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Original Article

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Disturbance of sleep maintenance, but not sleep duration, activates nuclear factor-κB and signal transducer and activator of transcription family proteins in older adults: sex differences

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Abstract

Study Objectives: Disturbances of sleep maintenance and sleep duration are common in older adults and associated with an increased risk for age-related mortality and morbidity. Converging evidence implicates inflammation as an underlying mechanism, especially in females. However, it is unknown what specific aspects of sleep disturbance impact inflammatory mechanisms in older adults.

Methods: Using data from community-dwelling older adults who participated in the Sleep Health and Aging Research (SHARE) field study (n = 262, mean age 71.9 ± 8.0 years), we conducted a secondary analysis to examine whether disturbance of sleep maintenance (i.e. greater amount of wake time after sleep onset [WASO]) and sleep duration (i.e. shorter total sleep time [TST]) assessed by sleep diary and actigraphy are associated with greater activation of nuclear factor (NF)-κB and signal transducer and activator of transcription (STAT) family proteins STAT1, STAT3, and STAT5 in peripheral blood monocytic cells. In addition, moderation effects of sex were explored.

Results: Data were available for sleep diary (n = 82), actigraphy (n = 74), and inflammatory signaling and transcriptional measures (n = 132). As assessed by sleep diary, greater amount of WASO (β = 0.39, $p < 0.01$), but not TST, was associated with higher levels of NF-κB. Whereas diary-assessed sleep measures were not associated with STAT family proteins, a moderation analysis revealed that greater diary-assessed WASO was associated with higher levels of STAT1 ($p < 0.05$), STAT3 ($p < 0.05$), and STAT5 ($p < 0.01$) in females, but not in males. Actigraphy-assessed sleep measures were not associated either with NF-κB or STAT activation.

Conclusions: In older adults, self-reported disturbance of sleep maintenance assessed by sleep diary was uniquely associated with higher levels of NF-κB, along with higher levels of STAT family proteins in females, but not in males. Our data suggest that improvingself-reported sleep maintenance might mitigate age-related increases in inflammatory signaling and transcriptional pathways, possibly more strongly in females, with the potential to reduce mortality risk in older adults.

Key words: WASO; TST; NF-κB; STAT family proteins

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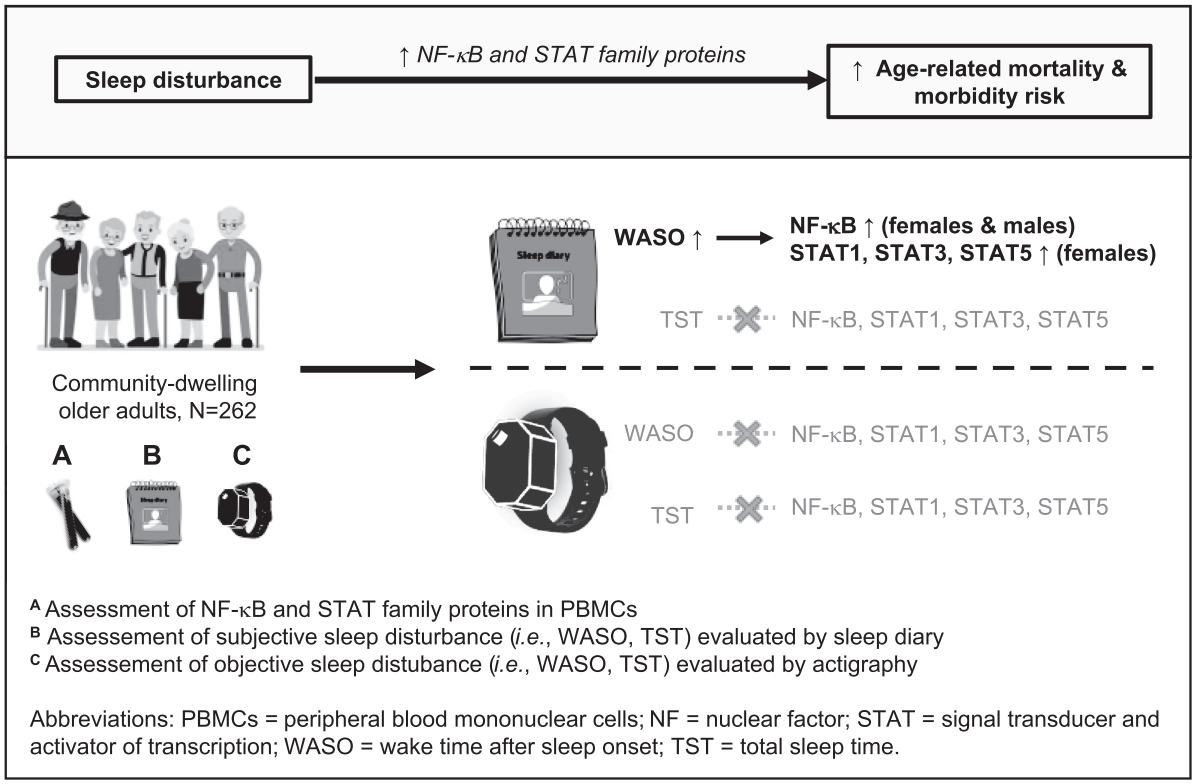
Graphical Abstract



Statement of Significance

Disturbances of sleep maintenance and sleep duration are common in older adults and associated with an increased risk for age-related mortality and morbidity, with converging evidence implicating inflammation as an underlying mechanism, especially in females. The present study aimed to examine whether disturbance of sleep maintenance and sleep duration assessed by sleep diary and actigraphy differentially impact inflammatory mechanisms in older adults, and to explore sex differences. Results showed that poor self-reported sleep maintenance, but not sleep duration, was uniquely associated with activation of transcriptional signaling mechanisms especially in females. Treatment that improves sleep maintenance might mitigate age-related increases in inflammation, possibly more strongly in females, with the potential to reduce mortality risk in older adults.

Introduction

Disturbances in sleep maintenance and sleep duration are a common health complaint among older adults [1]. Large epidemiological studies have shown that poor sleep maintenance (i.e. greater amount of wake time after sleep onset [WASO]) [2] as well as short sleep duration (<6 hours of nocturnal sleep) [3] both predict increases in mortality risk in older adults. Moreover, a recent analysis of the Whitehall II prospective cohort study found that sleep duration ≤5 hours is associated with higher multimorbidity and that this risk increases with age (i.e. hazard ratio [HR] at age 50 = 1.30, HR at age 60 = 1.32, and HR at age 70 = 1.40) [4]. However, despite these adverse health outcomes linked to sleep disturbance in older adults, the underlying mechanisms that link poor sleep maintenance and short sleep duration to increases in age-related mortality and morbidity risk are still poorly understood. Thus, given evidence that the number of older adults is increasing at an unprecedented pace (i.e. the number of people aged 60 years and older was 1 billion in 2019, and this number is projected to increase to 2.1 billion by 2050 [5]), there is an urgent need to identify molecular mechanisms that can be targeted by behavioral and pharmacological interventions to improve health outcomes in older adults.

A growing body of evidence suggests that the association of poor sleep maintenance and short sleep duration with age-related mortality and morbidity risk is driven by a dysregulation of inflammatory pathways. For example, meta-analytic analyses of observational studies found that poor sleep maintenance and short sleep duration are both associated with elevations of systemic markers of inflammation, including C-reactive protein and interleukin (IL)-6 [6], which—in turn—are known to predict mortality and morbidity risk in older adults [7]. Paralleling these findings from observational research, prior experimental work from our group demonstrated that disruption of sleep maintenance (i.e. forced awakening) [8] as well as reduction of sleep duration (i.e. partial sleep deprivation [PSD]) both activate up-stream mechanisms of systemic inflammation, including Toll-like receptor-4-stimulated production of IL-6 and tumor necrosis factor-α by monocytes [9]. In addition, we previously showed that a single night of PSD induces robust increases in the activation of up-stream signaling and transcription pathways

underlying systemic inflammation, such as the nuclear factor (NF)-κB [10] and signal transducer and activator of transcription (STAT) protein families [11], two signaling and transcriptional protein families that play a critical role in the regulation of the inflammatory response. For example, activation of NF-κB induces transcription of proinflammatory immune response genes and the translation, production, and release of proinflammatory mediators (e.g. cytokines) [12], whereas STAT proteins convey the extracellular inflammatory signal from the cell surface receptor to the nucleus to directly bind DNA and regulate cytokine-inducible gene transcription [13]. Of note, different proinflammatory cytokines have been found to display different propensities for specific STAT proteins: for example, binding of interferons to their receptors induces STAT1 signaling [14–16], binding of IL-6 to its receptor induces STAT3 signaling [17–19], and binding of IL-2 binding to its receptor induces STAT5 signaling [20–22]. Interestingly, we also previously found that PSD induces activation of NF-κB signaling primarily in females, but not in males [10], which might contribute to the heightened autoimmune and inflammatory-disease risk found in females [23]. However, it is not known whether poor sleep maintenance and short sleep duration play differential roles for NF-κB and STAT signaling and transcriptional activity, whether subjective versus objective measures differentially capture these inflammatory signaling and transcriptional pathways, or whether sex moderates these mechanisms.

Thus, to further understand what specific aspects of sleep disturbance might drive increases in activation of inflammatory signaling and transcriptional pathways in older adults, we performed a secondary analysis using data from community-dwelling older adults who participated in the Sleep Health and Aging Research (SHARE) field study. The present study aimed to examine: (1) whether disturbance of sleep maintenance (i.e. greater amount of WASO) and sleep duration (i.e. shorter total sleep time [TST]) assessed by sleep diary and actigraphy are differentially associated with increased activation of NF-κB and STAT family proteins in peripheral blood monocytic cells (PBMCs), and (2) whether sex had a potential moderation effect on these relationships.

Methods

Participants

The present study was a secondary analysis of data collected in the SHARE field study, which was conducted at the Cousins Center for Psychoneuroimmunology at the University of California, Los Angeles (UCLA) [24]. Participants were community-dwelling older adults aged 60 years and above who were free from current medical disorders, and free from psychiatric and sleep disorders (including insomnia disorder) as evaluated by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IV). Participants underwent an in-person visit, which included a medical interview to evaluate an array of sociodemographic, clinical, behavioral, and socio-emotional measures. Moreover, the in-person visit included a blood draw to assess activation of NF-κB and STAT family proteins STAT1, STAT3, and STAT5 in PBMCs. Following the in-person visit, participants completed a 1-week period of ambulatory sleep monitoring to evaluate self-reported and objective sleep maintenance and sleep duration, as measured by sleep diary and actigraphy, respectively. All participants provided written consent prior to enrollment. All procedures received oversight and approval from the UCLA Institutional Review Board and were carried out in accordance with the Helsinki Declaration.

Measures

Sociodemographic, clinical, behavioral, and socio-emotional measures.

The present analysis included sociodemographic, clinical, behavioral, and socio-emotional measures, which are known to be related to inflammation [25] and sleep disturbance [26]. Sociodemographic measures included age, sex, race, and years of education. Clinical measures included body mass index (BMI) and chronic disease as assessed by medication use (i.e. Chronic Disease Score [CDS] [27]). Behavioral measures included levels of perceived loneliness (i.e. revised UCLA Loneliness Scale [R-UCLA] [28]) and stress (i.e. Perceived Stress Scale [PSS-14]) [29]. Finally, socio-emotional measures included presence and severity of anxiety (i.e. Beck Anxiety Inventory [BAI] [30]) and depressive symptoms (Beck Depression Inventory-II [BDI-II] [31]).

Assessment of sleep maintenance and sleep duration. Subjective sleep measures.

Subjective amounts of sleep maintenance and sleep duration were evaluated using the Pittsburgh Sleep Diary [32]. Sleep diaries were completed over a 1-week period in the participants' home environment. Participants were instructed to keep the sleep diary next to their bed and to journal their sleep information within 10 minutes after arising in the morning. After completing this period, sleep diary data were reviewed to calculate amounts of WASO (in min) and TST (in min) averaged across the nights of collection.

Objective sleep measures.

Objective amounts of sleep maintenance and sleep duration were evaluated using actigraphy (Octagonal Sleepwatch-L, Ambulatory Monitoring, Inc. Ardsley, NY). Along with concurrent sleep diary assessments, actigraphy was completed over a 1-week period in the participants' home environment. Actigraphs recorded activity and illumination exposure in 1-minute epochs using an accelerometer [33, 34]. After completing the 1-week period, actigraphy data quality were scanned for technical and situational artifacts. The Time Above Threshold method within the Action4 software package (Ambulatory Monitoring, Inc. Ardsley, NY) was used to estimate amount of WASO (in min) and TST (in min), which were again averaged across the nights of recording.

Assessment of inflammatory signaling and transcriptional activity.

During the in-person visit, participants also underwent a blood draw in order to evaluate levels of nuclear activation of the following inflammatory signaling and transcriptional pathways. Blood samples were obtained by a study phlebotomist via venipuncture between 08:00 am and 10:00 am of the in-person visit to minimize influences of circadian factors. Levels of activated NF-κB, STAT1, STAT3, and STAT5 were assessed in PBMCs by flow cytometry, as previously described [10, 11]. Briefly, purified PBMCs from heparinized whole blood were fixed, then treated with 90% methanol to permeabilize the nuclear membrane. Intranuclear levels of activated (phosphorylated) NF-κB (p65), STAT1 (pTyr-701), STAT3 (pTyr-705), and STAT5 (pTyr-694) were determined by single-color flow cytometry (FACScan, BD Immunocytometry) using phycoerythrin-labeled antibodies specific for the phosphorylated forms of each transcription factor. Cell Quest software was used

to gate on total PBMCs. A histogram analysis plot was then used to determine the nuclear levels of each activated transcription factor based on the mean fluorescence intensity (MFI).

Statistical analysis

Data were analyzed using SPSS version 28.0 (IBM Inc., USA) and GraphPad Prism version 9.0 (GraphPad Software Inc., USA). The present analysis focused on WASO and TST as measures of sleep maintenance and sleep duration, given the relationship of these variables with inflammation, as well as mortality risk [2, 3]. Both WASO and TST measures were used as continuous variables without specific cutoffs. Other sleep diary- and actigraphy-derived sleep variables, such as sleep efficiency or time in bed, were not included in this study to limit number of comparisons. Due to non-normal distribution, sleep diary and actigraphy data were naturally log-transformed. Prior to conducting our main analysis, we performed a bivariate correlational analysis to test whether diary-assessed and actigraphy-assessed sleep measures correlated with each other. As a first step of our main analysis, a multivariate regression approach was used to test whether poor sleep maintenance (as indexed by greater amount of WASO) and short sleep duration (as indexed by shorter TST) assessed by sleep diary and actigraphy were differentially associated with inflammatory signaling and transcriptional outcomes. Multivariate regression models were separately computed for diary-assessed and actigraphy-assessed sleep measures and controlled for sociodemographic (age, sex, race, and years of education), clinical (BMI and CDS), behavioral (R-UCLA and PSS), and socio-emotional factors (BAI and BDI-II). We created 5 different models: Model 1 was the unadjusted model (which included WASO and TST); Model 2 included sociodemographic variables (Model 1 + adjusted for sex, age, race, and years of education); Model 3 additionally included clinical variables (Model 2 + adjusted for BMI and CDS); Model 4 additionally included behavioral variables (Model 3 + adjusted for R-UCLA and PSS); finally, Model 5 additionally included socio-emotional variables (Model 4 + adjusted for BAI and BDI-II). Strengths of associations were indexed by the standardized regression coefficient (β), which facilitated comparison across models. Given that both long and short sleep duration are associated with increases in inflammation, we also explored whether there might be a non-linear (i.e. quadratic) relationship between sleep duration (i.e. diary- and actigraphy-assessed TST) and inflammatory signaling and transcriptional outcomes. As a second step of our main analysis, a moderation analysis was conducted to explore moderation effects of sex. To test for moderation, we computed separate multivariate regression models, which included a predictor variable (i.e. diary-assessed WASO; or diary-assessed TST; or actigraphy-assessed WASO; or actigraphy-assessed TST), the sex variable, a computed interaction variable term (i.e. "diary-assessed WASO × sex"; or "diary-assessed TST × sex"; or "actigraphy-assessed WASO × sex"; or "actigraphy-assessed TST × sex"), and an inflammatory signaling and transcriptional outcomes variable (i.e. NF-κB; or STAT1; or STAT3; or STAT5). Moderation models were again adjusted for sociodemographic (i.e. age, race, and years of education), clinical (i.e. BMI and CDS), behavioral (i.e. R-UCLA and PSS), and socio-emotional factors (i.e. BAI and BDI-II).

Results

Sample characteristics

Table 1 gives an overview of the sociodemographic, clinical, behavioral, and socio-emotional sample characteristics. Data were available for sleep diary (n = 82), actigraphy (n = 74), and inflammatory signaling and transcriptional measures (n = 132). Overall, the older adult sample showed measures of sleep maintenance and sleep duration that were comparable with other population-based studies in older adults [1]. A bivariate correlational analysis showed correlations between diary-assessed and actigraphy-assessed WASO measures (r = 0.44, p < 0.001) and between diary-assessed and actigraphy-assessed TST measures (r = 0.71, p < 0.001).

Table 1. Sample Characteristics (n = 262)

Sociodemographic variables	
Age, mean years (SD)	71.9 (8.0)
Sex, female %	50.8
Race, nonwhite, %	13.0
Education, mean years (SD)	16.4 (2.8)
Clinical variables	
Body mass index, mean kg/m^2 (SD)	26.3 (4.2)
Chronic disease score, mean score (SD)	2.0 (2.2)
Behavioral variables	
Revised UCLA Loneliness Scale, mean score (SD)	37.8 (11.6)
Perceived Stress Scale, mean score (SD)	31.3 (8.2)
Socio-emotional variables	
Beck anxiety inventory, mean score (SD)	4.7 (5.4)
Beck depression inventory, mean score (SD)	5.5 (5.5)
Sleep variables	
Sleep diary (n = 82)	
WASO, median minutes (IQR)	39 (17 – 59)
TST, median minutes (IQR)	412 (364 – 463)
Actigraphy (n = 74)	
WASO, median minutes (IQR)	30 (20 – 49)
TST, median minutes (IQR)	431 (400 – 471)
Inflammatory variables (n = 132)	
NF-κB, MFI (SD)	27.4 (6.7)
STAT1, MFI (SD)	35.9 (14.4)
STAT3, MFI (SD)	26.0 (8.7)
STAT5, MFI (SD)	29.1 (10.9)

WASO, wake time after sleep onset; MFI, mean fluorescence intensity; SD, standard deviation; IQR, interquartile range.

Subjective sleep measures and inflammatory signaling and transcriptional outcomes

Poor subjective sleep maintenance, as indexed by greater amount of WASO assessed by sleep diary, was associated with higher levels of NF-κB (Model 1: β = 0.34, p < 0.01), but not with levels of STAT family proteins, including STAT1, STAT3, and STAT5 (p's > 0.05). Importantly, the association between poor subjective sleep maintenance and higher levels of NF-κB remained statistically significant after adjusting for sociodemographic (Model 2: β = 0.35, p < 0.01), clinical (Model 3: β = 0.36, p < 0.01), behavioral (Model 4: β = 0.40, p < 0.01), and socio-emotional factors (Model 5: β = 0.42, p < 0.01). In contrast, short subjective sleep duration, as indexed by shorter TST assessed by sleep diary, was not associated with levels of NF-κB or STAT family proteins in unadjusted or adjusted

Table 2. Associations Between Subjective Sleep Measures and Inflammatory Signaling and Transcriptional Outcomes

			NF-κB		
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**
$WASO^{ln}$, min	0.34**	0.35**	0.36**	0.40**	0.42**
TST^{ln}, min	–0.02	0.05	0.05	0.07	0.06
			STAT1		
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**
$WASO^{ln}$, min	–0.09	–0.11	–0.09	–0.09	–0.06
TST^{ln}, min	0.07	0.01	0.01	0.01	0.01
			STAT3		
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**
$WASO^{ln}$, min	–0.03	–0.05	–0.02	–0.01	0.01
TST^{ln}, min	0.06	–0.09	–0.08	–0.08	–0.08
			STAT5		
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**
$WASO^{ln}$, min	0.10	0.05	0.10	0.08	0.14
TST^{ln}, min	–0.08	–0.14	–0.12	–0.13	–0.12

Shown are standardized regression coefficients (β) from multivariate regression models. Model 1 was the unadjusted model (which included WASO and TST); Model 2 included sociodemographic variables (Model 1 + adjusted for sex, age, race, and years of education); Model 3 additionally included clinical variables (Model 2 + adjusted for BMI and CDS); Model 4 additionally included behavioral variables (Model 3 + adjusted for R-UCLA and PSS); finally, Model 5 additionally included socio-emotional variables (Model 4 + adjusted for BAI and BDI-II). *Abbreviations:* WASO, wake time after sleep onset; R-UCLA, revised UCLA Loneliness Scale; PSS, Perceived Stress Scale; BDI-II, Beck Depression Inventory-II.
** $p < 0.01$.

models (p's > 0.05). Associations between subjective sleep measures and inflammatory signaling and transcriptional outcomes are shown in **Table 2**. As noted, we also explored whether there might be a non-linear (i.e. quadratic) relationship between subjective sleep duration assessed by sleep diary and inflammatory signaling and transcriptional outcomes; this possibility was not supported.

Objective sleep measures and inflammatory signaling and transcriptional outcomes

Actigraphy-assessed WASO was not associated with levels of NF-κB, and also was not associated with levels of STAT family proteins (p's > 0.05; data not shown). Similarly, there were no associations between actigraphy-assessed measures of TST and inflammatory signaling and transcriptional outcomes (p's > 0.05; data not shown). Furthermore, exploratory analyses showed no evidence for a non-linear (i.e. quadratic) relationship between objective sleep duration as indexed by actigraphy-assessed TST and inflammatory signaling and transcriptional outcomes.

Moderation analysis

Given evidence that sleep loss induces greater increases in NF-κB in females compared to males [9], we explored moderation effects of sex on the associations between sleep measures and inflammatory signaling and transcriptional outcomes. Adjusting for sociodemographic, clinical, behavioral, and socio-emotional factors, results showed that sex moderated the association between poor subjective sleep maintenance (i.e. greater sleep diary-assessed WASO) and higher levels of STAT1 ($p < 0.01$), STAT3 ($p < 0.01$), and STAT5 ($p < 0.001$), but not NF-κB ($p = 0.11$). Separate follow-up analyses confirmed that poor subjective sleep maintenance (i.e. greater sleep diary-assessed WASO) was associated with higher levels of STAT family proteins in females (STAT1: β = 0.47, $p < 0.05$; STAT3: β = 0.52, $p < 0.05$; STAT5: β = 0.64, $p < 0.01$), but not in males (p's > 0.05). Significant moderation effects are summarized in **Figure 1**. While sex moderated the relationships between poor subjective sleep maintenance and STAT family proteins, there were no moderation effects of sex on the relationship between short self-reported sleep duration (i.e. short sleep diary-assessed TST) and inflammatory signaling and transcriptional outcomes (p's > 0.05). As noted, amounts of objective sleep measures (i.e. actigraphy-assessed WASO and TST) were not associated with levels of NF-κB or STAT family proteins; further, sex had no moderating effect on the relationship between objective sleep measures (i.e. actigraphy-assessed WASO and TST) and inflammatory signaling and transcriptional outcomes (p's > 0.05; data not shown).

Discussion

This study of community-dwelling older adults examined whether disturbance of sleep maintenance and sleep duration differentially contribute to inflammatory signaling and transcriptional pathways. Our results showed that poor self-reported sleep maintenance assessed by sleep diary was uniquely associated with higher levels of NF-κB, along with higher levels of STAT family proteins in females, but not in males. Conversely, self-reported sleep duration assessed by sleep diary was not associated with inflammatory signaling and transcriptional outcomes. Similarly, poor objective sleep maintenance and sleep duration assessed by actigraphy were not associated with inflammatory signaling and transcriptional outcomes, neither in the overall sample, and there were no sex moderation effects.

First, our results showed that poor self-reported sleep maintenance, but not shortself-reported sleep duration, was associated with higher levels of NF-κB. Importantly, the relationship between poor self-reported sleep maintenance and higher levels of NF-κB

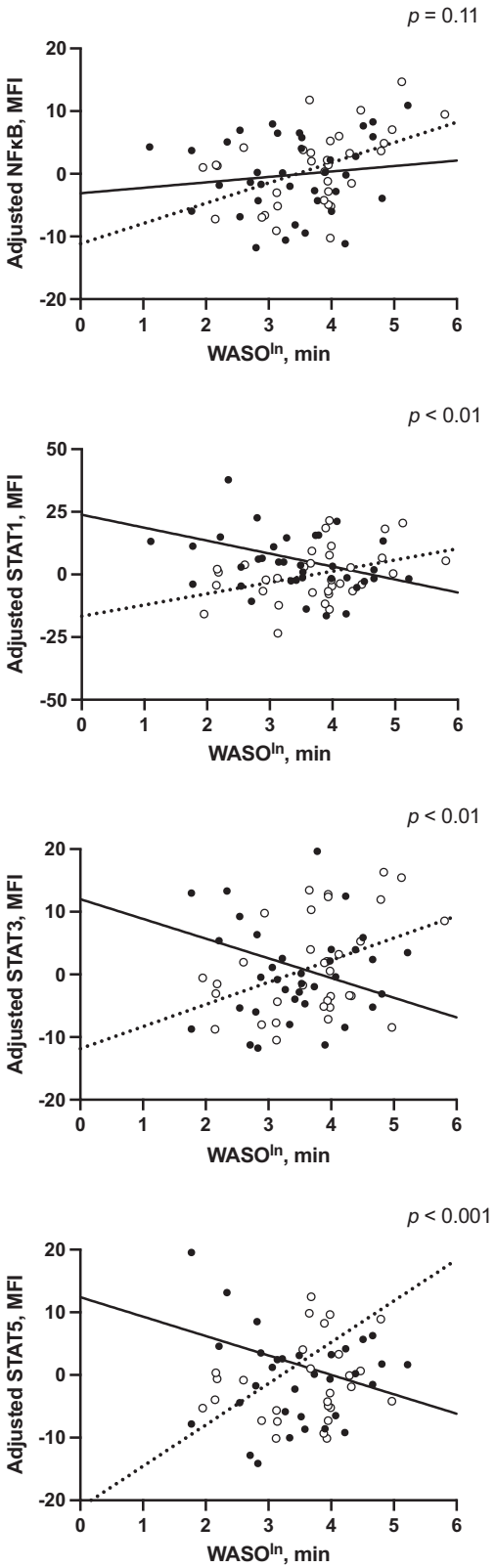


Figure 1. Moderation analysis. Shown are scatterplots and linear prediction lines for associations between sleep diary-derived WASO and inflammatory signaling and transcriptional outcomes in PBMCs in females (dashed line) and males (solid line). All scatterplots are adjusted for sociodemographic (age, race, and years of education), clinical (BMI and CDS), behavioral (R-UCLA and PSS), and socio-emotional factors (BAI and BDI-II). Residualized levels (i.e. error between predicted and observed value) of NF-κB, STAT1, STAT3, and STAT5 are plotted on the y-axis. Shown *P*-values represent significant interactions. *Abbreviations:* NF, nuclear factor; STAT, signal transducer and activator of transcription; WASO, wake time after sleep onset; MFI, mean fluorescence intensity; PBMCs, peripheral blood mononuclear cells; R-UCLA, revised UCLA Loneliness Scale; PSS, Perceived Stress Scale; BDI-II, Beck Depression Inventory-II.

remained significant even after adjusting for sociodemographic, clinical, behavioral, and socio-emotional factors. This observation parallels previous findings by our group, which showed that older adults with clinical sleep disturbance (i.e. insomnia disorder) exhibit a higher prevalence of transcription factor binding motifs of genes related to NF-κB signaling as compared to those without insomnia [35]. However, diary-assessed sleep measures, including sleep maintenance and sleep duration, were not associated with levels of STAT family proteins, which was surprising, given that previous work of our group showed that experimental sleep loss increases activation of STAT family proteins [11]. Similarly, actigraphy-assessed sleep measures were not associated either with NF-κB or STAT activation.

Second, our results showed a moderating role of sex for the relationship between sleep measures and inflammatory signaling and transcriptional outcomes. Poor self-reported sleep maintenance was associated with higher levels of STAT1, STAT3, and STAT5 in females, but not in males, whereas sex did not moderate the relationship between sleep measures and levels of NF-κB. These results are in contrast to findings in a previous study by our group, which suggested experimental sleep loss induces an increase in the activation of NF-κB only in females [10], and increases activation of STAT proteins in both males and females [11]. However, it is noteworthy, that the assessment of associations between naturalistic aspects of sleep disturbance and activation of inflammatory signaling and transcriptional measures—as conducted in the present study—is a substantially different approach compared to employing experimental sleep deprivation to induce NF-κB and STAT activation. While prior human research on sex differences in NF-κB and STAT protein activity is sparse, previous work in animals has suggested that estrogen, a sex hormone responsible for the development and regulation of the female reproductive system and secondary sex characteristics, promotes activation of NF-κB and STAT proteins in the inflammatory response [36], and that females show greater activation of STAT protein signaling compared to males [37]. Paralleling these findings in animals, our data might provide a better understanding of potential sex differences regarding the association between sleep disturbance and age-related mortality and morbidity.

Given that sleep, disturbance induces NF-κB and STAT signaling, that both signaling and transcription factor families play a role in inflammatory-disease risk, and that poor sleep maintenance and short sleep duration predict increases in mortality and morbidity risk in older adults, self-reported sleep maintenance might play a salient role for age-related mortality and morbidity risk. Indeed, this framework is in line with prior research which proposed inflammatory markers as mediators of the relationship between sleep duration and mortality in community-dwelling adults [3, 38]. However, why would poor self-reported sleep maintenance be more strongly related to inflammatory signaling and transcriptional activity, rather than short self-reported sleep duration or objective measures of sleep disturbance? Indeed, several lines of research have highlighted the importance of perception of sleep maintenance over self-reported sleep duration or objective evaluation of sleep complaints. For example, the DSM-5 diagnosis

of insomnia is solely based on the self-reported complaints of difficulty initiating or maintaining sleep, early awakening, interrupted or non-restorative sleep, and associated impairments in daytime functioning. Furthermore, the American Academy of Sleep Medicine does not recommend polysomnography (PSG) for assessment of insomnia, although it is typically used in the evaluation of other sleep disorders such as sleep apnea [39]. Finally, objective measures of sleep (i.e. PSG) are known to provide relatively little information to confirm or exclude insomnia in a study of patients with insomnia and controls [40]. To further demonstrate the complexity of our findings, it is important to note that sleep disturbance and inflammation share a reciprocal relationship [41]. In the context of sleep maintenance (i.e. greater WASO) and inflammation, previous research has shown that experimental activation of the inflammatory response, such as exposure to typhoid vaccination [42] or endotoxin [43], have the potential to increase WASO. Conversely, administration of cytokine antagonists, such as the tumor necrosis factor-α antibody infliximab, is reported to decrease WASO [44, 45]. Paralleling these findings from experimental research, clinical observational studies have reported that patients with an inflammatory disorder, such as inflammatory bowel disease, multiple sclerosis, and rheumatoid arthritis, present with increases in WASO [46–48]. However, no prior study has evaluated whether inflammation induces alterations in WASO in older adults.

Strengths and limitations

This is the first study to examine whether disturbance of sleep maintenance and sleep duration assessed by sleep diary and actigraphy play differential roles for inflammatory signaling and transcriptional pathways in older adults, a population that is well-known to frequently report sleep disturbance and present with increases in inflammation. However, the present study does not come without limitations. While subjects who reported a current or lifetime history of sleep disorder, including sleep apnea, nocturnal myoclonus, phase-shift disorder, or primary insomnia, were excluded from participation, this information was based on self-report and not on apparatus-based diagnostics. Moreover, given the secondary nature and the observational cross-sectional design of our analysis, the overall interpretations regarding a cause-effect relationship are limited and future studies are needed to evaluate our findings.

Clinical implications

Our data might have important clinical implications, given that poor self-reported sleep maintenance and inflammation are thought to jointly contribute to various age-related health adversities, such as depression [49], cognitive impairment [50], and Alzheimer's disease [51]. Importantly, poor self-reported sleep maintenance, as indexed by greater amount of WASO, is an important measure of sleep maintenance that can be targeted by behavioral interventions, such as cognitive behavioral therapy for insomnia (CBT-I). For example, consistent evidence from randomized controlled trials has not only shown that CBT-I has promising effects to improve sleep outcomes [52–55], but that CBT-I can also revert the inflammatory dynamics that emerge in association with sleep disturbance [56, 57]. A robust body of evidence has shown that poor sleep maintenance and short sleep duration also activate inflammatory pathways in young adults [58]. However, older adults might be particularly at risk for sleep-immune dysfunction for several reasons: first, "usual" aging is associated with various changes in sleep behavior, including increases in WASO and decreases in TST [59]; second, up to 50% of older adults are estimated to experience difficulties maintaining sleep [60]; third, usual aging is associated with low-level increases in systemic inflammation, a condition commonly referred to as "inflammaging" [24, 61–64]; fourth, experimental studies have suggested that cellular aging may be particularly sensitive to sleep loss, and that a single night of PSD increases leukocyte gene expression indicative of DNA damage responses and the senescence-associated secretory phenotype in older adults [65]. However, whether improving sleep maintenance also reduces age-related mortality risk by mitigating inflammatory signaling and transcriptional pathways is unknown and future research is needed to test this question.

Supplementary Material

Supplementary material is available at *SLEEP* online.

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Disclosure Statement

None declared.

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