

The Association Between Bereavement and Biomarkers of Inflammation

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Bereavement is a major life event that has been associated with a range of negative health outcomes. To assess whether levels of inflammation markers and cortisol vary significantly by bereavement status and/or number of recent bereavements. The study was based on a secondary analysis of data from the Midlife in the United States (MIDUS) II biomarkers project. After excluding participants suffering from conditions which directly affect immune functions, 529 participants were included (age 34–84 years), of whom 260 experienced the death of a person close to them 5–63 months prior to assessment. Levels of interleukin 6 (IL-6), C-reactive protein (CRP), soluble intercellular adhesion molecule-1 (sICAM-I), Soluble E-selectin (sE-selectin) and cortisol were examined controlling for demographic and health characteristics. Bereaved respondents had higher levels of inflammatory biomarkers IL-6 and sE-selectin, but not CRP and sICAM-I than the non-bereaved. Number of recent bereavements significantly predicted levels of IL-6 in the unadjusted and adjusted regression models. Body Mass Index (BMI) and number of chronic conditions partially mediated the association between number of bereavements and IL-6 levels. Number of recent bereavements is associated with higher levels of inflammation, particularly among individuals with higher BMI and/or chronic health problems.

Keywords: bereavement, biomarkers, inflammation, C-reactive protein, interleukin 6

Those who are grieving may experience a number of psychological problems in reaction to the death of a significant person.¹ Research suggests that psychological grief reactions are often most pronounced in early bereavement, although for some, the suffering becomes much more intense and prolonged.² There are a number of factors that affect the nature and intensity of the grieving process, including relationship (eg parent, spouse), nature of the death,² and previous psychiatric history of the bereaved.³ In bereavement, individuals often experience a range of distressing emotions (anguish, loss, sadness, sorrow, yearning, regrets, loneliness) which are part of normal grief⁴ and sometimes depression, anxiety, and post-traumatic stress disorder (PTSD) symptoms, or a combination of these

disorders.² Several studies have shown a relationship between grief and depressive symptoms.^{5,6}

In addition to the mental health consequences of grief, there is a substantial literature indicating potential negative physical health outcomes. Bereaved adults are more vulnerable to high blood pressure,⁷ exacerbation of type II diabetes⁸ and arthritis symptoms⁸ than their non-bereaved peers. Furthermore, bereavement is associated with elevated rates of cancer, heart disease,⁷ myocardial infarction,² strokes⁹ and higher mortality.¹⁰

The exact pathway through which bereavement impacts health has not been fully clarified. In Figure 1, we propose a conceptual model of potential pathways through which psychological grief could impact chronic inflammation. An important, but understudied, potential pathway between bereavement and negative health outcomes may be due to the emotional distress, depression and reduced well being experienced during normal and complicated grief. Distress in reaction to chronic stressors, depression and reduced

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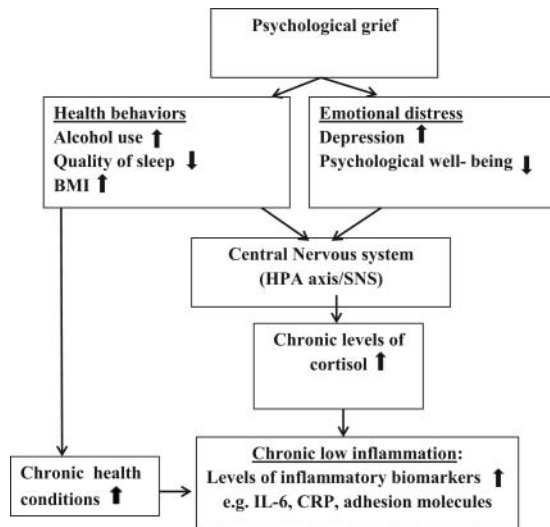


FIGURE 1 A conceptual model for the possible pathways that may explain the cascade of associations between psychological grief and chronic inflammatory state. Abbreviations: HPA, hypothalamic-pituitary-adrenal; SNS, sympathetic nervous system.

well-being activate the hypothalamic-pituitary-adrenal (HPA) axis or the sympathetic nervous system.¹¹ This activation results in increased secretion of catecholamines and cortisol^{11,12} which may cause dysregulation of the immune system, including a decrease in cytotoxic activity, especially decreased natural killer cytotoxic activity and T-cell proliferation in reaction to mitogenic *in vitro* stimulation^{12,13} and increased inflammation.^{12,14} Interleukin-6 is a major pro-inflammatory cytokine secreted during infection, trauma, and psychological stress.^{14,15} It stimulates hepatocyte production of C-reactive protein (CRP) during inflammation. Adhesion molecules, intercellular adhesion molecule-1 (ICAM-1), and E-selectin, which are expressed on the surface of endothelial cells and play an important role in recruiting leukocytes, are also up-regulated during inflammation.¹⁶

Distress in reaction to stressors and depression had been linked with increased secretion of cortisol and catecholamines, increased and sustained secretion of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF- α ,^{13,17–20} and higher inflammatory markers, such as CRP.^{17,18,20,21} Two recent meta-analyses showed a dose–response associations between depression and inflammatory markers such as CRP, IL-1, and IL-6 (22,23). However, several studies have reported a lack of association, or even an inverse association between depression and IL-6.^{24–26} Increased levels of pro-inflammatory cytokines (especially IL-6) and CRP and adhesion molecules are known risk factors for the development and progression of certain aging-related diseases including type 2 diabetes, cardiovascular diseases, some cancers, osteoporosis, and rheumatoid arthritis.^{12,14} Also, inflammation markers such as CRP, ICAM-1, and E-selectin are associated with the development or acceleration

of various diseases, such as diabetes and cardiovascular diseases.¹⁶

There are substantial inconsistencies in the findings of studies examining immune functions in relation to bereavement. New widows have been found to have reduced cytotoxic activity, especially Natural Killer Cell Activity (NKCA) and mitogen induced T-cell proliferation²⁷ in comparison to controls.²⁸ However, other studies found no difference in NKCA²⁹ or T-cell proliferation^{5,30} and one study even found elevated rates of T-cell proliferation.²⁹ Irwin and colleagues found that plasma cortisol levels were significantly elevated among new widows as compared to controls,²⁸ while other studies found cortisol levels in recently widowed women were similar to matched controls.²⁷ These ground breaking studies had very limited power to detect statistical significance due to their very small samples of bereaved respondents ranging from 9^{28,30} to 26 respondents.²⁷

In a more recent study of 75 elderly women, a lifetime history of sudden loss was positively associated with serum IL-6 levels and negatively associated with insulin-like growth factor-1 levels. The number of unexpected losses was linearly related to levels of IL-6 and insulin-like growth factor-1.³¹ Another pathway between bereavement and immune dysregulation may be via changes in health behaviors. There is a large body of evidence suggesting that grief can negatively impact health behaviors including smoking,³² alcohol abuse^{2,9,32} and prescription drug abuse. Furthermore, bereavement is associated with changes in activity level and nutrition which often results in increase in weight and high body mass index (BMI).^{2,32,33} Negative health behaviors and especially increased BMI may be directly related to impaired health^{34,35} or indirectly related through heightened inflammation.³³

Empirical information is lacking: It is unclear if there is an association between bereavement and inflammatory markers and if so, which factors, if any, link bereavement with inflammatory markers. The very few existing studies have yielded contrasting results, possibly due to differences in age of respondents and other demographic variables and in the immune parameters studied. The small samples in many of the studies limited the power to detect an association, especially in light of the high variability in the normal range of immune and cortisol parameters. Most studies had too few participants to permit controlling for many confounding health (eg, chronic diseases, medications, health behaviors, BMI) and demographic variables (eg, socioeconomic status, age, education) and depression, all variables which may affect hormonal and immune functions. Thus, larger scale studies are needed in order to control for confounding variables and to better understand the effect of interpersonal loss on immune functions.

The aim of the present study was to assess, using the large sample of the MIDUS II biomarker project, the association between bereavement and levels of inflammation

markers and cortisol levels. In addition we examined whether there was a dose-response relationship between the number of deaths experienced and inflammatory biomarkers. In response to the literature suggesting that bereavement may be associated with increased BMI, we investigated whether cortisol, number of chronic conditions and BMI mediated the relationship between the number of bereavements and inflammation. Therefore our hypotheses were:

- (a) Hypothesis 1: Higher levels of cortisol and of the inflammatory markers will be found among those who have experienced the death of a person close to them at some point within the preceding five years in comparison to those who had not experienced a bereavement in this period.
- (b) Hypothesis 2: A positive association will be found between the number of bereavements experienced in the preceding five years and the level of inflammatory biomarkers; the greater the number of deaths, the higher the level of inflammatory biomarkers.
- (c) Hypothesis 3: Levels of cortisol, BMI and number of chronic conditions will mediate the associations between the number of bereavements and inflammation.

In order to minimize confounding, we excluded participants taking particular medications and those suffering from conditions known to have a direct effect of hormonal and immune parameters. In addition, we statistically controlled for depression, quality of sleep, smoking, alcohol use and demographic characteristics.

METHODS

Participants

This study was based on a secondary analysis of data from the Midlife in the United States (MIDUS) II biomarkers project. In 1995 and 1996, the original MIDUS I survey collected data on a nationally representative sample of Americans aged 25 to 74, obtained through random digit dialing,³⁶ a sample of siblings of many respondents, and national sample of twins. The 1,255 participants in the MIDUS II biomarkers study were comprised of a subsample drawn from the longitudinal follow-up of the MIDUS I study (666 drawn from the random digit dialing respondents and 388 from the twins study). An additional 201 respondents were drawn from a supplementary survey of new participants of African Americans from Milwaukee, Wisconsin. Data on demographic and psychosocial variables was obtained through phone interviews and self-administrated questionnaires (called Project 1, MIDUS II). Biomarker data was gathered during 2004–2009, through a

two day clinic visit. (Project 4, MIDUS II). The participation rate in the biomarkers study was 39.3%.³⁷ Details on study protocol and content are available elsewhere.³⁷

The present analysis was based on a subsample of the 1255 participants in the Biomarkers study. As shown in Figure 2, the sibling sample was not included in the present analysis (all 6 participants from the sibling group were not included due to having specific conditions and/or medications that were part of our exclusion criteria) and only the first member of each twin pair was included because genetic relatedness might influence the results.

In consultation with a clinical immunologist MD, we developed the following exclusion criteria to remove the majority of participants who had chronic conditions or/and regularly took medications that have a direct effect on the immune system: Participants were excluded if they regularly took medications that could affect the immune or the endocrine system (ie, antihistamines [targeting various allergies], steroid and non-steroid anti-inflammatory [targeting inflammatory diseases such as arthritis or asthma], immune suppressive medications, hormone replacements, psychiatric medications, oral contraceptive medications or insulin for diabetes, adrenergic blockers, calcium channel blocking, antibiotics or antiviral, chemotherapy, and cardiac drugs). Patients with the following conditions that could affect neuro-immune parameters were also excluded: fibromyalgia, lupus, Parkinson's, HIV, cirrhosis, thyroid dysfunction, and insulin-dependent diabetes. The final sample consisted of 529 participants (Figure 2). The number of individuals in the final sample who reported each of the main chronic conditions and the main prescribed medications are presented in the Appendix.

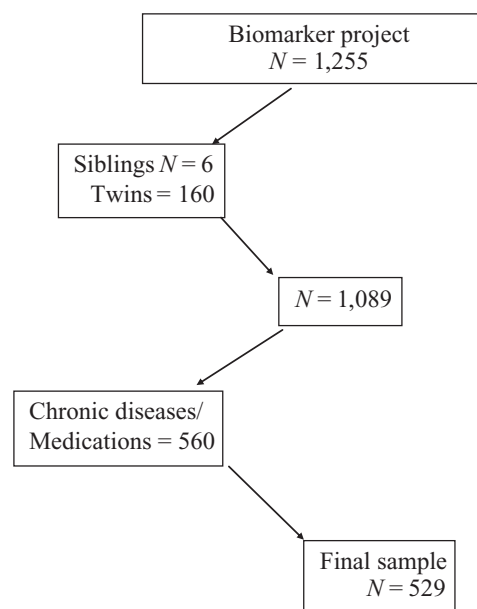


FIGURE 2 Diagram of sample exclusions and inclusion criteria.

Of these 529 respondents in the biomarkers study (Project 4, MIDUS II) who met our inclusion criteria, 260 reported (49.1%) that they had experienced the death of a person close to them since the baseline interview (Project 1) of the MIDUS II study. The average time since the bereavement was 1.04 years (SD = 0.99 years) with a range from 5 to 63 months. Of the 260 participants who had experienced death of a person, one death event was reported by 171 (32.3%) participants, two death events were reported by 47 participants (8.9%), 3 death events were reported by 18 participants (3.4%), 4 and 5 death events were reported by 15 (2.8%) and 9 (1.7%) participants, respectively.

The study was approved by the Institutional Review Board at each participating MIDUS center, and informed written consent was obtained for all participants.³⁷

Measures

Demographic Details

Demographic details included age, sex, level of education, and combined income of respondents and spouses/partners. The last two variables were retrieved from MIDUS II Project 1 data. Unless otherwise stated, all data were gathered from MIDUS II (Project 4, the Biomarkers study).

Bereavement

Bereavement was measured by two questions. During the MIDUS II (Project 4, Biomarkers) wave of data collection (conducted between 2004 and 2009), individuals were asked whether anyone close to the participant had died since their MIDUS II (Project 1) interview was completed. If the participant reported that they had experienced a death since the MIDUS II (Project 1) interview, the number of persons close to the participant who had died since the last interview was recorded.

Health Status: Number of Chronic Conditions

Health status was determined by respondents' number of chronic conditions. This measure consisted of a list of 23 medical conditions. Respondents were asked to indicate (yes/no) whether they were ever diagnosed with each of the following conditions: glaucoma, heart diseases, cancer, arthritis, liver diseases, high blood pressure, cholesterol problems, or asthma. As described above, the study excluded participants with illnesses or those taking medications that could directly affect the immune system.

Depression

Depression was assessed using the Center for Epidemiological Studies Depression (CES-D) 20-item scale.³⁸ Response items are given on a scale from 1 = rarely or none of the time to 4 = most or all the time. It is a widely

used scale for measuring depression^{39,42} with good psychometric properties.⁴⁰ All items were recoded to a 0–3 scale. Four items were reversed so that high scores reflected higher depression levels. Scores were computed by summing across all items for which there was no missing data. If respondents had missing data on only one of these four items, mean substitution was used. Respondents with two or more questions with missing data were excluded from the analysis. A cut-off score of 16 was used to define severe depression. Internal consistency of the scale in the present analysis was 0.88.

Subjective Well-being

Subjective well-being was measured using the five-item Satisfaction With Life Scale.⁴³ Participants were asked to rate their evaluation of life in general from 1 = strongly disagree to 7 = strongly agree (eg, "In most ways my life is close to my ideal," "The conditions of my life are excellent"). A mean score was calculated. Internal consistency of the scale in the present analysis was 0.89.

Quality of Sleep

Quality of sleep was assessed using the Pittsburgh Sleep Quality (PSQ) index.⁴⁴ It consists of 19 items measuring 7 components of sleep: Subjective Sleep Quality, Sleep Latency, Sleep Duration, Habitual Sleep Efficiency, Sleep Disturbance, Use of Sleeping Medications, and Daytime Dysfunction. A Global Sleep score was constructed by summing the 7 sleep components for each case with complete data. Global Sleep scores were not computed for cases with a Habitual Sleep Efficiency greater than 100%. Internal consistency of the scale in the present analysis was 0.65.

Body Mass Index

Body mass index (BMI) was calculated using weight (kg) and height (m) measures gathered by the General Clinical Research Centers (GCRC) staff as part of the physical exam. The BMI formula is weight in kilograms divided by height in meters squared. The values were log-transformed to normalize the distribution.

Three Health Behaviors

Three health behaviors were used in the present analysis: (1) the average number of days per week the respondent had consumed alcohol during the past month; (2) whether currently smoking (0, no; 1, yes), and; (3) whether the respondent was exercising regularly, defined as at least 20 min 3 × week, as reported by the participants (0, no; 1, yes).

Inflammation Measures

Interleukin-6 (IL6)

Interleukin-6 (IL6) levels in serum was measured using the Quantikine® High-sensitivity ELISA kit (R & D Systems, Minneapolis, MN). The laboratory inter-assay variability was 13% and the intra-assay coefficient of variance was 4.09%. Reference range was 0.45–9.96 pg/mL.⁴⁵

C-Reactive Protein (CRP)

C-reactive protein (CRP) levels were measured using the BNII nephelometer from Dade Behring utilizing a particle enhanced immunonephelometric assay. Polystyrene particles are coated with monoclonal 239 antibodies to CRP, which in the presence of CRP results in an anti-240 gen agglutinate and increased light intensity that can be measured 241 on a BNII nephelometer (Siemens Healthcare Diagnostics, Deerfield, IL). The intra-assay coefficient of variance was 5.0%. Reference range was > 3 ug/mL.⁴⁵

Soluble Intercellular Adhesion Molecule-1 (sICAM-1)

Soluble intercellular adhesion molecule-1 (sICAM-1) levels were measured by an ELISA assay (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN). The inter-assay coefficient of variance was 2.1%–5.7% and the intra-assay coefficient of variance was 2.3%–4.4%. Reference range was 237–415 ng/mL.⁴⁵

Soluble E-selectin (sE-selectin)

Soluble E-selectin (sE-selectin) levels were measured by an ELISA assay (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN). The inter-assay coefficient of variance was 2.1%–5.7% and the intra-assay coefficient of variance was 2.3%–4.4%. Reference range was 29.1–63.4 ng/mL

Cortisol Levels in Urine

Cortisol levels in urine were measured with enzymatic colorimetric assay and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Deuterated cortisol [d (3)-cortisol] was added to a 0.1-mL urine specimen as an internal standard. Cortisol was extracted from the specimens using on-line turbulent flow HPLC and analyzed by liquid chromatography–tandem mass spectrometry using multiple reaction monitoring in positive mode. The inter-assay coefficient of variance were 5.7%–8.8% and intra assay coefficients of variance were 4.7%–5.0%. Reference range was 29.1–63.4 ng/ml (45).

Data Collection Procedure

Biomarkers were collected for participants of MIDUS II who had completed the telephone and mail surveys. The

biological data was collected during a two-day visit to General Clinical Research Centers. During the visit participants provided a complete medical history and medication information, underwent a physical exam and provided urine. Fasting blood samples were drawn on the morning of the second day, prior to any caffeine or nicotine consumption for the day. For more details please see Dienberg et al.³⁷

Statistical Analysis

Data was analyzed using SPSS 17 software. Means (SD) or frequencies were obtained for demographic, health behaviors, health and depression measures. For CES-D, means (SD) and Cronbach's alpha were calculated for the sample. Due to the non-normal distribution of biomarkers and BMI, these were log transformed in order to obtain a normal distribution. Differences in demographic and study variables between the bereaved and non-bereaved respondents were assessed with independent t-test for continuous variables and chi-square tests for categorical variables. A hierarchical regression analysis of each biomarker was conducted to assess the independent contribution of the number of bereavements experienced, controlling for demographic, depression, health and health behavior variables. Multicollinearity was assessed and ruled out. The income variable has not been included in the regression analysis due to a large number of missing answers. More than 90% of the sample were White, therefore, ethnicity was not been included as a variable in the analysis. Furthermore, there were some missing values for this variable. Finally, in cases in which significant associations existed between the independent, mediating and dependent variables, mediation tests were conducted using the Baron and Kenny guidelines⁴⁶ and the Sobel test⁴⁷ to assess the possible mediating role of BMI and the number of chronic conditions in the relationship between the number of deaths experienced and the biomarkers. The level of significance was set at $p < .05$.

RESULTS

Bivariate Comparison of the Bereaved and Non-bereaved

The demographic characteristics of the bereaved and non-bereaved respondents were similar for both groups (see Table 1). The mean age was approximately 54, and 70% had at least a college education. Over 90% of the respondents were White. Of the 37 non-White respondents, 16 were African Americans. Both groups had a mean household income above \$70,000 per annum.

Table 2 reports the means of biomarkers levels, health and health behaviors measures, depression and well-being in the bereaved and non-bereaved groups. The levels of IL-6 and of sE-selectin in serum were significantly higher for

TABLE 1
Demographic Characteristics of Participants, by Bereavement Status ($N = 529$)

	Bereaved Respondents ($N = 260$)		Non-Bereaved Respondents ($N = 269$)		t -test/ χ^2
Sex (N,%)					
Male	135	51.9	130	48.3	.41
Female	125	48.1	139	51.7	
Age, y (M, SD)	54.27	11.72	53.23	11.05	
Range	34–84		34–82		
Education					.80
Graduated high school or less	77	29.6	77	28.6	
Some college or more	183	70.4	192	71.4	
Ethnicity ¹					.63
White	231	92.0	244	93.5	
Others	20	8.0	17	6.5	
Married or living with a partner (N,%)	193	74.2	195	72.5	.65
Mean combined income per month ² (M, SD)	77,521.21	62,454.80	72,979.71	60,107.82	.73

¹17 responses missing.

²Spouse and partner's combined mean income (reported at MIDUS II project 1 study).

participants who had been bereaved. The difference remained significant after controlling for age, using ANCOVA, $F(1, 523) = 4.62, p < .05$ for IL-6 and $F(1, 523) = 4.83, p < .05$ for sE-selectin. Levels of CRP, sICAM-1 and of urine cortisol were not significantly different between the two groups. Levels of depression were low and similar for both groups. Using the cut-off score of 16 indicative of severe depression, 28 (10.8%) of the bereaved respondents and 24 (8.9%) of the non-bereaved respondents were classified as having clinically relevant levels of depression symptoms. This difference was not statistically significant, $\chi^2(1) = .48, p > .05$. Levels of subjective well-being and quality of sleep were also high and similar for both groups.

However, the bereaved group reported more chronic conditions and had a higher mean BMI. These two differences remained significant when controlling for age in ANCOVA analyses, $F(1, 526) = 4.32, p < .05$; and $F(1, 526) = 5.48, p < .05$, respectively. Bereaved and non-bereaved respondent did not differ significantly on alcohol consumption or prevalence of current smokers or regular exercisers.

Unadjusted and adjusted regression analyses were conducted to further assess the relationship between number of bereavements experienced and each of the biomarkers. Potential confounders such as demographic characteristics, health status and health behaviors were controlled (see Table 3).

TABLE 2
Means (SDs) and Differences in Level of Depression, Log-Transformed Inflammatory Biomarkers and Health Behaviors by Bereavement Status

Variables	Bereaved Respondents ($N = 260$)		Non-Bereaved Respondents ($N = 269$)		t test
	M	SD	M	SD	
IL-6 (pg/mL)	2.56	2.00	2.40	2.56	2.33*
CPR (ug/ml)	2.12	2.85	2.44	3.99	-0.41
sICAM-1 (ng/ml)	272.17	101.83	280.62	93.12	-1.36
sE-selectin (ngmL/ml)	43.32	23.54	38.80	17.74	2.13*
Urine cortisol (ug/dL)	1.27	1.44	1.22	1.26	0.47
Depression (CES-D)	7.75	7.49	7.13	6.82	1.02
Subjective well-being	4.88	1.27	4.95	1.25	0.63
Quality of sleep	5.65	3.37	5.27	3.07	1.33
No. of chronic conditions	7.56	3.28	6.66	3.34	3.10**
BMI	28.96	5.98	27.91	5.17	2.05*
Alcoholic drinks	1.62	1.61	1.53	1.46	0.42
	<i>N</i>	%	<i>N</i>	%	
Currently smoking (N,%)	27	10.4	37	13.8	.23
Regular exercising (N,%)	209	80.4	221	82.2	.60

Notes. Presented are the means (SDs) of the biomarkers levels. The t tests and p values were based on the log-transformed values. Abbreviations: IL-6, interleukin-6; sIL-6r, soluble IL-r receptor; CPR, C-reactive protein; sI-CAM, soluble intercellular adhesion molecule-1; BMI, body mass index; Alcoholic drinks, days per week alcohol was consumed.

* $p < .05$, ** $p < .01$.

TABLE 3
Hierarchical Regression of the Number of Bereavements on Log-Transformed Inflammatory Biomarkers (N = 484)

	IL-6	CRP	sICAM-1	sE-selectin	Urine cortisol
Model 1 (unadjusted)					
No. of close family/friends who have died	.14**	.08	-.06	.12**	.02
R ²	.02 (.02)	.01 (.01)	.00 (.00)	.01 (.01)	.00 (.00)
F(1, 493)	9.39**	2.92	1.80	6.93**	0.14
Model 2 (Adjusted)					
No. of close family/friends who have died	.08*	.02	-.08	.08 ⁺	.05
Age	.20***	.02	.17***	-.11*	.01
Sex	.07	.11**	-.07	-.18**	-.12*
Education	.03	-.04	.07	.03	.07
BMI	.32***	.40***	.07	.20***	.00
# chronic conditions	.10*	.10*	.07	.16**	-.13**
Currently smoke	.11*	.03	.19***	.07	.11*
Regular exercising	-.11**	-.08*	.01	-.03	.01
Alcoholic drinks	-.05	-.06	-.06	-.02	-.10*
Depression	-.02	.06	.00	-.02	.05
Subjective well-being	-.04	.07	-.02	-.02	-.09
Quality of sleep	-.04	.00	.04	.03	-.15
Urine cortisol	-.06	.05	.04	.05	—
R ² (adjusted R ²)	.22 (.20)	.22 (.20)	.10 (.08)	.12 (.10)	.09 (.07)
F(13, 481)	10.65***	10.59***	4.25***	4.99***	4.10***

Notes. Sex: 0, male; 1, female. Currently smoke: 0, no; 1, yes. Regular exercising: 0, no; 1, yes. Depression: 0, ≥ 16 ; 1, < 16 .
⁺*p* = .054, **p* < .05, ***p* < .01, ****p* < .001.

In the unadjusted model, levels of IL-6 and of sE-selectin were positively and significantly associated with number of bereavements experienced by the respondent in the preceding five years. The association between number of bereavements and levels of CRP, sICAM-1, or cortisol were not significant.

The adjusted models, which included demographic, health, and health behaviors in addition to the bereavement variable, were significant for the biomarkers. The models explained 27% of levels of IL-6, 22% of CRP, and between 9%–12% of sICAM-1, sE-selectin and cortisol levels. After adjustment for these potentially confounding variables, the number of bereavements significantly predicted levels of IL-6 (*p* < .01) and also was in a borderline significant association with sE-selectin (*p* = .054).

Levels of IL-6, sICAM-1 and sE-selectin were higher with age. CRP was significantly higher among women, while sE-selectin and cortisol were significantly higher among men. The association of these biomarkers with education was not significant. Significant and strong associations were found for BMI and number of chronic conditions with IL-6, CRP, and sE-selectin. Also, number of chronic conditions was negatively associated with levels of cortisol. Current smoking was significantly associated with higher levels of IL-6, sICAM-1, and cortisol. Regular exercise was negatively associated with levels of IL-6 and CRP. while frequency of consuming alcoholic drinks was negatively associated with levels of cortisol. Depression, subjective well-being and

quality of sleep were not significantly associated with any of the dependent variables.

Finally, BMI and chronic conditions were associated with number of bereavements between the two waves of data collection, $\beta = .10, p < .05$ and $\beta = .12, p < .01$, respectively) and with IL-6 ($\beta = .34$ and $\beta = .22, p < .001$, respectively). Therefore, their mediating role was assessed. The association between the number of bereavements and IL-6 was $\beta = .14 (p < .01)$ and it changed to $\beta = .09 (p < .05)$ once BMI was entered into the regression. Thus, the Sobel test for mediation was applied. It indicated that BMI played a partial mediating role in the relationship ($Z = 2.25, p < .05$), but caution is needed in interpretation of mediation due to the cross-sectional nature of data collection. A similar procedure was used to assess the mediating role of number of chronic conditions. The initial association between number of bereavements and IL-6 changed from $\beta = .13, (p < .01)$ to $\beta = .10 (p < .05)$ after number of chronic conditions was added to the equation. The Sobel test for mediation showed that number of chronic conditions may have partially mediated the association between number of bereavements experienced and IL-6 levels ($Z = 2.50, p < .05$). The association between number of bereavements and sE-selectin was changed from $\beta = .12 (p < .01)$ to $.10 (p < .05)$ when BMI was entered to the regression. The Sobel test showed that BMI partially may have mediated the association ($Z = 2.16, p < .05$). The number of chronic conditions did not mediate the relationship between number of bereavements and levels of sE-selectin.

DISCUSSION

The present study shows that bereaved persons had higher levels of the inflammatory biomarkers IL-6 and sE-selectin, but not CRP and sICAM-I. The number of bereavements significantly predicted the level of IL-6 in the unadjusted and adjusted models. BMI and number of chronic conditions partially mediated the association between number of bereavements and IL-6 levels. These relationships were maintained even after adjusting for a large number of potential demographic, personal and health confounders.

The present results for IL-6 are in line with previous studies demonstrating higher levels of inflammatory markers in individuals undergoing stressors.^{14,48,49} Moreover, the findings of the present study indicated a dose-response relationship; the more individuals close to participant who had died during the study period, the higher were the levels of IL-6. There have been a few studies examining inflammation among the bereaved. One other study also showed higher levels of IL-6 among older women experiencing a sudden loss.³¹ Previous studies have been inconsistent with respect to the effect of bereavement on cytotoxic immune functions, such as t-cell proliferation and NKCA.^{27-29,50} It is possible that chronic stressors have an accumulative effect on individuals' biomarkers. Although some scholars propose that the experience of recurring negative events may improve coping strategies and therefore create resilience, multiple bereavements may drain personal resources for effective coping.⁵¹ Moreover, the accumulating effect of chronic stressors on inflammatory biomarkers may contribute to the development of chronic diseases and the well-documented higher morbidity and mortality related to bereavement.^{2,8} Future research is needed to determine if chronic inflammation plays an important pathway between bereavement and the long-documented elevated mortality, particularly cardiac mortality, among widowers.^{52,53}

In contrast to the significant associations found for IL-6, levels of CRP were not significantly related to bereavement status in our current study. These findings are in keeping with a recent meta-analysis of the effects of other stress conditions, such as acute psychological stress on levels of circulating inflammatory markers. The meta-analysis concluded that psychological stress had a robust effect with respect to increased IL-6 but only a marginal effect for CRP (54).

Only a few studies have examined adhesion molecules, which serve as biomarkers of level and severity of inflammation. In line with the present results, elevated levels of sE-selectin were found in a previous study to be associated with the chronic stress of discrimination.⁵⁵ The paucity of studies on adhesion molecules in relation to stress makes it difficult to draw conclusions. However, due to the role of adhesion molecules in atherosclerosis,¹⁶ further studies on psychological stressors, such as bereavement are important.

In this study, neither depression nor well-being levels differed between the bereaved and non-bereaved participants. Neither of these factors was related to the measured biomarkers. This finding may be due to the subsample we used. We deliberately excluded individuals receiving antidepressants or other medication for psychiatric conditions, thereby removing many of the depressed respondents from our analysis. This exclusion criterion may also explain our finding that levels of cortisol were not different between the bereaved and non-bereaved groups and did not predict levels of inflammatory biomarkers. Anisman's research suggests levels of cortisol were found to be particularly related to depression.¹¹ In our sample, the effect of bereavement on IL-6 may be mediated through pathways other than depression, such as through catecholamines.⁵⁶

Another major pathway through which bereavement may affect inflammatory markers is through health behaviors. Lack of physical exercise, smoking, alcohol use, unhealthy nutrition and lack of sleep are established risk-factors for elevated inflammation markers.^{34,35} Bereaved individuals are vulnerable to many of these problematic health behaviors,^{2,9,32} thus they may serve as mediators of inflammation among the bereaved. Increased BMI due to unhealthy nutrition and inadequate physical exercise, is associated with increased inflammation. In the present study, smoking, exercise and sleep did not differ by bereavement status, however smoking and exercise were significant predictors of most of the measured inflammatory markers.

Unhealthy nutrition and physical inactivity often result in an increase in BMI and worsening of chronic conditions. In line with the existing knowledge that BMI and chronic diseases are characterized by high inflammatory biomarkers,⁵⁷ in the present study, BMI and number of chronic diseases predicted higher levels of the inflammatory biomarkers (except sICAM-I). Also, they partially mediated the relationship between number of bereavements and levels of IL-6. It may be that the negative health behaviors among the bereaved, particularly those with multiple bereavements, are directly responsible for their poorer health. An alternative explanation is that those participants who already had existing chronic conditions and high BMI were more vulnerable to inflammation in response to bereavement. It is important to note that in order to minimize confounding, the study excluded individuals with conditions known to have a strong and direct effect on hormonal and immune parameters. Thus, the chronic conditions examined as mediators were restricted to relatively common conditions which are not thought to be as strongly associated to pro-inflammatory cytokines (eg, high blood pressure and high cholesterol).

However, as almost all chronic conditions may affect immune functions and inflammatory biomarkers and as most participants (88%) had at least one chronic disease, it was impossible to exclude all chronic diseases. In order to

be precise in the exclusion process, we did not rely solely on participants' reports of chronic conditions, but had an expert physician carefully inspect each of the medications participants reported to use regularly. Although this exclusion is not optimal, it is important to note that most of the studies that had analyzed the MIDUS data regarding the associations between inflammatory biomarkers and psychosocial factors, included participants with any chronic conditions in the study and controlled for the chronic conditions and types of medications.^{58,59} The exclusion criteria used in our study is more rigorous than simply controlling for potential confounding conditions and medication. It is possible, however, that confounding remains and therefore caution should be used in interpretation of the findings.

In contrast to previous reports from MIDUS study data,^{55,60,61} income and education were not related in the present study to inflammatory markers. This difference may emerge from the fact we excluded respondents with certain medical conditions and medications that may affect the inflammation process.

It is important to note that several of the statistically significant differences found in levels of pro-inflammatory biomarkers between the bereaved and non-bereaved respondents were of relatively small magnitude. Unfortunately, there is no clear consensus on what difference in magnitude in levels of cytokines and in other variables is clinically relevant. It is possible that these factors only reached statistical significance due to the large sample size of the current study. However, a study using MIDUS Biomarker data⁶¹ and other studies analyzing much smaller samples^{13,17} also found small absolute differences in levels of cytokines or other inflammatory markers that were statistically significant. It is important to mention that the analyses conducted in this study were log transformed and controlled for main confounders, steps that increase the soundness of the results. Although several studies link high levels of pro-inflammatory biomarkers to various health problems such as inflammatory diseases, slower wound healing, lower efficiency of vaccines (see Kiecolt-Glaser et al for a review¹²), the question of what magnitude of elevated levels of pro-inflammatory cytokines, is a risk factor for health problems should be addressed in future research.⁶²

There are several limitations which must be considered. This is a cross-sectional study, therefore causality cannot be inferred and results are merely correlational. In addition, we conducted bivariate analyses of five different biomarkers, therefore, the problem of multiple comparisons cannot be entirely ruled out. Therefore results should be interpreted with caution. In addition, the bereaved group had a substantial range in the time since bereavement (ie, from 5 to 63 months). More recent bereavements may have different biological consequences than those which had a longer-lasting impact.⁶³ Although we controlled for a wide range of potential risk factors (eg, demographic

characteristics, health status, and health behaviors) which might influence inflammatory markers and cortisol, we could not control for some important potential confounds such as pre-bereavement caregiving status, comorbid post-traumatic stress disorder (PTSD) and potentially important bereavement-related characteristics. Indeed, many bereaved individuals develop post-traumatic stress disorder (PTSD),² which was previously found related to higher levels of pro-inflammatory cytokines.^{64,66}

CONCLUSIONS

Despite these limitations, this study used a very large sample to examine the relationship between number of recent bereavements and inflammatory markers and cortisol in a healthy population. This study excluded individuals receiving certain medications (eg, psychiatric medications, antihistamines, hormone replacements, oral contraceptive medications) and specific conditions (eg, fibromyalgia, lupus, Parkinson's, HIV, cirrhosis, thyroid dysfunction, and insulin dependent diabetes) that may directly affect the immune and hormonal indices. The findings suggest that, even among this relatively healthy sample, the number of recent bereavements was significantly associated in a dose-response relationship with levels of IL-6 and sE-selectin. Future research should identify important bereavement related characteristics such as participant's relationship to the deceased, cause of death and time since death. These factors may play a role in the impact of the bereavement on the respondent.

The present results may serve to improve identification of bereaved individuals at risk—particularly those experiencing multiple bereavements in a relatively short time period, those with negative health behaviors and individuals with a high BMI or chronic medical conditions. Physicians and other health professionals should screen for bereavement with particular attention to those most vulnerable among the bereaved.

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APPENDIX

Prevalence of Main Medical Conditions and Prescribed Medications Regularly Used by Participants (N = 529)

	Prevalence of medical condition ¹		Prevalence of medication use ²	
	N	%	N	%
Main chronic conditions ³ (N,%)				
Ever had heart disease	34	6.4	0	0
Ever had circulation problems	36	6.8	0	0
Ever had heart murmur	60	11.3	0	0
Ever had TIA or CVA	13	2.5	0	0
Ever had anemia/blood disease	62	11.7	0	0
Ever had asthma	50	9.5	0	0
Ever had cancer	58	10.9	0	0
Hypertension	138	20.1	136	25.7
Cholesterol problems	185	35.0	103	19.5
Non-insulin dependent diabetes	14	2.6	4	0.7
Osteoporosis/osteopenia	42	7.9	42	7.9
Thyroid disease	27	5.1	0	0
Arthritis ⁴	132	25.0	0	0

¹Only conditions reported at least by 10 participants are described.

²Only use of prescribed medications taken on a regular basis is reported in the table.

³Some discrepancy may exist between self-reports on chronic conditions and regular medication use (eg, participants may report heart disease, but those included in the sample had not received any regular medications for heart problems).

⁴Participants included in this category may have experienced a range of different conditions of joint or limb pain. Participants receiving regular drug treatment for arthritis were excluded from the study because of the potential that these drugs could affect the immune or the endocrine system.

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