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Cortisol Response to Social Stress in Parentally Bereaved Youth

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Abstract

Background—Parental bereavement is associated with increased risk for psychiatric illness and functional impairment in youth. Dysregulated hypothalamic-pituitary-adrenal (HPA) axis functioning may be one pathway through which bereaved children experience increased risk for poor outcomes. However, few studies have prospectively examined the association between parental bereavement and cortisol response while accounting for psychiatric disorders in both youth and their caregivers.

Methods—One-hundred and eighty-one bereaved and nonbereaved offspring and their caregivers were assessed at multiple time points over a 5-year period after parental death. Offspring participated in an adaptation of the Trier Social Stress Task (TSST), and salivary cortisol samples were collected before and after exposure to social stressors. Mixed models for repeated measures were used to analyze the effects of bereavement status, psychiatric disorder in both offspring and caregiver, and demographic indices on trajectories of cortisol response.

Results—After controlling for demographic variables and offspring depression, bereaved offspring demonstrated significantly different trajectories of cortisol response compared with nonbereaved offspring, characterized by higher total cortisol output and an absence of cortisol reactivity to acute social stress. Within the bereaved group, offspring of parents who died by sudden natural death demonstrated significant cortisol reactivity to social stress compared with offspring whose parents died by suicide, who demonstrated more blunted trajectory of cortisol response.

Conclusions—Parentally bereaved youth demonstrate higher cortisol output than nonbereaved youth but are less able to mount an acute response in the face of social stressors.

Keywords

Adolescent; bereavement; cortisol; depression; HPA axis; TSST

The death of a parent is one of the most stressful events that a child can experience (1), and it is estimated that 1 in 25 youth will experience the death of a parent before age 18 (2). The consequences of parental loss on children are profound and may include increased family adversity (3, 4), increased emotional and behavioral problems (5), and increased likelihood of depression and suicide (6–10). Record linkage, retrospective, and prospective studies

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suggest trajectories of negative outcomes for children 5 to 12 years after parental death, including functional impairment compared to nonbereaved youth (3), and up to a 13-fold increased risk for major depression (11). Taken together, the effects of parental death on children strongly confer developmental risk for psychopathology and poor outcomes that extends beyond the period of acute bereavement (12, 13).

High levels of psychosocial stress may alter the functioning of the hypothalamic-pituitary-adrenal (HPA) axis, one of the physiologic systems that helps focus and sustain emotional, cognitive, behavioral, and metabolic activity in response to perceived threat (14–16). Neurons in the hypothalamic paraventricular nucleus secrete corticotropin-releasing hormone, which activates the production of adrenocorticotrophic hormone from the anterior pituitary. In turn, adrenocorticotrophic hormone stimulates the release of glucocorticoids, such as cortisol, from the adrenal cortex, which regulates cardiovascular, metabolic, immunologic, and homeostatic functions related to “fight or flight” response (17). Adaptive functioning of the HPA axis is related to “short, robust well orchestrated activations of these systems,” marked by acute reactivity and a rapid return to homeostasis (18). Chronic psychosocial stress has been hypothesized to place a burden on multiple physiologic systems that must constantly adapt, via reactivity or regulation, to meet environmental demands appropriately. Over time, the burden of ongoing adaptation may lead to alterations in these regulatory systems in which they function less efficiently and effectively (19). The effect of chronic psychosocial stress has been hypothesized to contribute to the dysregulation of the HPA axis, evidenced by hypo- or hypersecretion of cortisol (14, 20, 21), a blunted physiological response to acute stress (22), or an extended physiologic stress response due to impairment of the homeostatic function of the HPA system. Dysregulation of HPA the axis may also negatively influence the adaptive functioning of related cardiovascular, immunologic, and/ or metabolic systems (23).

There is evidence of altered HPA axis function in children who have experienced the death of a parent, although the direction of cortisol response has been largely inconsistent across prospective studies. Children who experienced the sudden death of a parent in the terrorist attack of September 11, 2001, demonstrated higher afternoon and evening basal cortisol levels compared with nonbereaved children (24). Young adults who experienced parental loss were found to show an exaggerated cortisol response to social stress compared with nonbereaved control subjects (25). In another sample of parentally bereaved youth, lower total cortisol output was associated with greater exposure to negative life events, and lower cortisol levels across an acute stress task was associated with externalizing symptoms (26, 27).

The relationship between bereavement and HPA axis dysfunction is further complicated by high rates of psychiatric disorder in parentally bereaved children. Bereavement is strongly associated with depression (28, 29), posttraumatic stress disorder (30, 31), alcohol/substance use (32), and behavioral disorders, some of which may actually antedate the loss of a parent (33–35). These conditions have been associated with alterations in diurnal cortisol secretion and response to acute stress compared with youth without these disorders. Exposure to parental psychopathology has also been associated with increased cortisol response to stress in children (36–38). Hence, psychiatric disorders in bereaved offspring and their surviving caregivers may account for associations between bereavement and alterations in cortisol secretion.

This study reports on cortisol response in a large, wellcharacterized sample of parentally bereaved and nonbereaved youth who have participated in a prospective, longitudinal study of the impact of sudden parental death on children and families (39–41). Both groups participated in an acute social stress procedure conducted approximately 5 years after time

of parental death, wherein salivary cortisol was sampled 5 times over a 50-minute period. It was hypothesized that parentally bereaved children would evidence a significantly different trajectory of cortisol response compared with nonbereaved controls, marked by greater reactivity to acute stress. In this article, psychiatric disorders associated with cortisol response in offspring were considered possible confounds and were controlled to isolate the influence of bereavement on the acute stress responses of participants. Exploratory analyses investigated differences in cortisol response to acute social stressors within the bereavement group depending on the type of proband death (sudden natural death, accidental death, or suicide).

Methods and Materials

Participants

The participants were 181 youth, aged 10 to 29, from 62 parentally bereaved families and 53 nonbereaved families; 66.3% of participants had at least 1 sibling in the study. The deceased parents (probands) died within 24 hours of definite verdicts of suicide ($n = 21$), accidental death ($n = 13$), or sudden natural death ($n = 28$). The accidental deaths consisted of five drug overdoses, three motor vehicle accidents, one accidental fall, and four others (e.g., drowning, exposure to cold). The sudden natural deaths were due to myocardial infarction ($n = 22$), infection ($n = 1$), and 5 less frequent causes (e.g., diabetes mellitus, stroke, aneurysm, gastric bypass surgery). Nonbereaved families were recruited by frequency matching to the deceased probands on sex, age, and neighborhood. Nonbereaved offspring had two living biological parents, lived in the home of at least one of them, and had no first-degree relatives who had died within the 2 years before recruitment. The caregivers of nonbereaved offspring were self-identified as primary caregivers. This study was approved by the University of Pittsburgh institutional review board, and all participants gave written consent or assent.

Out of 427 recruited at study entry, 284 youth were available for follow-up and 181 (64%) completed the acute laboratory social stress task. Youth who participated in the laboratory protocol were similar to youth who did not participate, except that youth who participated in the laboratory protocol were less likely to have a history of physical or sexual abuse (Fisher's Exact Test, $p = .001$) and were more likely to have a diagnosis of attention-deficit/hyperactivity disorder (ADHD) at study entry (Fisher's Exact Test, $p = .001$).

Procedures

Offspring and caregivers completed the 5-year follow-up assessment in their homes, which consisted of a structured diagnostic interview and questionnaires regarding psychiatric symptoms and other measures of well-being. At this time, the laboratory study of physiological responses to social stress was presented to all bereaved and nonbereaved offspring. Those who chose to participate attended a 1-hour laboratory visit, where salivary cortisol was collected before and after a 15-minute social stress task. Participants were asked to refrain from alcohol or smoking in the 24 hours before the test, and from eating and ingesting caffeine or dairy products in the 2 hours before the test. The social stress task was administered between 3:30 and 5:00 PM, and the initial cortisol sample, which served as relative baseline for participants, was collected approximately 15 minutes after their arrival.

Measures

Psychiatric Disorders—Psychiatric disorders in adult offspring and caregivers were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (42). Offspring younger than 18 years of age were interviewed using the Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime

Version (43). At study entry, caregivers and offspring reported on their history of psychiatric disorders before the time of proband death and a psychiatric assessment of the proband was conducted, using a psychological autopsy procedure (44) and the SCID-I (42). The course of disorders in the offspring and caregiver was documented by using the Longitudinal Interview for Follow-Up Evaluations (45), or the adolescent version. High interrater reliability was maintained on psychiatric diagnoses, as indicated by kappa values of .74 to .85.

We examined the relationship between offspring cortisol response and 1) previous history of psychiatric disorders before proband's death, 2) psychiatric disorders 0 to 33 months after death, and 3) psychiatric disorders 34 to 62 months after death. Six psychiatric disorders in offspring were investigated: depression (major depressive disorder, dysthymia, and depressive disorder not otherwise specified), anxiety (generalized anxiety disorder, social phobia), posttraumatic stress disorder, alcohol/substance disorders, ADHD, and behavior disorders (conduct disorder, oppositional defiant disorder). The same disorders were investigated for caregivers except for ADHD and behavior disorders because there were so few cases ($n = 2$ and $n = 0$, respectively). Because they have been presented in previous papers (29, 30), the demographic and psychiatric characteristics of the 90 bereaved and 91 nonbereaved offspring before and in the 0 to 33 months after proband death are included as Supplement 1. The psychiatric status of offspring and caregivers in the 34 to 62 months after proband death are described in Table 1.

Trier Social Stress Task—Bereaved and nonbereaved offspring participated in a modified version of the Trier Social Stress Test (TSST) (46), a procedure designed to induce a moderate stress response. Offspring watched a 10-minute travel video during which baseline cortisol sample was obtained (0 minutes). For the next 15 minutes, participants were asked to prepare and deliver a brief speech and then perform a mental arithmetic task while being observed by research staff. Salivary cortisol samples were obtained immediately after the social stress task (15 minutes after baseline), and 5, 10, and 20 minutes after the social stress task (20, 25, 35 minutes after baseline). Vials were frozen at -20°C until they were assayed. Saliva samples were sent to Salimetrics (State College, Pennsylvania), where they extracted and assayed in duplicate using a high-sensitive enzyme immunoassay with a range of sensitivity from .007 to 1.8 $\mu\text{g/dL}$. The average intra- and interassay coefficients of variation were 4.13% and 8.89%, respectively.

Cortisol response was operationalized as changes in cortisol levels across the five time points during the laboratory procedure. Total cortisol output was indexed by area under the curve (AUC) with respect to ground, computed according to the trapezoid method (47) using raw values, and then logtransformed to correct for skewness. Cortisol reactivity was defined as a statistically significant within-subjects increase in cortisol levels from baseline or relative minimum to estimated peak (minute 25) in bereaved and nonbereaved offspring.

Covariates—Age at time of TSST, sex, minority status, socioeconomic status as indexed by the Hollingshead's scale (48), and history of abuse were assessed via interview. Body mass index was calculated from height and weight obtained from participants at the time of the 5-year psychiatric follow-up assessment. Participants also completed an inventory of food, caffeine, tobacco, and medication usage on the day of cortisol collection (Table 2).

Analytic Strategy

Analysis of variance (ANOVA) and chi-square were conducted to provide descriptive statistics regarding differences between bereaved and nonbereaved offspring on

demographic, health, psychiatric indices, and mean cortisol levels during the laboratory procedure.

Multilevel linear modeling was used to evaluate group differences in cortisol levels over time using the xtmixed procedure in STATA 11.0 (2009; StataCorp LP, Stata Statistical Software, College Station, Texas), with repeated cortisol measures nested within subjects and subjects nested within families. A level 1 variable, cortisol sample order, modeled the pattern of within-subjects responses over time. Bereavement status served as the between-subjects dimension and nonbereaved offspring as the referent condition. Mean cortisol values (micrograms/deciliter units) were log transformed to correct for high rightskewness in the distribution. Because cortisol responses are typically not linear, we compared each set of mixed-effects models with time as linear versus time as a quadratic function (time2) using a likelihood ratio test. We used the linear or linear plus quadratic terms to test for change in cortisol over time, as indicated. Log-likelihood ratio test indicated that inclusion of the quadratic time variable significantly improved model fit ($\chi^2(1) = 26.89, p < .001$) for our primary analyses. Hence, two interaction terms, bereavement status by time and bereavement status by time2, were created to characterize cortisol response curves of bereaved and nonbereaved offspring. Secondary analyses involved exploring differences within the bereaved group, and in these models, the between-subjects dimension was death type with suicide as the reference group.

Analysis of variance was used to assess group differences in total cortisol output. Paired samples *t* tests were used to test for cortisol reactivity within bereaved and nonbereaved offspring and within each of the three bereaved offspring groups.

A comprehensive set of covariates were tested to ensure that any association between bereavement status and cortisol response was not due to demographic or health confounds. Univariate mixed-effects regression models were conducted to determine whether demographic and health variables were associated with cortisol response in the sample. There were main effects for age ($b = .12, SE = .04, z = 3.36, p < .001$), sex ($b = -.07, SE = .04, z = -1.87, p = .06$), and minority status ($b = -.16, SE = .06, z = -2.50, p = .01$), with higher cortisol levels for those participants who were older, male, or nonminorities, but there were no statistically significant interactions between time and any of these demographic variables. Hence, age, sex, and minority status were included as covariates in all of our analyses.

Univariate mixed-effects regression models were also conducted to examine associations between cortisol response and the psychiatric histories of offspring, caregivers, and probands before time of proband death, and the incident of psychiatric disorders in offspring and caregivers in the 0 to 33 months and 34 to 62 months after death (Table 3). Variables that continued to demonstrate a significant effect after controlling for age, sex, and minority status were included as covariates in our final models. Stringent error control strategies were not used for analyses to explore potential covariates because these did not inform the study's central hypotheses but were important to ensure validity of results.

Results

Cortisol Response, Output, and Reactivity in Bereaved and Nonbereaved Offspring

After controlling for demographic covariates, mixed-effects regression models revealed a significant interaction between bereavement status and time, indicating differences in the trajectories of cortisol response in bereaved and nonbereaved offspring (see Table 4). Post hoc repeated contrasts showed that the group differences in slope of cortisol levels occurred during the first 15 minutes of the TSST [$F(1,176) = 7.04, p = .01$], during which there was no

significant change in cortisol in the bereaved group [$t(89) = -1.26, p = .21$], whereas cortisol levels in the nonbereaved group significantly decreased [$t(90) = 2.34, p = .02$], as shown in Figure 1. Correspondingly, the total cortisol secretion, as measured by AUC, was higher in the bereaved than in the nonbereaved groups [$F(1,179) = 6.15, p = .01$]. Within-subjects pairwise contrasts conducted to assess cortisol reactivity revealed no significant changes in cortisol levels between minute 0 and 25 for bereaved offspring. For nonbereaved offspring, although there was no significant difference between minutes 0 and 25, there was a significant increase in the lowest cortisol level (minute 15) and minute 25 [$t(90) = -2.03, p = .05$].

Cortisol Response and Psychiatric Disorders in Offspring and Caregivers

After adjusting for age, sex, and minority status, offspring and caregivers' depression in the 34 to 62 months after proband death, and offspring ADHD before death and in the 34 to 62 months after proband death were significantly associated with higher cortisol levels compared with those offspring and caregivers who did not meet criteria for these diagnoses at corresponding time periods.

A significant bereavement by time interaction persisted when these diagnostic variables were incorporated into the final mixed-effects model and backward stepwise regression was used to consolidate the most parsimonious set of predictors of offspring cortisol response (see Table 4). In the final model, offspring depression 34 to 62 months after proband death remained a significant predictor of cortisol response.

Moderators—There were no significant interactions between bereavement status and age, sex, minority status, sex of proband, or offspring depression 34 to 62 months after proband death on offspring cortisol response over time. Offspring history of abuse was neither associated with offspring cortisol response in univariate analyses nor did it change the pattern of significance reported in our final model when included as a covariate.

Cortisol Response, Output, and Reactivity among Bereaved Offspring

Hierarchical mixed-effects models investigated differences in cortisol response in offspring bereaved by parental suicide, accident, or sudden natural death. A significant difference in cortisol response over time emerged between the offspring of sudden natural death and offspring of suicide probands (see Table 5). The significant group by time interaction between minutes 20 and 25 [$F(2,84) = 3.56, p = .03$] indicated that cortisol levels for offspring in the sudden natural death group significantly increased [$t(40) = -2.16, p = .04$], whereas there was no significant change in cortisol levels for offspring in the suicide group (see Figure 2). There were no significant differences among the three bereaved groups in total cortisol output [$F(2,87) = .73, p = .48$]. Paired comparisons within each of the three bereaved groups revealed that the sudden natural group demonstrated cortisol reactivity, as evidenced by a significant increase in cortisol levels between 0 minutes (baseline) and 25 minutes [$t(40) = -2.32, p = .03$], whereas the offspring bereaved by suicide and accidental death did not.

Within the bereaved group, three psychiatric variables were identified that showed main effects on cortisol levels, after adjusting for age, sex, and minority status: offspring ADHD before proband death ($b = .20, SE = .10, z = 2.10, p = .04$), offspring alcohol/substance disorder 34 to 62 months after proband death ($b = .17, SE = .08, z = 2.07, p = .04$), and caregivers' depression 34 to 62 months after proband death ($b = .22, SE = .08, z = 2.74, p = .01$). Inclusion of these variables in the final mixed effects regression model did not change the pattern of results, and the group by time interaction between offspring bereaved by sudden natural death and those bereaved by suicide remained significant (see Table 5). In

the final model, offspring alcohol/substance disorder and caregiver depression in the 33 to 62 months after proband death remained significant predictors of cortisol response.

Discussion

In this study, bereaved and nonbereaved offspring showed different patterns of cortisol response to social stress. Bereaved youth showed overall higher total cortisol output but showed no significant increase in cortisol levels over time in response to social stress. The nonbereaved group, on the other hand, evidenced a significant increase in cortisol levels in the 10 minutes after the TSST. Within the bereaved group, offspring bereaved due to sudden natural death showed a significant increase in cortisol relative to baseline, which was significantly different from the lack of cortisol reactivity evidenced by the suicide-bereaved group. These findings suggest that offspring bereaved by parental suicide do not appear to mount a significant response in reaction to acute social stress, whereas offspring bereaved by sudden natural death demonstrate a more typical trajectory of cortisol reactivity.

The results from this study need to be considered in light of its strengths and limitations. This is one of the few controlled longitudinal studies of bereaved youth with detailed data on the 5-year course of psychiatric disorder and adjustment postdeath and one of the few studies that can control for the potential confounds of psychiatric disorders in both offspring and caregivers on offspring cortisol responses. There are also some significant limitations. First, cortisol response to social stress was not assessed before proband death, preventing the examination of longitudinal changes in cortisol subsequent to bereavement. As such, we do not know when the differences in cortisol response for bereaved and nonbereaved offspring emerged or whether these differences were present before bereavement. Although we are able to adjust for the effects of psychiatric disorder, our study cannot determine whether changes in cortisol predated psychopathology or vice versa. Next, the long duration between bereavement and examination of cortisol response allows for many possible intervening variables that were not investigated in the current study, such as parenting, changes in family relationships, and social adversities subsequent to parental death. Although the procedures used were similar to those in other published reports (29, 49), in retrospect, the duration of the cortisol sampling may not have been long enough to establish a stable baseline or to document full recovery. However, we were able to compare acute cortisol response to a provocative social stressor in bereaved and nonbereaved offspring within the limits of the 50-minute protocol.

Bereaved offspring demonstrated significantly higher total cortisol output across the acute stress laboratory procedure than nonbereaved offspring and may suggest that bereaved offspring have higher basal cortisol than nonbereaved offspring. This profile is similar to other pediatric samples exposed to chronic stress (50–52). At the same time, bereaved youth did not demonstrate cortisol reactivity to a provocative challenge, which is also characteristic of individuals under chronic stress (53, 54). The blunted trajectory of cortisol response in bereaved offspring may suggest that the HPA axis cannot mount an acute response to social stress and may imply a less adaptive stress system (20, 21). Although the pathophysiology by which acute psychosocial stress is translated to a physiological level is unclear, it has been suggested that a blunted response of the HPA axis in reaction to acute stress may result from chronic hypersecretion of cortisol, which may, over time, decrease the effects of cortisol on its receptors and target cells (16). Although our findings in this sample of bereaved offspring in this study support this hypothesis, future prospective longitudinal studies are needed to test the pathophysiology of cortisol response in parentally bereaved youth.

Significant differences in the cortisol response trajectories of bereaved and nonbereaved offspring continued to be present after accounting for demographic variables and for psychiatric disorders in both offspring and their caregivers. Hence, the observed differences in cortisol responses to social stress in bereaved offspring were not explained by the psychiatric disorder in offspring and caregivers that are increased with bereavement. Although studies of bereaved college students have shown that the quality of postloss family environment moderates TSST response (55), we were not able to identify significant effects of family functioning or life events on cortisol response (Supplement 1).

Offspring of probands who died by sudden natural death demonstrated significant and expected cortisol reactivity in response to social stress compared with offspring of probands who died by suicide, who demonstrated no significant changes in cortisol levels. Differences in cortisol responses may suggest differences in chronic stress experienced by offspring bereaved by different death circumstances. The blunted reactivity pattern of offspring experiencing parental suicide may indicate exposure to more chronic stress before proband death than offspring whose parent died by sudden natural death. Bereaved offspring whose parent died by suicide may also have higher rates of familial psychopathology. However, the differences between the sudden natural death and suicide groups persisted even after controlling for salient psychiatric variables. Several retrospective studies of chronic stress in adults suggest that blunted responses of the HPA axis are associated with early childhood adversity, including maltreatment, poverty, and psychopathology (56–59), which has been shown to be increased in suicide bereaved offspring compared with a sudden natural death group (31, 32). These findings may suggest that the HPA axis of offspring bereaved by parental suicide cannot adaptively mount an acute response to stress.

Overall, findings suggest higher cortisol output combined with less vigorous cortisol reactivity to social stress in bereaved versus nonbereaved offspring. Bereaved youth may be exposed to adverse health effects of excess cortisol secretion (60) but may be deprived of the ability to adaptively respond to acute stressors. Future studies should examine changes in the HPA axis earlier in bereavement to understand how the evolving change in stress response and cortisol dynamics relate to mental and physical health of bereaved youth. In addition, future studies should monitor cortisol response as a potential biomarker of overall adaptation to the chronic stress of parental bereavement. These findings, taken together, also suggest that future intervention research with bereaved youth should consider health consequences of bereavement and how bereavement may present acute risk for health problems (61).

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Figure 1.
Cortisol Responses of Bereaved and Nonbereaved Offspring

Figure 2.
Cortisol Responses of Bereaved Offspring Whose Parent Died by Suicide, Accident, or Sudden Natural Death

Table 1

Psychiatric Characteristics of Offspring and Caregivers in Bereaved and Nonbereaved Comparison Families (34 – 62 months after time of proband death)

Characteristic	Families with Parental Death (n = 62)	Comparison Families (n = 53)	Statistic
Offspring Psychiatric Status (n = 181)			
Depression (% , n)	35.6% (32)	7.7% (7)	$\chi^2_1 = 20.78^{**}$
Anxiety (% , n)	22.2% (20)	8.8% (8)	$\chi^2_1 = 6.24^*$
Alcohol/ Substance Disorder (% , n)	7.8% (7)	2.2% (2)	$\chi^2_1 = 2.98$
PTSD (% , n)	13.3% (12)	0% (0)	FET ^{**}
ADHD (% , n)	12.4% (11)	0% (0)	FET ^{**}
Behavior Disorders (% , n)	2.2% (2)	0% (0)	FET
Caregiver Psychiatric Status (n = 107)			
Depression (% , n)	67.8% (n = 40)	20.8% (n = 10)	$\chi^2_1 = 23.45^{**}$
Anxiety (% , n)	30.5% (n = 18)	10.4% (n = 5)	$\chi^2_1 = 6.33^*$
Alcohol/ Substance Disorder (% , n)	6.8% (n = 4)	6.3% (n = 3)	$\chi^2_1 = 0.01$
PTSD (% , n)	25.4% (n = 15)	4.2% (n = 2)	FET ^{**}

Note. FET=Fisher's Exact Test

*
p < 0.05;

**
p < 0.01

Table 2

Descriptive Statistics of Bereaved and Nonbereaved Offspring

	Bereaved Offspring (n = 90)	Nonbereaved Offspring (n = 91)	Statistic
Offspring Demographic and Health Characteristics			
Sex (% male)	45.6% (n = 41)	47.3% (n = 43)	$\chi^2_1 = 0.05$
Age in years (Mean, SD)	17.5(3.9)	17.0(3.1)	$F(1, 179) = 0.65$
Adolescents ^I (% , n)	60.0% (n = 54)	63.7% (n = 58)	$\chi^2_1 = 0.27$
Adults ^I (% , n)	40.0% (n = 36)	36.3% (n = 33)	
Minority Status (% minority)	15.6% (n = 14)	13.2% (n = 12)	$\chi^2_1 = 0.21$
BMI (Mean, SD)	25.6 (6.4)	24.0 (5.4)	$F(1, 165) = 3.06^{\dagger}$
Medical Problems (% , n)	17.4% (15)	11.1 (10)	$\chi^2_1 = 1.45$
Abuse History (% , n)	15.6% (14)	1.1% (1)	FET ^{**}
Offspring Status on day of Cortisol Collection			
Time of Testing (mean, SD)	15: 03(1: 01)	15:03(1:09)	$F(1, 179) = 0.03$
Eating <= 2 hours prior to TSST (% , n)	34.5% (30)	40.4% (36)	$\chi^2_1 = 0.67$
Caffeine <= 2 hours prior to TSST (% , n)	19.0% (16)	13.3% (12)	$\chi^2_1 = 1.05$
Milk Products (% , n)	46.5% (40)	57.4% (54)	$\chi^2_1 = 3.22^{\dagger}$
Smoking (% , n)	14.8% (12)	8.0% (7)	$\chi^2_1 = 2.00$
Medications (% , n)	37.6% (32)	29.2% (26)	$\chi^2_1 = 1.39$
Offspring Mean Salivary Cortisol Levels			
0 minutes (before TSST)	0.16(0.02)	0.14(0.13)	$F(1, 179) = 1.63$
15 minutes (after TSST)	0.19(0.28)	0.13(0.09)	$F(1, 179) = 4.71^*$
20 minutes (5 minutes after TSST)	0.20 (0.27)	0.13(0.10)	$F(1, 179) = 5.70^*$
25 minutes (10 minutes after TSST)	0.21 (0.30)	0.16(0.26)	$F(1, 179) = 1.24$
35 minutes (20 minutes after TSST)	0.18(0.27)	0.12(0.10)	$F(1, 179) = 3.61^{\dagger}$
Offspring Total Cortisol Output			
AUC	-0.20 (0.30)	-0.30 (0.25)	$F(1, 179) = 6.15^*$

Note. FET=Fisher's Exact Test

^I Adolescents = offspring between ages of 10–18; Adults = offspring between ages of 19–29.

[†] p < 0.10;

^{*} p < 0.05;

^{**} p < 0.0

Table 3

Offspring, caregiver, and proband psychiatric status predicting offspring cortisol response

Before Proband Death				
		SE	z	p
Offspring Psychiatric History (n = 181)				
Depression	0.15	0.06	2.38	0.02
Anxiety	0.02	0.08	0.31	0.76
PTSD	0.42	0.27	1.57	0.12
Alcohol/ Substance Disorder	−0.09	0.18	−0.50	0.62
ADHD	0.23	0.09	2.59	0.01
Caregiver Psychiatric History (n = 106)				
Any Axis I Disorder	0.00	0.05	0.01	0.99
Proband Psychiatric History (n = 113)				
Any Axis I Disorder	0.01	0.05	0.21	0.84
0–33 months After Proband Death				
		SE	z	p
Offspring Psychiatric Status (n = 180)				
Depression	−0.01	0.05	−0.21	0.83
Anxiety	−0.05	0.05	−0.86	0.39
PTSD	0.10	0.08	1.19	0.23
Alcohol/ Substance Disorder	0.04	0.09	0.51	0.61
ADHD	0.02	0.05	0.05	0.62
Caregiver Psychiatric Status (n = 107)				
Depression	0.10	0.05	2.11	0.04
Anxiety	0.02	0.06	0.27	0.79
PTSD	0.01	0.07	0.16	0.87
Alcohol/ Substance Disorder	−0.04	0.10	−0.41	0.68
34–62 months after Proband Death				
		SE	z	p
Offspring Psychiatric Status (n = 178)				
Depression	0.12	0.05	2.27	0.02
Anxiety	−0.08	0.08	−1.06	0.29
PTSD	0.09	0.27	0.35	0.73
Alcohol/ Substance Disorder	0.10	0.06	1.52	0.13
ADHD	0.17	0.08	2.06	0.04
Caregiver Psychiatric Status (n = 107)				
Depression	0.13	0.07	1.95	0.05
Anxiety	−0.09	0.10	−0.85	0.39
PTSD	0.27	0.15	1.80	0.07
Alcohol/ Substance Disorder	0.09	0.11	0.86	0.39

All results unadjusted for demographic/ health covariates; PTSD = Post Traumatic Stress Disorder; ADHD = Attention Deficit Hyperactivity Disorder

Table 4

Bereavement status predicting offspring cortisol response during TSST

	Model 1 (n=181)				Model 2 (n= 178)			
		SE	Z	p		SE	Z	p
Time (minutes)	-.03	.02	0.93	.35	.02	.02	.92	.36
Time ² (minutes)	-.00	.00	-1.62	.11	-.00	.00	-1.56	.12
Bereaved	.02	.05	.39	.70	-.00	.05	-.09	.93
Bereaved × Time	.06	.02	2.39	.02	.06	.03	2.28	.02
Bereaved × Time ²	-.01	.00	-2.40	.02	-.01	.00	-2.30	.02
Age at time of TSST (years)	.02	.01	4.38	<.001	.02	.01	4.27	<.001
Sex	-.08	.03	-2.19	.03	-.09	.04	-2.48	.01
Minority Status	-.13	.06	-2.29	.02	-.13	.06	-2.04	.04
Offspring Depression (34-62 month after proband death)					.11	.05	1.97	.05

* Dummy variable for bereavement status: Bereaved = Bereaved offspring vs. Nonbereaved offspring

SE: standard error

Sex (0 = males, 1 = females); Minority Status (0 = no, 1 = yes)

Table 5

Bereaved offspring cortisol response during social stress task

	Model 1 (n=90)			Model 2 (n=81)		
	SE	Z	p	SE	Z	p
Time (minutes)	.02	.01	-.226	.02	-.03	-.266
ACC*	.01	.08	.09	.04	.07	.58
NAT*	-.03	.07	-.55	-.04	.06	-.57
ACC × Time	.00	.02	.29	.01	.02	.76
NAT × Time	.03	.01	2.34	.04	.01	2.82
Age at time of TSST (years)	.02	.01	3.66	.12	.06	2.18
Sex	-.15	.05	-2.80	.01	-.15	-.288
Minority Status	-.22	.08	-2.72	.01	-.21	-.268
Offspring Alcohol/ Substance (34–62 months after death)	--	--	--	.16	.08	2.06
Caregiver Depression (34–62 month after death)	--	--	--	.21	.07	2.99
						<.001

Note Inclusion of the quadratic time variable (time²) and its interaction terms with the two dummy coded variables, did not significantly improve the fit of the model (log-likelihood $\chi^2_1 = 1.52$, $p = 0.46$), and were subsequently removed from final mixed regression models

* Dummy variables for proband death type: ACC= Accidental vs. Suicide; NAT= Natural vs. Suicide,

SE: standard error

Sex (0 = males, 1= females); Minority Status (0 = no, 1= yes)