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SHORT COMMUNICATION

Diurnal cortisol in Complicated and Non-Complicated Grief: Slope differences across the day

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Summary Although grief has been described primarily as a psychological phenomenon, empirical evidence reveals that grief also has physiological correlates that have consequences for health. The present study investigates the diurnal cortisol production patterns in women who have been bereaved in the past 18 months. Specifically, the study compares women with Complicated Grief ($n = 12$) from those with Non-Complicated Grief ($n = 12$), testing whether cortisol slope distinguishes the two groups. Results demonstrate that the two groups do not differ on demographic variables (except education), but as hypothesized, those with Complicated Grief have a flatter slope across the day, controlling for education and body mass index.

1. Introduction

Complicated Grief (CG), a disorder under consideration for DSM-5, includes a prolonged set of symptoms including persistent intense yearning and longing for the person who died, disruptive preoccupation with thoughts and memories of this person, avoidance of reminders that the person is gone, deep relentless sadness, self-blame, bitterness, or anger in connection with the death, and an inability to gain satisfaction or joy through engaging in meaningful activities or relationships with significant others (Horowitz et al., 1997). This profoundly painful and disruptive disorder is distinct from acute grief, although acute grief also can be intensely emotional and distressing.

Although the past century of theory about grief has primarily described it as a psychological phenomenon (Freud, 1917/1957), empirical evidence reveals that grief also has physiological correlates that have consequences for health. The earliest evidence came from investigations of the "broken-heart phenomenon" (Kraus and Lilienfeld, 1959), demonstrating that recently bereaved individuals had higher mortality rates than those without such a loss. More recent and sophisticated studies that control for a host of potentially confounding covariates have continued to confirm this association between bereavement and morbidity/mortality (Stroebe et al., 2007).

Most prior research on the link between grief and health outcomes has included bereaved individuals without taking

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into consideration their psychological adjustment. Empirical data demonstrate that almost 50% of bereaved persons are resilient, showing negligible fluctuations in their mental health (Bonanno et al., 2002). The association between bereavement and morbidity/mortality may be stronger if constrained to those bereaved individuals with CG. For example, bereaved wives who show greater distress after the death of their spouse also show lower NK cell activity (Irwin et al., 1987). After the death of a child, there is a positive correlation between grief intensity and urinary cortisol output (Hofer et al., 1972). With the advent of diagnostic criteria for CG[1], research documents higher rates of high blood pressure among those with CG vs. Non-CG (Prigerson et al., 1997).

The physiological mechanisms that bridge bereavement and morbidity/mortality are an important area of inquiry because these pathways may underlie the association, and may allow identification of those who are most likely to experience the broken-heart phenomenon. In addition, there is an immediate relevance to understand the physiological mechanisms for diagnostic classification, in order to discriminate between CG and Non-CG reactions during bereavement.

The morbidity and mortality associated with bereavement comes from all causes (as opposed to a specific cause of death, such as cardiovascular causes) (Elwert and Christakis, 2008). Therefore, focusing on putative mechanisms that are involved in multiple health pathways are more promising candidates than are disease-specific pathways. Hypothalamic—pituitary—adrenal (HPA) dysregulation impacts multiple organ systems, and increases morbidity and mortality from many potential causes (Seeman et al., 2001). Flattened circadian slope specifically has been associated with coronary calcification (Matthews et al., 2006) and mortality from breast cancer (Sephton et al., 2000). Accordingly, our *a priori hypothesis* was that those with CG would have a flatter circadian pattern of cortisol secretion than those with Non-Complicated Grief (Non-CG).

2. Methods

Twenty-four women (CG = 12, Non-CG = 12) who had experienced the death of a mother or sister to breast cancer in the past 5 years were recruited to participate in a larger study including additional psychological and biological variables that are not relevant to the present report (O'Connor et al., 2008). Participants were recruited from the Revlon/UCLA Breast Center High Risk Clinic and from the community. Participants were excluded for current Axis I disorder (including major depressive disorder), as evidenced by the Structured Clinical Interview for the DSM-IV (SCID-I) (Spitzer et al., 1994). Other exclusion criteria included current smoking and anti-depressant use. The UCLA Institutional Review Board approved the study and all participants gave written informed consent.

CG was diagnosed with the Interview for Complicated Grief, a structured clinical interview (Prigerson and Jacobs, 2001). The interview consists of fifteen questions about emotional and behavioral responses to the death event. A cut-off score of 35 was used, based on a comparable cut-off score used for a self-report scale of CG symptoms in a large randomized control trial of CG treatment (Shear et al., 2005).

Free cortisol was sampled in saliva with Salivettes (Sarstedt Inc., Newton, NC) at waking, 45 min post-waking, 4 PM and 9 PM on three consecutive days. These time-points are based upon recommendations from a meta-analytic study (Hellhammer et al., 2007). Participants were instructed on the correct use of the Salivettes and were asked to refrain from drinking, eating, brushing their teeth and using mouthwash in the 15 min prior to salivary collection. Participants telephoned a voicemail number at the time for each sample. A timestamp was documented for each phone call at the time of each sample. This method reduced missed samples and achieved very high integrity for the sample times. Less than 4% of samples (11 individual sampling time points out of 288) were missing. Missing values were imputed using a multiple regression approach which uses analysis weights based on existing variables in the dataset to impute missing values (Missing Values Add-On Module; SPSS 18); BMI and all available cortisol values within the given day and within the given time/across day were the variables used to estimate each individual missing cortisol value.

Saliva samples were frozen at −70 °C. At the study's end, all salivary cortisol samples were assessed by high sensitivity enzyme-linked immunosorbent assay (ELISA). The lower detection limit was less than 0.007 μg/dL. The mean intra-assay coefficient was 5.5 (range, 4—6%). The mean inter-assay coefficient was 8.2 (range, 7—11%). All samples from a participant were analyzed in duplicate in the same assay to minimize variability. The assay values were log transformed prior to statistical analysis to resolve the skewed nature of cortisol data.

Each evening, participants answered a previously developed questionnaire. Items included questions about the day's activities and experiences, including exercise, general wellness, stress level, physical pain, sleep quality, alcohol and caffeine intake. Those participants who abstained from alcohol and caffeine were not asked to answer these last two questions. Three participants did not return questionnaires.

3. Results

Descriptive characteristics. Symptoms that were significantly different between CG and Non-CG groups according to ANOVA analyses ($p < 0.01$) included intrusive thoughts, yearning, loneliness, avoidance of reminders of the death, emotional numbness, feeling shocked, disbelief, feeling that life had lost its meaning, and feeling that part of oneself had died along with the deceased loved one. The CG and Non-CG groups did not differ significantly in age, race, body mass index (BMI) or the usual number of alcoholic drinks per week (Table 1). Those in the CG group were somewhat more likely to have lost a sister (as opposed to a mother). CG group participants had significantly lower educational attainment, which replicates prior work (Stroebe and Schut, 2001). CG and NCG groups did not differ significantly on any of the activities listed on the daily questionnaire (F values < 1.6,

[1] In 1997, the diagnostic category was termed Traumatic Grief and later changed to CG.

Table 1 Demographic comparisons of Complicated and Non-Complicated Grief groups.

	CG (n = 12)	Non-CG (n = 12)	F value/X^2	p value
Age	42.67 (10.54)	46.91 (9.32)	1.36	0.26
Education	15.33 (2.84)	17.58 (2.11)	4.86	0.04
% White (vs. non-white)	67%	75%	0.20	0.65
Body mass index	24.90 (5.42)	24.60 (5.55)	0.02	0.90
Alcoholic drinks/week	2.43 (3.93)	2.41 (4.00)	.00	0.99
Months since death	23.58 (20.17)	32.50 (20.47)	1.16	0.29
% Mother (vs. sister) loss	58%	92%	3.56	0.06

$p > 0.21$), although there was a trend for the CG group to report greater alcohol consumption than usual on the saliva sampling days in comparison to the NCG group ($F(1, 15) = 4.34$, $p < 0.06$). CG and Non-CG groups did not differ in the types of medications they took, and conducting the statistical analyses after removing those participants on medications other than birth control and allergy medications did not change the results reported below.

Group differences in diurnal cortisol. In order to determine whether the two groups differed in the slope of their cortisol values across the four time points, multilevel modeling was performed using Day, Time of day and Group as fixed variables, and controlling for years of education and BMI. Education was included in the model because it was the only variable to show a group difference, and BMI because it is strongly associated with cortisol levels. CG and Non-CG groups were significantly different, controlling for years of education and BMI (Time of day $\times$ Group: $F(3, 20) = 3.65$, $p = 0.03$). As graphically depicted in Fig. 1, the diurnal slope for the CG group was lower at the beginning of the day and higher at the end of the day (i.e., flatter) than for the NCG. Because there were trends toward significance between groups in alcohol use and in relationship to the deceased (sister versus mother), alcohol use on each day and relationship were included in separate multi-level model analyses. The interaction between Time of day and Group remained significant.

In order to determine which of the time points contributed most to the slope differences between groups, post-hoc t tests were conducted. As compared to the NCG group, the CG group had a significantly lower cortisol level at 45-min post-wake ($t(2, 22) = 1.98$, $p = .05$) and higher at 4 PM ($t(2, 22) = -1.84$, $p = 0.08$). The partial eta squared for the 45-min post-wake time point was 0.15, and for the 4 PM time point was 0.13. In other words, lower cortisol levels at 45-min post-waking and higher cortisol levels at 4 PM were associated with CG.

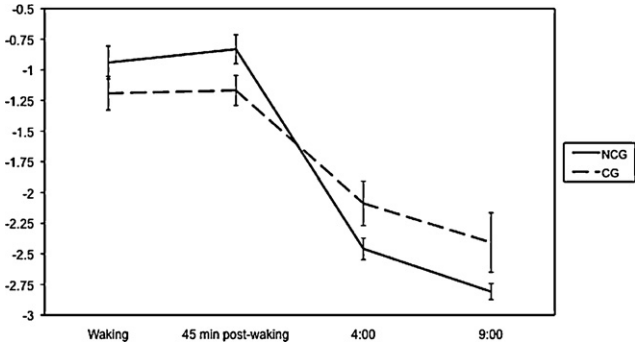

Figure 1 Log-transformed circadian values of cortisol presented with no covariates for Complicated and Non-Complicated Grief groups.

4. Discussion

As hypothesized, the present study demonstrates that the CG group showed a flatter diurnal cortisol slope than the Non-CG group. More specifically, cortisol was lower at 45 min post-wake and higher at 4 PM in the CG group than in the NCG group. Although the study's sample is small, this finding adds to a growing literature that distinguishes between those who have CG and those who are not diagnosed with this disorder. Accordingly, the present study contributes some support for the case to include CG as a diagnostic category in the DSM-5.

A flatter cortisol slope for those with CG as compared to those with Non-CG is consistent with our hypothesis, based on the literature of other groups undergoing stressful experiences. Meta-analytic research demonstrates that higher subjective distress is associated with a higher, flatter cortisol slope, and those who experience a traumatic and uncontrollable stressor evidence a higher, flatter slope than those with a non-traumatic or controllable stressor (Miller et al., 2007).

Sensitivity to glucocorticoids is an individual difference, but also differs within a person depending on the organ tissue in question. Genetic analyses have demonstrated that the traditional idea of a single glucocorticoid receptor is too simplistic (Oakley and Cidlowski, 2011). Cells can produce a variety of receptor isoforms, which contributes to the tissue- and even cell-specific effects of glucocorticoids. The effect of glucocorticoids on health has predominantly been discussed as due to their regulatory effect on the inflammatory immune response (for a review, see Rohleder et al., 2010). Relevant to psychological disorders, higher levels of pro-inflammatory cytokines (e.g., IL-6, IL-1) are associated with depressive symptoms. However, the brain is also acutely sensitive to the effects of cortisol, with effects on memory, emotion and motivation, and these effects could logically be at work in psychological disorders such as CG. A limitation of the present study is that it is unclear what target tissues the cortisol is affecting in those with CG vs. those with Non-CG, and this should be investigated in future research.

The present findings serve both to identify a biological marker that appears to distinguish individuals with and without CG and to suggest a mechanism through which bereavement, and CG specifically, might confer risk for untoward health outcomes. In order to further delineate the mechanism linking CG and morbidity/mortality, future research should

investigate whether HPA dysregulation influences immune cells, through NFKB or other inflammatory mechanisms, consistent with findings in other populations who are experiencing life stress (Miller et al., 2008). In addition, future research should include longitudinal study of the association between CG and disease morbidity and mortality, and whether this relationship is mediated in part by flatter diurnal cortisol.

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Conflict of interest

All other authors declare that they have no conflicts of interest.

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