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Spousal Loss Predicts Alterations in Diurnal Cortisol Activity Through Prospective Changes in Positive Emotion

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Objective: The objective of this study was to examine the role of spousal bereavement and positive emotion in naturally occurring levels of daily cortisol. ***Methods:*** Analyses were conducted using data from the Midlife in the United States survey and the National Study of Daily Experiences. Baseline assessments of extraversion, neuroticism, trait positive emotion, and trait negative emotion were obtained, as were reports of demographic and health behavior covariates. Salivary cortisol levels were measured at wakeup, 30 min after awakening, before lunch, and at bedtime on each of 4 successive days. ***Results:*** Multilevel growth curve analyses indicated that independent of age, gender, education, extraversion, neuroticism, negative emotion, medication use, and smoking, spousal bereavement was associated with lower levels of cortisol at wakeup and a flattening of the diurnal cortisol rhythm. Mediation analyses revealed that prospective changes in positive emotion accounted for the impact of bereavement on diurnal cortisol slopes. ***Conclusion:*** The current prospective study is among the first to provide evidence for a role for positive emotion as a mechanism by which bereavement influences hypothalamic–pituitary–adrenal axis dysregulation in older adults.

Keywords: bereavement, positive emotion, diurnal cortisol, spousal loss

The death of a spouse or life partner is associated with increase prevalence of physical illness and psychiatric morbidity, as well as excess risk of mortality in later adulthood (Christakis & Allison, 2006; Stroebe, Schut, & Stroebe, 2007). Accumulating evidence suggests that the health consequences of bereavement are associated with changes in underlying neuroendocrine stress response systems, particularly the hypothalamic–pituitary–adrenal (HPA) axis. More specifically, bereavement has been linked with various patterns of cortisol dysregulation, including increased stressor reactivity, lower cortisol awakening responses (CARs), and attenuated cortisol diurnal slopes (Hagan, Luecken, Sandler, & Tein, 2010; Luecken, 1998; Meinlschmidt & Heim, 2005). Alterations in HPA axis function resulting from bereavement, in turn, have been implicated in the pathogenesis of psychiatric disorders, including posttraumatic stress disorder (e.g., Pfeffer, Altemus, Heo, & Jiang, 2009; Tyrka et al., 2008). Despite findings of a link between bereavement and changes in HPA axis activity, the emotion regulatory mechanisms by which the loss of a partner contributes to long-term patterns of cortisol dysregulation remain largely unexplored.

In the current study, we examined the extent to which deficits in positive emotion following spousal loss contribute to cortisol dysregulation in later adulthood. Cortisol levels are typically highest in the morning on awakening; increase, on average, 50–60% in the first 30–40 min postawakening (CAR); drop rapidly in the next few hours; and then decline gradually across the day to near-zero levels by bedtime (Kirschbaum & Hellhammer, 1989, 1994). Emerging evidence suggests a link between positive emotion and favorable diurnal cortisol functioning. For instance, naturally occurring cortisol levels, both total output and the awakening response, have consistently been shown to be lower among individuals with higher levels of trait positive emotion (e.g., Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005; Smyth et al., 1998; Steptoe, O'Donnell, Badrick, Kumari, & Marmot, 2008; Steptoe, Wardle, & Marmot, 2005). Moreover, a recent prospective study revealed that compared with married controls, recently widowed individuals experienced a significant decline in positive emotion following spousal loss (Ong, Fuller-Rowell, & Bonanno, 2010).

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Deficits in positive emotion, thus, may play an important role in the pathway leading from spousal loss to diurnal cortisol dysregulation.

With respect to the neuroendocrine effects of positive emotion among bereaved adults, surprisingly few studies have been conducted. Furthermore, despite empirical research demonstrating an association between positive emotion and HPA axis activity, evidence regarding the extent to which associations between cortisol and positive emotion are independent of negative emotion has been inconsistent (Jacobs et al., 2007; van Eck, Berkhof, Nicolson, & Sulon, 1996). Accounting for potential confounding variables may be especially relevant in the context of bereavement research. Psychological morbidity, particularly psychological distress, increases the risk of death among recently bereaved partners independent of age or bereavement (Schulz, Beach, & Ives, 2000; Unutzer, Patrick, Marmon, Simon, & Katon, 2002). The study of bereavement and cortisol has likewise been hampered by inadequate assessment and control of demographic (e.g., age, gender), personality (e.g., extraversion, neuroticism), and health behavior covariates (e.g., smoking), which might be associated with HPA axis dysregulation (Adam & Kumari, 2009; Almeida, Piazza, & Stawski, 2009; Luecken & Appelhans, 2006; Nicolson, 2004).

In the present study, we used prospective data from a national sample of adults to examine the relationship between positive emotion and neuroendocrine function, as measured by diurnal salivary cortisol levels, among bereaved spouses and nonbereaved controls. We tested the hypothesis that spousal bereavement is associated with variation in multiple parameters of diurnal cortisol activity, including lower levels of cortisol at wakeup, higher CAR, and flatter cortisol slopes across the waking day. In addition, we examined a potential mechanism that may account for the relationship between spousal bereavement and disruptions in the abovementioned parameters characterizing the diurnal cortisol rhythm. We hypothesized that the effect of spousal loss on each cortisol parameter (wakeup level, CAR, diurnal slope) would be mediated by pre- to postloss changes in positive emotion.

Method

The data for this study are from (a) the National Survey of Midlife Development (MIDUS), a two-wave panel survey of adults between the ages of 25 and 74 in 1994–1995 in the coterminous United States, and (b) the National Study of Daily Experiences (NSDE), a telephone diary study of a representative subsample of the MIDUS survey participants. Phone interviews and self-administered questionnaires were conducted in 1995–1996 (Wave 1) and again in 2004–2006 (Wave 2). Wave 1 MIDUS data comprised four subsamples: a national random digit dialing (RDD) sample (n = 3,487), oversamples from five metropolitan areas (n = 757), siblings of individuals from the RDD sample (n = 950), and a national RDD sample of twin pairs (n = 1,914). A longitudinal follow-up was conducted in 2004–2006 (Wave 2). Of those who participated in Wave 1, 4,963 completed a Wave 2 telephone interview (70% response rate; 75%, when adjusted for mortality), and 81% of individuals who completed the telephone interview also completed self-administered questionnaires. A subset of participants from Wave 2 of the MIDUS (n = 2,022) was assessed in the second wave of the NSDE, which is the source of data for the present analyses (see Almeida, McGonagle, & King, 2009, for a more detailed description).

Sample

Between 1994 and 2006, 132 adults in the sample experienced the death of a spouse. In the current study, inclusion criteria for the bereaved group consisted of individuals who were (a) widowed within 3 years of the follow-up interview,[1] (b) unmarried at the time of recruitment in Wave 2 (2004–2006), and (c) included in the second wave of the NSDE. Of the total 132 bereaved spouses, 22 met eligibility criteria; average time since loss was approximately 17.5 months (SD = 10.3). We compared this group of individuals (bereaved group) with a random sample of 22 continuously married controls (control group). Control participants from the Wave 1 sample were selected to match the widowed respondents in age, gender, and education. The final sample consisted of 44 (14% male) adults between the ages of 48 and 80 years (M = 65.8 years, SD = 8.9), with a little over a third (36%) having completed some college or more. The majority were White/Caucasian (93.2%), with the remainder being Black/African American (4.5%) or other (2.3%).

Measures

Positive and negative emotion. Assessments of positive emotion and negative emotion were obtained by self-administered questionnaires (Mroczek & Kolarz, 1998). Participants rated the amount of time they experienced various emotional states over the past 30 days on a 5-point scale, ranging from 1 (*none of the time*) to 5 (*all of the time*). The six-item positive emotion scale (e.g., "cheerful," "extremely happy," "calm and peaceful") comprised items from several well-validated measures of positive affect including the Affect Balance Scale (Bradburn, 1969) and General Well-Being Schedule (Fazio, 1977). The scale demonstrated excellent reliability in the MIDUS samples (Cronbach's alphas for the six-item scale at Waves 1 and 2 were .91 and .90, respectively). Negative emotion was assessed with the following six items: "so sad nothing could cheer you up," "nervous," "restless or fidgety," "hopeless," "that everything was an effort," "worthless" (α = .88; items were recoded so that higher scores indicated more negative emotion).

Extraversion and neuroticism. Personality markers for extraversion and neuroticism were rated on a 4-point scale, ranging from 1 (*a lot*) to 4 (*not at all*). Extraversion was assessed with the following items: "outgoing," "friendly," "lively," "active," "talkative" (α = .79). Neuroticism was assessed with the items "moody," "worrying," "nervous," and "calm" (α = .77; items were recoded so that the higher scores indicated greater neuroticism). These personality scales were developed from a pool of established Big Five trait adjectives (Goldberg, 1992) and have been used in a number of prior studies (e.g., Keyes, Shmotkin, & Ryff, 2002; Staudinger, Fleeson, & Baltes, 1999).

Salivary cortisol. Samples of salivary cortisol were collected four times on each of 4 successive days using salivette collection devices (Sarstedt, Nümbrecht, Germany). The cortisol collection

[1] Because bereavement studies have rarely collected data beyond 2 years postloss (for a review, see Bonanno & Kaltman, 2001), we limit our analyses to data gathered on average 18 months after the death of a spouse.

occurred, on average, 3 to 6 months after the questionnaire assessment. Sixteen numbered and color-coded salivettes were included in the collection kit. In addition to written instructions that accompanied each collection kit, telephone interviewers reviewed the collection procedures and answered participant questions. Respondents provided four saliva samples per day on Days 2–5 of the 8-day study period that were later assayed for cortisol. Saliva was collected once on awakening, 30 min after waking, before lunch, and at bedtime. Cortisol concentrations were quantified with a commercially available luminescence immunoassay (IBL, Hamburg, Germany), with intra-assay and interassay coefficients of variations below 5 (Polk et al., 2005). Compliance data on the exact time respondents provided each saliva sample were determined by both nightly telephone interviews and paper–pencil logs sent with the collection kit (see Almeida, McGonagle, et al., 2009; Almeida, Piazza, et al., 2009). Cortisol values were natural logarithmically transformed prior to analysis to correct for positive skew in the cortisol distribution.

Demographic and health behavior covariates. Prior to the telephone diary study, demographic and health behavior covariates including age, gender, education, smoking status, and medication use were obtained. Smoking status was determined by respondents identifying themselves as regular smokers as well as by the number of cigarettes individuals reported consuming on a daily basis during the study period. A dichotomous variable (0 = nonsmoker, 1 = smoker) was used as an index of smoking status. Medication use was determined by participants reporting their current use of medications known to influence cortisol, including steroid inhalers, steroid medications, medications containing cortisone, birth control pills, other hormonal medications, and antidepressant or antianxiety medications. A dichotomous variable was created to indicate whether a participant reported taking any of the abovementioned medications (0 = did not use medications, 1 = used medications).

Analytic Strategy

A three-level hierarchical growth curve analysis (Raudenbush & Bryk, 2002) was used to estimate latent diurnal (daytime) cortisol profiles for each person, and to examine predictors of the key parameters defining individual differences in diurnal cortisol activity. Analyses control for the effects of extraversion, neuroticism, negative emotion, demographic variables, and health behavior covariates. Use of multilevel modeling adjusts for the nonindependence of observations associated with nested data structures, thereby permitting the simultaneous modeling of the shape of the diurnal cortisol rhythm for each individual, along with an examination of day-level and person-level factors predicting differences in these rhythms (for a discussion, see Adam, 2006; Hruschka, Kohrt, & Worthman, 2005). In these models, level of cortisol for each person at each moment was the criterion variable and was predicted by moment-level predictors (Level 1), day-level predictors (Level 2), and person-level predictors (Level 3).

The individual growth of cortisol at time t on day i of person j is shown in Level 1 as

$$\begin{aligned}\text{Cort}_{tij} = {} & \pi_{0ij} + \pi_{1ij}(\text{Time Since Waking})_{tij} \\ & + \pi_{2ij}(\text{Time Since Waking}^2)_{tij} + \pi_{3ij}(\text{CAR})_{tij} + e_{tij},\end{aligned} \tag{1.10}$$

where Cort_{tij} is the outcome at time t on day i of person j; π_{0ij}, the intercept, represents a latent estimate of each person's average cortisol level at wakeup; $(\text{Time Since Waking})_{tij}$ reflects a latent estimate of linear change in each person's diurnal cortisol rhythm across the day; $(\text{Time Since Waking}^2)_{tij}$ represents a latent estimate of the acceleration or the rate of curvilinearity in each person's diurnal cortisol rhythm across the day; $(\text{CAR})_{tij}$, the cortisol awakening response, reflects a latent estimate of the average magnitude of each person's change in cortisol 30 min after waking.

Time of day values were expressed as number of hours since awakening for each person each day. The CAR was coded as a dummy variable (with 1 as the second cortisol sample taken approximately 30 min after waking and 0 for the other samples). Scaling time and the CAR in this manner defines the intercept at the time of waking and allows for the diurnal slope to be unaffected by the magnitude of the CAR (Adam, Hawkley, Kudielka, & Cacioppo, 2006; Adam & Kumari, 2009).

The Level 2 model represents the variability in each of the Level 1 intercept and growth parameters across days within individuals:

$$\pi_{0ij} = \beta_{00j} + r_{0ij}, \tag{2.10}$$

$$\pi_{1ij} = \beta_{10j} + r_{1ij}, \tag{2.11}$$

$$\pi_{2ij} = \beta_{20j} + r_{2ij}, \tag{2.12}$$

$$\pi_{3ij} = \beta_{30j} + \beta_{31j}(\text{Wakeup Time})_{ij} + r_{3ij}, \tag{2.13}$$

where β_{00j} is the mean cortisol intercept for person j; β_{10j} is the mean linear rate of decline in cortisol for person j; β_{20j} is the mean deceleration in the rate of decline in cortisol for person j; β_{30j} is the mean cortisol awakening (30 min postawakening) for person j; β_{31j} is the mean deviation in cortisol level at wakeup time for person j. Thus, each person's intercept, wakeup level, response to awakening, and afternoon slope parameters on a given day are predicted by each person's average intercept and slope across the 4 days, as well as a person-centered time-varying covariate (wake-up time).

The Level 3 model represents the variability among persons in each of the Level 2 intercept and slope parameters. In addition to the primary predictor variables of spousal loss and pre- to postloss changes in positive emotion, the Level 3 model included extraversion, neuroticism, negative emotion, and demographic and health covariates (i.e., age, gender, level of education, smoking status, and medication use). As shown below, Level 3 predictors were included in models estimating the three main criterion variables: the average level of cortisol at wakeup (β_{00j}), the average linear slope of the diurnal cortisol curve (β_{10j}), and the average magnitude of the CAR (β_{30j}).

$$\begin{aligned}\beta_{00j} = {} & \gamma_{000} + \gamma_{001}(\text{Age})_j + \gamma_{002}(\text{Gender})_j + \gamma_{003}(\text{Education})_j \\ & + \gamma_{004}(\text{Smoking})_j + \gamma_{005}(\text{Medication})_j \\ & + \gamma_{006}(\text{Extraversion})_j + \gamma_{007}(\text{Neuroticism})_j \\ & + \gamma_{008}(\text{Negative Emotion})_j + \gamma_{009}(\text{Loss})_j \\ & \pm \gamma_{010}(\Delta\text{Positive Emotion})_j + u_{00j},\end{aligned} \tag{3.10}$$

$$\beta_{10j} = \gamma_{100} + \gamma_{101}(\text{Age})_j + \gamma_{102}(\text{Gender})_j + \gamma_{103}(\text{Education})_j + \gamma_{104}(\text{Smoking})_j + \gamma_{105}(\text{Medication})_j + \gamma_{106}(\text{Extraversion})_j + \gamma_{107}(\text{Neuroticism})_j + \gamma_{108}(\text{Negative Emotion})_j + \gamma_{109}(\text{Loss})_j \pm \gamma_{110}(\Delta\text{Positive Emotion})_j + u_{10j}, \quad (3.11)$$

$$\beta_{20} = \beta_{200} + u_{20j}, \quad (3.12)$$

$$\beta_{30j} = \gamma_{300} + \gamma_{301}(\text{Age})_j + \gamma_{302}(\text{Gender})_j + \gamma_{303}(\text{Education})_j + \gamma_{304}(\text{Smoking})_j + \gamma_{305}(\text{Medication})_j + \gamma_{306}(\text{Extraversion})_j + \gamma_{307}(\text{Neuroticism})_j + \gamma_{308}(\text{Negative Emotion})_j + \gamma_{309}(\text{Loss})_j \pm \gamma_{310}(\Delta\text{Positive Emotion})_j + u_{30j}, \quad (3.13)$$

$$\beta_{31} = \beta_{310}. \quad (3.14)$$

All models were fitted using HLM software, Version 6.08 (Raudenbush, Bryk, Cheong, Congdon, & du Toit, 2004).

Results

Descriptive Analyses

Of a possible 704 person-observations days (44 persons × 4 observations × 4 days), participants provided time-logged cortisol samples on 636 person-observations days (90%). Bereaved and nonbereaved participants did not differ significantly in their reports of baseline positive emotion, negative emotion, extraversion, neuroticism, or health behavior covariates. The first set of descriptive analyses examined the distribution of cortisol values across moments, days, and persons. These analyses are referred to as fully unconditional because no predictor variables are specified at any level. The ratio of between- to within-person variance (i.e., the intraclass correlation) for this model is $\rho_{\text{person}} = .13$. Thus, most of the explainable variation in cortisol occurred at Levels 1 and 2 (within days and persons). Moreover, the variance components for the intercept ($u_{00j} = .114$), slope ($u_{10j} = .004$), and CAR ($u_{30j} = .034$) were all significantly different from zero ($p < .05$), implying that variations exist to be potentially explained by adding predictor variables to the model (Raudenbush & Bryk, 2002). Comparison of the variance components between the fully unconditional model and the partially unconditional model (described in Equation 1.10) revealed that 72% of the total within-person variance in cortisol was accounted for by the Level 1 model parameters (i.e., linear slope, quadratic coefficient, and CAR).

Multilevel Growth Curve Analyses

Multilevel growth curve analyses of key parameters defining the shape of the diurnal cortisol curve are presented in Table 1. The outcome of interest, cortisol level, was transformed by natural logarithm; thus, the effect sizes for all coefficients can be interpreted as a percentage change per 1-unit change in the independent variable, after applying the following transformation: $B_{\%\text{change}} = [\exp(B_{\text{raw}})] - 1$ (Adam, 2006; Adam et al., 2006). As illustrated in Model 1, participants showed the expected diurnal cortisol pattern. Cortisol levels, on average, were appropriately high at wakeup ($\gamma_{000} = 2.65$, $p < .001$; equivalent to 14.11 nmol/L),[2] and was followed by a 53% rise 30 min after awakening ($\gamma_{300} = .423$, $p < .001$; equivalent to 1.53 nmol/L). As expected, cortisol levels decreased throughout the day at a rate of 12% per hour at wakeup time ($\gamma_{100} = -.125$, $p < .001$; equivalent to 0.88 nmol/L), with a significant declining rate of change thereafter due to the positive quadratic effect ($\gamma_{200} = .003$, $p < .01$), reflecting a greater rate of deceleration in the diurnal cortisol slope.

Spousal Loss and Salivary Cortisol Patterns

Table 1 provides a summary of the multilevel growth curve models. Of the health behavior covariates, only smoking status was significantly associated with wakeup levels of cortisol. Compared with nonsmokers, smokers exhibited significantly higher cortisol levels at wakeup (75% higher, $\gamma_{004} = .557$, $p < .05$; equivalent to 1.75 nmol/L). Furthermore, baseline levels of negative emotion were significantly associated with basal salivary cortisol activity, with higher levels of negative emotion predicting lower levels of cortisol at wakeup (22% lower, $\gamma_{008} = -.244$, $p < .05$; equivalent to 0.78 nmol/L). As shown in Model 2, spousal loss demonstrated distinct associations with several diurnal cortisol parameters. Compared with nonbereaved controls, bereaved respondents demonstrated significantly lower average wakeup levels of salivary cortisol (21% lower, $\gamma_{009} = -.233$, $p < .05$; equivalent to 0.79 nmol/L). As illustrated in Figure 1, the slope of the diurnal cortisol curve was significantly flatter among bereaved spouses ($\gamma_{109} = .021$, $p < .05$; equivalent to 1.02 nmol/L). There was no main effect of spousal loss on the size of the CAR ($\gamma_{309} = .063$, $p = .551$).

Mediating Effect of Positive Emotion

To test the hypothesis that prospective changes in positive emotion would mediate the associations between loss and diurnal cortisol parameters, we examined both bereavement status and pre- to postloss changes in positive emotion at Level 3 using a 2–2–1 multilevel mediation model (Krull & MacKinnon, 2001). Using this model, analyses indicated that prospective changes in positive emotion did not account for the association between spousal loss and participants' cortisol levels at wakeup, suggesting that this effect is not solely due to intraindividual changes in positive emotion. In contrast, the coefficient for the previously observed spousal loss effect on average diurnal cortisol slopes was reduced in size. Table 1 shows that the coefficient for spousal loss fell from .021 to .016. The latter coefficient is the direct effect of loss on the diurnal cortisol slope, and it is nonsignificant ($\gamma_{109} = .016$, $p = .092$). The indirect effect of spousal loss through changes in positive emotions is .007 units and reflects how much spousal loss affects changes in the diurnal cortisol slope through positive emotions. The proportion of the indirect effect to the total effect is .29, indicating that pre- to postloss changes in positive emotion ex-

[2] Given the logarithmically transformed outcome variable (natural log of cortisol values), the exponential function of that transformation was applied to return the intercept to its original scale of measurement (nmol/L).

Table 1
Multilevel Growth Curve Model of Diurnal Cortisol Parameters

Fixed effect	Model 1 Estimate	Model 1 *SE*	Model 2 Estimate	Model 2 *SE*	Model 3 Estimate	Model 3 *SE*
Wake-up cortisol, π_0						
Average wakeup level, β_{00}						
Intercept, γ_{000}	2.647***	.084	2.756***	.083	2.762***	.082
Age, γ_{001}	.050	.066	.062	.062	.039	.058
Gender, γ_{002}	−.044	.166	−.0376	.181	.010	.179
Education, γ_{003}	−.111	.110	.118	.103	−.119	.100
Smoker, γ_{004}	.557*	.204	.675**	.205	.689**	.196
Medication, γ_{005}	.017	.101	−.008	.087	.003	.086
Extraversion, γ_{006}	.071	.076	.101	.077	.105	.073
Neuroticism, γ_{007}	−.017	.091	−.037	.084	−.043	.083
Negative affect, γ_{008}	−.244*	.109	−.207*	.086	−.232**	.081
Spousal loss, γ_{009}	—	—	−.233*	.090	−.266**	.086
Δ in positive emotion, γ_{010}	—	—	—	—	−.054	.038
Time since waking, π_1						
Average linear slope, β_{10}						
Intercept, γ_{100}	−.125***	.016	−.133***	.017	−.132***	.017
Age, γ_{101}	−.002	.008	−.004	.007	−.008	.007
Gender, γ_{102}	.012	.015	.011	.012	.019	.012
Education, γ_{103}	−.007	.009	−.007	.009	−.006	.008
Smoker, γ_{104}	−.007	.020	−.017	.020	−.016	.019
Medication, γ_{105}	−.013	.010	−.011	.011	−.009	.011
Extraversion, γ_{106}	−.008	.007	−.011	.007	−.010	.007
Neuroticism, γ_{107}	−.020	.011	−.018	.011	−.019	.010
Negative affect, γ_{108}	.019*	.009	.016	.008	.011	.008
Spousal loss, γ_{109}	—	—	.021*	.009	.016	.009
Δ in positive emotion, γ_{110}	—	—	—	—	−.009**	.003
Time since waking squared, π_2						
Average curvature, β_{20}						
Intercept, γ_{200}	.003**	.001	.003**	.001	.003**	.001
Awakening response, π_3						
Average awakening response, β_{30}						
Intercept, γ_{300}	.423***	.068	.394***	.082	.392***	.081
Age, γ_{301}	−.054	.066	−.060	.067	−.043	.074
Gender, γ_{302}	−.169	.140	−.169	.143	−.196	.151
Education, γ_{303}	.196	.103	.200	.105	.195	.101
Smoker, γ_{304}	−.192	.161	−.221	.173	−.221	.174
Medication, γ_{305}	−.063	.103	−.057	.103	−.058	.104
Extraversion, γ_{306}	−.107	.075	−.114	.073	−.118	.068
Neuroticism, γ_{307}	−.119	.097	−.115	.096	−.109	.095
Negative affect, γ_{308}	.271*	.100	.262*	.097	.266*	.103
Spousal loss, γ_{309}	—	—	.063	.100	.077	.094
Δ in positive emotion, γ_{310}	—	—	—	—	.025	.042
Waking time, β_{31}	−.199**	.067	−.199**	.067	−.200**	.067

Note. All momentary-level (Level 1) predictors are uncentered; day-level (Level 2) and person-level (Level 3) predictors are grand mean centered.
* $p < .05$. ** $p < .01$. *** $p < .001$.

plained 29% of the effect of spousal loss on diurnal cortisol slopes (see MacKinnon, Lockwood, & Williams, 2004).

The significance of the mediation effect was evaluated following the procedure outlined by Mackinnon and colleagues (MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002; MacKinnon et al., 2004).[3] Specifically, asymmetric confidence limits were formed using the upper and lower critical values of the distribution of the product of two standard normal variables (Meeker, Cornwell, & Aroian, 1981). If zero was not in the 95% interval of the upper and lower confidence limits, we concluded that the mediation effect was statistically significant. Using this method, the lower and upper 95% confidence limits based on the distribution of the product were .0011 and .0138, suggesting that the mediated effect

[3] The upper and lower confidence limits for the indirect effect were calculated using PRODCLIN (distribution of the PRODuct Confidence Limits for Indirect effects). The SAS and R macro programming languages used to run PRODCLIN are described in MacKinnon et al. (2004).

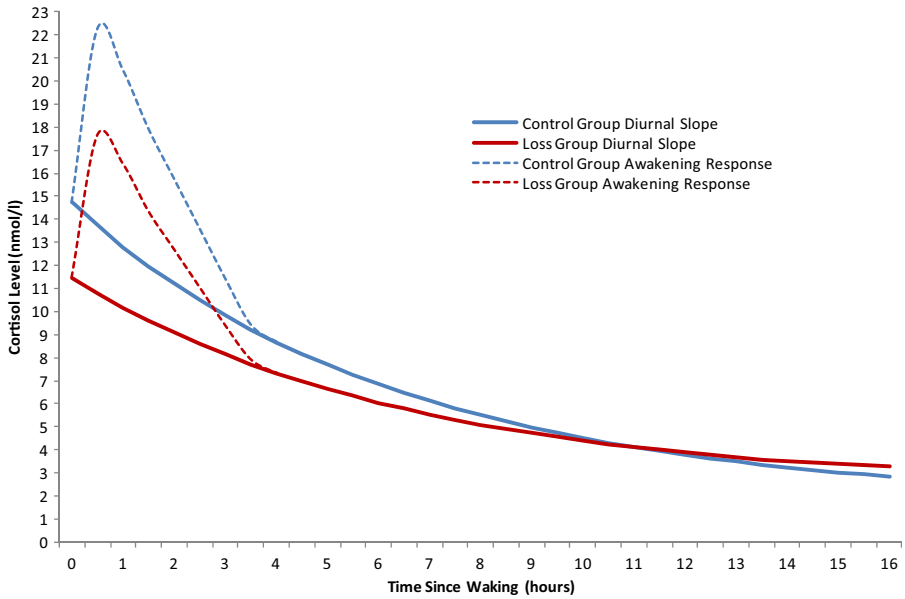

Figure 1. Average cortisol rhythms across the waking day by bereavement status.

of changes in positive emotion was statistically significant.[4] This effect was significant even when possible confounding factors, such as awakening time, demographic variables, extraversion, neuroticism, negative emotion, and health covariates, were included in the model.

Discussion

This prospective longitudinal study identified significant differences in HPA axis function and positive emotion among adults recently bereaved by spousal loss compared with nonbereaved controls. Although prior research has examined longitudinal relationships between spousal bereavement and cortisol levels (e.g., Pfeffer et al., 2009), the current study is among the first to document a potential mechanism (i.e., positive emotion) by which bereavement may affect the diurnal rhythm of salivary cortisol in older adults.

Findings from the current study are consistent with the hypothesis that bereavement is associated with altered diurnal cortisol activity. As predicted, bereaved partners had significantly flatter diurnal cortisol slopes. This finding is in accordance with other research illustrating the chronic impact of bereavement on HPA axis dysregulation (Hagan et al., 2010; Tyrka et al., 2008) and provides further support for flattened diurnal cortisol patterns as a marker of increased vulnerability to stress (Adam & Gunnar, 2001; Elzinga et al., 2008; Miller, Chen, & Zhou, 2007; Sephton, Sapolsky, Kraemer, & Spiegel, 2000). Of note, this association was independent of potential confounding factors, including demographic variables (e.g., age, socioeconomic status), personality (e.g., extraversion and neuroticism), negative emotion, and health behavior covariates (e.g., smoking status), that have been previously found to be related to flatter diurnal slopes (Adam & Kumari, 2009; Cohen et al., 2006).

In the current investigation, spousal bereavement was associated with lower levels of cortisol at wakeup. Prior evidence for associations between basal cortisol levels and bereavement has been mixed, however, with some studies reporting higher basal cortisol levels, and others reporting no or negative associations with bereavement (Bloch, Peleg, Koren, Aner, & Klein, 2007; Gunnar, Morison, Chisholm, & Schuder, 2001; Pfeffer, Altemus, Heo, & Jiang, 2007). Future studies might address these inconsistencies by examining differences in characteristics of the loss experience (type, duration, intensity), sample composition (developmental age, history of psychiatric illness), and cortisol measurements (variability in awakening time, defining cortisol slopes with vs. without the CAR period).

Our meditational findings for the diurnal cortisol slopes highlight how positive emotions may play an important role in the pathway leading from bereavement to HPA axis dysregulation. In particular, deficits in positive emotion following loss fully accounted for the differences in observed diurnal cortisol slopes. As noted, observational studies that have focused on the health effects of positive emotions have often failed to include adequate controls for negative mood states. Thus, it is important to note that consistent with previous research (e.g., Steptoe et al., 2005, 2008), the associations between positive emotion and cortisol were independent of negative emotion in the current study.

In general, these findings add to the growing body of evidence suggesting that positive emotions confer a wide range of benefits during bereavement (Bonanno & Keltner, 1997; Coifman & Bo-

[4] To ensure that the multilevel coefficients we report are robust, we reanalyzed the data on (a) the full sample of nonbereaved individuals and (b) a random augmented sample of matched nonbereaved controls that was 3 times the bereaved group. We note that the pattern of findings remains unchanged and is virtually identical when using either the full sample (n = 994) or random augmented matched sample (n = 66) of nonbereaved controls. Given this, we opted to select a random sample that was comparable in size to the bereaved group.

nanno, 2010; Ong, Bergeman, & Bisconti, 2004). It is important to note that the findings also highlight the need for future investigations to clarify the mechanism by which an overall disturbance in positive emotion following loss is linked to later HPA vulnerability. If the failure to mobilize adequate positive emotional resources in the aftermath of loss represents a significant risk factor for the development of HPA dysregulation, then interventions (Luecken et al., 2010) designed to bolster bereaved individuals' capacity for emotional awareness and positive emotional engagement during times of stress may prove to be beneficial.

There are several limitations to this study. First, generalizability is limited by a sample of predominantly female Caucasians. In addition, the data on cortisol were obtained over a 4-day period at a single point in bereavement, on average 17.5 months postloss. Thus, it will be important for future studies to examine cortisol responses during bereavement at multiple time points, both closer and farther away from the loss event. Similarly, data for the current study were based on CARs measured over two occasions (waking and 30 min later). A recent meta-analytic review (Chida & Steptoe, 2008) found that the association between positive psychological traits (e.g., positive emotion) and CAR was moderated by the number of cortisol assessments: Positive psychological traits were associated with reduced CAR only in studies that assessed cortisol three or more times across the waking period. Future investigations should include greater number of cortisol assessments to increase measurement reliability. A final limitation of the study is the fact that we could not objectively assess the effects of participant noncompliance with the saliva sampling protocol. Whether compliance is enhanced by research that incorporates monitoring of sample timings using objective devices, such as "smart-cap" salivettes and actigraphy (Adam & Kumari, 2009; Almeida, McGonagle, et al., 2009), remains to be determined.

Despite these limitations, this is the first prospective study, to our knowledge, to examine the effect of spousal bereavement and positive emotion on naturally occurring daily cortisol levels. The pattern of observed findings is consistent with evidence from other studies suggesting that major psychosocial stressors, such as the death of a partner, can produce long-term dysregulation of the HPA axis, and deficits in positive emotion following loss may represent a key mechanism by which bereavement is linked to HPA dysfunction.

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