	−1.32
BDI-TST Index (BTI) Model									
BTI	−0.265	0.047	5.44***	0.004	0.002	1.65	1.18E − 05	3.18E − 05	−0.37
BDI	0.005	0.006	0.83	2.37E − 04	2.04E − 04	1.16	−6.03E − 06	3.04E − 06	−1.99
BTI × BDI	0.041	0.012	3.42***	−0.002	0.001	−3.61***	1.28E − 05	7.82E − 06	1.63
Gender Model									
Gender[a]	−0.134	0.064	−2.10*	0.001	0.002	0.44	−2.20E −05	3.00E − 05	−0.73
BDI (females)	−0.003	0.008	−0.38	6.86E − 04	2.45E − 04	2.80**	−9.40E − 06	3.58E − 06	−2.62**
BDI (males)	0.013	0.009	1.42	−0.001	3.00E − 04	−1.94[†]	3.01E − 07	4.38E − 06	0.07
Gender × BDI[b]	0.016	0.012	1.34	−0.001	3.88E − 04	−3.27**	9.70E − 06	5.66E − 06	1.71

*$p < .05$;
**$p < .01$;
***$p < .001$
[†]$p = .053$
[a]Estimates represent the relative difference in morning cortisol among males compared to females.
[b]Estimates represent the difference in the impact of depressive symptoms on morning cortisol among males compared to females. BTI: BDI-TST Index scores; BDI: Beck Depression Inventory scores; TST: Total Sleep Time.

Three-way interaction: BTI, Gender, and depressive symptoms predicting CAR

There was a significant three-way interaction predicting awakening cortisol, Gender × BTI × Depressive Symptoms $b = -0.073$, $t(103) = 2.88$, $p < .01$. Specifically, among males in which greater depressive symptoms were associated with shorter sleep, greater depressive symptoms were associated with lower cortisol at awakening, BTI × Depressive Symptoms $b = 0.073$, $t(104) = 4.23$, $p < .001$. A similar effect was not seen among females, BTI × Depressive Symptoms $b = -0.001$, $t(100) = -0.03$, $p > .20$. No other three-way interactions, including our hypothesized three-way interaction predicting individual differences in post-awakening cortisol rise, were significant. All estimates for our final model are summarized in Table IV.

Discussion

The CAR has the potential to be a useful biomarker for large-scale studies on depression. In this study, we aimed to increase the potential utility of CAR in depression research by identifying factors that could explain past inconsistencies in the CAR–depression literature. We found that the association between depressive symptoms and TST moderated the link between depressive symptoms and CAR. That is, the impact of depressive symptoms on CAR was a function of how depressive symptoms were related to TST. In addition, gender also moderated the association between depressive symptoms and CAR, such that greater depressive symptoms were associated with a greater CAR in females, yet a blunted CAR among males. This raises the possibility that the biopsychosocial meaning of CAR in relation to depressive symptoms might also differ by gender, or alternatively, that the manner in which depressive symptoms is associated with this specific index of neuroendocrine functioning is gender specific.

We found no overall link between depressive symptoms and CAR. This finding is consistent with a number of studies showing no relationship between depression and CAR (e.g. Ellenbogen, Hodgins, Walker, Couture, & Adam, 2006; Mommersteeg, Heijnen, Verbraak, & van Doornen, 2006; Therrien et al., 2008), yet inconsistent with studies showing atypical CAR among individuals with depression (Dedovic et al., 2010; Dietrich et al., 2013; Kern et al., 2011; Mangold et al., 2011). Thus, in isolation, this finding would only further contribute to an equivocal understanding of the relationship between depression and CAR. However, our results revealed that the link between depressive symptoms and CAR may be a function of how depressive symptoms relate to sleep. Greater depressive symptoms

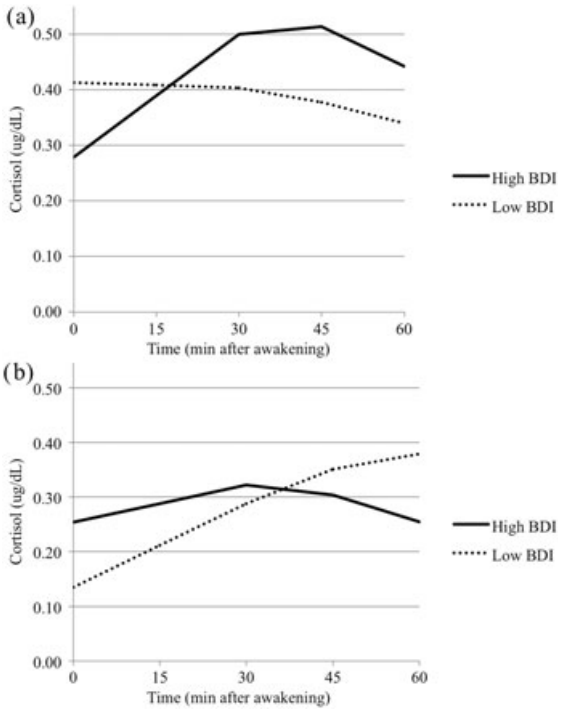

Figure 2. Estimated CAR as a function of the *association* between BDI and TST (i.e. BDI-TST index). BDI-TST index (BTI) curves are based on the estimated BTI mean score of the sample. Figure 2a represents those closer to the perfect negative correlation (i.e. −1SD from mean BTI; TST decreases as BDI increases), and Figure 2b represents those closer to the perfect positive correlation (i.e. +1SD from mean BTI; TST increases as BDI increases). Curves represent high and low BDI scores based on the estimated means. High BDI = 1 SD above the mean BDI (11.27); Low BDI = 1 SD below the mean BDI (0.37)

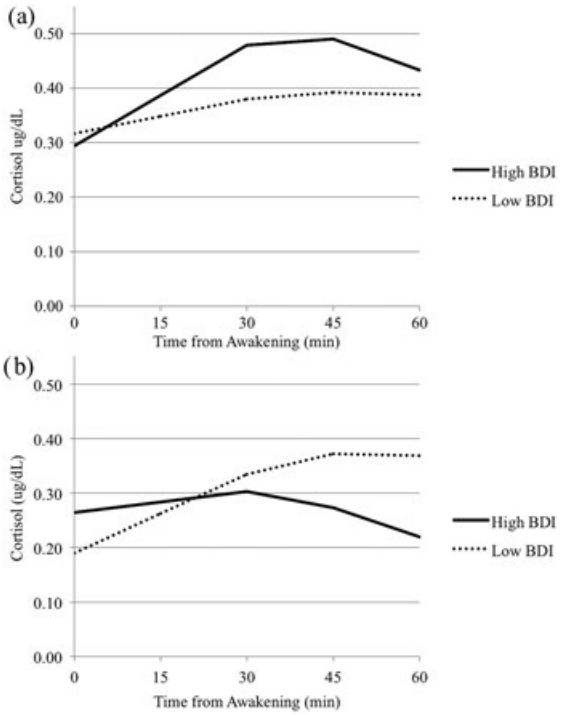

Figure 3. Estimated CAR as a function of depressive symptoms among females (3a) and males (3b). Curves represent high and low BDI scores based on the estimated means. High BDI = 1 SD above the mean (11.27); Low BDI = 1 SD below the mean (0.37)

were associated with lower cortisol at awakening and a steeper CAR when depressive symptoms were linked to a shorter TST. The opposite was true when depressive symptoms were linked to a longer TST. These findings suggest that variability in CAR may instead reflect individual differences in sleep, and the extent to which depressive symptomatology leads to either greater or reduced sleep time at the individual level.

While the mechanisms by which sleep is associated with CAR, and consequently the CAR–depression link, remain unclear, our findings provide some insight into potential mechanisms modulating this link. Compared to long sleepers, short sleepers generally show lower cortisol at awakening (Vargas & Lopez-Duran, 2014), as they may be waking up during an earlier phase of the pre-awakening cortisol rise (Steiger, 2002). Cortisol follows a robust circadian rhythm as adrenal sensitivity, and consequently cortisol release, is strongly driven by the suprachiasmatic nucleus (SCN), or the brain's endogenous 'clock' (Gunnar & Vasquez, 2001; Buckley & Schatzberg, 2005; Clow et al., 2010). This circadian drive may therefore interact with a person's awakening time (in relation to their sleep onset time) to dictate the intensity of CAR. Depressed individuals who sleep less may have lower awakening cortisol, and must compensate by increasing the rate of activation during the post-awakening cortisol rise. Because depressed individuals who sleep more may have greater awakening cortisol, they have less cortisol to produce in order to reach similar homeostatic levels. Alternatively, consistent with the hypothesis that CAR may be a stress response to awakening (Wilhelm et al., 2007), the process of awakening may be more stressful following short sleep versus long sleep. This effect may be particularly strong among those with high depressive symptoms given the link between depression and increased stress sensitivity (Monroe & Harkness, 2005; Spijker & van Rossum, 2012).

Additionally, we found that the link between depressive symptomatology and CAR varied by gender. Greater depressive symptoms were associated with an elevated CAR among females only, which is consistent with studies showing an elevated CAR among females with elevated depression symptoms (e.g.Tops, Riese, Oldehinkel, Rijsdijk, & Ormel, 2008) and studies oversampled with females (e.g. Mannie, Harmer, & Cowen, 2007). CAR is often considered an adaptive response to the demands of the upcoming day (Eller,

Table IV. Adjusted random effects model with depressive symptomatology and BDI-TST index (BTI) predicting morning cortisol

	Cortisol at awakening			Linear slope			Quadratic slope		
	b	SE	*t*-value	*b*	SE	*t*-value	*b*	SE	*t*-value
Females									
BDI	−1.29E − 03	7.24E − 03	−0.18	0.001	2.70E − 04	2.16*	−9.18E − 06	4.05E − 06	−2.27*
BTI	−0.166	0.084	−1.97†	0.003	0.004	0.93	−5.18E − 05	5.44E − 05	−0.95
BDI × BTI	−0.001	0.019	−0.03	−0.002	0.001	−1.81†	6.75E − 06	1.32E − 05	0.51
Males									
BDI	2.04E − 04	0.009	0.02	−2.57E − 04	3.62E − 04	−0.71	−3.02E − 06	5.44E − 06	−0.56
BTI	−0.301	0.062	−4.87***	0.004	0.003	1.29	1.90E − 05	4.19E − 05	0.45
BDI × BTI	0.073	0.017	4.23***	−0.001	0.001	−1.88†	1.10E − 05	1.15E − 05	0.96
Interactions[a]									
BDI	0.001	0.012	0.13	−0.001	4.52E − 04	−1.86†	6.15E − 06	6.78E − 06	0.91
BTI	−0.135	0.104	−1.30	2.05E − 04	0.005	−0.05	7.08E − 05	6.86E − 05	1.03
BDI × BTI	0.073	0.026	2.88**	1.44E − 04	0.001	0.12	4.25E − 06	1.75E − 05	0.24

†$p < .10$
*$p < .05$;
**$p < .01$;
***$p < .001$.
This table includes the two-way and three-way interactions with gender.
[a]Estimates for the relative difference between females and males on each model parameter. Direction of effects reflect relative change from females to males.

Netterstrøm, & Hansen, 2006; Rohleder et al., 2007); thus, atypically elevated CAR may reflect a physiological vulnerability, or increased sensitivity to particular types of stress (Hankin, Mermelstein, & Roesch, 2007; Kuehner, 2003; Shih, Eberhart, Hammen, & Brennan, 2006). There is evidence of increased vulnerability to stress (Maciejewski et al., 2001; Weiss et al., 1999) and stress sensitization (Chopra et al., 2009; Nolen-Hoeksema, 2001) in depressed females. Therefore, in females, CAR may be an index of sensitivity to stress. The awakening process or anticipation to the upcoming day may trigger an elevated CAR in vulnerable individuals and such atypical responses may differentiate depressed from non-depressed females. Alternatively, CAR may not be an index of stress sensitivity but instead may reflect a circadian process under control of the SCN (Fries et al., 2009). It is possible that depressive symptomatology may maximize such circadian driven processes in females, given the known sexual dimorphisms in the structure and function of the SCN (Hofman, Fliers, Goudsmit, & Swaab, 1988; Swaab, Fliers, & Partiman, 1985).

In contrast, among males, depressive symptomatology was associated with a blunted CAR. Some researchers suggest that, among depressed individuals, the regulatory mechanisms that modulate variability in CAR can become exhausted over time (Dedovic et al., 2010), presumably as a function of chronic stress exposure. Specifically, after repeated exposure to high levels of stress, the HPA-axis can down-regulate, resulting in a blunted endocrine stress response (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Hellhammer & Wade, 1993). Relative to females, males are less sensitive to the depressogenic effects of stress (Shih et al., 2006), and thus a greater amount of lifetime stress exposure may be needed for males to reach comparable depressogenic effects. Consequently, one possibility is that the blunted CAR observed among males with higher depressive symptoms reflects down-regulation of the HPA-axis because of greater stress exposure. However, such interpretation is inconsistent with several recent studies showing *greater* HPA-axis activation among depressed males, relative to non-depressed males, suggesting sensitization rather than down-regulation of the axis in depressed males (Binder et al., 2009; Grant, Friedman, Haskett, Riso, & Thase, 2007; Hinkelmann et al., 2011; Owens et al., 2014). Thus, an alternative possibility is that blunted CAR observed among males with high depressive symptoms reflects group differences in circadian processes unrelated to the stress response. Specifically, CAR begins prior to awakening and may be modulated by homeostatic pressure to maintain certain levels of cortisol throughout the day (Meerlo, Sgoifo, & Suchecki, 2008; Thorn, Hucklebridge, Evans, & Clow, 2009; Tops, van Peer, Wijers, & Korf, 2006). Thus, post-awakening cortisol changes are influenced by awakening levels, such that high awakening levels are associated with lower CAR presumably in order to prevent reaching levels beyond homeostatic range or because high awakening levels reflect a later stage in the awakening response. Therefore, males with greater depressive symptoms may have a blunted CAR because their cortisol at awakening may be significantly elevated compared to their non-depressed counterparts (Hinkelmann et al., 2011; Owens et al., 2014).

Limitations and strengths

An important limitation of the current study was that the sample was composed of only college students. Depressive symptoms and sleep patterns among college students may not be representative of the general population nor clinical samples. Indeed, our sample did not consist of clinically depressed participants and thus follow-up studies with clinical samples are warranted. Yet, the increasing rates of depression on college campuses is an important public health concern (Buchanan, 2012). Approximately 15% of college students report a lifetime history of depression, with 32% reporting an episode within the last year (ACHA-NCHA, 2009). Furthermore, 21% of our sample reported mild-to-moderate depressive symptoms (i.e. BDI $\geq$ 10), suggesting enough variability in depressive symptoms to examine the link between depressive symptoms and CAR. However, it is important to note that the BDI was used as a unidimensional construct, which is inconsistent with some literature suggesting it has a two-factor model (Storch, Roberti, & Roth, 2004). Unfortunately, we were unable to confirm its structure via factor analysis because of our small sample size.

Circadian phase preference and insomnia symptoms —factors that have been associated with CAR in previous studies (Backhaus, Junghanns, & Hohagen, 2004; Kudielka, Federenko, Hellhammer, & Wüst, 2006; Randler & Schaal, 2010)—were not directly assessed and need to be controlled for in future studies examing the link between depressive symptoms and CAR. Similarly, differences in light levels during the awakening phase can impact morning cortisol levels (Elder et al., 2014). Our naturalistic study design was not set up to control for awakening light levels in the participants' bedroom. In addition, sleep patterns were assessed via self-reported sleep diaries only. While some sleep parameters (i.e. TST) correlate highly with objective measures of sleep (e.g. actigraphy, polysomnography), others parameters (i.e. WASO or sleep onset latency) do not (Monk et al., 1994). Replication with actigraphy- and polysomnography-assessed sleep patterns is needed in future studies, as well as research investigating the variables and/or mechanisms that explain the heterogeneous relationship between depression and sleep.

Likewise, we collected saliva using self-sampling techniques. This has the limitation that objective methods of assessing compliance (i.e. MEMS caps) were not used, and therefore, information regarding the actual timing of sample collection may be missing or inaccurate. Research suggests that delays greater than 15 min may sufficiently impact cortisol values (Okun et al., 2010), and therefore, self-reported wake/collection times should be interpreted with caution. However, we made an effort to increase sampling adherence by utilizing text messages to remind participants of the correct sample times. The group's CAR response rate (69%) was similar to previously reported response rates among healthy populations (Wüst et al., 2000); therefore, our efforts to facilitate correct sample collection were likely successful. Lastly, our sample size was relatively small and replication with a larger sample is needed.

Despite these limitations, our study had some important strengths. For example, this was the first study to investigate the impact of gender, sleep parameters and depressive symptoms on CAR simultaneously. This provides some insight into how individual (i.e. gender) and contextual (i.e. sleep) factors modulate the link between depressive symptomatology and CAR. We used a growth curve modelling framework to address limitations of more traditional statistical approaches, such as repeated measures ANOVA or AUC, allowing us to better characterize changes cortisol (Gueorguieva & Krystal, 2004; Hruschka et al., 2005). Our analyses controlled for day of collection in all models. Furthermore, we also initially ruled out the effect of any other potentially confounding variables (e.g. age, menstrual cycle, oral contraceptive use, global sleep quality, time of awakening or other sleep parameters) on CAR.

Conclusions

In conclusion, these results advance our current understanding of the factors that may contribute to the variability in the association between depressive symptoms and CAR. We provide evidence suggesting the link between depressive symptoms and CAR varies as a function of both gender and how depressive symptoms relate to sleep, potentially providing an explanation for the previously mixed findings. These findings have theoretical implications for understanding the observed gender differences in depression. Our data indicated that, in a sample of healthy young adults, females had a greater CAR compared to males. CAR is often considered a marker of stress sensitivity (Chida & Steptoe, 2009), and therefore, these data support the hypothesis that females may have an increased [physiological] sensitivity to stress, although alternative conceptualizations of CAR have been proposed (e.g. Wilhelm et al., 2007). Furthermore, they have practical implications for future research, as our findings highlight the importance of conceptually accounting for sleep (i.e. actually considering sleep's role in this model as opposed to simply 'controlling' for it) when examining the relationship between depression and CAR. Ultimately, a better understanding of the factors that impact the relationship between depressive symptomatology and CAR may provide evidence for the utilization of CAR as a potential field biomarker for depression, as well as help explain some of the commonly reported gender differences in depression.

Conflict of interest

The authors have declared that they have no conflict of interest.

REFERENCES

Adam, E. K., Doane, L. D., Zinbarg, R. E., Mineka, S., Craske, M. G., & Griffith, J. W. (2010). Prospective prediction of major depressive disorder from cortisol awakening responses in adolescence. *Psychoneuroendocrinology*, *35*(6), 921–931.

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology*, *34*(10), 1423–1436.

American College Health Association-National College Health Assessment [ACHA-NCHA] (2009). American College Health Association—National College Health Assessment Spring 2008 Reference Group Data Report (Abridged). *Journal of American College Health*, *57*(5), 477–488.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders: DSM-5*. Washington, D.C.: American Psychiatric Association.

Angst, J., Gamma, A., Gastpar, M., Lépine, J.-P., Mendlewicz, J., & Tylee, A. (2002). Gender differences in depression. Epidemiological findings from the European DEPRES I and II studies. *European Archives of Psychiatry and Clinical Neuroscience*, *252*(5), 201–209.

Backhaus, J., Junghanns, K., & Hohagen, F. (2004). Sleep disturbances are correlated with decreased morning awakening salivary cortisol. *Psychoneuroendocrinology*, *29*(9), 1184–1191.

Baji, I., Lopez-Duran, N. L., Kovacs, M., George, C. J., Mayer, L., Kapornai, K., … Vetró, A. (2009). Age and sex analyses of somatic complaints and symptom presentation of childhood depression in a Hungarian clinical sample. *The Journal of Clinical Psychiatry*, *70* (10), 1467–1472.

Beck, A., Steer, R., & Brown, G. (1996). *Manual for the beck depression inventory-II*. San Antonio, TX: Psychological Corporation.

Bhagwagar, Z., Hafizi, S., & Cowen, P. J. (2003). Increase in concentration of waking salivary cortisol in recovered patients with depression. *The American Journal of Psychiatry*, *160*(10), 1890–1891.

Bhagwagar, Z., Hafizi, S., & Cowen, P. J. (2005). Increased salivary cortisol after waking in depression. *Psychopharmacology*, *182*(1), 54–57.

Binder, E. B., Künzel, H. E., Nickel, T., Kern, N., Pfennig, A., Majer, M., … Holsboer, F. (2009). HPA-axis regulation at in-patient admission is associated with antidepressant therapy outcome in male but not in female depressed patients. *Psychoneuroendocrinology*, *34*(1), 99–109.

Born, J., Hansen, K., Marshall, L., Mölle, M., & Fehm, H. L. (1999). Timing the end of nocturnal sleep. *Nature*, *397*(6714), 29–30.

Breslau, N., Roth, T., Rosenthal, L., & Andreski, P. (1996). Sleep disturbance and psychiatric disorders: A longitudinal epidemiological study of young adults. *Biological Psychiatry*, *39*(6), 411–418.

Buchanan, J. (2012). Prevention of depression in the college student population: A review of the literature. *Archives of Psychiatric Nursing*, *12*(1), 21–42.

Buckley, T. M., & Schatzberg, A. F. (2005). On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. *The Journal of Clinical Endocrinology and Metabolism*, *90*(5), 3106–3114. doi:10.1210/jc.2004-1056

Gunnar, M. R., & Vazquez, D. M. (2001). Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. *Development and Psychopathology*, *13*, 515–538.

Carney, C. E., Buysse, D. J., Ancoli-Israel, S., Edinger, J. D., Krystal, A. D., Lichstein, K. L., & Morin, C. M. (2012). The consensus sleep diary: Standardizing prospective sleep self-monitoring. *Sleep*, *35*(2), 287–302.

Carney, C. E., Harris, A. L., Falco, A., & Edinger, J. D. (2013). Rumination about insomnia symptoms. *Journal of Clinical Sleep Medicine*, *9*(6), 567–575.

Chida, Y., & Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: A systematic review and meta-analysis. *Biological Psychology*, *80*(3), 265–278.

Chopra, K., Ravindran, A., Kennedy, S., Mackenzie, B., Matthews, S., Anixman, H., … Levitan, R. (2009). Sex differences in hormonal responses to a social stressor in chronic major depression. *Psychoneuroendocrinology*, *34*, 1235–1241.

Clow, A., Hucklebridge, F., Stalder, T., Evans, P., & Thorn, L. (2010). The cortisol awakening response: more than a measure of HPA axis function. *Neuroscience and Biobehavioral Reviews*, *35*(1), 97–103. doi:10.1016/j.neubiorev.2009.12.011

Cohen, J., Cohen, P., West, S., & Aiken, L. (2013). *Applied multiple regression/correlation analysis for the behavioral sciences*. Mahwah, New Jersey: Lawrence Eribaum Associates, Inc.

Dedovic, K., Engert, V., Duchesne, A., Lue, S. D., Andrews, J., Efanov, S. I., … Pruessner, J. C. (2010). Cortisol awakening response and hippocampal volume: Vulnerability for major depressive disorder? *Biological Psychiatry*, *68*(9), 847–853.

Dietrich, A., Ormel, J., Buitelaar, J. K., Verhulst, F. C., Hoekstra, P. J., & Hartman, C. A. (2013). Cortisol in the morning and dimensions of anxiety, depression, and aggression in children from a general population and clinic-referred cohort: An integrated analysis. The TRAILS study. *Psychoneuroendocrinology*, *38*(8), 1281–1298.

Dozois, D. J. A., Dobson, K. S., & Ahnberg, J. L. (1998). A psychometric evaluation of the Beck Depression Inventory-II. *Psychological Assessment*, *10*(2), 83–89.

Elder, G. J., Wetherell, M. A., Barclay, N. L., & Ellis, J. G. (2014). The cortisol awakening response: Applications and implications for sleep medicine. *Sleep Medicine Reviews*, *18*(3), 215–224.

Ellenbogen, M. A., Hodgins, S., Walker, C.-D., Couture, S., & Adam, S. (2006). Daytime cortisol and stress reactivity in the offspring of parents with bipolar disorder. *Psychoneuroendocrinology*, *31*(10), 1164–1180.

Eller, N. H., Netterstrøm, B., & Hansen, A. M. (2006). Psychosocial factors at home and at work and levels of salivary cortisol. *Biological Psychology*, *73*(3), 280–287.

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): Facts and future directions. *International Journal of Psychophysiology*, *72*(1), 67–73.

Fries, E., Hesse, J., Hellhammer, J., & Hellhammer, D. H. (2005). A new view on hypocortisolism. *Psychoneuroendocrinology*, *30*(10), 1010–1016.

Grant, M. M., Friedman, E. S., Haskett, R. F., Riso, L. P., & Thase, M. E. (2007). Urinary free cortisol levels among depressed men and women: Differential relationships to age and symptom severity? *Archives of Women's Mental Health*, *10*(2), 73–78.

Gueorguieva, R., & Krystal, J. H. (2004). Move over ANOVA: Progress in analyzing repeated-measures data and its reflection in papers published in the Archives of General Psychiatry. *Archives of General Psychiatry*, *61*(3), 310–317.

Hammen, C. L. (2005). Stress and depression. *Annual Review of Clinical Psychology*, *1*, 293–319.

Hankin, B. L., Mermelstein, R., & Roesch, L. (2007). Sex differences in adolescent depression: Stress exposure and reactivity models. *Child Development*, *78*(1), 279–295.

Hellhammer, D. H., & Wade, S. (1993). Endocrine correlates of stress vulnerability. *Psychotherapy and Psychosomatics*, *60*(1), 8–17.

Hinkelmann, K., Botzenhardt, J., Muhtz, C., Agorastos, A., Wiedemann, K., Kellner, M., & Otte, C. (2011). Sex differences of salivary cortisol secretion in patients with major depression. *Stress*, *15*(1), 105–109.

Hofman, M. A., Fliers, E., Goudsmit, E., & Swaab, D. F. (1988). Morphometric analysis of the suprachiasmatic and paraventricular nuclei in the human brain: Sex differences and age-dependent changes. *Journal of Anatomy*, *160*, 127–143.

Hruschka, D. J., Kohrt, B. A., & Worthman, C. M. (2005). Estimating between- and within-individual variation in cortisol levels using multilevel models. *Psychoneuroendocrinology*, *30*(7), 698–714.

Jorgensen, E., & Pedersen, A. R. (1998). How to obtain those nasty standard errors from transformed data—And why they should not be used.

Kern, S., Schultheiss, T., Schneider, H., Schrempf, W., Reichmann, H., & Ziemssen, T. (2011). Circadian cortisol, depressive symptoms and neurological impairment in early multiple sclerosis. *Psychoneuroendocrinology*, *36*(10), 1505–1512.

Kroenke, K., & Spitzer, R. L. (1998). Gender differences in the reporting of physical and somatoform symptoms. *Psychosomatic Medicine*, *60*, 150–155.

Kudielka, B. M., Federenko, I., Hellhammer, D. H., & Wüst, S. (2006). Morningness and eveningness: The free cortisol rise after awakening in "early birds" and "night owls". *Biological Psychology*, *72*(2), 141–146.

Kuehner, C. (2003). Gender differences in unipolar depression: An update of epidemiological findings and possible explanations. *Acta Psychiatrica Scandinavica*, *108*, 163–174.

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. *The Journal of Clinical Endocrinology and Metabolism*, *94*(12), 4801–4809.

Liu, X., Buysse, D. J., Gentzler, A. L., Kiss, E., Mayer, L., Kapornai, K., … Kovacs, M. (2007). Insomnia and hypersomnia associated with depressive phenomenology and comorbidity in childhood depression. *Sleep*, *30*, 83–90.

Lopez-Duran, N. L., Kovacs, M., & George, C. J. (2009). Hypothalamic–pituitary–adrenal axis dysregulation

in depressed children and adolescents: A meta-analysis. *Psychoneuroendocrinology*, *34*(9), 1272–1283.

Lopez-Duran, N. L., Mayer, S. E., & Abelson, J. L. (2014). Modeling neuroendocrine stress reactivity in salivary cortisol: Adjusting for peak latency variability. *Stress*, *17*(4), 285–295.

Maciejewski, P. K., Prigerson, H. G., & Mazure, C. M. (2001). Sex differences in event-related risk for major depression. *Psychological Medicine*, *31*(4), 593–604.

Mangold, D., Marino, E., & Javors, M. (2011). The cortisol awakening response predicts subclinical depressive symptomatology in Mexican American adults. *Journal of Psychiatric Research*, *45*(7), 902–909.

Mannie, Z. N., Harmer, C. J., & Cowen, P. J. (2007). Increased waking salivary cortisol levels in young people at familial risk of depression. *The American Journal of Psychiatry*, *164*(4), 617–621.

Meerlo, P., Sgoifo, A., & Suchecki, D. (2008). Restricted and disrupted sleep: Effects on autonomic function, neuroendocrine stress systems and stress responsivity. *Sleep Medicine Reviews*, *12*(3), 197–210.

Mommersteeg, P. M. C., Heijnen, C. J., Verbraak, M. J. P. M., & van Doornen, L. J. P. (2006). Clinical burnout is not reflected in the cortisol awakening response, the day-curve or the response to a low-dose dexamethasone suppression test. *Psychoneuroendocrinology*, *31*(2), 216–225.

Monk, T. H., Reynolds, C. F., Kupfer, D. J., Buysse, D. J., Coble, P. A., Hayes, A. J., … Ritenour, A. M. (1994). The Pittsburgh Sleep Diary. *Journal of Sleep Research*, *3*(2), 111–120.

Monroe, S. M., & Harkness, K. L. (2005). Life stress, the "kindling" hypothesis, and the recurrence of depression: Considerations from a life stress perspective. *Psychological Review*, *112*(2), 417–445.

Nolen-Hoeksema, S. (2001). Gender differences in depression. *Current Directions in Psychological Science*, *10*(5), 173–176.

Nolen-Hoeksema, S. (2012). Emotion regulation and psychopathology: The role of gender. *Annual Review of Clinical Psychology*, *8*(1), 161–187.

Okun, M. L., Krafty, R. T., Buysse, D. J., Monk, T. H., Reynolds, C. F., Begley, A., & Hall, M. (2010). What constitutes too long of a delay? Determining the cortisol awakening response (CAR) using self-report and PSG-assessed wake time. *Psychoneuroendocrinology*, *35*(3), 460–468.

Osbourne, J. (2002). Notes on the use of data transformation. *Practical Assessment, Research & Evaluation*, *8*(6), 1–8.

Owens, M., Herbert, J., Jones, P. B., Sahakian, B. J., Wilkinson, P. O., Dunn, V. J., … Goodyer, I. M. (2014). Elevated morning cortisol is a stratified population-level biomarker for major depression in boys only with high depressive symptoms. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(9), 3638–3643.

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: Classical theories and new developments. *Trends in Neuroscience*, *31*(9), 464–468.

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., Von Auer, K., Jobst, S., … Kirschbaum, C. (1997). Free cortisol levels after awakening: A reliable biological marker for the assessment of adrenocortical activity. *Life Sciences*, *61*(26), 2539–2549.

Randler, C., & Schaal, S. (2010). Morningness–eveningness, habitual sleep–wake variables and cortisol level. *Biological Psychology*, *85*(1), 14–18.

Riemann, D., Berger, M., & Voderholzer, U. (2001). Sleep and depression — Results from psychobiological studies: an overview. *Biological Psychology*, *57*(1–3), 67–103.

Rohleder, N., Beulen, S. E., Chen, E., Wolf, J. M., & Kirschbaum, C. (2007). Stress on the dance floor: The cortisol stress response to social-evaluative threat in competitive ballroom dancers. *Personality & Social Psychology Bulletin*, *33*(1), 69–84.

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. *Psychosomatic Medicine*, *66*(2), 207–214.

Schulz, P., Kirschbaum, C., Prubner, J., & Hellhammer, D. H. (1998). Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. *Stress Medicine*, *14*(1998), 91–97.

Shih, J. H., Eberhart, N. K., Hammen, C. L., & Brennan, P. A. (2006). Differential exposure and reactivity to interpersonal stress predict sex differences in adolescent depression. *Journal of Clinical Child & Adolescent Psychology*, *35*(1), 103–115.

Spijker, A. T., & van Rossum, E. F. C. (2012). Glucocorticoid sensitivity in mood disorders. *Neuroendocrinology*, *95*(3), 179–186.

Steiger, A. (2002). Sleep and the hypothalamo–pituitary–adrenocortical system. *Sleep Medicine Reviews*, *6*(2), 125–138.

Stetler, C., & Miller, G. E. (2005). Blunted cortisol response to awakening in mild to moderate depression: Regulatory influences of sleep patterns and social contacts. *Journal of Abnormal Psychology*, *114*(4), 697–705.

Storch, E. A., Roberti, J. W., & Roth, D. A. (2004). Factor structure, concurrent validity, and internal consistency of the Beck Depression Inventory—Second Edition in a sample of college students. *Depression and Anxiety*, *19*(3), 187–189.

Subica, A., Fowler, J., & Elhai, J. (2014). Factor structure and diagnostic validity of the Beck Depression Inventory—II with adult clinical inpatients: Comparison to a gold-standard diagnostic interview. *Psychological Assessment*, *26*(3), 1–10.

Swaab, D. F., Fliers, E., & Partiman, T. S. (1985). The suprachiasmatic nucleus of the human brain in relation to sex, age, and senile dementia. *Brain Research*, *342*, 37–44.

Therrien, F., Drapeau, V., Lupien, S. J., Beaulieu, S., Doré, J., Tremblay, A., & Richard, D. (2008). Awakening cortisol response in relation to psychosocial profiles and eating behaviors. *Physiology & Behavior*, *93*(1–2), 282–288.

Thomsen, D. K., Yung Mehlsen, M., Christensen, S., & Zachariae, R. (2003). Rumination—Relationship with negative mood and sleep quality. *Personality and Individual Differences*, *34*(7), 1293–1301. DOI:10.1016/S0191-8869(02)00120-4.

Thorn, L., Hucklebridge, F., Evans, P., & Clow, A. (2009). The cortisol awakening response, seasonality, stress and arousal: A study of trait and state influences. *Psychoneuroendocrinology*, *34*(3), 299–306.

Tops, M., van Peer, J. M., Wijers, A. A., & Korf, J. (2006). Acute cortisol administration reduces subjective fatigue in healthy women. *Psychophysiology*, *43*(6), 653–656.

Tops, M., Riese, H., Oldehinkel, A. J., Rijsdijk, F. V., & Ormel, J. (2008). Rejection sensitivity relates to hypocortisolism and depressed mood state in young women. *Psychoneuroendocrinology*, *33*(5), 551–559.

Tsuno, N., Besset, A., & Ritchie, K. (2005). Sleep and depression. *Journal of Clinical Psychiatry*, *66*(10), 1254–1269.

Ulrike, S., Reinhold, L., & Dirk, H. (2013). Major depression in young girls is related to altered cortisol awakening response. *European Child & Adolescent Psychiatry*, *22*(6), 379–384.

Vargas, I., & Lopez-Duran, N. L. (2014). Dissecting the impact of sleep and stress on the cortisol awakening response among healthy young adults. *Psychoneuroendocrinology*, *40*(1), 10–16.

Veen, G., van Vliet, I. M., DeRijk, R. H., Giltay, E. J., van Pelt, J., & Zitman, F. G. (2011). Basal cortisol levels in relation to dimensions and DSM-IV categories of depression and anxiety. *Psychiatry Research*, *185*(1–2), 121–128.

Vreeburg, S. A., Hoogendijk, W. J. G., van Pelt, J., DeRijk, R. H., Verhagen, J., van Dyck, R., … Penninx, B. W. J. H. (2009). Major depressive disorder and hypothalamic–pituitary–adrenal axis activity: Results from a large cohort study. *Archives of General Psychiatry*, *66*(6), 617–626.

Vrshek-Schallhorn, S., Doane, L. D., Mineka, S., Zinbarg, R. E., Craske, M. G., & Adam, E. K. (2013). The cortisol awakening response predicts major depression: predictive stability over a 4-year follow-up and effect of depression history. *Psychological Medicine*, *43*(3), 483–493.

Ward, L. C. (2006). Comparison of factor structure models for the Beck Depression Inventory—II. *Psychological Assessment*, *18*(1), 81–88.

Wardenaar, K. J., Vreeburg, S. A., Van Veen, T., Giltay, E. J., Veen, G., Penninx, B. W. J. H., & Zitman, F. G. (2011). Dimensions of depression and anxiety and the hypothalamo–pituitary–adrenal axis. *Biological Psychiatry*, *69*(4), 366–373.

Weiss, E., Longhurst, J., & Mazure, C. (1999). Childhood sexual abuse as a risk factor for depression in women: Psychosocial and neurobiological correlates. *The American Journal of Psychiatry*, *156*, 816–828.

Wilhelm, I., Born, J., Kudielka, B. M., Schlotz, W., & Wüst, S. (2007). Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology*, *32*(4), 358–366.

Wilson, K. G., Watson, S. T., & Currie, S. R. (1998). Daily diary and ambulatory activity monitoring of sleep in patients with insomnia associated with chronic musculoskeletal pain. *Pain*, *75*(1), 75–84.

Wüst, S., Wolf, J. M., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000). The cortisol awakening response—Normal values and confounds. *Noise & Health*, *2*(7), 79–88.