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Negative emotionality, depressive symptoms and cortisol diurnal rhythms: Analysis of a community sample of middle-aged males

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ABSTRACT

Prior research suggests that individuals with particular personality traits, like negative emotionality, are at greater risk for adverse health outcomes. Despite bivariate associations between negative emotionality, depressive symptoms and the hypothalamic pituitary adrenal axis (HPA axis), few studies have sought to understand the biological pathways through which negative emotionality, depressive symptomatology and cortisol—one of the primary hormonal products of the HPA axis—are associated. The present study explored whether negative emotionality influenced cortisol dysregulation through current depressive symptomatology and whether negative emotionality served as a moderator of the relationship between depressive symptoms and cortisol. In the community-based Vietnam Era Twin Study of Aging, 783 male twins completed two days of cortisol saliva sampling in their natural environments. Three measures of cortisol were analyzed: waking levels, the cortisol awakening response, and the peak to bed slope. Depressive symptoms significantly mediated the associations between negative emotionality and the peak to bed slope. A 2-way interaction between depressive symptoms and negative emotionality was significant for the peak to bed slope and for waking levels of cortisol. Exploration of the interactions illustrated that depressive symptoms only affected cortisol slopes at average or high levels of negative emotionality and only affected waking levels at low levels of negative emotionality. Negative emotionality and depressive symptoms were not related to the cortisol awakening response. This is the first study to find indirect associations between negative emotionality and peak to bed cortisol slopes through depressive symptoms. These findings illustrate the complex interplay between personality characteristics, depressive symptoms and different indices of the cortisol diurnal rhythm.

Negative emotionality, depressive symptoms and cortisol diurnal rhythms: mediating and moderating pathways

Prior research suggests that individuals with particular personality traits, like negative emotionality, are at greater risk for adverse health outcomes (for reviews see Friedman, 2000; Kern and Friedman, 2011; Smith and Gallo, 2001). Studies have found that higher levels of negative emotionality were associated with risky health behaviors (e.g. Caspi et al., 1997), inflammatory markers and subsequent disease (Black, 2003; Sutin et al., 2010), and subjective well-being (e.g. Friedman et al., 2010). Negative emotionality has been shown to be stable over adulthood and consistent across situations; thus it has been conceptualized as "trait" marker of vulnerability toward general distress.

An indicator of health that has not been closely examined in relation to negative emotionality is the hypothalamic pituitary adrenal axis (HPA axis). The HPA axis is one of the body's major stress responding systems. This association is particularly important

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as negative emotionality may influence how an individual perceives the stressors in their environment. The HPA axis reacts to stressors in the environment by activating a complex cascading of events, eventually resulting in the release of cortisol. Under normal circumstances (i.e., in the absence of specific stressors), cortisol follows a daily rhythm. Specifically, cortisol follows a diurnal pattern by which cortisol levels are high upon awakening in the morning, peak about thirty minutes after waking (commonly called the cortisol awakening response; CAR), and decline across the waking day (Kirschbaum and Hellhammer, 1989; Pruessner et al., 1997). Prior research has found elevations of cortisol in relation to negative or depressed mood in non-clinical populations (Knight et al., 2010; Pruessner et al., 2003; Sjögren et al., 2006) and more persistent, chronic life stressors (Miller et al., 2007), but its associations with the personality trait negative emotionality has yet to be examined.

Negative emotionality and cortisol

To our knowledge, there are no studies of adults that have investigated associations between negative emotionality and cortisol. However, negative emotionality is closely associated with neuroticism (Tellegen, 1985), and is linked with broad negative affectivity (Patrick et al., 2002), which both have been linked with HPA axis activity. Although not all studies have found differences in diurnal cortisol rhythms in subjects high in neuroticism as compared to those with low neuroticism (Kirschbaum et al., 1992a, 1992b; Schommer et al., 1999; van Santen et al., 2010), neuroticism has been associated with differences in HPA axis regulation in everyday life (e.g. diurnal measures; Hauner et al., 2008; Schlotz et al., 2006). Some studies have found increased levels of cortisol at waking in individuals high in neuroticism (Portella et al., 2005; Wetherell et al., 2006), while others reported positive associations between neuroticism and higher evening levels of cortisol (Gerritsen et al., 2009). A recent study found higher average level of cortisol across the whole day in subjects high in neuroticism (Nater et al., 2010).

Relationships among depression, negative emotionality and cortisol

There is evidence that personality traits are associated with depression (Fanous et al., 2007; Kahn et al., 2005, Kendler et al., 1993). Because negative emotionality is a propensity to experience psychological distress that has been shown to be stable over adulthood and consistent across situations, it has been conceptualized as a "trait" marker of vulnerability toward general distress and may predispose an individual to experiencing higher levels of depressive symptoms. Therefore, negative emotionality may impact HPA axis regulation through higher levels of depressive symptomatology. In addition, there is both cross-sectional and prospective evidence that cortisol is associated with depressive symptoms, although relationships with salivary cortisol may be weaker (for review see Stetler and Miller, 2011). van den Bergh et al. (2008) argued that by looking at symptom levels rather than simply the presence of a disorder we may be able to identify pre-disease pathways in all parts of the depressive symptoms continuum by using biological markers as "vulnerability markers." Indeed, Pruessner et al. (2003) found that higher levels of depressive symptoms were associated with greater cortisol awakening responses, and others report associations between depressed mood and flatter diurnal cortisol rhythms (Knight et al., 2010; Sjögren et al., 2006). A recent meta-analysis investigating chronic stress and alterations in HPA-axis regulation argued that even when a person does not develop a psychiatric condition, greater emotional distress is associated with flatter diurnal cortisol (Miller et al., 2007).

Despite bivariate associations between negative emotionality, depressive symptoms and cortisol, no studies have examined these three constructs simultaneously. In the present study we examine whether a stable personality characteristic, negative emotionality, influences cortisol dysregulation through current depressive symptomatology. In addition, previous studies have not specifically examined whether the relationship between depressive symptoms and HPA axis dysregulation is modified by personality characteristics such as negative emotionality. To the extent that negative emotionality can be considered a marker of a liability to general distress, it may exacerbate the effects of depressive symptomatology on cortisol. Thus, experiences of depressive symptomatology may be more strongly associated with HPA axis dysregulation among individuals with high levels of negative emotionality.

The present study

In this study we examined the potential pathways among negative emotionality, depressive symptoms and indices of the cortisol diurnal rhythm. The present study extends previous work by considering the joint pathways through which negative emotionality and depressive symptoms are associated with diurnal rhythms of cortisol and by testing whether negative emotionality moderates the association between depressive symptoms and diurnal rhythms of cortisol in a large sample of community-dwelling men. We focused on three measures of the diurnal pattern of cortisol — waking levels, slope of the diurnal rhythm across the day and the CAR because of the findings between these parameters and major depression or depressive symptoms in prior research (as outlined above). We hypothesized the following: 1) negative emotionality and depressive symptoms would be independently associated with indicators of the cortisol diurnal rhythm; 2) depressive symptoms would partially mediate the associations between negative emotionality and cortisol; and 3) levels of negative emotionality would moderate the associations between depressive symptoms and cortisol such that the relationship between depressive symptoms and cortisol dysregulation is higher among people with higher levels of negative emotionality.

Method

Participants

The sample for this study included a subset of male monozygotic and dizygotic twins who took part in the Vietnam Era Twin Study of Aging (VETSA). The VETSA has been described in detail elsewhere (Kremen et al., 2006). VETSA twins were randomly selected for recruitment from a pool of 3322 twin pairs in the Vietnam Era Twin Registry who served in the US military at some time during the Vietnam era (1965–1975), were aged 51–60 during the VETSA data collection, and who took part in a prior study of psychological health in 1992 (Tsuang et al., 2001). These individuals were not selected as Veteran Affairs patients and most were never in combat or in Vietnam (Kremen et al., 2006). There were 1237 participants in the VETSA (mean age = 55.9, SD = 2.58). Eighty-eight percent of the sample was Caucasian, 4.3% African-American, 2.9% Hispanic, .9% Native American, and .4% Pacific Islander. Compared to national data for men in their 50s (National Health and Nutrition Examination Survey, NHANES III, 2007; National Center for Disease Statistics. Health and United States, 2003), the VETSA participants are similar to the larger US population of men in this age range based on demographic (age, education, income, marital status, employment) and health data (prevalence of chronic health problems, diabetes, and hypertension). Two years after the VETSA study began, an additional study was initiated to understand the role of cortisol and other hormonal dysregulation as a risk factor for cognitive aging. Approximately one-third (N = 442) of VETSA participants had already been studied and were ineligible for the additional cortisol study. Of the remaining 795 subjects, 786 (98.9%) participated in the hormone data collection study between 2005 and 2007. The analyses reported herein were non-twin analyses.

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Procedures

Participants completed salivary collection in their naturalistic settings on two non-consecutive "typical" working days (for full description of saliva collection procedures see Franz et al., 2010). Cortisol kits were mailed via courier and participants received a reminder call the day before they were to begin sampling to ensure that materials were received, to go over procedures and to answer any questions. Materials included: 4.5 mL Cryotube vials, original Trident sugarless gum, straws, tissues, detailed instructions, a daily log, pen, a reminder watch set for all of the sampling times, and a storage container with a MEMS 6™ (Aardax) track cap for detecting compliance with protocol. The materials were tested to ensure that they did not alter the assays. On each day participants were asked to provide samples at waking, 30 min post waking, 10:00 am, 3:00 pm, and 9:00 pm or bedtime for a total of 10 samples per participant. Participants with non-standard schedules (i.e., night shift workers) provided samples at equivalent time periods based on their wake time. At each sampling point, participants provided a saliva sample, placed it in the storage container (with the track cap so the entry was logged) and filled out a written log reporting on their mood, food and drink consumption, medication use, alcohol use and whether or not they smoked or exercised in the last hour. Lastly, participants also filled out an extensive psychosocial questionnaire at home and brought the questionnaire to their study site.

Measures

Cortisol

Participants provided five samples a day for two days. Cortisol was collected by passive drool until the participant had provided at least 2.25 mL of saliva. If necessary, they chewed Trident gum to stimulate saliva and removed the gum prior to providing the sample. Participants stored and refrigerated their saliva samples in an insulated lunch bag included with the cortisol kits. At the end of the two days, they sent the saliva samples via overnight mail to the University of California, Davis to be assayed. Salivary assays were estimated in duplicate using commercial radioimmunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA). The sensitivity of the cortisol assay is 1.39 nmol/L and the intra- and inter-assay coefficients of variation are 3.96 and 5.66 respectively. All saliva samples from each individual participant were assayed together in batches containing one to three individuals. Three individuals were excluded due to lost (N = 1) or contaminated (N = 2) samples, resulting in a final sample of N = 783. There was very little missing data due to participant lapses or technical problems (~1.0%) although 0.1% of samples were re-coded as missing because they had values greater than 50 nanomoles per liter (nmol/L; Hellhammer et al., 2009; Nicolson, 2008; see Franz et al., 2010 for more details). Individual cortisol values were positively skewed (skewness range = 1.7 to 20.1) and were log transformed to approximate normality. Analyses focused on three indices of cortisol patterns throughout the day, as described in detail below.

Negative emotionality

Negative emotionality was assessed using Tellegen's Multidimensional Personality Questionnaire (MPQ) factor-form NZ; the NZ version is considered to be very similar to the Brief Form (Caspi, 2000; Caspi et al., 1997; Krueger et al., 2000; Patrick et al., 2002). Validity of the psychometric properties of the MPQ is well documented (Krueger et al., 2000; Patrick et al., 2002; Tellegen, 1985). The MPQ is a self-report questionnaire and consists of 11 scales. The three subscales of interest in this study are Stress Reaction, Alienation, and Aggression, which when summed together make up the broad trait of Negative Emotionality (NEM). This trait was our primary interest for these analyses because of overlap with measures of trait neuroticism (Tellegen, 1985). The stress reaction scale has 14 items and assesses whether someone is prone to negative emotions or is easily worried or anxious ($\alpha = .86$). The alienation scale has 17 items and detects whether someone often feels victimized, mistreated or pushed around ($\alpha = .83$). Lastly, the aggression scale has 18 items and assesses whether someone enjoys aggression, is physically aggressive, or is vindictive ($\alpha = .75$). These three subscales were summed to create a total NEM score with a possible range of 0 to 49.

Depressive symptoms

Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977). The scale is made up of 20 items designed to measure levels of depressive symptoms experienced in the past week. Participants circled responses ranging from 0 to 3, with 0 indicating that they had experienced that symptom rarely or <1 day of the week and 3 indicating that they had experienced that symptoms most of that week or 5–7 days. The CES-D has been checked for reliability across several age groups, has good internal consistency within the VETSA sample ($\alpha = .90$), and has been found to be highly correlated with indicators of major depression based on accepted cut-off scores (Rush et al., 2000; Shafer, 2006). Items were summed to create a composite scale with a possible range of 0 to 60. This measure was positively skewed (skewness = 1.72) and therefore was log transformed prior to analysis.

Covariates

As part of the cortisol collection procedure, participants were asked to record whether they had smoked, eaten, drunk alcohol, taken medication, or exercised before each sample. Responses at each time were coded as 0 (no) or 1 (yes). In accordance with prior research (Kirschbaum et al., 1992a, 1992b), smoking prior to the sample collection was the only variable consistently related to cortisol level. In addition, data were collected on several other variables known to be associated with cortisol including wake time and number of hours slept prior to the days of sampling (see Doane et al., 2010 for details).

Data preparation and manipulation

Cortisol follows a predictable diurnal rhythm with relatively high levels at wake time, followed by an increase of 50–60% in the 20–40 min after waking (known as the cortisol awakening response), a rapid decline in the subsequent few hours, and a slower decrease throughout the remainder of the day to reach the lowest point near midnight (Pruessner et al., 1997). We simulated this growth curve pattern using the statistical program WinBUGS (Spiegelhalter et al., 2004). WinBUGS uses Markov Chain Monte Carlo (MCMC) which allowed us to control for the non-independence of samples introduced when individuals were assayed in batches, and further allowed us to include nicotine use as a time-varying covariate in the analysis. To account for the batch effects, we set the ith assayed value of the jth twin by Y_{ijk}, where the subscript k further indicates the batch in which the value was assayed such that assays in the same batch have the same value of k. We used each participant's log-transformed cortisol values at each time point to model the waking value of cortisol (WAKE), the cortisol slope from wake up to the peak (cortisol awakening response, CAR), and the exponential decay after the peak until bedtime or peak-to-bed slope for each day, while also accounting for batch and nicotine use. A larger CAR indicates a greater difference between waking and peak levels of cortisol at the wake and wake + 30 samples, while a lower PTB slope indicates "flatter" cortisol slopes across the day. Parameter estimates for WAKE, CAR, and PTB for each participant were then exported for use as the main dependent variables in all analyses.

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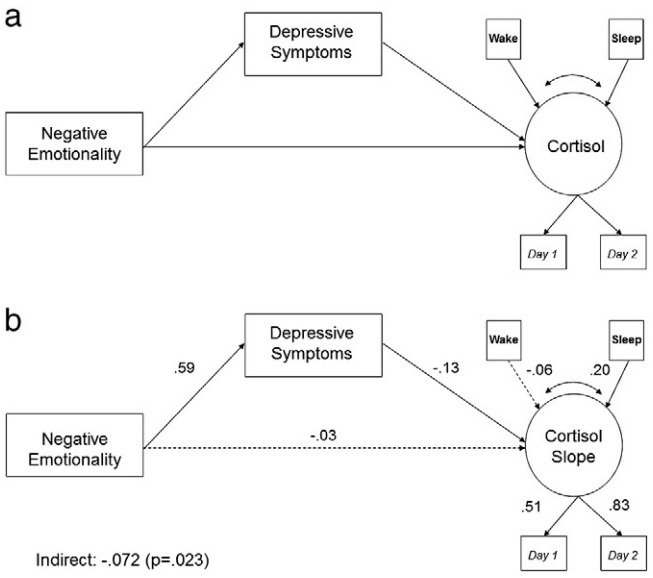

Fig. 1. Latent factor path model testing the direct and indirect effects of negative emotionality and depressive symptoms on cortisol. Dotted lines represent nonsignificant paths and full lines represent significant paths ($p<.05$). Panels represent: a) generalized model and b) latent factor path model for peak to bed cortisol diurnal slope estimates. There is also a significant indirect effect of negative emotionality through depressed mood to the peak to bed slope.

Analytic strategy

Analyses were conducted on 735 (94% of the original 783) participants who had complete data for all cortisol parameters, predictors and covariates. The sample consisted of 340 "complete" twin pairs (N = 680 individuals) and 55 men whose twin did not participate in the study. Analyses were performed in three stages: 1) mediational models; 2) moderation analyses; and 3) post hoc exploration of moderation effect.

Mediation

The first stage of analysis used a latent factor path model to test models of WAKE, CAR and PTB as predicted by depressive symptoms and NEM. These models were run to determine if negative emotionality affected cortisol through its effect on depressive symptoms. To accomplish this we fit a full model specifying direct and indirect effects of NEM on the cortisol parameters as well as direct effects of depressive symptoms on the cortisol parameters (see Fig. 1a). Cortisol parameters were defined as latent variables with corresponding indicators measured over the two days. Hours of sleep and wake time (averaged across the two days) were also included as covariates of the cortisol latent factors. Paths were estimated from NEM to depressive symptoms and cortisol. A path was also specified from depressive symptoms to cortisol. For ease of interpretation, all variables were standardized in the analysis. The cluster command was used to account for the correlated observations of participants from the same family (i.e., twins within pairs).

Mediation models were fit using MPlus 3.0 (Muthén and Muthén, 1998–2004). Maximum likelihood estimation was used and absolute model fit was assessed with the chi-squared statistic, the comparative fit index (CFI) and the root mean square error of approximation (RMSEA). The criterion of acceptable fit was >.95 for the CFI and <.06 RMSEA (Hu and Bentler, 1999).

Moderation

The role of NEM as a significant moderator of the depressive symptoms–cortisol associations was tested using three-level hierarchical linear models to account for the nesting of days within persons, and persons nested within family (Raudenbush and Bryk, 2002; Singer and Willett, 2003). In these models the cortisol parameters (WAKE, CAR, PTB) for each person at each day were the outcome variables, and were predicted by day-varying covariates of hours of sleep and wake time (Level 1), and the person level predictors of depressive symptoms, NEM, and the interaction between NEM and depressive symptoms (Level 2). Clustering for individuals nested within families occurred at Level 3. Predictors were grand mean centered prior to creating interaction terms and were then standardized for ease of interpretation and for comparison of estimates with other studies.

Testing interaction between negative emotionality and depressive symptoms

Significant two-way interactions between depressive symptoms and NEM were further explored using the simple slopes technique for hierarchical linear modeling as outlined in Preacher et al. (2006) for interactions between two Level 2 predictors. This method expands on typical simple slopes techniques (e.g., Aiken and West, 1991), as it accounts for the nesting of the data and possible covariation both within and between levels of the nested data. Briefly, we utilized the online calculator developed by Preacher et al. (http://people.ku.edu/~preacher/interact/hlm2.htm) to produce simple slopes estimates, standard errors and *p*-values for associations of depressive symptoms and cortisol at low, average, and high levels of NEM. The online calculator also produced an estimate of the range of NEM where the relationship between depressive symptoms and cortisol was statistically significant.

Results

Preliminary and descriptive analyses

Descriptive statistics for our primary independent and dependent variables and covariates are presented in Table 1. For simplicity, cortisol values are shown averaged across Day 1 and Day 2. Note that the mean raw cortisol values follow the expected diurnal rhythm with levels high in the morning, peaking 30 min after waking and slowly declining across the day. Simple correlations among the predictors, the various cortisol parameters and covariates are shown in Table 2.

Table 1
Descriptive statistics for dependent and independent variables (N = 735).

Variable	Mean	SD	Minimum	Maximum
Average wake time cortisol (nmol/L)[a,b]	9.698	5.437	1.385	38.066
Average wake + 30 min cortisol (nmol/L)[a,b]	13.591	6.952	1.611	46.729
Average 10:00 am cortisol (nmol/L)[a,b]	5.835	3.323	1.392	24.720
Average 3:00 pm cortisol (nmol/L)[a,b]	4.123	2.307	1.390	23.582
Average bedtime cortisol (nmol/L)[a,b]	2.707	2.239	1.390	25.160
Negative emotionality	10.168	7.755	0.000	45.000
Depressive symptoms from the CES-D[b]	8.104	7.943	0.000	52.000

[a] Day 1 and Day 2 values have been averaged.
[b] Raw values are presented for descriptive purposes but log transformed values are used in all analyses.

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Table 2
Intercorrelation table of main predictors, covariates and cortisol parameters (N = 735).

	2	3	4	5	6	7
1. Wake time cortisol (nmol/L)	−0.314**	−.600**	.047	.070*	−.018	−.069*
2. Cortisol awakening response		−0.212**	.009	−.007	−.204**	−.046
3. Peak to bed cortisol slope			−.077*	−.104**	.164	−.044
4. Negative emotionality				.578**	.037	.011
5. Depressive symptoms					.009	−.026
6. Hours of sleep the night before						.273**
7. Wake time						

** $p<.01$.
* $p<.05$.

As expected, NEM was significantly associated with depressive symptoms ($r=.58$, $p=.000$). WAKE was associated with depressive symptoms ($r=.070$, $p=.05$) and PTB was associated with NEM and depressive symptoms ($r=-.077$, $p=.05$; $r=.104$, $p=.005$). This indicated that higher levels of depressive symptomatology were associated with higher levels of cortisol at waking and with flatter cortisol slopes from peak to bedtime, and NEM was associated with flatter cortisol slopes from peak to bedtime. CAR was not significantly associated with either of the primary predictors of interest (NEM: $r=.009$, $p=.80$; depressive symptoms: $r=-.007$, $p=.85$).

Mediation analyses

Structural equation models predicting WAKE and CAR from NEM and depressive symptoms did not reveal significant meditation pathways. Neither depressive symptoms nor NEM were associated with WAKE (NEM: $\beta=.025$, $SE=.045$, $p=.58$; depressive symptoms: $\beta=.067$, $SE=.045$, $p=.13$) or with CAR (NEM: $\beta=.052$, $SE=.050$, $p=.30$; depressive symptoms $\beta=-.034$, $SE=.049$, $p=.49$).

In contrast, there was significant mediation for PTB. Results from the mediation models are shown in Fig. 1b. All estimates presented in Fig. 1b are standardized estimates. There was good absolute fit of the model to the data for PTB ($X^2(5)=5.014$, $p=.41$; RMSEA = .002, CFI = 1.000). There were significant direct effects of NEM on depressive symptoms ($\beta=.59$, $SE=.027$, $p=.00$) indicating positive associations. There was a significant direct path from depressive symptoms to PTB ($\beta=-.13$, $SE=.061$, $p=.021$) indicating that higher levels of depressive symptoms were associated with flatter PTB slopes (see Fig. 1b). There were no significant direct effects of NEM on PTB; however there was a significant indirect effect of NEM through depressive symptoms on PTB ($\beta=-.072$, $SE=.036$, $p=.023$), indicating that depressive symptomatology was a significant mediator of the effects of NEM on PTB. Additionally, hours of sleep was associated with PTB slopes ($\beta=.20$, $SE=.059$, $p=.001$).

Moderation analyses

Standardized estimates from the HLM moderation analyses are presented in Table 3. There were no main effects of depressive symptoms ($\beta_{01}=.066$, $SE=.041$, $p=.11$) or NEM ($\beta_{02}=.010$, $SE=.041$, $p=.74$) with waking levels of cortisol. However, the 2-way interaction between depressive symptoms and NEM was significantly associated with WAKE ($\beta_{03}=-.062$, $SE=.031$, $p=.04$) indicating that NEM was a significant moderator of the depressive symptoms–WAKE associations. For CAR, there were no main effects of depressive symptoms ($\beta_{01}=-.019$, $SE=.034$, $p=.58$) or NEM ($\beta_{02}=.031$, $SE=.034$, $p=.42$), and the 2-way interaction between NEM and depressive symptoms was not significant ($\beta_{03}=-.014$, $SE=.024$, $p=.59$). Depressive symptoms were significantly associated with PTB ($\beta_{01}=-.076$, $SE=.037$, $p=.04$) while NEM as a main effect was not associated with PTB ($\beta_{02}=-.021$, $SE=.038$, $p=.06$). The 2-way interaction between depressive symptoms and NEM was significant ($\beta_{03}=-.021$, $SE=.010$, $p=.05$) indicating that NEM was a significant moderator of the depressive symptoms–PTB associations.

Interactions between negative emotionality and depressive symptoms

In order to explore the significant interactions between depressive symptoms and NEM for WAKE and PTB, we calculated simple slopes using the online calculator developed by Preacher et al. (2006). Slopes were calculated at low (1 SD below the sample mean), average (sample mean), and high (1 SD above the sample mean) values of NEM. These calculations revealed that there was a significant association between depressive symptoms and WAKE at low levels of NEM ($\beta=.128$, $SE=.05$, $t=2.53$, $p=.01$) but not at average ($\beta=.066$, $SE=.04$, $t=1.62$, $p=.11$) or high levels ($\beta=.003$, $SE=.05$, $t=.07$, $p=.94$). The online calculator indicated that the relationship between depressive symptoms and WAKE was statistically significant at values of NEM less than −.239 (51.7% of the sample).

In contrast, the simple slopes calculations for PTB indicated that there was a significant association between depressive symptoms and PTB slope at average ($\beta=-.076$, $SE=.04$, $t=2.04$, $p=.04$) and high ($\beta=-.097$, $SE=.04$, $t=2.56$, $p=.01$) levels of NEM, but not at low levels of NEM ($\beta=-.055$, $SE=.04$, $t=1.41$, $p=.16$). This was confirmed via the region of significance values: the relationship between depressive symptoms and PTB was significant at values of NEM values greater than −0.13 which corresponds to 43.2% of the sample. An illustration of the simple slopes for relationships between

Table 3
Moderator models for waking level and peak to bed slopes (N = 735).

	Waking levels of cortisol		Cortisol awakening response		Peak to bed slopes	
	Coefficient	*SE*	Coefficient	*SE*	Coefficient	*SE*
Level 1 intercept, Π0						
Average level, β_{00}	.037	.036	.009	.034	−.013	.036
Depressive symptoms, β_{01}	.066	.041	−.019	.034	−.076*,**	.037
Negative emotionality, β_{02}	.010	.041	.031	.034	−.021	.038
Depressive symptoms negative emotionality, β_{03}	−.062*	.031	−.014	.024	−.021*	.010

Note: Analyses covaried for the effects of hours of sleep and wake time at Level 1.
* $p<.05$.
** $p<.01$.

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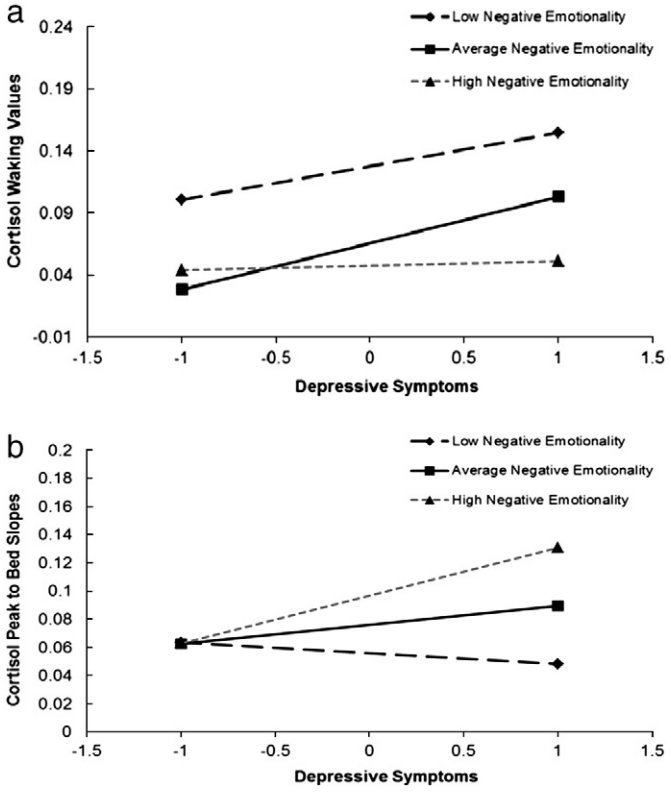

Fig. 2. Simple slope plots for a) wake values of cortisol estimates by levels of depressive symptoms at low, average and high levels of negative emotionality and b) peak to bed cortisol diurnal slope estimates by levels of depressive symptoms at low, average and high levels of negative emotionality. All slope estimates have been adjusted for batch, nicotine, hours of sleep and wake time.

depressive symptoms with WAKE and PTB at low, average, and high levels of NEM can be seen in Figs. 2a and b, respectively.

Discussion

To our knowledge this is the first epidemiological study to examine relationships between NEM, depressive symptoms and measures of diurnal cortisol in a sample of men studied in their naturalistic settings. In this large community-dwelling sample of middle-aged men, significant correlations were found between both NEM and depressive symptoms with PTB cortisol slopes and between depressive symptoms and wake values of cortisol. The CAR was not associated with any of the predictors. Mediation analyses revealed that the relationship between NEM and PTB slope was mediated through depressive symptoms. Thus, depressive symptoms had a direct effect on cortisol, while the effects of NEM on cortisol were indirect. Furthermore, NEM significantly moderated the associations between depressive symptoms and PTB slopes and waking values. Specifically, individuals with high levels of depressive symptoms *and* average or above average levels of NEM had the flattest PTB slopes, while individuals with high levels of depressive symptoms and low levels of NEM had the highest waking values.

To our knowledge no studies have looked specifically at the associations between NEM, depressive symptoms and diurnal measures of cortisol concurrently. In some studies investigating the role of neuroticism, HPA axis activity has been primarily based on laboratory pharmacological or psychological stress-response paradigms (McCleery and Goodwin, 2001; Oswald et al., 2006; Zobel et al., 2004) or single day cortisol measures (Gerritsen et al., 2009; Portella et al., 2005). No prior study has sought to test whether depressive symptoms played a significant meditational role. Previous findings looking at the direct effects between neuroticism or NEM and diurnal cortisol rhythms from naturalistic settings have found *direct* associations between neuroticism and greater cortisol in adults (Nater et al., 2010; Polk et al., 2005;) and flatter cortisol slopes and neuroticism in adolescents (Hauner et al., 2008). Our findings differ in that we found an *indirect* association between NEM and flatter cortisol slopes through depressive symptoms suggesting that depressive symptoms is a significant mediator of the relationship between NEM and cortisol.

In addition, we found two significant moderator effects of NEM on the depressive symptom–cortisol associations. First, as hypothesized, our findings indicated that the association between PTB slopes and depressive symptoms was stronger in subjects scoring average or high on NEM. Moreover, while there were no differences between subjects in PTB slopes based on their scores of NEM at low levels of depressive symptoms when depressive symptoms were high, men with higher NEM scores had significantly flatter PTB slopes than men with lower NEM scores. These results may suggest that NEM and depressive symptomatology both contribute to high levels of allostatic load within individuals (McEwen, 2003). That is, the repeated stressors associated with having high levels of NEM and high levels of depressive symptoms interact over time resulting in a continuous daily activation of the HPA-axis that can become "biologically embedded" (e.g., Bhagwagar and Cowen, 2008). Thus, there is a "flattening" of the diurnal cortisol curve in those who experience high levels of both NEM and depressive symptoms. The flattening of the diurnal cortisol curve over the course of the day has implications for both physical and mental health over the life course. Prior research has shown that flatter diurnal curves are risk factors for cardiovascular disease and diabetes (Rosmond and Bjorntorp, 2000), cognitive decline (Beluche et al., 2010) and decreased life expectancy among cancer patients (Sephton et al., 2000).

An unexpected finding was that depressive symptoms were only associated with waking values of cortisol at low levels of NEM. An intriguing interpretation of this result is that disruptions in waking levels of cortisol are "normative" adaptations to stress among individuals without an underlying liability to distress (i.e. individuals with low levels of NEM), as these individuals show predictable physiological changes associated with high depressive symptoms. In contrast, individuals who have an underlying liability to distress (i.e. individuals with high levels of NEM) may not show the short term physiological changes because of chronic activation of the HPA axis or allostatic load (McEwen, 2003). However, given that this is an unexpected finding, coupled with the fact that there were no main effects of NEM or depressive symptoms on waking levels of cortisol, the interaction between NEM and depressive symptoms for wake values of cortisol should be replicated in other samples.

Limitations and future directions

There are several limitations to the current research. First, the magnitude of the effects found in this study between our primary predictors and cortisol were small. It is not uncommon to find effect sizes of this magnitude when studying biomarkers like cortisol within large community samples. For example, a recent publication from the Whitehall Study (N = 2968 men; Kumari et al., 2010) reported a difference in diurnal cortisol slopes based on level of depressive symptoms, such that men who scored higher than 16 on the CES-D had flatter slopes than men who had lower scores on the CES-D, similar to patterns in the present study. However, the absolute difference between groups in diurnal cortisol slopes for the Whitehall Study was quite small (>16 on CES-D = −.129; <16 on CES-D = −.127). Indeed, a recent meta-analysis found that the associations among depression and cortisol were smallest in studies that used salivary measures of cortisol which was the methodology used in both the Whitehall Study and the present study (Stetler and Miller, 2011).

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It is worth noting that cortisol dysregulation is only a small part of a complex, biological system whereby stress and depression "get under the skin" to impact health and well-being. While any individual effect on biomarkers is likely to be small in absolute terms, together small perturbations in the system can lead to clinically relevant dysfunction over time. Another explanation for the relatively small effect sizes is that short-term indicators such as depressive symptoms over the past week may be insufficient to fully capture the complete range of environmental and psychosocial factors that could account for individual differences in HPA axis activity. Significant variations in HPA axis activity are likely best explained by multiplicative indicators of stress and depression over time that may, in fact, contribute to clinically meaningful dysregulation. Nevertheless, the fact that significant differences in diurnal patterns of cortisol across the day can be detected in a community sample of men is important. In particular, our finding that personality characteristics moderate the effects of depressive symptoms on cortisol regulation provides insight into potential sources of individual differences in the dynamic relationship between depression and cortisol, and may help us to understand why stress and depression have more severe consequences for some individuals than others.

A second limitation is that our study population consists solely of men. Given that women have a higher likelihood of experiencing depression and depressive symptoms (Kessler, 2006; for review see Hyde et al., 2008) our results regarding the mediating and moderating pathways relating to cortisol dysregulation may not generalize to women. Nevertheless, depression is less well-studied in men, and our study is based on a large national community dwelling sample of men, rather than relying on small clinical or selected samples.

Finally, we are limited by the cross-sectional nature of the data used in this study. This may have particular implications for our mediation analyses, as our underlying assumption was that depressive symptoms mediated the effects of NEM on cortisol; however, it is possible that the direction of causality could be reversed. Our assumption regarding the causal relationship between NEM and depression was grounded primarily by the fact that our measure of NEM was based on questions regarding how the individual usually feels, whereas our measure of depression was based symptoms during the previous week. In addition, there is evidence from other samples that negative emotionality and related characteristics such as neuroticism have substantial stability over time (e.g. Costa et al., 2000; Johnson et al., 2005), supporting our assumption that NEM is an enduring personality characteristic. Thus, we assumed that NEM reflects a more trait-like measure, while depressive symptoms scores are likely to capture variations in the shorter term. Of course, the true nature and direction of effects within the biological pathways underlying these associations can only be understood through the analysis of prospective data.

We also note that there was substantial phenotypic overlap ($r = 0.58$) between NEM and depressive symptoms in the present study. Consistent with the literature on genetic overlap between neuroticism and depression, previous results from our sample (Franz et al., in press) indicate almost complete genetic overlap between the subscales of NEM and depressive symptoms, although the phenotypic correlations were also due, in part, to common nonshared environmental factors. As such, the observed moderation and mediation effects in the present study may, in part, be a function of perturbations in depressive symptomatology due to relatively short-term (e.g., week-to-week) fluctuations in environmental factors, or they may be due to disruptions in underlying systems that are partially controlled by genetic factors. If genetic factors do, in fact, contribute to the comorbidities between NEM and depressive symptoms, future research using genetically informative analyses should strive to understand how the stability of negative emotionality relates to changes in both depressed mood and diurnal cortisol rhythms, as well as understand further the interplay of both genetic and environmental contributions to these associations.

Conclusions

To our knowledge this is the first epidemiological study to demonstrate the dynamic associations between negative emotionality, depressive symptoms and diurnal cortisol rhythms collected from men in their naturalistic settings. We believe that our findings not only extend prior work by beginning to deconstruct the pathways through which NEM and cortisol are associated, but that they also may explain some of the inconsistencies of past literature. If past research has not accounted for levels of depressive symptoms and only looked for the *direct* associations between personality traits and cortisol, they may have missed significant pathways through depressive symptomatology. Results of this study should encourage future research in population based samples of both men and women to understand if there are varying gendered pathways through which both personality characteristics and short term depressive symptomatology influence alterations in HPA-axis regulation.

Uncited references

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Kendler et al., 2006

Raudenbush et al., 2004

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