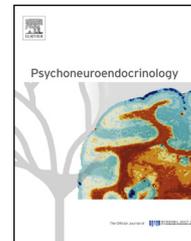




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Associations among daily stressors and salivary cortisol: Findings from the National Study of Daily Experiences

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Summary While much research has focused on linking stressful experiences to emotional and biological reactions in laboratory settings, there is an emerging interest in extending these examinations to field studies of daily life. The current study examined day-to-day associations among naturally occurring daily stressors and salivary cortisol in a national sample of adults from the second wave of the National Study of Daily Experiences (NSDE). A sample of 1694 adults (age = 57, range = 33–84; 44% male) completed telephone interviews detailing their stressors and emotions on eight consecutive evenings. Participants also provided saliva samples upon waking, 30 min post-waking, before lunch and before bed, on four consecutive interview days resulting in 5995 days of interview/cortisol data. Analyses revealed three main findings. First, cortisol AUC was significantly higher on stressor days compared to stressor-free days, particularly for arguments and overloads at home, suggesting that daily stressors are associated with increased cortisol output, but that not all daily stressors have such an influence. Second, individuals reporting a greater frequency of stressor days also exhibited a steeper diurnal cortisol slope. Finally, daily stressor–cortisol associations were unaltered after adjustment for daily negative affect and physical symptoms. Our discussion focuses on the influence of naturally occurring daily stressors on daily cortisol and the role of daily diary approaches for studying healthy cortisol responses to psychosocial stressors outside of traditional laboratory settings.

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1. Introduction

Daily stressors are minor events, such as arguments or work deadlines that arise out of the routines of day-to-day living, and have both immediate and cumulative effects on physical and psychological well-being (Lazarus, 1999; Zautra, 2003; Almeida, 2005). Previous research has shown that the experience of daily stressors is associated with increases in negative affect and physical symptoms (Almeida et al., 2005; Stawski et al., 2008; Piazza et al., 2013; Charles et al., 2013). While the self-reported emotional and physical toll of daily stressors has been well-demonstrated, evidence demonstrating the effects of daily stressors on stress physiology, specifically naturally occurring cortisol levels, is comparatively scant. Using data from the National Study of Daily Experiences (NSDE), which combines daily telephone interviews with saliva collection from adults ages 33–84, the current study examines prevalence of daily stressors across midlife and old age and their associations with naturally occurring levels and diurnal rhythms of salivary cortisol.

Stressors contribute to disease risk by taxing biological resources (McEwen, 1998). The hypothalamic–pituitary–adrenal (HPA) axis is thought to be a primary biological system for understanding the effects of psychosocial stressors on health and disease (Kemeny, 2003). Cortisol, a product of the HPA axis, has received considerable attention as a highly useful biomarker because of its sensitivity to psychosocial stress, utility as an indicator of neuroendocrine/HPA-axis health and function (Miller et al., 2007; Hellhammer et al., 2009), and predictor of general health and mortality (Wrosch et al., 2008; Schoorlemmer et al., 2009; Kumari et al., 2011). An important feature of cortisol is its diurnal pattern, reaching its peak within an hour after waking and declining thereafter, until reaching a nadir at approximately midnight (Pruessner et al., 1997; Kudielka et al., 2003). The initial rise is referred to as the cortisol awakening response (CAR), and the decline as the diurnal cortisol slope (DCS). A robust CAR and DCS are thought to reflect healthy HPA axis function (Stone et al., 2001; Adam and Kumari, 2009), whereas hypo- or hyper-activity of these two components are related to adverse physiological outcomes, such as hypertension (e.g., Wirtz et al., 2008) and coronary calcification (Matthews et al., 2006). Moreover, evidence suggests that ongoing stressors, such as low socioeconomic status (e.g., Steptoe et al., 2005) and burnout (e.g., Pruessner et al., 1999; De Vente et al., 2003) are associated with alterations in the CAR and DCS. The current study extends this research by examining associations between minor daily stressors and both the CAR and DCS.

1.1. Field studies of stress and salivary cortisol

Considerable experimental evidence has documented that experiencing moderate psychosocial stressors results in a transient increase in salivary cortisol (Dickerson and Kemeny, 2004). Additionally, researchers have moved outside of laboratory settings, using ecological momentary assessments (EMA), to better understand the temporal covariation of naturally occurring stressful experiences and cortisol (see Kudielka et al., 2012 for review). van Eck et al. (1996), for example, showed that stressors experienced at one sampling

occasion were associated with higher cortisol levels at the next occasion. Similarly, Jacobs et al. (2007) and Smyth et al. (1998) observed that cortisol levels were significantly higher on occasions when participants reported experiencing stressors; however, these effects were accounted for by respondents' momentary affect reports, suggesting that the influence on cortisol operated through emotional responses.

1.2. Stressors, affect and cortisol

Previous studies have examined associations between daily affect and the diurnal rhythm of cortisol among healthy adults. Using EMA designs, higher levels of negative mood are shown to be associated with higher levels of cortisol, with limited evidence for stressors having a separate influence (Hanson et al., 2000; Jacobs et al., 2007; Smyth et al., 1998). Using a daily diary design, Adam et al. (2006) showed that days older adults reported experiencing more intense negative affect were associated with a steeper CAR and a flatter DCS. However, Adam et al. (2006) utilized measures of emotional experience, which can be influenced by the experience of antecedent stressors as well as other psychosocial factors (i.e., personality) and sociodemographic characteristics (i.e., age, gender). Without separate assessments of stressors and affect, it is impossible to distinguish whether cortisol is influenced by stressors or from negative affect possibly related to or independent of the experience of stressors (e.g., Jacobs et al., 2007; Smyth et al., 1998). Thus, it is important to consider the unique influence of stressors and affect for predicting day-to-day variation in cortisol.

We address these issues using a daily diary approach that offers a complementary intensive repeated measures design which typically utilize end-of-day assessments, characterizing a person's day (Bolger et al., 2003). Whereas EMA approaches aim to characterize moments or hours, daily diary approaches aim to characterize days, providing an ideal landscape for examining day-to-day associations among experienced stressors and cortisol levels across the day, as well as the diurnal components of daily cortisol (i.e., CAR and DCS) and indices of total daily cortisol output such as area under the curve (AUC; Pruessner et al., 2003). The present study used the Daily Inventory of Stressful Events (DISE; Almeida et al., 2002) that was developed to combine checklist and interview-based assessments of stressful daily experiences emanating from different domains of life (e.g., interpersonal interactions, work- and home-related responsibilities, and social networks).

1.2.1. Aims of present study

The current study was conducted to investigate the effects of daily stressors on naturally occurring cortisol levels and rhythms using a daily diary approach and drawing on a national sample of midlife and older adults who completed telephone interviews detailing the stressors they experienced. We focus on three specific aims. First, we examine the prevalence and distribution of daily stressors experienced throughout midlife and later adulthood, including interpersonal tensions, work- and home-related overloads, and network stressors. Second, we test the hypothesis that minor daily stressors impact naturally occurring cortisol profiles resulting in higher overall cortisol levels, greater

total cortisol output (AUC), a steeper CAR and flatter DCS. Finally, we explore the unique effect of daily stressors on cortisol after taking daily affect and physical health symptoms into account.

2. Methods

2.1. Participants

Participants in the current study completed the second wave of the Midlife in the United States survey (MIDUS; [Friedman et al., 2009](#)), as well as the National Study of Daily Experiences (NSDE; [Almeida et al., 2002](#); [Almeida, 2005](#)). At wave 1 (1994–1995) MIDUS respondents ranged in age from 25 to 74 years with an oversample of people between the ages of 40–59 years, with a second wave of data collection occurring approximately 10 years later. The original data collection and follow-up included a combination of telephone interviews and self-administered questionnaires. As part of the second wave of data collection, additional measures of physiological and biological function were collected. The current study utilizes data from the second wave of data collection, as cortisol was not assessed during the first wave.

The second wave of the NSDE included 2022 participants with a mean age of 56 ($SD = 12$, range = 33–84), who were 57% female. Participants had an average household income of approximately \$53,000, and were fairly well educated, with 30% having a high school diploma or less, 51% having some college or a bachelor's degree, and 19% having beyond a bachelor's degree. The ethnic composition of the sample was 93% Caucasian and 3% African American, with the remaining 4% comprised of other ethnic groups.

2.2. Procedure

Across eight consecutive evenings, participants completed brief telephone interviews, during which they were asked about the events they experienced throughout the previous 24 h. This interview included questions regarding participants' affective state, their physical health status, and the stressors they encountered ([Almeida et al., 2002](#)). The day of the week for the first interview day was randomly selected, with the remaining interview days following consecutively. On four of the interview days (days 2–5) participants also provided saliva samples upon waking, 30-min post-waking, before lunch and before bedtime.

2.3. Daily stressors

Daily stressors were assessed using the Daily Inventory of Stressful Events (DISE; [Almeida et al., 2002](#)). The inventory consists of a series of stem questions asking whether certain types of daily stressors had occurred. For the purposes of the current study, we relied on questions which represented interpersonal tensions ("Did you have an argument or disagreement with anyone since (this time/we spoke) yesterday?"; Did anything happen that you could have argued about but you decided to let pass in order to avoid a disagreement?"); work-related overloads (Did anything happen at work or school that most people would consider stressful?); home-related overloads ("Did anything happen at home that

most people would consider stressful?"); and network stressors ("Did anything happen to a close friend or relative that turned out to be stressful for you?"). Participants also rated the severity of each stressor (0 = not at all stressful; 3 = very stressful). Dichotomous variables indicating whether each type of stressor occurred in the past 24 h, as well as a summary variable indicating whether any of the stressors endorsed, were used as measures of exposure. Stressor severity was indexed by summing the severity ratings of the reported events for each day.

2.4. Negative affect

Daily negative affect was assessed using scales developed for the MIDUS study ([Mroczek and Kolarz, 1998](#); [Kessler et al., 2002](#)). Participants indicated how they were feeling today, by responding on a 5-point scale (0 = none of the time, 1 = a little of the time, 2 = some of the time, 3 = most of the time, 4 = all of the time). The negative mood scale consisted of 14 items (restless or fidgety, nervous, worthless, so sad nothing could cheer you up, everything was an effort, hopeless, lonely, afraid, jittery, irritable, ashamed, upset, angry, and frustrated). Total scores were obtained by summing across items for each scale with higher scores reflecting higher negative affect. The alphas for the negative affect scale at the between- and within-person levels were .92 and .76, respectively.

2.5. Physical symptoms

Daily physical symptoms were assessed using a checklist of 25 physical symptoms ([Larsen and Kasimatis, 1991](#)) assessing pain and musculoskeletal symptoms (e.g., headache), gastrointestinal (e.g., nausea), flu and respiratory symptoms (e.g., cough), and other physical symptoms (e.g., teeth-related symptoms). Physical symptoms were quantified as a sum of the number of symptoms, where higher scores reflect a greater number of physical symptoms. The alphas for the symptoms checklist at the between- and within-persons levels were .80 and .60, respectively.

2.6. Covariates

We included a series of covariates in all models which previous research has identified as possible confounds with respect to analyses linking psychosocial factors to cortisol. These included age, sex, education, smoking status (smokers versus non-smokers), medication use (i.e., steroid inhalers, steroid medications, medications containing cortisone, birth control pills, other hormonal medications, and/or antidepressant/anti-anxiety medications), menopause status, and self-rated health (5 = poor, 4 = fair, 3 = good, 2 = very good, 1 = excellent). For cortisol levels at each sampling occasion, wakeup time and the appropriate sample collection time were included as covariates. For AUC, CAR, and DCS, wakeup time was included as a covariate.

2.7. Salivary cortisol sampling and collection

Respondents received a Home Saliva Collection Kit one week prior to their initial phone call. Saliva was obtained using salivette collection devices (Sarstedt, Nümbrecht,

Table 1 Descriptive statistics for salivary cortisol.

Sample collection times	M	SD(BP) in minutes	SD(WP) in minutes
Waking cortisol	06:44 h	63	51
30-min post-waking	07:19 h	64	52
Before lunch	12:40 h	53	66
Before bed	22:31 h	65	47
Cortisol samples (nmol/L)	M	SD(BP)	SD(WP)
Waking cortisol	15.24	5.84	6.80
30-min post-waking	21.17	7.69	8.15
Before lunch	7.18	3.32	4.06
Before bed	3.48	3.11	4.02
AUC	162.14	57.82	80.00

Note: SD(BP), standard deviation between-persons; SD(WP), standard deviation within-person across days.

Germany). Sixteen numbered and color-coded salivettes and instructions were included in the collection kit. In addition to written instructions, telephone interviewers reviewed the procedures and answered any of the participant's questions. On days two through five, respondents provided four saliva samples per day that were later assayed for cortisol. Saliva was collected immediately upon waking, 30 min after waking, before lunch, and before bed (Table 1). Data on the exact time respondents provided each saliva sample was obtained from the nightly telephone interviews, as well as on a paper-pencil log sent with the collection kit. The correlation between these two reports was above .90 for each of the four sampling occasions.

Upon completion of the saliva sampling procedure, the salivettes were shipped to the MIDUS Biological Core at the University of Wisconsin, where they were stored at -60°C . For analysis, salivettes were thawed and centrifuged at 3000 rpm for 5 min, yielding a clear fluid with low viscosity. Cortisol concentrations were quantified with a commercially available luminescence immunoassay (IBL, Hamburg, Germany), with intra-assay and inter-assay coefficient of variations below 5% (Dressendorfer et al., 1992). Almeida et al. (2009a,b) provide additional information regarding the assessment of cortisol in this study.

2.8. Analytic indices for cortisol

Multiple indices for cortisol were used in the current study including cortisol levels at each of the four sampling occasions, area under the curve (AUC), cortisol awakening response (CAR) and diurnal cortisol slope (DCS). AUC was calculated using raw cortisol values for all four daily samples, and with respect to ground (Pruessner et al., 2003). The CAR and DCS were estimated using a 3-level multilevel growth curve model (described below; see also Stawski et al., 2011). Information regarding the cortisol samples and sample times is shown in Table 1.

2.9. Analytic strategy

Multilevel modeling (MLM; Snijders and Boskers, 2012) was used to examine the effects of daily stressors on cortisol using

SAS PROC MIXED (v9.2). MLM provides a flexible framework for analyzing repeated measures data where observations are non-independent, as well as for examining both time-varying and time-invariant predictors of interest. Here, our primary hypotheses and statistical tests involve the covariation of daily stressors and cortisol across days and across people. Our index of daily stressors (whether any stressor occurred, and also each stressor type individually), was group-mean centered (Enders and Tofighi, 2007; Hoffman and Stawski, 2009). Such centering yields separate estimates of the association between daily stressors and cortisol both within- (across days) and between-persons (across individuals).

For the four cortisol levels and AUC, 2-level models were used to account for the nesting of days within persons (Eqs. (1a)–(1c)).

$$\begin{aligned} \text{Level 1 : } \text{AUC}_{di} \\ = a_{0i} + a_{1i}(\text{Stress}_{di} - \text{Stress}_{.i}) + e_{di} \end{aligned} \quad (1a)$$

$$\text{Level 2 : } a_{0i} = B_{00} + B_{01}(\text{Stress}_{.i}) + U_{0i} \quad (1b)$$

$$a_{1i} = B_{10} + U_{1i} \quad (1c)$$

At level 1, Eq. (1a) states that AUC on day d for individual i is a function of an intercept (a_{0i}), which reflects an individual's average daily AUC, and a slope (a_{1i}), reflecting the change in AUC associated with the experience of daily stressors. e_{di} is the residual variance across days. At level 2, Eqs. (1b) and (1c), the level 1 intercept and slope are given as a function of the sample average AUC (B_{00}) and daily stress effect (B_{10}), as well as random effects allowing for variation in the AUC (U_{0i}) and time-varying daily stress effects (U_{1i}). Furthermore, B_{01} reflects the person-mean frequency of stressors and reflects the association between individual differences in stressor frequency and AUC. The same models and logic were used for predicting cortisol levels at each of the four sampling occasions.

For the CAR and DCS, we used a 3-level model to account for the nesting of samples within days within persons (Eqs. (2b)–(2e)).

Table 2 Prevalence and distribution of daily stressors.

	Total sample	Females	Males	<i>p</i>	HS diploma or less	Some college or 4-year degree	More than 4-year degree	<i>p</i>	<i>r</i> _{age}	<i>p</i>
Any stressors	35%	36%	32%	<.01	27%	36%	43%	<.01	-.24	<.01
Arguments	9%	10%	8%	.10	7%	9%	11%	<.01	-.22	<.01
Avoided arguments	15%	16%	14%	<.01	13%	15%	17%	<.01	-.18	<.01
Work overloads	8%	8%	9%	.05	5%	9%	13%	<.01	-.26	<.01
Home overloads	9%	10%	7%	<.01	5%	9%	12%	<.01	-.05	.02
Network stressors	5%	6%	4%	<.01	4%	5%	7%	<.01	.02	.33

$$\text{Level 1 : Cortisol}_{odi} = a_{0di} + a_{1di}(\text{CAR}_{.di}) + a_{2di}(\text{DCS}_{.di}) + a_{3di}(\text{DCS}_{.di}^2) + e_{odi} \quad (2a)$$

$$\text{Level 2 : } a_{0di} = B_{00i} + B_{01i}(\text{Stress}_{di} - \text{Stress}_{.i}) + U_{0di} \quad (2b)$$

$$a_{1di} = B_{10i} + B_{11i}(\text{Stress}_{di} - \text{Stress}_{.i}) + U_{1di} \quad (2c)$$

$$a_{2di} = B_{20i} + B_{21i}(\text{Stress}_{di} - \text{Stress}_{.i}) + U_{2di} \quad (2d)$$

$$a_{3di} = B_{30i} + B_{31i}(\text{Stress}_{di} - \text{Stress}_{.i}) \quad (2e)$$

$$\text{Level 3 : } B_{00i} = C_{000} + C_{010}(\text{Stress}_{.i}) + V_{0di} \quad (2f)$$

$$B_{10i} = C_{100} + C_{110}(\text{Stress}_{.i}) + V_{1di} \quad (2g)$$

$$B_{20i} = C_{200} + C_{210}(\text{Stress}_{.i}) + V_{2di} \quad (2h)$$

$$B_{30i} = C_{300} + C_{310}(\text{Stress}_{.i}) \quad (2i)$$

At level 1, Eq. (2) serves as the growth curve model for the rhythm of cortisol across the day. Cortisol on occasion *o* on day *d* for individual *i* is a function of an intercept (*a*_{0*di*}), reflecting an individual’s cortisol level upon awakening (sample 1), and three slope parameters modeling changes in cortisol per hour time elapsed since awakening. *a*_{1*di*} represents the CAR and is the time elapsed between sample 1 (awakening) and sample 2. The resultant estimate yields the rate of change in cortisol, per hour, from awakening (sample 1) to sample 2. *a*_{2*di*} and *a*_{3*di*} represent the DCS and model linear and quadratic change in cortisol across samples 1, 3 and 4 per hour since awakening (sample 1). Thus, the linear component of the DCS reflects the initial and instantaneous rate of change in cortisol, per hour, at the time of sample 1, and the quadratic component reflects the rate of deceleration in DCS per additional hour elapsed since awakening. *e*_{odi} is the residual.

At level 2, Eqs. (2b)–(2e), the level 1 intercept and slopes become the outcomes and reflect an individual’s

average awakening cortisol level (*B*_{00*i*}), CAR (*B*_{10*i*}) and DCS (*B*_{20*i*} and *B*_{30*i*}) across the study days. Additionally, *B*_{10*i*}, *B*_{20*i*}, *B*_{30*i*} and *B*_{40*i*} reflect the change in the intercept, CAR and DCS associated with time-varying (within-person) effect of daily stressors. *U*_{0*di*}, *U*_{1*di*} and *U*_{2*di*} are the random effects allowing the intercept, CAR and DCS to vary within-persons across days. Finally, at level 3, Eqs. (2f)–(2i), the level 2 intercepts and slopes become the outcomes and reflect the sample average awakening cortisol level (*C*₀₀₀), CAR (*C*₁₀₀) and DCS (*C*₂₀₀ and *C*₃₀₀). *C*₀₁₀, *C*₁₁₀, *C*₂₁₀ and *C*₃₁₀ reflect the effect of individual differences in daily stressor frequency on the awakening cortisol level, CAR and DCS, respectively. *V*_{0*di*}, *V*_{1*di*} and *V*_{2*di*} are the random effects allowing the intercept, CAR and DCS to vary across persons. As preliminary results indicated that random slopes for time-varying daily stressor effect were not significant, only the fixed effects were estimated and shown in the equations.

The covariates, age, sex, education, smoking status, medication use,¹ menopause status, and self-rated health were included at the person-level in all models, whereas wakeup time (and sampling time for analyses with specific cortisol levels) was included as both day-level and person-level characteristics using person-mean centering (Hoffman and Stawski, 2009). All models were estimated using full information maximum likelihood estimation, and the variances and covariances among random effects were freely estimated using unstructured variance–covariance matrices. Furthermore, cortisol levels exhibited positive skew. As such we explored the influence this may have had on parameter estimates and our use of a linear multilevel model. While inspection of residuals did exhibit some evidence of the violation of normality, re-estimation using log-transformed cortisol values or robust standard errors revealed that there were negligible differences in the pattern of results and statistical significance across the methods. As such, we present our results using raw cortisol levels.

¹ We examined differences in cortisol levels among individual reporting using medications and those who did not. Participants reporting using medications exhibited slightly lower awakening cortisol levels (15.50 nmol/l vs. 14.60 nmol/l, *p* = .02) and AUC (165.1 vs. 156.8, *p* = .03), but did not differ in their cortisol levels 30-min post-waking, before lunch, before bed, CAR or DCS. Furthermore, the pattern of results was identical when including medication use as a covariate or excluding these individual from the analyses.

2.10. Data reduction

Of the 2022 participants in NSDE II, 287 (14%) were excluded, as they did not provide cortisol samples. Thirty nine (2.3%) participants were identified as shift workers with highly variable wake and bedtimes, leaving the analytic sample of 1694 persons. We excluded cortisol samples where the sample was missing (.09%), the assay value was deemed to be unreliable (.10%), not provided (1.49%), empty (1.41%), >60 nmol/l (1.46%), missing a time stamp (1.28%), or if the before lunch sample was ≥ 10 nmol/l more than the 30-min post-waking sample suggesting that participants may have consumed food prior to taking their sample (1.78%). Thus, of the possible 27,104 cortisol samples (1694 persons \times 4 samples \times 4 days), 25,045 (92.4%) were useable. For analyses involving AUC, CAR, and DCS, we also excluded days where <15 or >60 min elapsed between the first two samples as an indicator of non-compliance and potentially missing the CAR (11.53%), resulting in 5995 useable days of data.

3. Results

The prevalence and distribution of daily stressors is shown in Table 2. Stressors were reported on 35% of the study days with interpersonal tensions (i.e., overt and avoided arguments) occurring most frequently followed by home-related overloads, work-related overloads and network stressors. Females experienced daily stressors more frequently than males ($ps < .01$) across all stressor types except work-related overloads, which males experienced more frequently ($p < .05$). Daily stressor exposure increased across the education gradient with the most highly educated participants reporting the highest frequency of exposure across all

stressor types ($ps < .01$). Age was associated with a decreased frequency of daily stressors across all stressor types ($ps < .01$) except network stressors (*n.s.*).

Descriptive statistics regarding salivary cortisol sample collection times, levels, and AUC are shown in Table 1. There was considerable variability both across persons as well as within-persons across days in both the times individuals provided their samples and the absolute levels of cortisol and AUC. Additionally, we estimated an unconditional multilevel model for CAR, and DCS to provide estimates of sample averages for these slopes, reflecting the rate of change in cortisol per hour. The sample average level of cortisol upon awakening was 16.08 nmol/l, CAR was 8.80 nmol/l per hour (or 4.40 nmol/l over 30 min), while the linear and quadratic DCS slopes indicated that cortisol declined at an initial rate of 2.97 nmol/l per hour from awakening, and the rate of decline decelerated .12 nmol/l per hour thereafter.

Our models examining associations between daily stressors and cortisol levels and AUC are shown in Table 3 and CAR and DCS in Table 4. With respect to cortisol levels and AUC, Table 3 shows that within-persons across days, the experience of daily stressors was associated with significantly higher cortisol levels 30-min post-waking and before lunch, and a greater AUC compared to non-stressor days ($ps < .01$). The only significant between-person effect of daily stressors indicated that individuals who experience daily stressors more frequently also exhibit significantly higher levels of cortisol 30-min post-waking ($p < .05$). With respect to associations between daily stressors and the CAR and DCS, as shown in Table 4, the only significant effects of daily stressors were between-persons. Individuals experiencing daily stressors more frequently exhibit a significantly steeper DCS that decelerates more rapidly across the day ($ps < .05$).

Table 3 Associations between any daily stressors and salivary cortisol (nmol/L) – levels and AUC.

	Waking		30-min post-waking		Before lunch		Before bed		AUC	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Intercept	16.64**	.66	22.32**	.90	6.57**	.43	2.15**	.39	157.83**	6.59
Wakeup time (WP)	-.06	.14	3.06**	1.15	.11	.09	.02	.08	-18.19**	1.24
Wakeup time (BP)	-.16	-.15	3.50**	1.23	.36**	.11	-.07	.10	-11.29	1.51
Sample time (WP)	–	–	-4.58**	1.14	-.45**	.07	.28**	.08	–	–
Sample time (BP)	–	–	-3.97**	1.21	-.44**	.12	-.04	.10	–	–
Age	.04**	.01	.12**	.02	.08**	.01	.05**	.01	1.19**	.15
Sex (1 = female)	1.63**	.39	.61	.52	.71**	.26	.24	.23	13.20**	3.91
Education (ref. = high)	–	–	–	–	–	–	–	–	–	–
Middle	-.50	.44	.38	.59	.28	.29	.20	.26	4.36	4.37
Low	-1.42**	.51	.06	.68	-.27	.33	.13	.30	1.83	5.07
Smoker (1 = yes)	-.41	.63	.56	.84	1.68**	.40	.68	.37	25.83**	6.24
Medications (1 = yes)	-.55	.37	-.67	.50	-.11	.24	.36	.22	-3.50	3.71
Self-rated health	-.47**	.18	-1.09**	.24	.23*	.11	.36**	.11	-2.84	1.77
Menopause (1 = yes)	-.06	.47	.28	.63	-.59	.31	-.19	.28	-3.38	4.72
Daily stressors (WP)	.29	.23	.76**	.28	.36*	.17	.09	.14	6.30**	2.14
Daily stressors (BP)	.20	.73	2.30**	.97	.04	.48	.19	.43	10.20	7.29

Note: WP: within-persons, BP: between-persons. Daily stressors (WP) – 95% confidence interval: (30-min post-waking: .21–1.32; before lunch: .03–.69; AUC: 2.11–10.48). Daily stressors (BP) – 95% confidence interval: (30-min post-waking: .39–4.21).

* $p \leq .05$.

** $p \leq .01$.

Table 4 Associations between any daily stressors and salivary cortisol (nmol/L) – diurnal rhythm.

	Intercept		Cortisol Awakening Response		Diurnal Cortisol Slope (Linear)		Diurnal Cortisol Slope (Quadratic)	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Intercept	16.08**	.18	8.80**	.28	-2.97**	.03	.12**	.001
Wakeup time (WP)	-.23*	.12	1.90**	.27	.01	.04	.01**	.002
Wakeup time (BP)	-.21	.16	-.62*	.26	-.05*	.02	.01	.001
Sample time (WP)	—	—	—	—	—	—	—	—
Sample time (BP)	—	—	—	—	—	—	—	—
Age	.06**	.02	.12**	.02	-.01**	.003	.001**	.000
Sex (1 = female)	1.66**	.41	-2.12**	.66	.12	.07	-.009*	.004
Education (ref. = high)	—	—	—	—	—	—	—	—
Middle	-.28	.46	1.29	.74	-.04	.08	.002	.004
Low	-1.07*	.53	1.56	.86	-.03	.09	.005	.005
Smoker (1 = yes)	-.08	.66	2.40*	1.07	.24*	.11	-.02**	.006
Medications (1 = yes)	-.71	.39	.41	.63	.09	.07	-.003	.004
Self-rated health	-.54**	.18	-.29**	.30	.26**	.03	-.01**	.002
Menopause (1 = yes)	-.01	.49	.19	.80	-.08	.09	.004	.005
Daily stressors (WP)	.28	.21	.75	.79	-.07	.07	.002	.004
Daily stressors (BP)	0.65	.76	1.54	1.23	-.31*	.14	.02*	.01

Note: WP: within-persons, BP: between-persons. Intercept: model estimate cortisol level upon awakening. Daily stressors (BP) – 95% confidence interval: (diurnal cortisol slope – linear: -.58 to -.05; diurnal cortisol slope – quadratic: .001–.036).

* $p \leq .05$.

** $p \leq .01$.

Table 5 Daily stressor effects on cortisol covarying for negative affect and physical symptoms.

	Waking		30-min post-waking		Before lunch		Before bed	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Model 1								
Daily stress (WP)	.29	.23	.76**	.28	.36*	.17	.09	.14
Daily stress (BP)	.20	.73	2.30*	.97	.03	.48	.19	.43
Model 2								
Daily stress (WP)	.34	.23	.77**	.29	.37*	.17	.09	.14
Daily stress (BP)	.19	.73	2.31*	.97	.02	.47	.16	.43
Model 3								
Daily stress (WP)	.37	.23	.76**	.29	.37*	.17	.11	.15
Daily stress (BP)	.28	.75	2.59**	1.01	-.05	.50	.02	.45
AUC								
	AUC		Cortisol awakening response		Diurnal cortisol slope (linear)		Diurnal cortisol slope (quadratic)	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Model 1								
Daily stress (WP)	6.30**	2.14	.75	.79	-.07	.07	.002	.004
Daily stress (BP)	10.20	7.29	1.54	1.23	-.31*	.14	.02*	.007
Model 2								
Daily stress (WP)	6.45**	2.15	.30	.21	-.07	-.07	.002	.004
Daily stress (BP)	9.90	7.28	.56	.76	-.32*	.13	.013	.007
Model 3								
Daily stress (WP)	6.52**	2.20	-.50	.53	-.04	.08	.000	.004
Daily stress (BP)	10.99	7.59	-2.86*	1.34	-.48**	.15	.02*	.01

Note: WP: within-persons, BP: between-persons. Model 1: estimate from Tables 3 and 4 (all covariates). Model 2: Model 1 + negative affect (WP and BP). Model 3: Model 1 + physical symptoms (WP and BP).

* $p \leq .05$.

** $p \leq .01$.

While the within-person associations may seem small in absolute value, it is worth noting that the increase in cortisol levels and AUC on stressor days compared to stressor-free days is 6.3 times larger than the effect of age (1-year age difference) for cortisol levels 30-min post-waking, 4.8 times larger for lunchtime cortisol, and 5.5 times larger for AUC. Thus, the effect of a daily stressor on cortisol levels and total output is comparable to a 5–6 year age difference.

Next, we re-ran our models with daily stressors predicting cortisol, covarying for negative affect and physical symptoms both within- and between-persons. The results are shown in Table 5. Model 1 shows the estimates for daily stressor effects on cortisol from the models shown in Table 3, while Models 2 and 3 show the within- and between-person daily stressor effects covarying for negative affect (Model 2) and physical symptoms (Model 3), respectively. As can be seen in Table 5, the effects of daily stressors on cortisol levels, AUC, CAR, and DCS all remained significant and changed very little covarying for either negative affect or physical symptoms. Furthermore, results remained unchanged if negative affect and physical symptoms were included as covariates simultaneously.

We also moved beyond our use of a simple composite index of daily stressors indicating whether any stressors had occurred, and explored whether certain types of daily stressors were particularly potent with respect to cortisol. We reran our previous daily stress-cortisol models categorizing each type of stressor (arguments, avoided arguments, work-related overloads, home-related overloads, and network stressors); however, such analyses were only conducted when our initial models using the composite daily stressor index revealed a significant association with cortisol (i.e., cortisol levels 30-min post-waking and before lunch, AUC, CAR, and DCS). We found marginally significant within-person associations between network stressors and cortisol levels 30-min post-waking (Est. = 1.18, $SE = .60$, $p = .06$), as well as arguments and lunchtime cortisol levels (Est. = .43, $SE = .23$, $p = .06$). We also found that AUC was significantly higher on days when arguments (Est. = 6.62, $SE = 3.30$, $p = .05$) or home-related overloads were experienced (Est. = 6.83, $SE = 3.40$, $p = .05$). We did not observe any significant between-person associations when considering daily stressors and cortisol by stressor type.

Finally, we examined the effects of stressor severity on cortisol. However, stressor severity did not have an incremental effect on cortisol over and above the within-person effect of stressor exposure for cortisol levels 30-min post-waking (Est. = .68, $SE = .83$, $p = .41$), before lunch (Est. = $-.67$, $SE = .49$, $p = .17$), or on AUC (Est. = -7.27 , $SE = 6.23$, $p = .24$). Furthermore, individual differences in stressor severity did not have an incremental effect on cortisol over and above the between-person effect of stressor exposure for cortisol levels 30-min post-waking (Est. = 3.76, $SE = 3.08$, $p = .22$), the linear (Est. = $-.55$, $SE = .42$, $p = .19$) or quadratic (Est. = .02, $SE = .02$, $p = .28$) components of the DCS.

4. Discussion

The current study produced a number of notable results. First, daily stressors are prevalent during midlife and older

age occurring on 35% of study days, but there is heterogeneity in the types of stressors reported, their frequency, and their sociodemographic distribution. Second, between-person effects indicated that individuals who reported more frequent stressor exposure exhibited significantly higher levels of cortisol 30-min post-waking, a steeper CAR, and a steeper DCS. Third, within-persons across days, cortisol levels at 30-min post-waking and before lunch, as well as total cortisol output (AUC) were significantly higher on stressor days compared to stressor-free days, particularly for arguments and home-related overloads. Finally, the effects of daily stressors on cortisol were not appreciably attenuated after taking daily negative affect and physical health into account.

Our finding of substantial heterogeneity in the prevalence and distribution of daily stressors during midlife and older age is consistent with previous research showing that exposure to daily stressors is less frequent among males (Almeida and Kessler, 1998), older adults (Zautra et al., 1991), and individuals with fewer years of education (Grzywacz et al., 2004). While any particular type of stressor may occur relatively infrequently, the prevalence of stressor days is considerable, suggesting daily stressors may be important contextual factors influencing health. Previous research has shown the negative effects of daily stressors on daily affect (Sliwinski et al., 2009) and physical health symptoms (Almeida et al., 2005; Hoffman and Stawski, 2009). Indeed, recent research has shown that exposure to daily stressors is not predictive of global mental and physical health outcomes, but rather emotional responses to stressors, and these emotional responses, in turn, are predictive of emotional and physical health outcomes (Charles et al., 2013; Ong et al., 2013; Piazza et al., 2013). The current study compliments this work by demonstrating that the reported experience of daily stressors is associated with increased cortisol output. Whereas previous research has shown links between daily stressors and cortisol using a sample of chronically stressed individuals (Barker et al., 2012), momentary stressor–cortisol associations (e.g., Smyth et al., 1998; Jacobs et al., 2007), and emotion-diurnal cortisol links (Adam et al., 2006), we have extended this research and shown similar associations at a daily level in a broader, healthier, and more heterogeneous national sample of adults.

4.1. Between-versus within-person associations between daily stressors and cortisol

A complex and different pattern of results emerged depending on whether the associations between daily stressors and cortisol were between-person or within-person over time. Between-person associations showing that individuals who experience daily stressors more frequently exhibit higher levels of cortisol 30-min post-waking, and a steeper DCS, may seem counterintuitive, as this indicates that having fewer stressors is associated with a flatter DCS. One explanation is that while stressors in and of themselves may have negative consequences for health and well-being, the contexts and conditions that are reflected in the stressors assessed may be health-promoting resources. For example, interpersonal stressors such as arguments and network stressors may not be positive, health promoting experiences, but having a social network within which to experience these

types of events is likely good for health (Smith and Christakis, 2008). Similarly, feeling overloaded at work and home due to having too many demands and not enough resources to meet those demands may stretch a person thin, but may also reflect an active and engaged lifestyle, which is related to better health (Seeman and Crimmins, 2001). Individual differences in daily stressor exposure may, in part, be capturing aspects of a person's social network and lifestyle (being social and professionally engaged) that may have positive influences on HPA axis function and health more generally. Consistent with these notions, we observed the greatest frequency of daily stressors among the most-educated respondents. Together with our findings of a relatively normal and appropriate HPA-axis response to the stressors of daily life, the results of the current study appear to be consistent with the broader literature on biological stress responses among healthy individuals and better health overall among individuals at the upper end of the socioeconomic gradient.

Within-person associations revealed that cortisol levels at 30-min post-waking and before lunch, and total cortisol output (AUC) were significantly higher on stressor days compared to non-stressor days. These findings are consistent with previous research from EMA studies showing that naturally occurring minor daily stressors are associated with higher cortisol levels (van Eck et al., 1996; Smyth et al., 1998; Jacobs et al., 2007), and also suggest that the minor stressors arising out of daily living have an impact on HPA axis activation and total cortisol output over the course of a day. This pattern of findings is important because it is one of the first empirical demonstrations to temporally link naturally occurring daily stressors and cortisol output in a national field study—providing evidence of everyday stressors having an impact on the biological/endocrinological stress response. Furthermore, analyses indicated that the effects of daily stressors on increased cortisol AUC were somewhat specific to arguments and home-related overloads. It is unclear why these particular stressors are more potent stimuli of the HPA axis. One possibility is that characteristics of the stressors (e.g., who was involved, is the issue resolved) may influence their potency. Some researchers have suggested that the same nominal type of stressor can have differential effects on health and well-being depending on the characteristics of the stressor and circumstances surrounding it (Dohrenwend, 2006; Almeida et al., 2010). Stressors involving stakes for social relationships (e.g., arguments) or potentially having ramifications for one's social network (e.g., home-related overloads), for example, appear to have the most potent effect on cortisol (Gruenewald et al., 2004). We did examine associations among subjective reports of stressor severity with cortisol, but it did not have an influence either at the level of the day or individual differences. This pattern of results suggests that it is the presence of daily stressors more than the differential severity of different events that influence cortisol. Subjective evaluations of stressful events are likely important for understanding stressor–cortisol associations, as gradations of severity of minor daily stressors may pale in comparison to gradations across major life events and traumas. Thus, future research aimed at understanding the mechanisms underlying the specificity of event potency is a promising area for stress research.

While we predicted that, within-persons across day, cortisol levels and AUC would be higher and CAR and DCS would

be flatter on stressor days compared to stressor-free days, only levels at 30-min post-waking and before lunch, and total cortisol output (AUC) were higher on stressor days compared to stressor-free days. These higher levels of cortisol on stressor days could reflect anticipation of stressful events over the course of the day, and would be consistent with previous research showing associations among anticipation of stressors and elevations in cortisol (Smyth et al., 1998; Powell and Schlotz, 2012). The lack of a within-person association between daily stressors and the CAR and DCS could be due to the time-dependent nature of HPA axis reactions to stressors. Cortisol increases 20–40 min after exposure to stressors (Dickerson and Kemeny, 2004), and end-of-day retrospective reports of stressors and multiple-sample cortisol assessments schemes may lack sensitivity to detect such precise associations. Our study design allowed more nuanced characterizing of diurnal cortisol over four consecutive days, but is more weakly poised to capture more fine-grained time-dependent stressor–cortisol associations within the day. Given that the strength of daily diary studies is to characterize the day, the emergence of cortisol AUC, an index of total *daily* output, being sensitive to the effects of daily stressors reflects its particular utility for examining daily stress–cortisol linkages. It is important to note, however, that while EMA and daily diary-type studies are both intensive repeated measures/within-person designs, assessments are done over completely different time courses (i.e., moments versus days). Thus, while stressors and cortisol can and do exhibit significant associations over multiple different temporal cadences (e.g., moments, days, etc.), this does not mean that the same process is being captured or the mechanisms driving these associations are the same (Molenaar, 2004). As such, it is likely unreasonable to expect the results of within-person designs using substantially different temporal intervals for assessments to yield identical results or be directly comparable. Instead, these different study designs provide complementary strengths for examining and understanding stressor–cortisol links.

Whereas previous EMA research has shown that stressors themselves have no or little effect on cortisol levels after controlling for affect (Smyth et al., 1998; Jacobs et al., 2007), and daily diary research has shown fluctuations in daily affect to predict diurnal cortisol (Adam et al., 2006), our results provide strong evidence of a direct effect of daily stressors on cortisol output that could not be accounted for by daily negative affect or physical symptoms. Despite well-known effects of daily stressors on affect and self-reported physical symptoms, this study provides evidence of daily stressors getting under the skin to influence the biological stress response. Thus, the results of the current study may reflect relatively normal, healthy and adaptive responses to challenge, regardless of the extent to which one experiences increases in negative affect or physical symptoms. Furthermore, the lack of influence of negative affect on stressor–cortisol is inconsistent with previous research using EMA. This discrepancy could be due to differences in the discrete emotions assessed, as not all emotions influence health to the same extent (Consedine and Moskowitz, 2007). Furthermore, our end-of-day affect reports may lack temporal sensitivity for predicting cortisol compared to momentary reports or experimental studies where cortisol and affect are tied to a specific event (Buchanan et al., 1999). While the

time course of stressor-affect-cortisol associations has been investigated in the laboratory (Schlotz et al., 2008; Oldenhinkel et al., 2011; Campbell and Ehler, 2012), future research aimed at fleshing out the specific and differential effects of stressors and experienced emotions on cortisol, as well as their respective time courses in the context of daily diary designs, would be valuable.

4.2. Limitations

While this study was successful in demonstrating a within-person association between daily stressors and naturally occurring cortisol levels, there are limitations worth noting. Our approach where affirmative responses to stem questions are probed for more detailed information may have underestimated daily stressor exposure by inadvertently discouraging respondents from endorsing stressors in order to avoid being probed further. Further, our operational definitions of CAR and DCS relied on 2 and 3 cortisol samples, respectively, and we only had 4 samples per day to capture the diurnal rhythm. Previous research on CAR and DCS has shown the shapes of the CAR and DCS to possibly be more complex than what we were able to quantify given our data. More frequent samples of cortisol may provide greater sensitivity for detecting daily stressor–cortisol links. Similarly, we only had 4 days of data per participant limiting our ability to examine how cortisol is associated with the accumulation and carryover of daily stressors, as well as more complex bidirectional associations between stress and cortisol. It is possible that certain daily biological profiles predispose an individual to have more stressful days. Thus, utilizing advancing technology to improve the assessment of biologically informative information in field-based population studies will allow researchers to improve their assessments of biomarkers and yield a larger scientific return on investment. Finally, times for cortisol samples were based on self-report. Use of electronically monitored timing would improve the precision and accuracy of sample timing, as well as the ability to screen for potentially invalid samples.

5. Conclusion

Despite these limitations, this study was successful in documenting the prevalence and distribution of daily stressors in a national sample of midlife and older adults, demonstrating that relatively minor stressors that arise out of daily living do influence biological markers of the stress process, in particular cortisol. Importantly, the results of the current study show that stressors arising out of daily life have a demonstrable impact on naturally occurring cortisol output. The results suggest that healthy, community-dwelling midlife and older adults exhibit relatively normal, healthy and adaptive HPA-axis reactions to stressors, and that diary designs with integrated biomarker collections are particularly valuable for understanding stress and health in an individual's natural contexts.

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Conflict of interest

None of the authors have any conflicts of interest to declare.

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