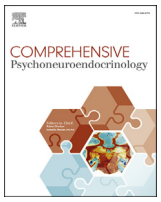
Comprehensive Psychoneuroendocrinology 7 (2021) 100056

Contents lists available at ScienceDirect

Comprehensive Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/cpnec



Socioeconomic disparities in health: Changes in sleep quality and inflammation during bereavement

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ARTICLE INFO

Keywords:
Widowhood
Inflammation
Sleep disturbance
Subjective social status
Socioeconomic status
Stressful life event

ABSTRACT

Widow(er)s experience significant sleep disruption that may dysregulate immune functioning. This longitudinal study aimed to determine 1) whether changes in sleep quality were associated with changes in pro-inflammatory cytokine production during the first six months of bereavement and 2) whether these relationships depended on objective socioeconomic status (SES) and/or subjective social status. One hundred and six bereaved spouses (M = 68.49 years, SD = 9.35, 69 females) completed the following assessments at approximately three months post-death and six-month post-death: a venous blood draw and self-report questionnaires on sleep quality (Pittsburgh Sleep Quality Index), SES (MacArthur Sociodemographic Questionnaire), health, and demographic information. T-cell stimulated pro-inflammatory cytokines were assessed, including IL-6, TNF-α, IFN-γ, IL-17A, and IL-2. Worsening sleep quality was associated with increased levels of pro-inflammatory activity even after adjusting for confounding variables. The present study also identified SES as an important factor for understanding health following spousal bereavement: individuals with low SES were more susceptible to sleep-related changes in immune function. Compared to more educated widow(er)s, less educated widow(er)s showed greater increases and decreases in inflammation when sleep quality worsened or improved, respectively, over time. Findings provide evidence for a biobehavioral pathway linking bereavement to disease risk, highlight SES disparities in late adulthood, and identify individuals who may require tailored interventions to offset SES-related burden that impedes adaptive grief recovery.

1. Introduction

Losing a spouse ranks as one of life's greatest stressors. It elicits significant psychological, physiological, and behavioral changes that can last weeks to months [1]. Bereaved spouses are at excess risk for mortality [1], with cardiac events accounting for a substantial amount of deaths during the first six months [2]. A growing body of research suggests that the psychological stress associated with bereavement promotes autonomic and immune dysregulation [3]. Altered health behaviors, such as sleep disruption, frequently accompany psychological responses to chronic stress, and they are commonly observed in recently bereaved spouses [4]. Sleep disruption may further exacerbate physiological systems in grieving individuals, given that poor sleep is associated with chronic diseases [5] and grief maladjustment [6].

Inflammatory mechanisms likely underlie mortality risk in bereaved individuals. When the body is confronted with a physical stressor (i.e., injury or infection), a rapid inflammatory response followed by down-regulation of the response is imperative for effective healing and maintaining homeostasis [7]. However, when inflammatory responses are sustained for extended periods (termed chronic inflammation), homeostatic set points shift to accommodate abnormal physiological conditions, which can be detrimental to the integrity of tissues and organs [7]. Thus, chronic low-grade inflammation is a key predictor for the onset and progression of many age-related illnesses [8]. Notably, in a recent

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https://doi.org/10.1016/j.cpnec.2021.100056
Received 28 December 2020; Received in revised form 2 April 2021; Accepted 12 April 2021

systematic review of 33 publications, the authors found that bereaved individuals exhibited higher levels of systemic inflammation and weakened adaptive immune function compared to non-bereaved controls [9] – these findings suggest that the stress associated with losing a close loved one negatively impacts immune functioning.

The quality of sleep during bereavement may profoundly impact the immune system, as sleep serves a homeostatic role in regulating immune functioning [5]. While a full night's sleep prepares the immune system for infectious challenges, sleep impairment promotes elevated day-time inflammation [5]. Indeed, sleep loss, extreme sleep durations (short and long), and sleep disturbance are all associated with increased levels of inflammation, which may be due to the effects of sleep on sympathetic nervous activity (i.e., adrenergic signaling) that drive inflammatory gene expression [5].

Prolonged periods of sleep disruption may therefore pose significant implications for the immunological health of grieving individuals. Still, longitudinal work in the context of bereavement remains sparse. The absence of one's spouse may be felt most saliently during bedtime routines because many bereaved seniors spend decades co-regulating their sleep with their spouse. Dyadic sleep patterns significantly impact bedtime, sleep onset latency, and wake bouts [10]. In fact, compared to nonbereaved normal sleepers, widow(er)s experience significant sleep disruption (e.g., longer sleep onset latency, longer nighttime wakefulness) in the early months of grief [4]. In a cross-sectional study, sleep disturbance was associated with elevated inflammation among bereaved spouses [11]. Though one study suggests that persistent sleep disturbance is associated with worse grief trajectories and poorer health [6], no work to date has examined how sleep patterns change with inflammatory activity during early bereavement.

Biobehavioral changes may look different across widow(er)s depending on their social context. Socioeconomic status (SES) is important to health and impacts people at all levels of SES [12]. Because SES is associated with stress appraisal and physiology [13], SES may differentially influence how biobehavioral health changes during early bereavement. Lower SES individuals are more strongly impacted by emotionally undesirable events than higher SES individuals; this vulnerability has been attributed to differences in financial resources and nonfinancial coping resources such as social support and psychological traits [14].

Psychological perceptions of SES (i.e., subjective social status) also predict physiological functioning and capture dimensions of inequality and health beyond traditional (i.e., objective) SES measures. Higher subjective social status is associated with better health outcomes even after controlling for objective SES [15,16]. Lower subjective social status is also associated with exaggerated inflammatory responses to acute stressors [17], suggesting that social hierarchies shape physiological reactions to threatening stimuli.

Objective SES and subjective social status sometimes influence sleep and immune functioning differently. Subjective social status, but not objective SES, is associated with cold susceptibility [18] and also influences the relationship between sleep duration and cold severity – among infected individuals who reported low subjective social status, short sleep duration was associated with more mucus production [19]. The relationship between persistent sleep problems and immunological health may differ between low and high objective SES and/or subjective social status during bereavement.

To determine the longitudinal relationship between sleep and inflammation during bereavement, the current study investigated changes in sleep quality and inflammation across two time points among recently bereaved individuals. Because lower objective SES and subjective social status are consistently associated with adverse physical health outcomes, this study evaluated whether the relationship between sleep and inflammation depended on one's objective socioeconomic status or subjective social status. We hypothesized that 1) worsening sleep quality over time would predict increased levels of inflammation over time and 2) low objective SES and low subjective social status, but not high SES or high subjective social status, would exacerbate the relationship between sleep quality and levels of inflammation. In post-hoc tests, we explored which measure of social status (objective or subjective) more strongly impacted the relationship between sleep quality and inflammation and did not make any a priori hypotheses.

2. Materials and methods

2.1. Study design

One hundred and eight bereaved spouses from an ongoing longitudinal study were included in the original sample. Inclusion criteria required subjects to be recently bereaved (spousal death within three and half months), married to their partner for at least three years before the death, not be divorced within the past year, and English-speakers. Interested subjects were excluded if they 1) experienced the additional loss of a very close individual (rated as 75 or above on a 0–100 closeness scale) within the last year, 2) divorced within the past year 3) were currently undergoing cancer treatment, 4) had an auto-immune disease, or 5) had a pacemaker. Only subjects who went to bed between 8pm and 2am were included in the final sample. As such, two participants were excluded from analyses due to irregular sleep schedules: one subject slept between 8:30am-3:30pm and another subject reported sleeping 4 hours in the evening and 4 hours in the afternoon. The final sample size used for the current analyses was 106. All subjects provided informed consent, and Rice University's Institutional Review Board approved all recruitment methods and study procedures.

Research personnel conducted assessments at the Bioscience Research Collaborative in the Texas Medical Center or at the participant's home. Participants in this study completed two visits, one approximately three months post-death (Time 1) and one about six months post-death (Time 2). These are clinically relevant timepoints for bereavement, specifically, as widow(er)s are at most significant risk for cardiovascular events during the first 6 months after spousal death [2]. Thus, analyzing health behavior-changes and changes in inflammation between 3- and 6-months post-loss may be critically important to understand who is at greater risk for morbidity and mortality. For blood draw purposes, participants were asked to follow a regimented breakfast list, avoid strenuous exercise 48 hours prior, limit caffeine and alcohol intake, and reschedule their visit if they were experiencing acute illness symptoms. At both visits, participants completed self-report questionnaires, including demographic and clinical (e.g., mental and physical health) questionnaires. Blood samples were collected between 7:30 and 11:00 a.m. to control for diurnal variation.

2.2. Measures

2.2.1. Primary variables of interest

Circulating cytokines at the plasma level are often beyond detection and subject to extreme variability as a result of a variety of factors including diurnal variation, changes in plasma volume, and enlargement of the cell pool. Rather than measuring cytokines at the plasma level, we measured the reactivity of T cells to mitogenic stimulation using whole blood cell cultures to induce cytokine production. By measuring the capacity of immune cells to produce inflammatory mediators after ex-vivo stimulation, a representation of the in-vivo response of the immune system to stress and infection is obtained [20]. Whole blood, diluted 1:10 with RPMI-1640 (Gibco, Grand Island, NY), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine was stimulated with anti-CD2/CD28 monoclonal antibodies final concentration anti-CD2.1/anti-CD2.2 0.33 μg/ml and anti-CD28 1.33 μg/ml at 37 °C/5% CO_2 in 96-well round-bottomed plates. Supernatants were collected after 72 h of culture and stored at −80 °C until they were analyzed using multiplex assays according to the manufacturer's instructions (R&D Biosystems). T cell mitogen-induced secretion of IL-6, TNF-α, IFN-γ, IL-17A, and IL-2 was assessed. All inflammatory

cytokines were assayed in a single run.

Multiple cytokines serve as indicators of pro-inflammatory signaling. To minimize Type I error associated with repeated hypothesis testing of individual biomarkers, a composite index of pro-inflammatory markers was created. Composite variables are commonly used to prevent Type I error, especially when small sample sizes preclude testing for multiple comparisons or organizing highly correlated variables into a meaningful construct [21]. For each cytokine, z scores derived from log transformed values were calculated and averaged to create a summary inflammatory construct for each participant. Markers were analyzed individually if the composite index was found to be significant. In this study, the proinflammatory composite consisted of IL-6, TNF-α, IFN-γ, IL-17A, and IL-2 (Time 1, $\alpha = 0.92$; Time 2, $\alpha = 0.93$). These cytokines were selected to make up the composite index to maintain consistency with previous work among the bereaved population [22].

Sleep quality was assessed using the *Pittsburgh Sleep Quality Index (PSQI)*. The PSQI is a self-rated questionnaire designed to measure sleep quality in clinical populations [23]. It has demonstrated good reliability and validity across diverse sample populations, including psychiatric and sleep disorder patients and healthy elderly subjects [23]. The PSQI is comprised of 19 items that contribute to seven sleep components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. A global sleep quality score (range: 0–21) is computed from the sum of the seven components. Higher scores indicate poorer overall sleep quality, with scores greater than five yielding good sensitivity and specificity for distinguishing poor sleepers from good sleepers (scores less than 5) [23]. Overall reliability coefficient (Cronbach's alpha) for the seven component scores of the PSQI were .72 and .69 at Time 1 and Time 2 testing, respectively.

Objective socioeconomic status and subjective social status were assessed with the *Sociodemographic Questionnaire* from the *MacArthur Research Network* on SES and Health [15]. Years of education served as an indicator of objective SES and was also included as a covariate in all adjusted models. Subjects selected between 1 and 20+ years of school. For older adult samples, education is considered a stable indicator of SES. Compared to income and occupation, education is less impacted by disease in adulthood and is fairly stable beyond early adulthood, as it is not influenced by age-related changes in employment (i.e., retirement and housemaker) [24]. Across all racial and ethnic groups in the United States, 37.9% of adults aged 65+ years have a college degree; among the White population in the United States, 41.3% of adults aged 65+ years have a college degree [25]. Subjective social status was assessed using the pictorial of a "social ladder". Subjects were asked to place an "X" on the rung of a 10-rung ladder that represented their standing in society. Individuals ranked themselves relative to other people in the United States. Higher rungs indicate higher subjective social status. This measure of subjective social status has been previously validated as a measure with good test-retest reliability and predictive utility for examining links between subjective social status and health [26].

2.2.2. Covariates in primary analyses

Age, sex (male or female), years of education (1–20+), and body mass index (BMI) were included as covariates. Sex was coded as a binary variable (male = 0, female = 1). BMI was computed as weight in kilogram divided by height in meters squared.

2.2.3. Covariates in post-hoc analyses

Researchers in the field of psychoneuroimmunology recommend controlling for additional covariates known to influence inflammatory markers [27]; as such, we controlled for the following additional covariates in post-hoc analyses: days since the death at enrollment, days between visits, race, ethnicity, smoking status, post-menopausal status, anti-inflammatory medication use, depressive symptoms, comorbid conditions, physical activity. Race (White or Non-white), ethnicity (Hispanic or non-Hispanic), smoking status (yes or no), post-menopausal status (yes, no, not applicable), and anti-inflammatory medication use were acquired from a self-report demographic questionnaire. Anti-inflammatory medication consisted of statins, metformin, non-steroidal anti-inflammatory drugs (NSAIDS), COX-2 inhibitor drugs, RANKL inhibitors, and steroids; for data analysis, anti-inflammatory medication was coded as a binary variable.

Depressive symptomology was assessed using the Center for Epidemiological Studies Depression Scale (CES-D) [28]. Higher scores on the scale indicate greater depressive symptomology. The reliability of the CESD scale was high ($\alpha = 0.92$).

Comorbid health conditions were assessed using the Charlson Comorbidity Index, which is the most widely used comorbidity index for predicting mortality [29]. Weights are assigned to 19 comorbid conditions based on their potential influence on one-year mortality.

Physical activity was assessed using the International Physical Activity Questionnaire [30]. Metabolic equivalent (MET) minutes a week were calculated to represent the amount of energy expended while carrying out low, moderate and vigorous activity.

2.3. Statistical analyses

Descriptive statistics were assessed, and inflammatory markers were natural log-transformed, as they were positively skewed. Before conducting linear regression analyses, we examined assumptions of linearity, normality, homoscedasticity, and multicollinearity (VIF). All assumptions were met.

Missing data were accounted for using random forest imputation, a machine learning technique that outperforms traditional multiple imputation methods, addresses limitations imposed by traditional multiple imputation, and performs well under moderate and high missingness [31]. Random forest uses decision trees and bootstrap aggregation (i.e., bagging) to develop a single predictive model [32]. Random forest accommodates complex interactions, nonlinear variables and addresses issues of overfitting when imputing multiple variables on moderate sample sizes [32]. The 'bagImpute' method of the 'preProcess' function within the *caret* package in R was used, which utilizes bagged trees to impute [33]. Specifically, for each predictor, bagged tree models are developed from all other predictors; bagged trees are subsequently used to predict missing predictor values [33]. Prior to imputation, difference scores were computed from Time 2 (6 months post-death) to Time 1 (3 months post-death) for every time-varying variable: PSQI, inflammatory cytokine values, CES-D scores, BMI, physical activity. Missing data points among categorical variables (i.e., ethnicity, race, smoking, anti-inflammatory medication) were assigned a factor level "missing" value (i.e., −1). The following variables were included in the imputation model: PSQI (Time 1, Time 2, difference score), inflammatory values (Time 1, Time 2, difference score for all 5 cytokines), BMI (Time 1, Time 2, difference score), sex, age, ethnicity, race, anti-inflammatory medication use, smoking, physical activity (Time 1, Time 2, difference score), CES-D (Time 1, Time 2, difference score), subjective social status, education, days since passing, days between visits, and comorbidities. Subject identification was not included in imputation models. Missing data comprised 12% of the overall dataset, ranging from 0.9% for subjective social status up to 31% for physical activity. Due to incomplete follow-ups, 19% of the sample had missing data for sleep and inflammatory variables at Time 2.

To assess change across 2 critical time points, change-regression analyses were used [34,35]. In post-hoc analyses (see below), when additional covariates were included, the following time-varying covariates were entered as arithmetic difference scores into models: depressive symptoms and physical activity. Regression analyses and simple slope tests were performed using the *stats* package and *MeMoBootR* package [36] in R, respectively. Both unadjusted (without covariates) and adjusted models were assessed. In adjusted models, variables were entered in a hierarchical manner: 1) all covariates, 2) sleep, 3) subjective social status, 4a) sleep x education OR 4b) sleep x subjective social status.

Moderators were examined independently in separate models to preserve power but were examined simultaneously in the same model in post-hoc analyses. Baseline inflammation was not included in any models reported here because the interpretation of change differs slightly from the primary research questions of interest. Instead, models with baseline inflammation accounted for can be found in supplemental materials. The same analytic procedures were applied to non-imputed data, and detailed results for these analyses can be found in supplemental materials.

Several post-hoc analyses were conducted. First, if a priori adjusted models using the composite inflammatory index were significant, the index was subsequently deconstructed and individual cytokines were assessed; this method aims to minimize Type I error that comes with multiple testing. Second, moderators (i.e., education, subjective social status) were examined simultaneously in the same model to examine which moderator had a stronger effect on the association between sleep and inflammation. Third, regression models were re-run with additional covariates to examine whether study findings remained after controlling for factors known to influence inflammatory outcomes [27]. Fourth, sensitivity analyses were conducted to examine the impact of outliers on study findings. Outliers were defined as exceeding 3 standard deviations for inflammatory values after values underwent natural log transformation and were z-scored. Analyses examining the effect of specific sleep components on the composite inflammatory index are not reported here but can be found in supplemental materials.

3. Results

3.1. Primary analyses with composite inflammatory index

Study sample characteristics are summarized in Table 1. In correlation analyses, sleep quality at Time 1 was significantly correlated with IFN- γ ($p = .04$), marginally correlated with TNF- α ($p = .06$), and not correlated with remaining cytokines at Time 1. Sleep quality at Time 2 was marginally correlated with IL-2 ($p = .08$) inflammatory markers at Time 2. Change in inflammation was significantly correlated with changes in the composite inflammatory index ($p = .04$), TNF- α ($p = .02$), IFN- γ ($p = .02$), and IL-17A ($p = .05$), marginally correlated with changes in IL-6 ($p = .06$), and not correlated with changes in IL-2 ($p = .40$). Detailed correlational data for continuous variables examined in this study can be found in Tables S1 and S2. Unadjusted regression models can be found in Table S3. In sensitivity analyses, two subjects were labeled as outliers. Study findings remained robust after excluding outliers; thus, all results are reported with the original sample size (N = 106).

In the main effect unadjusted model, regression analyses revealed that changes in sleep quality were positively associated with changes in inflammation, such that worsened overall sleep quality was associated with increased levels of composite inflammation at follow-up; the unadjusted model significantly explained some of the variance in inflammation ($F(1,104) = 6.41$, $R^2 = 0.06$, Adj. $R^2 = 0.05$, $p = .01$). The relationship between change in sleep quality and change in inflammation remained even after controlling for age, sex, BMI, and education ($\beta_{PSQI} = .23$, $sr^2 = 0.05$, $p = .02$; see Table 2, Model 2 for details). Compared to the unadjusted model, the adjusted model explained an additional 2% of the variance in composite inflammation ($F(5,100) = 2.55$; $R^2 = 0.11$; Adj. $R^2 = 0.07$; $p = .03$).

Unadjusted and adjusted models revealed that the relationship between change in sleep quality and change in inflammation was moderated by years of education (Adjusted model: $\beta_{PSQI\ x\ Education} = -1.55$, $sr^2 = 0.07$, $p < .01$). Compared to the unadjusted model ($F(3,102) = 5.47$, $R^2 = 0.14$, Adj. $R^2 = 0.11$, $p < .01$), the adjusted model explained an additional 2% of the variance in inflammation ($F(7,98) = 3.25$, $R^2 = 0.19$, Adj. $R^2 = 0.13$, $p < .05$). Simple slopes analyses revealed that worsened sleep quality was significantly associated with increased levels of inflammation over time only in individuals with fewer years of education ($-1\ SD = 13.78$ years; $b = 0.14$, $p < .001$) and average years of education ($M = 16.39$ years; $b = 0.07$, $p = .008$) but not in those with more years of education ($+1\ SD = 19$ years, $b = -0.003$, $p = .93$). Simple slopes with education as a moderator are depicted in Fig. 1a. These relationships were found even after controlling for subjective social status.

Similarly, in both unadjusted and adjusted models, the relationship between change in sleep quality and change in inflammation also depended upon subjective social status (Adjusted model: $\beta_{PSQI\ x\ subjective\ social\ status} = -1.27$, $sr^2 = 0.05$, $p = .02$). Compared to the unadjusted model ($F(3, 102) = 4.00$, $R^2 = 0.11$, Adj. $R^2 = 0.08$, $p < .01$), the adjusted model explained an additional 4% of the variance in inflammation ($F(7,98) = 2.95$, $R^2 = 0.17$, Adj. $R^2 = 0.11$, $p < .01$). Simple slopes tests revealed that worsened overall sleep quality over time was significantly related to increased levels of inflammation (composite index) over time only in those who reported lower subjective social status ($-1\ SD$) ($b = 0.14$, $p < .001$) but not in those who perceived themselves to be at higher standing ($+1\ SD$) ($b = -0.03$, $p = .49$), relative to others in the United States. In individuals who reported average levels of subjective social standing, there was a marginally significant association between sleep quality and levels of inflammation ($b = 0.05$, $p = .06$). Simple slopes with

Table 1
Study sample characteristics.

Variable	Number (%) or mean (SD)		
	Time 1 (3 months post-death)	Time 2 (6 months post-death)	Average change
Age (years)	68.49 (9.35)	–	–
Sex (female)	69 (65.1%)	–	–
Race (White)	96 (90.6%)	–	–
Ethnicity (Hispanic/Latinx)	10 (10.2%)	–	–
Postmenopausal	64 (92.8%)	–	–
Smoker	4 (3.8%)	–	–
Anti-inflammatory medication[a]	62 (58.5%)	–	–
Comorbidities	0.34 (1.0)	–	–
Depressive symptoms[b,h]	17.93 (11.4)	13.9 (10.5)	−3.20 (8.26)[b]
Body mass index (kg/m^2)	27.67 (5.2)	27.63 (5.3)[b]	.24 (3.05)[b]
Physical activity[b,c]	2699.05 (3327.9)	2758.80 (3397.5)	-.04 (1.02)[b]
Days since death[d]	84.4 (17.8)	195.04 (13.5)[b]	–
Days between visits[e]	110.9 (22.3)[b]	–	–
Subjective social status[h]	7.11 (1.7)	–	–
Education (in years)	16.39 (2.6)	–	–
Sleep Quality[b,h]	8.25 (4.0)	7.04 (3.5)	−1.21 (2.8)[b]
Composite index[b,f]	−0.08 (0.8)	-.01 (.9)	.13 (.83)[b]
IL-6[b,g]	4.40 (1.4)	4.44 (1.3)	.14 (1.27)[b]
TNF-α[b,g]	6.38 (1.5)	6.45 (1.2)	.19 (1.28)[b]
IL-17A[b,g]	5.79 (1.0)	5.96 (1.0)	.22 (.95)[b]
IFN-γ[b,g]	8.05 (1.6)	8.27 (1.5)	.28 (1.48)[b]
IL-2[b,g]	7.23 (0.9)	7.38 (1.0)	.23 (1.04)[b]

Note. All reported values are based on a sample size of N = 106 unless otherwise mentioned.

[a] Anti-inflammatory medication consisted of statins, metformin, non-steroidal anti-inflammatory drugs (NSAIDS), COX-2 inhibitor drugs, steroids, and RANKL inhibitors.

[b] Indicates variables or values that were calculated based on an N < 106.

[c] Physical activity = metabolic minutes per week.

[d] Days since death = days since spousal death at the time of enrollment (Time 1).

[e] Days between visits = number of days between Time 1 and Time 2.

[f] Composite inflammatory index is an average of all z-scored proinflammatory cytokines.

[g] Reported values for proinflammatory cytokines (IL-6, TNF, IL-17A, IFN-γ, IL-2) are natural log-transformed.

[h] Range of possible scores for the measures are as follows: Depressive symptoms using Center for Epidemiological Studies Depression Scale (0–60), sleep quality using the Pittsburgh Sleep Quality Index (0–21), subjective social status using the MacArthur Sociodemographic Questionnaire (1–10).

Table 2
Summary of hierarchical regression analyses for variables predicting change in levels of inflammation, including 4 covariates (N = 106).

	Composite			IL-2			IL-6			IL-17A			IFN-γ			TNF-α		
	ΔR^2	*b*	95% CI	ΔR^2	*b*	95% CI	ΔR^2	*b*	95% CI	ΔR^2	*b*	95% CI	ΔR^2	*b*	95% CI	ΔR^2	*b*	95% CI
Model 1																		
Covariates	.06			.05			.12*			.04			.04			.03		
BMI		−0.06*	[-0.11, −0.01]		−0.04	[-0.10, 0.03]		−0.14**	[-0.22, −0.06]		−0.05	[-0.11, 0.01]		−0.09	[-0.19, 0.01]		−0.07	[-0.15, 0.01]
Age		−0.01	[-0.02, 0.01]		−0.01	[-0.03, 0.01]		−0.01	[-0.03, 0.02]		−0.01	[-0.03, 0.01]		−0.00	[-0.03, 0.03]		−0.01	[-0.03, 0.02]
Sex		0.07	[-0.24, 0.38]		0.29	[-0.09, 0.68]		0.01	[-0.45, 0.47]		0.05	[-0.30, 0.41]		0.12	[-0.45, 0.70]		0.11	[-0.38, 0.60]
Educ.		0.02	[-0.04, 0.07]		0.03	[-0.04, 0.10]		−0.01	[-0.09, 0.08]		0.03	[-0.04, 0.09]		0.03	[-0.07, 0.14]		0.01	[-0.08, 0.10]
Model 2																		
PSQI	.05*	0.07*	[0.01, 0.12]	.01	0.04	[-0.03, 0.11]	.04*	0.09*	[0.00, 0.17]	.04*	0.07*	[0.01, 0.13]	.05*	0.12*	[0.02, 0.23]	.06*	0.11*	[0.02, 0.20]
Model 3																		
SSS	.01	0.05	[-0.05, 0.14]	.00	−0.04	[-0.16, 0.08]	.01	0.07	[-0.07, 0.21]	.01	0.05	[-0.06, 0.16]	.02	0.12	[-0.05, 0.29]	.02	0.11	[-0.03, 0.26]
Model 4a																		
PSQI x Educ.	.07**	−0.03**	[-0.05, −0.01]	.05*	−0.03*	[-0.05, −0.00]	.05*	−0.04*	[-0.06, −0.01]	.02	−0.02	[-0.04, 0.00]	.06*	−0.05*	[-0.08, −0.01]	.06**	−0.04**	[-0.07, −0.01]
Total R^2	.19**			.11			.22**			.12†			.17*			.17**		
Model 4b																		
PSQI x SSS	.05*	−0.05*	[-0.09, −0.01]	.05*	−0.06*	[-0.12, −0.01]	.06**	−0.09**	[-0.15, −0.03]	.03	−0.04	[-0.09, 0.01]	.05*	−0.10*	[-0.17, −0.02]	.07**	−0.09**	[-0.16, −0.03]
Total R^2	.17**			.11			.22**			.12†			.16*			.18**		

Note. In Models 2-4a/b, variables from preceding models were included but are not shown for brevity. All inflammatory variables were natural log transformed prior to analyses. The composite proinflammatory index is comprised of a z-scored average of T-cell stimulated cytokines (i.e., IL-6, IL-2, IL-17, TFN-α, IFN-γ). PSQI = change in sleep quality such that positive values indicate an increase in PSQI scores (i.e., worsening sleep quality from Time 1 to Time 2) and negative values indicate a decrease in PSQI scores (i.e., improving sleep quality from Time 1 to Time 2). Educ = years of education (higher values indicate more years of education). Sex = Male (0), Female (1). SSS = subjective social status (higher values indicate higher subjective social status). The comprehensive version of this table can be found in Table S9 in the supplemental material.
†p < .10, *p < .05, ** p < .01.

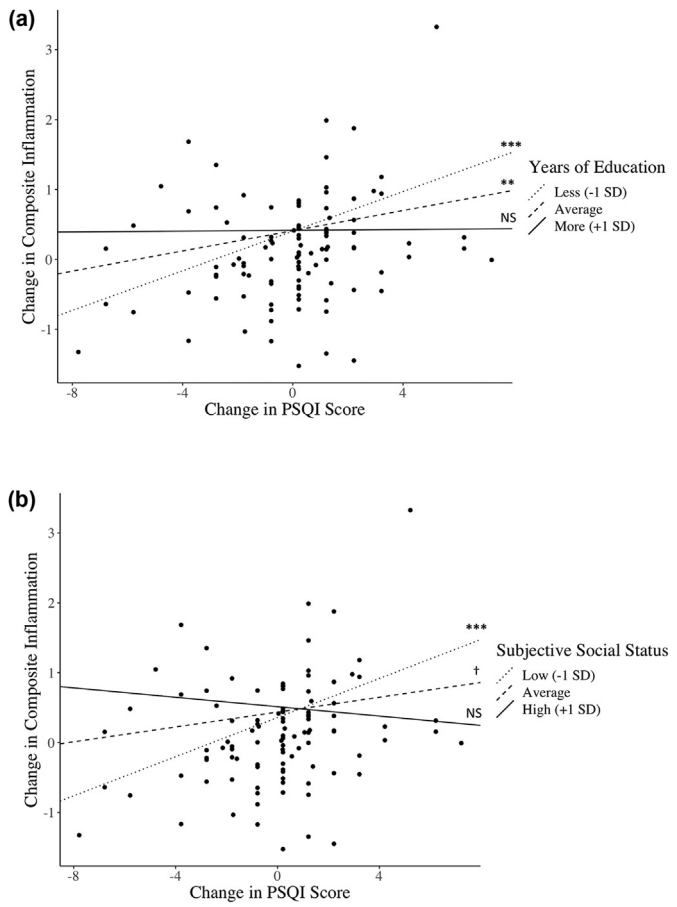

Fig. 1. aChange in sleep quality (PSQI) and composite inflammation as a function of less, average, and more years of education.
Note: For PSQI, positive values indicate an increase in PSQI score (i.e., worsening sleep quality) from Time 1 to Time 2 and negative values indicate a decrease in PSQI score (i.e., improving sleep quality) from Time 1 to Time 2. For composite inflammation, positive values indicate an increase in proinflammatory activity from Time 1 to Time 2 and negative values indicate a decrease in proinflammatory activity from Time 1 to Time 2.
*** p < .001.
**p < .01.
NS = not significant.

subjective social status as a moderator are depicted in Fig. 1b. Primary analytic findings using composite inflammation as the dependent variable were also supported by nonimputed data (see supplemental materials).

3.2. Post-hoc analyses with individual pro-inflammatory cytokines and additional covariates

In post-hoc main effect analyses using each individual cytokine as the dependent variable, change in sleep quality was associated with change in levels of IL-6, TNF-α, IFN-γ, and IL-17A in unadjusted and adjusted models (see Table 2). Change in sleep quality was not associated with change in levels of IL-2 in both unadjusted and adjusted models.

In post-hoc adjusted analyses testing moderation, education and subjective social status significantly moderated the relationship between sleep quality and levels of IL-6, TNF-α, IFN-γ, and IL-2 but not sleep quality and levels of IL-17 (see Table 2). Simple slopes for each individual cytokine are depicted in supplemental materials (Figs. S3 and S4).

When both interactions were simultaneously entered into the same model ($F(8,97) = 3.20$, $R^2 = 0.21$, Adj. $R^2 = 0.14$, $p < .01$), education x sleep quality ($\beta = -1.21$, $b = -0.02$, 95% CI b [-0.04, −0.00]; $sr^2 = 0.04$, $p = .04$), but not subjective social status x sleep quality ($\beta = -0.86$, $b = -0.04$, 95% CI b [-0.08, 0.01]; $sr^2 = 0.02$, $p = .11$), significantly predicted change in composite inflammation.

Importantly, main effect and moderation analyses remained robust after controlling for additional covariates (i.e., race, ethnicity, comorbidity, anti-inflammatory medication, depressive symptoms, smoking status, post-menopausal status, physical activity, days since death, time between visits) in the models. Because the addition of these 10 covariates did not change the aforementioned findings reported above, models are delineated in detail in supplemental materials (Tables S4, S5, S6).

4. Discussion

This study was the first to investigate how sleep and inflammation change over time in a sample of bereaved spouses: changes in sleep quality were associated with changes in pro-inflammatory cytokine production. Specifically, bereaved individuals who reported worsened sleep quality from three months post-death to six months post-death also exhibited increased levels of IL-6, TNF-α, IFN-γ, and IL-17A over time. Not all widow(er)s shared similar biobehavioral risks. The present study also identified SES as an important factor for understanding physiological vulnerability following spousal bereavement: individuals with low SES may be more susceptible to physiological changes that interact closely with sleep behavior. Indeed, widow(er)s who perceived themselves to be in lower social standing or were less educated exhibited greater increases in pro-inflammatory activity as sleep quality worsened over a 3-month period. These findings support the well-established associations between sleep and immune health [5] and highlight sociodemographic differences in health following spousal bereavement.

Bereavement is associated with maladaptive patterns of immune activity [9]. The present findings provide preliminary evidence that persistent sleep problems are related to stress-induced changes in immune function. In addition to the distress associated with the death, grieving spouses experience secondary stressors resulting from the loss, such as financial strain, loss of community/work roles, and loss of daily routine [37]. These stressors coupled with age-related declines in physiological functioning [38] may further exacerbate immune functioning [39]. Then, sleep becomes a valuable necessity for restoring immune functioning back to homeostatic levels [5]. However, significant sleep impairment often follows bereavement [4]. The stress of bereavement compounds the relationship between sleep quality and inflammatory activity at three months post-death [11]. Further analyses with specific sleep components revealed that the association between changes in sleep quality and inflammation was primarily driven by changes in sleep onset latency (see supplemental materials for details). Longitudinal work with age-matched comparisons are needed to 1) confirm whether *persistent* sleep disturbance differentially relates with immune processes in widow(er)s and nonbereaved adults and 2) whether clinically significant levels of sleep disturbance are needed to promote a rise in inflammation during the first 6 months of bereavement.

Poor sleep quality in bereaved individuals may be attributed to elevated inflammatory activity. Due to the observational nature of the current study, causal assumptions about directionality cannot be made. While most human subject research on sleep and immune function focuses on the role of sleep in regulating immune activity [5], some work suggests that inflammatory activity can modulate physiological correlates of sleep. For example, intravenous injections of bacterial endotoxin, which stimulate the secretion of inflammatory cytokines without affecting body temperature and neuroendocrine systems, promote changes in the duration and intensity of non-rapid eye movement (NREM) sleep [40]; these changes in NREM are mediated by TNF-α and IL-1 [40]. Hence, it is also likely that increasing levels of TNF-α during bereavement, as observed in the current study, promote worsening sleep quality over time.

Temporal ordering may be gleaned by conducting experimental paradigms that manipulate sleep quality or inflammatory activity (i.e.,

inducing inflammation via vaccine injection or bacterial endotoxin or inhibiting inflammatory activity via an antagonist) and subsequently examine how inflammatory levels and sleep quality change following the manipulation. For example, Weinberger et al. [41] randomly assigned subjects with treatment-resistant depression to receive a TNF antagonist (infliximab) or placebo; they found that blocking TNF improved sleep continuity in subjects with high inflammation, but not those with low inflammation. Due to the sensitive nature of the bereaved population, inducing poor sleep quality in the laboratory may not be appropriate or ecologically valid. Instead, future studies may consider randomly administering a sleep-facilitating drug to bereaved spouses reporting severe sleep impairment and observing how inflammatory levels change thereafter in those who report improved sleep quality. However, given the known reciprocal pathways between the brain and the immune system, the relationship between sleep behavior and immune functioning is likely bidirectional [42]. Interventions that target either sleep and inflammatory activity may effectively improve sleep quality and reduce systemic inflammation in widow(er)s.

Less-educated bereaved spouses may be at a more significant disadvantage because they report more stress and have fewer resources to offset the compounded stress of bereavement and socioeconomic disadvantages. Education instills a set of cognitive skills, abilities, attitudes, and habits that foster motivation, effort, dependability, confidence, effective problem-solving, and learning - all of which *cultivate* a sense of personal control over one's circumstances [43]. Less-educated individuals experience more severe daily stressors [44], report less control over their environment [43], and employ less effective coping strategies [45]. In contrast, highly educated older adults may more easily seek out and engage in coping strategies that reduce daily stressors and mitigate the negative consequences of bereavement-related changes in health because they have the skillset to acquire and use information effectively, the self-efficacy to confront challenging events, and the economic capital to preserve health. Sleep is invaluable for restoring physiological stress systems to homeostatic conditions. Still, it may be insufficient due to the additional stressors imposed by low SES. To address the SES health gap, additional, tailored strategies that minimize daily stress may be needed. For example, cognitive reappraisal ability, which promotes *self*-regulation of emotions, is more beneficial for lower SES (measured using education, income, occupation, and as a composite index) individuals than high SES individuals in the context of improving psychological health [46].

Despite showing greater sleep-related increases in inflammation, less educated widow(er)s also showed greater sleep-related decreases in inflammation than more educated widow(er)s. Less and more educated widow(er)s showed marked differences in inflammatory changes when their sleep quality improved at least 3.6 points on the PSQI or when their sleep quality worsened by at least 2.3 points on the PSQI (see supplemental materials). In other words, less educated widow(er)s may be more sensitive to sleep-related inflammatory changes during the first 6 months of bereavement than more educated widow(er)s. Because stressful life events and adverse social environments (e.g., socioeconomic status) influence neuroplasticity in brain regions associated with memory, affect regulation, threat appraisal, and the stress response, alterations in neuroplasticity can affect patterns of stress reactivity, recovery, coping, and aging [47]. Thus, stress-responsive symptoms in low SES widow(er)s (in particular, sleep and immune activity) may be primed to respond more strongly to major life stressors or daily hassles. Our findings suggest that targeting sleep quality or inflammation to improve either or both sleep quality and immune health may be a more effective strategy for lower SES individuals than higher SES individuals. In fact, previous studies have demonstrated that health behavior interventions (i.e., eating behavior, physical activity) can differ in effectiveness according to socioeconomic factors [48,49].

Both education and subjective social status influenced the relationship between sleep quality and inflammation. Still, education was a stronger moderator than subjective social status. These findings differ from previous work by Prather et al. [19]; they found that only subjective social status, not education, moderated the association between sleep duration and cold susceptibility among older adults. Several studies report that, relative to objective indicators of SES, subjective social status more strongly predicts physiological health outcomes like cold susceptibility [18], respiratory illness [16], and resting heart rate [15]. Subjective social status reflects an aggregation across different objective indicators of SES *within* an individual's social context. It captures health-relevant constructs that are sometimes not adequately assessed by objective measures of SES [16]. The effect of subjective social status on health may be less pronounced than objective SES in high SES samples. In studies that found that subjective social status had a stronger effect than objective SES, the socioeconomic range was wider and less skewed toward higher SES [15,18,19]. Compared to the sample in Prather et al. [19], subjects from the current sample on average, were more educated (3+ years) and reported higher rankings of subjective social status (3+).

The strengths of this study include a longitudinal and meticulous research design. All subjects experienced the same type of loss (i.e., death of a spouse) and were recruited within stringent time frames during the first six months of bereavement. Blood samples were consistently collected in the morning. Moreover, assessment at two time points during a period in which mortality rates are highest [2] provides a unique perspective into the behavioral and physiological changes that undermine mental and physical health during bereavement. Lastly, the relationships between sleep, inflammation, and SES remained robust even after accounting for factors known to impact inflammatory outcomes [27] (see Tables S4 and S5). For example, sleep disturbance is considered a core symptom of major depression [50], and inflammation and major depression are causally linked [51]. Our findings suggest that the relationship between poor sleep quality and pro-inflammatory activity is not solely attributed to psychological distress (i.e., depressive symptoms).

Sleep quality was assessed using a self-report questionnaire, which is a significant limitation to acknowledge. Despite high internal homogeneity, the PSQI may be less reflective of actual sleep parameters and more representative of sleep dissatisfaction. Previous work has demonstrated that the PSQI correlates with a sleep diary and depressive symptoms but not actigraphic sleep parameters [52]. Future work should simultaneously assess sleep using subjective and objective measures (i.e., polysomnography, actigraphy) to evaluate whether both subjective and objective sleep disturbance similarly impact inflammation longitudinally. However, in numerous bereavement studies comparing bereaved to nonbereaved controls, no significant differences were found using objective sleep measures, despite bereaved individuals self-reporting greater sleep impairment than controls [4].

The modest socioeconomic and sociodemographic variability in the bereaved sample may limit the generalizability of the present findings. About 69% of the current sample had at least a college education (i.e., 16+ years), a statistic much higher than the overall older adult population in the U.S [25]. Ninety percent of the sample was White compared to the 61% of the U.S. population being White [25]. Given that health disparities are most evident when comparing across the lowest and highest in society [12], the influence of socioeconomic status on biobehavioral health may be even stronger in the general U.S. population. However, it is also possible that the reported associations between sleep, inflammation, and SES in recently bereaved spouses only apply to a highly educated and predominantly White sample and may look very different in a low-income or less educated sample. Future work examining a more diverse and heterogeneous sample is warranted.

For some people, the first year of bereavement may be a period of recovery rather than a period of stress reactivity. In the current study, the average widow(er) showed improvements in sleep quality, increases in inflammation, and decreases in depressive symptoms from 3 months to 6 months post-spousal loss. Previous studies have found that widow(er)s who anticipated the death of their spouses (i.e., dementia caregivers) showed declines in depressive symptoms [53] and cardiovascular risk [54] and improvements in immune function after the death [55]. In

contrast, those whose spouses died unexpectedly experienced increases in depressive symptoms and mortality risk [56–58]. Unfortunately, the current dataset did not contain sufficient information to examine the bereavement context. No study thus far has attempted to study whether death anticipation alters sleep trajectories post-bereavement. Future work may investigate whether post-bereavement biobehavioral outcomes differ by the decedent's death.

5. Conclusion

In conclusion, prolonged sleep impairment may be a mechanism through which bereavement dysregulates immunological health and increases mortality risk; inflammation increased with worsening sleep quality during the first six months following spousal loss. This association depended on both objective SES (i.e., years of education) and subjective social status (e.g., perception of social standing). This study highlights socioeconomic disparities in health that extend into late adulthood and identify those who be more susceptible to sleep-related changes following spousal bereavement. In light of the present findings, researchers, therapists, and clinicians should consider socioeconomic factors when developing and implementing interventions for improving the biobehavioral health of recently bereaved spouses.

Declaration of competing interest

None.

Acknowledgements

This work was supported by the National Heart, Lung, and Blood Institute (Grant No. 1R01HL127260-01) and the National Science Foundation (Grant No. SMA-1853936 and SMA-1559393). We would like to acknowledge Ms. Kristi Parker and Ms. Patricia Morales for project management and the bereaved individuals who offered their valuable time to be in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2021.100056.

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