The Unique Impact of Late-Life Bereavement and Prolonged Grief on Diurnal Cortisol

Jason M. Holland,¹ Vincent Rozalski,¹ Kara L. Thompson,¹ Roanne Joy Tiongson,¹ Alan F. Schatzberg,² Ruth O’Hara,² and Dolores Gallagher-Thompson²

¹Department of Psychology, University of Nevada, Las Vegas.
²Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, California.

Objectives. This study expands on previous research by examining the effects of prolonged grief disorder (PGD) symptoms and bereavement on diurnal cortisol patterns above and beyond depressive symptomatology.

Methods. Drawing on information from 56 depressed older adults, 3 groups were compared: (1) a depressed nonbereaved group, (2) a depressed bereaved without elevated PGD symptoms group, and (3) a depressed bereaved with elevated PGD symptoms group. Multilevel modeling was used to examine differences in diurnal cortisol profiles between these 3 groups, controlling for demographic factors and depressive symptoms.

Results. Results revealed that those who were bereaved had more dysregulated cortisol patterns, but PGD symptomatology seemed to have little effect. Subsidiary analysis with just the bereaved participants suggests that those who were recently widowed may have had greater cortisol dysregulation compared with other bereaved individuals in the sample.

Discussion. These findings suggest that the circumstance of being bereaved may be associated with more dysregulated cortisol, regardless of PGD symptomatology. This pattern of results might reflect greater disturbance in daily routines among bereaved individuals and acute stress in the case of those experiencing the recent loss of a spouse, which leads to disruption in circadian rhythms and the diurnal cycle of cortisol.

Key Words: Biomarkers—Death and dying—Widowhood—Salivary cortisol—Complicated grief.

The loss of a loved one can have serious health consequences (Jones, Bartrop, Forcier, & Penny, 2010; Stroebe, Schut, & Stroebe, 2007). Most notably, widowed and other bereaved individuals are at significantly higher risk for mortality—a trend which is often referred to as the “broken-heart” phenomenon (Moon, Kondo, Glymour, & Subramanian, 2011). However, the precise mechanisms by which this phenomenon occurs are poorly understood. Studies have documented a number of more intermediary health outcomes among bereaved individuals, including depressed immune functioning (Bartrop, Luckhurst, Lazarus, Kiloh, & Penny, 1994), cardiovascular risk factors (Buckley, McKinley, Tofler, & Bartrop, 2010), physical pain (Bradbeer, Helme, Yong, Kendig, & Gibson, 2003), and increased medication usage (Thompsoon, Breckenridge, Gallagher, & Peterson, 1984). Although these studies certainly point to the gravity of loss for health and well-being, it remains unclear the extent to which differences in physical health outcomes reflect increased depressive symptoms, severe grief reactions, or the stress of the bereavement itself. Thus, the purpose of this study is to disentangle the unique effects of bereavement and grief on cortisol—an objective biomarker of stress and physical health.

Cortisol is regarded by many as a crucial biological intermediary by which chronic stress gets “underneath the skin” and leads to disease (Sapolsky, Romero, & Munch, 2000). In particular, stressful life circumstances, like bereavement, often stimulate hypothalamic-pituitary-adrenocortical (HPA) axis functioning, and activation of this hormonal response system ultimately results in increased blood levels of cortisol, frequently referred to as the “stress hormone” (Lovallo, 2005). A daily pattern characterized by peak levels of cortisol at wake and a steep negative slope is generally regarded as “normal”; whereas, a shallower or flatter cortisol slope is typically considered an indication of dysregulation. The recent meta-analysis of Miller, Chen, and Zhou (2007) supported this idea and found that, compared with nonstressed controls, chronically stressed groups generally had a dysregulated pattern of hormone secretion, with lower than normal morning output but higher than expected secretion across the rest of the day, yielding a flattened diurnal pattern. These prolonged elevations in cortisol can increase allostatic load and compromise the immune system, ultimately increasing one’s risk for autoimmune-related and metabolic disorders (Sapolsky et al., 2000).

With regard to bereavement and cortisol in particular, previous studies generally suggest that the loss of a loved one is associated with more dysregulated cortisol patterns (Miller et al., 2007; Stroebe et al., 2007). Among these studies, most have compared cortisol patterns between bereaved and nonbereaved individuals, sometimes matching for demographic factors (e.g., age, sex; Gerra et al., 2003) and sometimes not matching (Luecken, 1998). Despite the methodological advantages of many of these studies...
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Those who reported active bereavement (i.e., “actively grieving”) the loss of a loved one, and these individuals were considered depressed non-bereaved individuals (Group 1).

Method

Participants

This study draws upon data collected from 60 depressed older adults recruited as part of a larger study on predictors of response to cognitive-behavioral therapy for late-life depression. In the present analysis, 56 participants were included, given that 4 participants did not provide one or more reliable saliva samples. These four excluded participants did not significantly differ from the rest of the sample in terms of demographic factors or depressive symptoms.

Following Institutional Review Board approval, participants were recruited throughout the San Francisco Bay Area, via radio, newspaper and Internet advertisements, flyers, free talks in senior centers, and through referrals from other professionals. Participants were eligible for the study if they were English speaking, had a score of 16 or higher on the Center for Epidemiologic Studies Depression scale (Radloff, 1977), and met diagnostic criteria for some type of current depressive disorder (e.g., major depression, dysthymic disorder, adjustment disorder with depressed mood), as assessed by the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998). Those who reported active suicidal ideation or plan, a history of psychosis or mania, or active substance abuse at baseline were excluded from the study, as well as any individuals who showed frank evidence of some type of dementia. Participants’ demographic and background information are presented in Table 1.

Measures

Loss status.—For the present analyses, participants were divided into three groups based on their bereavement status and the severity of their grief. Thirty-two participants indicated that they were not “actively grieving” the loss of a loved one, and these individuals were considered depressed non-bereaved individuals (Group 1).
Twenty-four individuals responded that they were actively grieving the loss of a loved one. Among the bereaved, the loss of a spouse or partner was most commonly reported (n = 8; 33.0%), followed by the loss of a parent (n = 4; 16.7%), sibling (n = 3; 12.5%), friend (n = 3; 12.5%), or child (n = 1; 4.2%). The vast majority of deaths were due to natural causes (n = 21; 87.5%) and on average losses occurred 3.1 years ago.

Bereaved individuals were divided into two subgroups: depressed bereaved individuals without elevated PGD symptoms (Group 2) and depressed bereaved individuals with elevated PGD symptoms (Group 3). PGD symptoms were assessed with a self-report version of the PG-13 (Prigerson & Maciejewski, 2008), which is based on a set of empirically derived criteria that have demonstrated internal consistency (Cronbach’s α = .82 to .93) and incremental validity (Delalibera, Coelho, & Barbosa, 2011; Prigerson et al., 2009). For the purposes of this study, if a participant reported experiencing a particular PGD symptom “at least once a week” or more frequently (i.e., at least a 3 on a 5-point scale), the symptom was considered to be present for that individual. Bereaved participants who met at least two of the three symptom-based PGD criteria (i.e., separation distress; cognitive, emotional, and behavioral symptoms; and functional impairment) were considered to have elevated PGD symptoms. Using this scoring procedure, 15 participants were categorized as depressed bereaved individuals without elevated PGD symptoms, and 9 participants were categorized as depressed bereaved individuals with elevated PGD symptoms. No significant differences were found between Groups 1, 2, and 3 in terms of depressive symptoms, number of stressful life events in the past year, or demographic factors (see Table 1). However, depressed bereaved individuals without elevated PGD symptoms were significantly less likely to be taking antidepressant medications compared with the other two groups. (The multivariate analysis was run controlling for antidepressant medication and hormone replacement therapy usage, and an identical pattern of results was obtained as that reported in this article.)

Depression.—Depressive symptoms were assessed with the Hamilton Rating Scale for Depression (HRSD). The HRSD is an interviewer-rated tool composed of 17 questions, 8 on a 5-point scale from 0 to 4, and 9 on a 3-point scale from 0 to 2, with higher scores representing greater severity of depressive symptoms. A recent meta-analytic study showed that the HRSD has good test–retest reliability (mean r = 0.87), internal consistency (mean α = .79), and inter-rater reliability (mean r = 0.87; Trajković et al., 2011), in addition to correlating highly with other established depression measures (Whisman et al., 1989).

Cortisol.—Salivary cortisol served as our primary dependent variable in this study. Saliva was collected using an oral swab kit at wake, 5:00 p.m., and 9:00 p.m. across 2 consecutive days. Participants were instructed to freeze each sample immediately after collection and return it to research staff at their next appointment, where it was then stored in a laboratory freezer before being assayed. They were also told not to eat, drink, smoke, brush their teeth, or use mouthwash in the 30 min before collection and not to drink alcohol during the 8–10 hr before collecting samples or during the 2 days of collection. Participants were asked to record the exact time of saliva collection and any events that might have influenced the sample (e.g., recent smoking

### Table 1. Demographic and Background Information (N = 56)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Overall</th>
<th>Depressed nonbereaved</th>
<th>Depressed bereaved without elevated PGD</th>
<th>Depressed bereaved with elevated PGD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Men</td>
<td>22</td>
<td>39.3</td>
<td>12</td>
<td>37.5</td>
</tr>
<tr>
<td>Women</td>
<td>34</td>
<td>60.7</td>
<td>20</td>
<td>62.5</td>
</tr>
<tr>
<td>Ethnicity/race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>40</td>
<td>71.4</td>
<td>25</td>
<td>78.1</td>
</tr>
<tr>
<td>African American</td>
<td>2</td>
<td>3.6</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Asian American</td>
<td>6</td>
<td>10.7</td>
<td>4</td>
<td>12.5</td>
</tr>
<tr>
<td>Latino/Hispanic</td>
<td>5</td>
<td>8.9</td>
<td>2</td>
<td>6.3</td>
</tr>
<tr>
<td>Native American</td>
<td>3</td>
<td>5.4</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Antidepressants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>25.0</td>
<td>11</td>
<td>34.4</td>
</tr>
<tr>
<td>No</td>
<td>42</td>
<td>75.0</td>
<td>21</td>
<td>65.6</td>
</tr>
<tr>
<td>Mean</td>
<td>69.9</td>
<td>7.6</td>
<td>70.4</td>
<td>7.5</td>
</tr>
<tr>
<td>SD</td>
<td>15.0</td>
<td>2.6</td>
<td>15.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Mean</td>
<td>14.2</td>
<td>5.6</td>
<td>14.4</td>
<td>4.9</td>
</tr>
<tr>
<td>SD</td>
<td>6.0</td>
<td>3.2</td>
<td>5.5</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Notes. ELSI = Elders Life Stress Inventory; HRSD = Hamilton Rating Scale for Depression; PGD = prolonged grief disorder.
or alcohol use) in a sample collection log. Samples that were collected improperly were not used in this analysis.

All samples were assayed for salivary cortisol using a highly sensitive enzyme immunoassay (Salimetrics, State College, PA). The test uses 25 μL of saliva per determination, has a lower limit of sensitivity of 0.003 μg/dL, standard curve range from 0.012 to 3.0 μg/dL, an average intraassay coefficient of variation of 3.5%, and an average interassay coefficient of variation of 5.1%. Method accuracy determined by spike and recovery averaged 100.8%, and linearity determined by serial dilution averaged 91.7%. Values from matched serum and saliva samples show the expected strong linear relationship, \( r(47) = 0.91, p < .0001 \). To correct for positive skewness, cortisol values were log transformed. (This log transformation was performed by adding 1 to the raw cortisol values and then taking the natural logarithm of that value. We added a constant (i.e., 1) beforehand because many of the raw cortisol values, measured in micrograms per deciliter, were less than 1. In these cases, a log transformation can yield very large negative numbers, thereby skewing the distribution further.)

**Plan of Analysis**

Multilevel modeling was used to evaluate differences between the nonbereaved and two bereaved groups (with and without elevated PGD symptoms) in terms of their cortisol patterns across the day (Singer & Willett, 2003). This statistical procedure allows parameters to vary at more than one level—in this case, both between and within individuals. All analyses were conducted using the SPSS Mixed program, and parameters were estimated using maximum likelihood. Log-transformed cortisol (log-cortisol) values were used as the dependent variable.

In these analyses, the passage of time was measured as the number of hours since wake, and wake was considered initial status (i.e., time zero). Data collected from the saliva samples across the 2 consecutive days were combined in order to allow for a more reliable estimate of each participants typical diurnal pattern (Kraemer et al., 2006).

Loss status was treated as a dummy-coded variable with depressed nonbereaved individuals serving as the reference group. In addition to examining loss status as an independent variable, demographic factors and depressive symptoms (as measured by the HRSD) were also included as statistical controls. Continuous independent variables (i.e., age, years of education, HRSD scores) were centered by subtracting the sample mean. Dichotomous variables were coded as follows: gender (−1 = women, 1 = men), race/ethnicity (−1 = ethnic/racial minority, 1 = Caucasian). In order to examine the relationship of each of these variables to initial levels of log-cortisol at wake as well as to the slope of diurnal log-cortisol trajectories across the day, all independent variables were entered as main effects and as interaction effects with time.

**Results**

**Main Analysis**

Findings from the main multilevel modeling analysis are presented in Table 2. This analysis revealed that the overall mean for log-cortisol at wake was significantly greater than zero (coefficient = .305, \( p < .001 \)), and that log-cortisol values tended to significantly decrease across the day (coefficient = −.015, \( p < .001 \)), which is consistent with cortisol’s known diurnal pattern. It should be noted that the coefficients in this model can be interpreted similar to beta coefficients in standard linear regression. Specifically, these estimates represent the degree of change in the dependent variable (in this case log-cortisol) for every one unit increase in the independent variable. For example, since time was measured in hours, an estimate of −.015 means that, on average, for every passing hour log-cortisol levels decreased by .015.

Consistent with our hypothesis, findings revealed that those classified as depressed bereaved without elevated PGD symptoms had significantly lower levels of log-cortisol at wake (coefficient = −.114, \( p = 006 \)) and flatter diurnal slopes (coefficient = .007, \( p = .02 \)), compared with those categorized as depressed nonbereaved. This pattern is consistent with a more dysregulated cortisol profile. Surprisingly, no significant differences were found between the depressed bereaved with elevated PGD symptoms and

<table>
<thead>
<tr>
<th>Table 2. Multilevel Model Examining the Association Between Loss Status and Diurnal Cortisol Controlling for Depressive Symptoms and Demographic Factors (N = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coefficient</strong></td>
</tr>
<tr>
<td>Intercept (cortisol at wake)</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Race/ethnicity</td>
</tr>
<tr>
<td>Education</td>
</tr>
<tr>
<td>HRSD</td>
</tr>
<tr>
<td>Bereaved group without elevated PGD symptoms</td>
</tr>
<tr>
<td>Bereaved group with elevated PGD symptoms</td>
</tr>
<tr>
<td>Time</td>
</tr>
<tr>
<td>Time × age</td>
</tr>
<tr>
<td>Time × gender</td>
</tr>
<tr>
<td>Time × race/ethnicity</td>
</tr>
<tr>
<td>Time × education</td>
</tr>
<tr>
<td>Time × HRSD</td>
</tr>
<tr>
<td>Time × bereaved group without elevated PGD symptoms</td>
</tr>
<tr>
<td>Time × bereaved group with elevated PGD symptoms</td>
</tr>
</tbody>
</table>

*Notes. HRSD = Hamilton Rating Scale for Depression; PGD = prolonged grief disorder.*

Dichotomous variables were coded as follows: Gender (−1 = women, 1 = men), race/ethnicity (−1 = ethnic/racial minority, 1 = Caucasian). The loss status variable was dummy coded, and nonbereaved individuals were treated as the comparison group.
the depressed nonbereaved groups although the depressed bereaved with elevated PGD symptoms group trended toward having more dysregulated cortisol profiles (see Figure 1 for a graphical depiction of the cortisol profiles for the three groups). Considering this surprising result, the main multilevel modeling analysis was rerun using the depressed bereaved with elevated PGD symptoms group as the reference category, and in this reanalysis no significant differences were observed between bereaved individuals with and without elevated PGD symptoms in terms of waking cortisol (coefficient = −.101, \( p = .07 \)) or diurnal slope (coefficient = .006, \( p = .14 \)). Taken together, these results suggest that the loss of a loved one is predictive of more dysregulated cortisol, irrespective of one’s level of PGD symptoms. It should be noted that in both of these analyses, demographic factors (e.g., age and ethnicity/race) and depressive symptoms were included as statistical controls.

Subsidiary Analysis

Though not a central focus of our study, women and those who had higher levels of depressive symptoms trended toward having more dysregulated cortisol profiles, characterized by significantly lower log-cortisol levels at wake and somewhat flatter diurnal slopes. However, differences in diurnal slope for gender and HRSD scores were not statistically significant.

Discussion

Consistent with our hypothesis, the bereaved older adults in this study tended to have more dysregulated cortisol patterns, and this finding fits with previous research on HPA-axis functioning among bereaved individuals and other chronically stressed populations (Miller et al., 2007; Stroebe et al., 2007). Surprisingly, PGD symptoms appeared to be a relatively weak predictor of cortisol, regardless of whether it was conceptualized as a categorical or continuous variable. Taken together, these findings suggest that there may be something about the circumstance of being bereaved that puts older adults at-risk for cortisol dysregulation, even though the severity of grief symptoms may play little role.
Although preliminary, the subsidiary analysis conducted in this study points to one possible explanation for this pattern of results. Specifically, older adults who had lost a spouse in the more recent past were found to have the most dysregulated cortisol patterns compared with other bereaved individuals in the sample. The recent loss of a spouse, irrespective of whether this loss is anticipated, is one of the most acutely stressful events that can occur during one’s life. Further, it seems likely that those who lost a cohabitating spouse and had not had time to adjust to this life transition experienced the most disruptions in daily activities, especially if the deceased fulfilled important instrumental functions in the relationship (e.g., transportation, monitoring health, cooking; Hansson & Stroebe, 2007).

Indeed, past research suggests that widowhood in late-life is associated with poor eating behaviors and nutrient intake (Rosenbloom & Whittington, 1993), disrupted social rhythms (Brown et al., 1996), and sleep impairment (Richardson, Lund, Caserta, Dudley, & Obray, 2003). In fact one study showed that, compared with healthy controls, distressed grievers are more likely to have fewer social contacts, skip meals, stay indoors, miss work, and have evening snacks (Monk, Houck, & Shear, 2006). This abrupt change in daily routines may be responsible for disruption of circadian rhythms and dysregulation of cortisol’s normal diurnal pattern (Stetler, Dickerson, & Miller, 2004). Such an explanation could help account for past findings indicating that the mortality rate among widowed populations is at its highest in the first 6 months of bereavement (Martikainen & Valkonen, 1996; Moon et al., 2011). Notably, irregularity of daily activities at 3-months postloss has been shown to be associated with mental health symptoms at 1- and 2-years postloss among older bereaved spouses (Prigerson et al., 1996), and it stands to reason that these psychological difficulties might be accompanied by physical health problems as well.

If our findings are replicated in studies with larger, more diverse samples, researchers may begin exploring new treatments for bereaved older adults (particularly those who are recently widowed) that focus on re-establishing daily routines. For example, some adaptation of social rhythm therapy for bereavement that focuses on maintaining regular daily rhythms in activities such as sleeping, waking, eating, and exercise could help ameliorate the deleterious health effects of losing a loved one who may have helped to maintain a sense of stability and structure in life (Frank, Swartz, & Kupfer, 2000). In addition, research that focuses on other biomarkers that may detect disruptions in biological rhythms could help improve early identification of bereaved older adults at greatest risk for negative physical health outcomes. This study suggests that cortisol may be one such biomarker, and future studies might focus on other factors such as melatonin levels (Mirick & Davis, 2008) or genes associated with circadian rhythms (e.g., the “Clock” gene; Herzog, Takahashi, & Block, 1998).

It should be noted that this study is limited in several ways. First, our small sample size limited statistical power and also prevented higher order interactions (i.e., three- or four-way interactions) from being adequately tested. The associations and interactions between demographic factors (e.g., gender, ethnicity/race), circumstances of the loss (e.g., time since loss, relationship to the deceased, cause of death), psychiatric distress (e.g., PGD symptoms, depression) and cortisol are likely complex, and future studies with large sample sizes could help identify specific subgroups of individuals who are most at-risk for cortisol dysregulation and other negative health outcomes. In particular, closer examination of gender differences would be a fruitful area for further investigation. Although it was not a central focus in this study, some gender differences were observed in our larger analysis with the full sample, and other studies have noted gender differences in mortality rates among bereaved individuals (Moon et al., 2011), suggesting that men and women may have different physiological reactions to loss.

This study also included a broad range of bereaved individuals in terms of time since loss and relationship to the deceased. Although this allowed for some examination of how these factors might influence cortisol, it seems entirely possible that a different pattern of results might have been found had we only focused on widowed individuals or those who experienced recent losses. A more fine-grained analysis (e.g., comparing cortisol patterns for those who lost a spouse with those who lost a child or sibling) with a larger sample also seems warranted, given findings that the loss of more intimate relationships are associated with greater separation and traumatic distress (Holland & Neimeyer, 2011). In addition, most of the participants classified as having elevated PGD symptoms did not meet full criteria for the disorder, which could partly account for null findings related to the severity of grief symptoms. In addition, the vast majority of bereaved participants in this study had lost a loved one to natural causes. Considering that past research has found that loss by violent means may have a particularly profound negative impact on the bereaved (Holland & Neimeyer, 2011), researchers would do well to examine the impact of cause of death on cortisol among surviving family members. Finally, it should be noted that the distinction between bereaved and nonbereaved in this study was based on participants’ perception, rather than a more formalized assessment, which could have yielded a somewhat different categorization of participants.

Notwithstanding these limitations, this study is the first to simultaneously examine the effects of PGD symptoms and more general effects associated with losing a loved one on cortisol. The finding that the circumstance of being bereaved (regardless of PGD symptomatology) is associated with more dysregulated cortisol patterns, particularly among those who recently lost a spouse, has important implications for future interventions and assessment practices with bereaved older adults.
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Correspondence

Correspondence should be addressed to Jason M. Holland, PhD, Department of Psychology, University of Nevada, Las Vegas, 4505 South Maryland Parkway, Box 455030, Las Vegas, NV 89154-5030. E-mail: jason.holland@unlv.edu.

References


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