Interferon-γ moderation of poor sleep maintenance and depressed mood in community-dwelling older adults

Dominique Piber[1,2,3] (iD), Richard Olmstead[1], Joshua H. Cho[1], Miguel Guzman[1] and Michael R. Irwin[1,4]

[1]Department of Psychiatry and Biobehavioral Sciences, Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and Human Behavior, David Geffen School of Medicine, University of California Los Angeles (UCLA), Los Angeles, CA, USA; [2]Department of Psychiatry, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; [3]Berlin Institute of Health (BIH), Berlin, Germany and [4]Department of Psychology, College of Arts and Sciences, University of California, Los Angeles, CA, USA

Abstract

Background. Depressive symptoms, such as depressed mood, are common in older adults and associated with an increased risk for morbidity and mortality. Given the evidence that sleep disturbance and alterations in interferon (IFN)-γ biology are associated with depression risk, this study examines the separate and joint contributions of poor sleep maintenance and IFN-γ to depressed mood in older adults.

Methods. Community-dwelling, non-depressed older adults ($n = 36$, 72.1 ± 6.8 years) underwent a night of polysomnography to assess sleep maintenance [i.e. wake time after sleep onset (WASO)]. The morning after polysomnography, plasma levels of IFN-γ were evaluated along with self-reported depressed mood throughout the day. Multivariate linear regression tested associations of WASO and IFN-γ with the severity of depressed mood. In addition, moderation and mediation models examined the role of IFN-γ for the relationship between WASO and depressed mood.

Results. A greater amount of WASO ($p < 0.05$) and higher levels of IFN-γ ($p < 0.01$) were both associated with the severity of depressed mood. Moreover, IFN-γ moderated the relationship between WASO and depressed mood ($p < 0.01$), such that WASO was more strongly related to the depressed mood among those with higher IFN-γ, than among those with lower IFN-γ. However, IFN-γ did not mediate the relationship between WASO and depressed mood.

Conclusion. In this study of older adults, poor sleep maintenance and higher levels of IFN-γ were both related to depressed mood. Moreover, IFN-γ moderated the relationship between poor sleep maintenance and depressed mood. Together, these findings suggest that older adults with higher IFN-γ are at heightened risk for depressive symptoms following sleep disturbance.

Introduction

Major depression is a highly prevalent psychiatric illness that occurs in approximately 7% of community-dwelling older adults (World Health Organization, 2017). In addition, another 10–15% of older adults are estimated to exhibit subclinical depressive symptoms, such as depressed mood, even in the absence of the clinical DSM-5 diagnosis of major depression (Kok & Reynolds, 2017). Importantly, in older adults, longitudinal studies have found that the presence of depressive symptoms not only leads to functional impairment (Grabovich, Lu, Tang, Tu, & Lyness, 2010), but also substantially increases the risk for morbidity and mortality (Ariyo et al., 2000; Schulz et al., 2000; Simonsick, Wallace, Blazer, & Berkman, 1995; Whooley & Browner, 1998). Given that roughly one in five adults in the United States is projected to be 60 years or older by 2030 (Centers for Disease Control and Prevention, 2013), there is an urgent need to identify the biological mechanisms that contribute to depressive symptoms in older adults, which could help improve prevention strategies to mitigate age-related risk for morbidity and mortality.

Poor sleep maintenance, as typically indexed by increased amounts of time spent awake in bed after initially falling asleep (also known as 'wake time after sleep onset' or WASO), is a common health complaint among older adults (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). Indeed, a large population-based survey by the National Sleep Foundation found that about two-thirds of community-dwelling older adults in the United States complain of poor sleep maintenance at least a few nights per week (National Sleep Foundation, 2003). Importantly, longitudinal data from community-dwelling, non-depressed older adults

have identified poor sleep maintenance, as indexed by actigraphy-measured WASO, as a salient risk factor that increases the odds of developing depressive symptoms over a 5-year follow-up period (Maglione et al., 2014). Moreover, evidence from a cross-sectional study in older adults revealed that the severity of depressive symptoms is specifically associated with an increased amount of WASO, but not with other dimensions of sleep, such as sleep efficiency, sleep onset latency, or total sleep time (Maglione et al., 2012). Whereas these data suggest that an increased amount of WASO may be a key sleep component that drives the development of depressive symptoms in older adults, little is known about the mechanisms that underlie this relationship.

Inflammation (i.e. the excessive activation of the immune system) has recently emerged as a biologically plausible pathway by which poor sleep maintenance might translate to depressive symptoms in older adults. Indeed, both experimentally induced as well as naturally occurring disturbances of sleep maintenance have previously been shown to activate inflammatory pathways at transcriptional, cellular, and systemic levels (Irwin, 2015; Irwin & Opp, 2017). In turn, several cross-sectional (Doyle et al., 2013; Glaser, Robles, Sheridan, Malarkey, & Kiecolt-Glaser, 2003; Penninx et al., 2003; White, Kivimäki, Jokela, & Batty, 2017) as well as prospective studies (Au, Smith, Gariépy, & Schmitz, 2015; Bell, Kivimäki, Bullmore, Steptoe, & Carvalho, 2017; Gimeno et al., 2009; Matheny et al., 2011; Niles, Smirnova, Lin, & O'Donovan, 2018; Valkanova, Ebmeier, & Allan, 2013; Zalli, Jovanova, Hoogendijk, Tiemeier, & Carvalho, 2016) have suggested that raised levels of systemic inflammatory markers, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α or C-reactive protein (CRP), promote the development of depressive symptoms in older adults, although some studies have also yielded mixed or negative results regarding such associations (Adriaensen et al., 2014a, 2014b; Bondy et al., 2021; Bremmer et al., 2008; Dimopoulos, Piperi, Psarra, Lea, & Kalofoutis, 2008; Stewart, Rand, Muldoon, & Kamarck, 2009; van den Biggelaar et al., 2007). Less is known about the role of endogenous interferon (IFN)-γ, a signature cytokine that is centrally involved in the cellular immune response to viral and microbial infections (Ivashkiv, 2018; Miller, Maher, & Young, 2009; Pollard, Cauvi, Toomey, Morris, & Kono, 2013; Zhang, 2007), in the link between poor sleep maintenance and depressive symptoms in older adults. Interestingly, some evidence suggests that poor sleep maintenance might alter various molecular components involved in IFN-γ-related pathways. For example, our group has previously demonstrated that experimentally induced disruption of sleep maintenance (i.e. partial sleep deprivation) leads to an increase in nuclear activation of signal transducer and activator of transcription 1 (STAT1), a cytoplasmatic protein that conveys the IFN-γ-related transcriptional signal (Irwin, Witarama, Caudill, Olmstead, & Breen, 2015). Moreover, observational studies found that self-reported difficulties maintaining sleep (Huang et al., 2017) as well as clinical sleep disturbance (Jain et al., 2012) are both associated with elevated plasma levels of IFN-γ-inducible protein 10 (IP-10), which is a chemokine produced in response to IFN-γ stimulation. In turn, evidence from clinical studies implicates that alterations in IFN-γ-related pathways play a role in the development of depressive symptoms. Several clinical studies, for example, have demonstrated that patients with major depression present with increased levels of IFN-γ compared to non-depressed healthy controls (Dahl et al., 2014; Maes et al., 1994; Schmidt et al., 2014; Seidel et al., 1995; Simon et al., 2008), and various antidepressant medications are

reported to suppress the production of IFN-γ (Brustolim, Ribeiro-dos-Santos, Kast, Altschuler, & Soares, 2006; Kubera et al., 2001; Maes et al., 1999; Mohr, Goodkin, Islar, Hauser, & Genain, 2001). Moreover, variations in the IFN-γ gene have previously been found to modify both depression risk (Oxenkrug et al., 2011) as well as the efficacy of antidepressant treatments (Myint et al., 2013). In addition, in clinical patient populations, the expression of IFN-γ ribonucleic acid (RNA) has been found to correlate with depressive symptoms (Kahl, Kruse, Faller, Weiss, & Rieckmann, 2002). Paralleling these findings from human studies, work in animals has also demonstrated an involvement of IFN-γ-related pathways in depressive-like behavior (Campos, Vaz, Saito, & Teixeira, 2014; Kwant & Sakic, 2004; Litteljohn, Nelson, & Hayley, 2014; O'Connor et al., 2009). However, no prior study has examined whether poor sleep maintenance and alterations in IFN-γ biology act in concert to jointly contribute to depressive symptoms. Indeed, such joint effects might be particularly salient in older adults, a population that is well-known to show a high prevalence of sleep disturbance (Li, Vitiello, & Gooneratne, 2018) as well as increases in inflammation (Campisi, 2013; Franceschi et al., 2000; Franceschi & Campisi, 2014; Kennedy et al., 2014; Piber et al., 2019).

To address this gap, we objectively measured sleep maintenance as indexed by amounts of WASO during a night of naturalistic sleep with polysomnography (PSG) in a sample of community-dwelling, non-depressed older adults. The following morning, plasma levels of IFN-γ were evaluated, along with repeated assessments of self-reported depressed mood throughout the day as a cardinal depressive symptom. We hypothesized: (1) that poor sleep maintenance (as indexed by a greater amount of WASO) and (2) higher morning levels of IFN-γ would both be associated with greater severity of depressed mood throughout the following day; and (3) that the association between WASO and depressed mood would be moderated by IFN-γ. In addition, we explored whether IFN-γ would mediate the link between WASO and depressed mood (i.e. whether WASO would negatively influence mood through altering levels of IFN-γ).

Materials and methods

Procedures

Participants were community-dwelling, non-depressed older adults, who were recruited from the *Sleep Health and Aging Research* (*SHARE*) field study sample (Piber et al., 2019). Of 262 older adults who participated in the *SHARE* field study, 40 older adults agreed to participate in an additional sleep laboratory component (15.3% of the field study sample). Of this sample, complete sleep, immune, and behavioral data were available from 36 participants (13.7% of the field study sample). Participants were older adults aged 60 years and above, and free of current medical disorders, psychiatric disorders (including major depression as evaluated by the Structured Clinical Interview for DSM-IV Axis I Disorders), and sleep disorders (including insomnia disorder, sleep apnea, restless leg syndrome). Moreover, participants were free of medications with known effects on inflammation, such as anti-inflammatory, analgesic, or psychotropic medications.

For the current study, participants underwent a baseline assessment to evaluate various socio-demographic and clinical characteristics, followed by 2 weeks of outpatient actigraphy to confirm regular sleep patterns. Subsequently, participants were

admitted to the sleep laboratory at the Clinical Translational Research Center (CTRC) at UCLA, where they underwent several nights of PSG, including an adaptation night (first night) and an evaluation night of naturalistic sleep (second night). In the morning after the evaluation night, blood samples were obtained for the assessment of plasma levels of IFN-γ, along with repeated evaluation of self-reported depressed mood throughout the day. Participants provided written consent prior to the enrollment. The study received oversight and approval from the UCLA Institutional Review Board (IRB#11-000656).

Baseline measures

During the baseline assessment, various socio-demographic and clinical characteristics were evaluated including the body mass index (BMI). The presence of medications other than the anti-inflammatory, analgesic, or psychotropic medications was evaluated using the chronic disease score (CDS) (Von Korff, Wagner, & Saunders, 1992). Furthermore, given the known effects of physical activity on depressive symptoms (Mura & Carta, 2013), amounts and patterns of physical activity were assessed using the Yale Physical Activity Survey for Older Adults (YPAS) Part II (Dipietro, Caspersen, Ostfeld, & Nadel, 1993). The YPAS Part II evaluates the frequency and duration of physical activity in the last month across five distinct dimensions: vigorous activities, leisurely walking, moving, standing, and sitting. For each of these five dimensions, an index score is calculated by multiplying the responses by a specific weight factor. Subsequently, the five index scores are summed to determine a YPAS summary score.

Laboratory-based measures

Assessment of sleep measures

Following the baseline assessment, participants underwent 2 weeks of outpatient actigraphy to screen for regular sleep patterns with sleep periods between 10:00 p.m. and 7:00 a.m. Subsequently, participants were admitted to the sleep laboratory at CTRC, where they underwent all PSG procedures. Participants underwent several nights of PSG, including an adaptation night (first night) and an evaluation night of naturalistic sleep (second night). During the evaluation night, sleep maintenance was assessed, as indexed by WASO [i.e. the time (in minutes) spent awake in bed after the initial onset of sleep]. During the daytime, subjects were not allowed to consume caffeine or engage in any exhausting physical activity in order to reduce potential stress. Amounts of WASO were scored by a trained sleep technician according to the American Academy of Sleep Medicine (Berry et al., 2012).

Assessment of inflammatory markers

Plasma levels of IFN-γ were evaluated at 8:00 a.m. following the evaluation night of PSG using the Meso Scale Discovery (MSD) MULTI-SPOT Assay System (Rockville, MD). Plasma samples were assayed in duplicate on a custom 5-plex from the Proinflammatory Panel 1 Human Kit. In addition to IFN-γ, MSD assays also evaluated plasma levels of TNF-α, IL-6, IL-8, and IL-10. Given the *a priori* hypothesis and specific focus on IFN-γ, analyses of TNF-α, IL-6, IL-8, and IL-10 were exploratory. Briefly, after a period of overnight fasting, blood samples were collected in EDTA tubes, and processed at 4 °C to plasma aliquots. Plasma aliquots were stored at −80 °C until assayed in a single

batch. Assays were performed according to the manufacturer's protocol. ECL signals were measured on the MESO QuickPlex SQ 120 instrument (Rockville, MD), and the DISCOVERY WORKBENCH software (Rockville, MD) was used to generate a 4-parameter logistic fit curve. The mean intra-assay coefficient of variation (CV) for IFN-γ was less than 6%, and the mean inter-assay CV less than 12.2%, with similar CVs for other inflammatory markers.

Assessment of depressed mood

Throughout the day following the evaluation night of PSG, presence and severity of depressed mood was evaluated at 8:00 a.m. directly following the blood draw, and again at 2:00 p.m. and at 10:00 p.m. using the short-form of the Profile of Mood States Depression Subscale (POMS-D) (Baker, Denniston, Zabora, Polland, & Dudley, 2002). POMS-D is a self-administered questionnaire that assesses the extent to which a person experiences a depressed mood in the present moment, including feelings such as unhappy, sad, blue, hopeless, discouraged, miserable, helpless, and worthless. For each feeling, a subjective self-rating is collected on a 0–4 scale (0 = not at all; 1 = a little; 2 = moderately; 3 = quite a lot; 4 = extremely), resulting in a sum score with higher scores indicating greater severity of depressed mood. To account for circadian fluctuations of depressed mood, POMS-D scores were averaged across the 3 time points.

Statistical analyses

Data were analyzed using SPSS version 28 (IBM Inc., USA). To reduce skewedness of sleep and immune data, WASO and IFN-γ values were natural log-transformed. Prior to examining our main hypotheses, a series of partial correlations were computed to examine the relationships between WASO and assessed immune markers (i.e. IFN-γ, TNF-α, IL-6, IL-8, IL-10), while controlling for age, sex and BMI. To test the first hypothesis, that poor sleep maintenance as indexed by a greater amount of WASO would be associated with greater severity of depressed mood, a multivariate linear regression approach was used with WASO as an independent variable, and averaged POMS-D scores across the 3 time points as a dependent variable. To control for socio-demographic and clinical factors as well as levels of physical activity, we computed four different models: Model 1 was the unadjusted model (WASO only); Model 2 further included socio-demographic variables (Model 1 + age, sex, race, education); Model 3 further included clinical variables (Model 2 + BMI and CDS); finally, Model 4 further included levels of physical activity (Model 3 + YPAS summary score). To test the second hypothesis, that higher levels of IFN-γ would be associated with greater severity of depressed mood, an identical multivariate approach was used: Model 1 was the unadjusted model (IFN-γ only); Model 2 further included socio-demographic variables (Model 1 + age, sex, race, education); Model 3 further included clinical variables (Model 2 + BMI and CDS); Model 4 further included levels of physical activity (Model 3 + YPAS summary score). Using an identical regression modeling approach, exploratory analyses examined whether any of the other inflammatory markers, including TNF-α, IL-6, IL-8, and IL-10, were associated with the severity of depressed mood. To test the third hypothesis, that the association between WASO and depressed mood would be moderated by IFN-γ, we conducted a moderation analysis (online Supplementary Fig. S1). To additionally explore a potential mediation effect of IFN-γ on the relationship between WASO

and depressed mood, we also conducted a mediation analysis (online Supplementary Fig. S2). Both moderation and mediation analyses were performed using the PROCESS macro for SPSS version 4.0 (Hayes, 2017). To test for moderation, we used WASO as an independent variable, IFN-γ as a moderator variable, and depressed mood (averaged POMS-D scores across the 3 time points) as an outcome variable. The PROCESS macro automatically created a WASO × IFN-γ interaction term and subsequently computed conditional follow-up simple slope tests, which allowed probing associations between WASO and depressed mood stratified by higher (i.e. one standard deviation above the mean) and lower IFN-γ (i.e. one standard deviation below the mean). To test for mediation, we used WASO as an independent variable, IFN-γ as mediator variable, and depressed mood (POMS-D scores across the 3 time points) as the outcome variable to conduct the following analyses: (1) depressed mood was regressed on WASO; (2) IFN-γ was regressed on WASO; (3) the depressed mood was regressed on IFN-γ; (4) depressed mood was regressed on both WASO and IFN-γ simultaneously. To estimate the indirect effect (i.e. whether WASO negatively influenced mood through altering levels of IFN-γ), the bootstrapping method was used with 10 000 bootstrapped resamples. The indirect effect was considered to be significant when the 95% confidence interval did not include 0.

Results

Sample characteristics

Table 1 summarizes the sample's baseline characteristics. The sample had a mean age of 72.1 ± 6.8 years (range: 61–86 years), with 52.8% females. Levels of physical activity in the last month were normal compared to other studies in older adult populations (Kruskall, Campbell, & Evans, 2004). There were no differences in socio-demographic variables, clinical characteristics or levels of physical activity between those who did ($n = 40$) and those who did not ($n = 222$) participate in the sleep laboratory component of the *SHARE* study (all p's > 0.05, online Supplementary Table S1). As shown in Table 2, the sample had modest disturbances of sleep maintenance, as indexed by a median WASO of 55 min, which was consistent with meta-analytical data on sleep characteristics in older adults (Ohayon et al., 2004).

Correlational analyses

Controlling for sex, age and BMI, partial correlational analyses showed that WASO correlated with IFN-γ ($r = 0.35$, $p < 0.05$), but not with any of the other immune markers, including TNF-α, IL-6, IL-8, and IL-10 (online Supplementary Table S2), which indicated that greater amount of WASO was associated with higher levels of IFN-γ (Fig. 1).

Associations between sleep maintenance and depressed mood

First, we examined whether poor sleep maintenance as indexed by WASO would be associated with greater severity of depressed mood throughout the following day, while controlling for socio-demographic, clinical and behavioral factors (online Supplementary Table S3). In the unadjusted model, a greater amount of WASO was associated with greater severity of depressed mood (Model 1: $\beta = 0.43$, $p < 0.05$). Importantly, this association remained significant after adjusting for age, sex, race, and education (Model 2: $\beta = 0.42$, $p < 0.05$), BMI and CDS

Table 1. Baseline measures ($n = 36$)

	%	Mean (s.d.)	Range
Socio-demographic variables			
Age, years		72.1 (6.8)	61–86
Sex, female	52.8		
Race			
American Indian/Alaskan Native	–		
Asian	–		
Black/African American	11.1		
Native Hawaiian/Pacific Islander	–		
White	86.1		
Other/Multiracial	2.8		
Ethnicity			
Hispanic/Latino	11.1		
Non-Hispanic/Non-Latino	88.9		
Education, years		16.1 (2.7)	12–24
Clinical variables			
Body mass index, kg/m^2		26.5 (4.1)	18.9–36.0
Chronic disease score		1.3 (1.3)	0–5
Physical activity			
YPAS summary score		62.8 (29.2)	17–125

YPAS, Yale Physical Activity Survey for Older Adults; s.d., standard deviation.

Table 2. Laboratory-based measures ($n = 36$)

Measures	Median (IQR)
WASO[a], min	55 (34–89)
IFN-γ[b], pg/mL	5.3 (3.4–8.8)
Averaged POMS-D score[c]	1 (0–2)

WASO, wake time after sleep onset; IFN, interferon; POMS-D, Profile of Mood States – Depression Subscale; PSG, polysomnography; IQR, interquartile range.
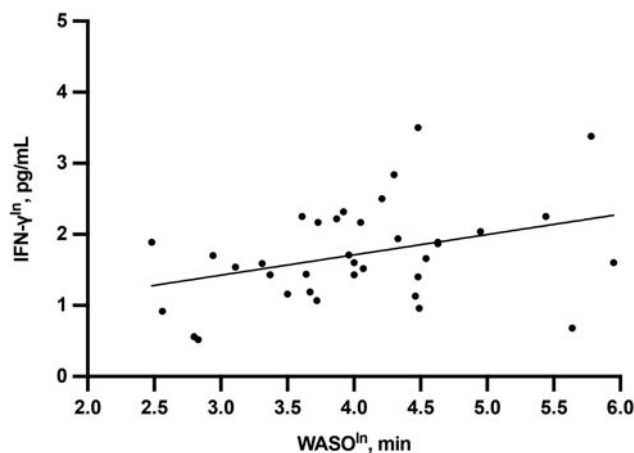[a]Assessed during the evaluation night of PSG.
[b]Assessed at 8:00 a.m. following the evaluation night of PSG.
[c]Assessed at 8:00 a.m., 2:00 p.m., and 10:00 p.m. following the evaluation night of PSG (POMS-D scores for each individual were averaged across the 3 time points).

(Model 3: $\beta = 0.43$, $p < 0.05$), as well as levels of physical activity (Model 4: $\beta = 0.43$, $p < 0.05$). Similar results were found for each of the three individual timepoints (data not shown).

Associations between IFN-γ and depressed mood

Second, we examined whether morning levels of IFN-γ would be associated with greater severity of depressed mood throughout the following day, while again controlling for socio-demographic, clinical and behavioral factors (online Supplementary Table S4). In the unadjusted model, heightened morning levels of IFN-γ were associated with greater severity of depressed mood (Model 1: $\beta = 0.47$, $p < 0.01$). Again, this association remained significant after adjusting for age, sex, race, and education (Model 2: $\beta = 0.44$, $p < 0.01$), BMI and CDS (Model 3: $\beta = 0.46$, $p < 0.01$), as well as physical activity (Model 4: $\beta = 0.47$, $p < 0.01$). Again, similar results were found for each of the three individual timepoints

Fig. 1. Association between WASO and levels of IFN-γ. Shown is a scatterplot of WASO and IFN-γ levels. WASO was assessed during the evaluation night of PSG; IFN-γ was assessed at 8:00 a.m. following the night of PSG. WASO, wake time after sleep onset; IFN, interferon; PSG, polysomnography.

(data not shown). However, exploratory analyses found that none of the other inflammatory markers, including TNF-α, IL-6, IL-8, and IL-10, were associated with severity of depressed mood (data not shown).

Moderation analysis

Third, to examine whether WASO would be more strongly associated with severity of depressed mood among those with higher levels of IFN-γ, than among those with lower levels of IFN-γ, we conducted a moderator analysis. As hypothesized, results showed that the association between WASO and depressed mood was moderated by levels of IFN-γ, as indicated by a significant WASO × IFN-γ interaction [$b = 2.59$, 95% CI (1.03–4.15), $p < 0.01$]. Follow-up simple slope tests confirmed that the slope for one standard deviation above the mean levels of IFN-γ was positive and significantly different from zero [$b = 3.67$, 95% CI (1.80–5.54), $p < 0.001$], while the slope for one standard deviation below the mean level of IFN-γ was not significantly different from zero [$b = 0.11$, 95% CI (−1.42 to 1.64), $p = 0.881$]. Together, this indicated that a greater amount of WASO was more strongly associated with severity of depressed mood among older adults with higher levels of IFN-γ, than among those with lower levels of IFN-γ (Fig. 2).

Mediation analysis

To further evaluate the directional nature of relationships among WASO, IFN-γ, and depressed mood, we additionally conducted an exploratory mediation analysis (Fig. 3). Results showed significant associations between WASO and IFN-γ (a-path: $\beta = 0.35$, $p < 0.05$), between IFN-γ and depressed mood (b-path: $\beta = 0.35$, $p < 0.05$), between WASO and depressed mood while controlling for IFN-γ (direct effect/c′-path: $\beta = 0.31$, $p < 0.05$), and between WASO and depressed mood without controlling for IFN-γ (total effect/c-path: $\beta = 0.43$, $p < 0.01$). Interestingly, the association between WASO and severity of depressed mood was attenuated by about 28% when accounting for IFN-γ, which suggested a mediation effect, although the model was not statistically significant [i.e. the 95% confidence interval of the

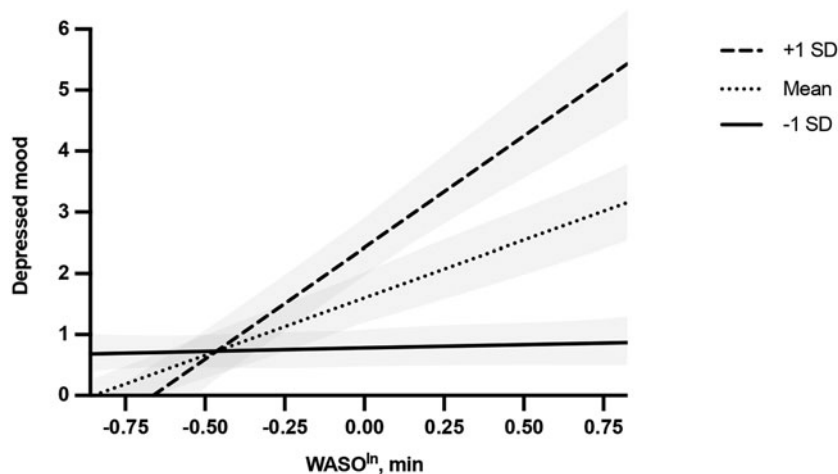association between WASO and depressed mood via IFN-γ (indirect effect/ab-path) included zero].

Discussion

This is the first study to show that in community-dwelling, non-depressed older adults, objectively measured disturbance of sleep maintenance as evaluated by PSG as well as heightened morning plasma levels of IFN-γ are associated with greater severity of depressed mood throughout the following day. Moreover, our data show that the link between poor sleep maintenance and depressed mood is moderated by IFN-γ, such that poor sleep maintenance is more strongly associated with severity of depressed mood among older adults with higher IFN-γ, than among those with lower IFN-γ. Whereas correlational analyses showed that WASO was related to IFN-γ, we did not find an 'indirect' (i.e. mediation) effect of IFN-γ on the relationship between WASO and depressed mood.

First, our data showed that even modest disturbance of sleep maintenance as assessed by PSG was associated with greater severity of self-reported depressed mood throughout the following day. These observations were consistent with previous data from community-dwelling older adults which demonstrated that increases in WASO assessed by actigraphy are associated with severity of depressive symptoms (Maglione et al., 2012, 2014). Moreover, our findings are in line with experimental data which have demonstrated that experimental disruption of sleep maintenance (i.e. sleep deprivation) induces depressed mood as assessed by POMS-D (Scott, McNaughton, & Polman, 2006). In addition, our findings parallel longitudinal data which have shown that the presence of clinical sleep disturbance is associated with an increased risk to develop major depression in both the general population (Baglioni et al., 2011) and in older adults (Cho et al., 2008; Lee et al., 2013).

Second, our data showed that heightened morning levels of IFN-γ were associated with greater severity of depressed mood throughout the following day. While no prior study specifically examined putative associations between levels of IFN-γ and depressed mood in older adults, our results are partially paralleled by previous clinical studies on the role of IFN-γ in major depression. Indeed, several studies have demonstrated that patients with major depression show increased production of IFN-γ as compared to non-depressed healthy controls (Dahl et al., 2014; Maes et al., 1994; Schmidt et al., 2014; Seidel et al., 1995; Simon et al., 2008), although meta-analytical work concluded that patients with major depression show comparable (Dowlati et al., 2010; Liu, Ho, & Mak, 2012) or even lower levels of IFN-γ compared to non-depressed individuals (Köhler et al., 2017). In addition, polymorphism in the IFN-γ gene in human subjects has been linked to increased activity of the indoleamine-2,3-dioxygenase (IDO) (Raitala, Pertovaara, Karjalainen, Oja, & Hurme, 2005), a key enzyme that catalyzes tryptophan to kynurenine, which in turn is known to contribute to the development of inflammation-driven depressive symptoms (Dantzer, O'Connor, Lawson, & Kelley, 2011; Vancassel, Capuron, & Castanon, 2018). Paralleling these findings from human studies, also data from mechanistic animal models have proposed a pathway in which IFN-γ induces depressive-like behavior through increasing IDO activation (O'Connor et al., 2009). Thus, our data extend previous work on the role of IFN-γ for mood regulation by demonstrating that higher morning levels of IFN-γ are associated with greater severity of self-reported

Fig. 2. Moderation analysis. Shown are simple slopes with 95% confidence bands of the associations between WASO and depressed mood in subjects with higher (i.e. one s.d. above the mean; dashed line) and lower levels of IFN-γ (i.e. one s.d. below the mean; solid line). The x-axis shows mean centered values of natural log-transformed WASO values, the y-axis shows averaged POMS-D score across the 3 time points. WASO, wake time after sleep onset; IFN, interferon; POMS-D, Profile of Mood States – Depression Subscale; s.d., standard deviation.
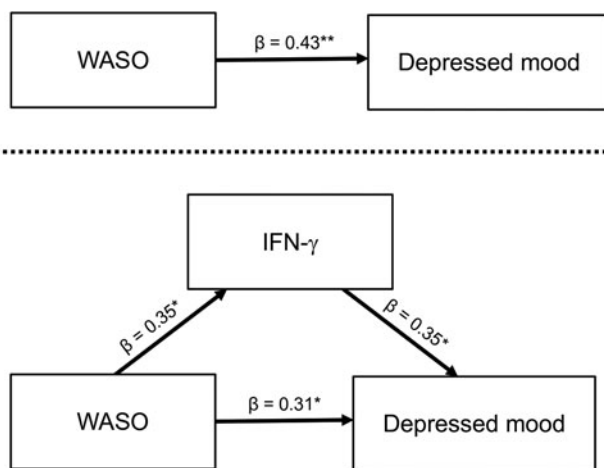
Fig. 3. Mediation analysis. Shown is the exploratory model of IFN-γ mediating the relationship between WASO and depressed mood. WASO was assessed during the evaluation night of PSG; IFN-γ was assessed at 8:00 a.m. following the night of PSG; the depressed mood was assessed at 8:00 a.m., 2:00 p.m., and 10:00 p.m. following night of PSG (POMS-D scores for each individual were averaged across the 3 time points). *$p < 0.05$, **$p < 0.01$. WASO, wake time after sleep onset; IFN, interferon; PSG, polysomnography.

depressed mood throughout the day in a non-clinical population of non-depressed older adults. Unlike IFN-γ, none of the other assessed inflammatory markers, including TNF-α, IL-6, IL-8, or IL-10, were associated with severity of depressed mood throughout the day. This was surprising, given that previous work has linked increased levels of inflammatory markers to depressive symptoms in older adults (Au et al., 2015; Bell et al., 2017; Doyle et al., 2013; Gimeno et al., 2009; Glaser et al., 2003; Matheny et al., 2011; Penninx et al., 2003; Valkanova et al., 2013; Zalli et al., 2016), although some studies have also yielded mixed or negative results regarding such associations (Adriaensen et al., 2014a, 2014b; Bondy et al., 2021; Bremner et al., 2008; Dimopoulos et al., 2008; Stewart et al., 2009; van den Biggelaar et al., 2007).

Finally, confirming our third hypothesis, our data showed that the association between WASO and depressed mood was moderated by IFN-γ, such that a greater amount of WASO was more strongly related to the severity of depressed mood among older adults with higher levels of IFN-γ, than among those with lower levels of IFN-γ. Although our exploratory mediation analysis did not demonstrate a significant indirect (i.e. mediation) effect, the association between WASO and severity of depressed mood was substantially attenuated when accounting for IFN-γ, which nonetheless suggested a 'mediation-like' effect. This lack of a significant mediation effect might have been due to the small sample

size and the related limitations in statistical power. Thus, future research should examine IFN-γ mediation pathways on the link between sleep disturbance and mood regulation in a larger sample. Although no prior study examined moderation and mediation effects of IFN-γ on associations between poor sleep maintenance and depressed mood, our findings are paralleled by previous research that linked sleep disturbance to increased activity of STAT1 (Irwin et al., 2015) and IP-10 (Huang et al., 2017; Jain et al., 2012). Moreover, our data support our previously conceptualized two-hit model of depression risk (Irwin & Piber, 2018), in which we hypothesized that sleep disturbance and increased inflammation serve as mutual vulnerability factors for depression risk, mirroring longstanding clinical observations that the risk of depression is increased when sleep disturbance and states of increased inflammatory activity coincide. However, we found no correlational association between WASO and any of the other inflammatory markers, including IL-6, IL-8, IL-10, and TNF-α.

While the present study is the first analysis to evaluate the joint contributions of poor sleep maintenance and IFN-γ biology to mood regulation in older adults, our work does not come without limitations. Our sample size was rather small which might have limited statistical power, although we observed a consistent statistical significance in the analyses including moderation analysis. Another limitation of our study is the lack of racial and ethnic

diversity, given that the majority of subjects self-identified as white and non-Hispanic/non-Latino. Thus, future research on the role of sleep disturbance, inflammation and mood regulation should include a broader range of diversity.

Taken together, our data suggest that sleep disturbance and increased levels of IFN-γ might jointly contribute to depressed mood in older adults. Indeed, our findings provide support for a moderating role of IFN-γ on the relationship between WASO and depressed mood, which implicates that higher levels of IFN-γ might serve as a biological vulnerability factor that augments the effects of sleep disturbance on mood regulation in older adults. Even though our exploratory mediation model was not statistically significant, the inclusion of IFN-γ in the model substantially reduced the association between sleep disturbance and depressive symptoms; the absence of significant mediation may be due to the small sample size and the respective lack of statical power. Indeed, such hypothetical mediation effect of IFN-γ on the relationship between WASO and depressed mood could follow various pathways. For example, longer periods of wakefulness during the night might subsequently raise levels of IFN-γ, which in turn might alter thresholds for affect responses. Alternatively, individuals with higher baseline levels of IFN-γ might be particularly vulnerable to the potentially depressing effects of sleep disturbance. Conversely, lower levels of IFN-γ might possibly serve as a resilience factor that could ameliorate the adverse effects of sleep disturbance on mood in older adults. However, to evaluate and test these hypotheses, further research in larger samples (and also clinical populations) with more repeated measures is needed. Indeed, gaining insight into the specific aspects of how sleep disturbance and IFN-γ biology jointly contribute to depressed mood in older adults could help improve prevention strategies to mitigate age-related risk for morbidity and mortality.

References

Adriaensen, W., Matheï, C., Vaes, B., van Pottelbergh, G., Wallemacq, P., & Degryse, J. M. (2014a). Interleukin-6 predicts short-term global functional decline in the oldest old: Results from the BELFRAIL study. *Age*, *36*, 9723. doi: 10.1007/s11357-014-9723-3.

Adriaensen, W., Matheï, C., van Pottelbergh, G., Vaes, B., Legrand, D., Wallemacq, P., & Degryse, J. M. (2014b). Significance of serum immune markers in identification of global functional impairment in the oldest old: Cross-sectional results from the BELFRAIL study. *Age*, *36*, 457–467. doi: 10.1007/s11357-013-9558-3.

Ariyo, A. A., Haan, M., Tangen, C. M., Rutledge, J. C., Cushman, M., Dobs, A., & Furberg, C. D. (2000). Depressive symptoms and risks of coronary heart disease and mortality in elderly Americans. Cardiovascular Health Study

Collaborative Research Group. *Circulation*, *102*, 1773–1779. doi: 10.1161/01.cir.102.15.1773.

Au, B., Smith, K. J., Gariépy, G., & Schmitz, N. (2015). The longitudinal associations between C-reactive protein and depressive symptoms: Evidence from the English Longitudinal Study of Ageing (ELSA). *International Journal of Geriatric Psychiatry*, *30*, 976–984. doi: 10.1002/gps.4250.

Baglioni, C., Battagliese, G., Feige, B., Spiegelhalder, K., Nissen, C., Voderholzer, U., … Riemann, D. (2011). Insomnia as a predictor of depression: A meta-analytic evaluation of longitudinal epidemiological studies. *Journal of Affective Disorders*, *135*, 10–19. doi: 10.1016/j.jad.2011.01.011.

Baker, F., Denniston, M., Zabora, J., Polland, A., & Dudley, W. N. (2002). A POMS short form for cancer patients: Psychometric and structural evaluation. *Psycho-Oncology*, *11*, 273–281. doi: 10.1002/pon.564.

Bell, J. A., Kivimäki, M., Bullmore, E. T., Steptoe, A., & Carvalho, L. A. (2017). Repeated exposure to systemic inflammation and risk of new depressive symptoms among older adults. *Translational Psychiatry*, *7*, e1208. doi: 10.1038/tp.2017.155.

Berry, R. B., Budhiraja, R., Gottlieb, D. J., Gozal, D., Iber, C., Kapur, V. K., … Tangredi, M. M. (2012). Rules for scoring respiratory events in sleep: Update of the 2007 AASM manual for the scoring of sleep and associated events. Deliberations of the sleep apnea definitions task force of the American Academy of Sleep Medicine. *Journal of Clinical Sleep Medicine*, *8*, 597–619. doi: 10.5664/jcsm.2172.

Bondy, E., Norton, S. A., Voss, M., Marks, R. B., Boudreaux, M. J., Treadway, M. T., … Bogdan, R. (2021). Inflammation is associated with future depressive symptoms among older adults. *Brain, Behavior, and Immunity – Health*, *13*, 100226. doi: 10.1016/j.bbih.2021.100226.

Bremmer, M. A., Beekman, A. T., Deeg, D. J., Penninx, B. W., Dik, M. G., Hack, C. E., & Hoogendijk, W. J. (2008). Inflammatory markers in late-life depression: Results from a population-based study. *Journal of Affective Disorders*, *106*, 249–255. doi: 10.1016/j.jad.2007.07.002.

Brustolim, D., Ribeiro-dos-Santos, R., Kast, R. E., Altschuler, E. L., & Soares, M. B. (2006). A new chapter opens in anti-inflammatory treatments: The antidepressant bupropion lowers production of tumor necrosis factor-alpha and interferon-gamma in mice. *International Immunopharmacology*, *6*, 903–907. doi: 10.1016/j.intimp.2005.12.007.

Campisi, J. (2013). Aging, cellular senescence, and cancer. *Annual Review of Physiology*, *75*, 685–705. doi: 10.1146/annurev-physiol-030212-183653.

Campos, A. C., Vaz, G. N., Saito, V. M., & Teixeira, A. L. (2014). Further evidence for the role of interferon-gamma on anxiety- and depressive-like behaviors: Involvement of hippocampal neurogenesis and NGF production. *Neuroscience Letters*, *578*, 100–105. doi: 10.1016/j.neulet.2014.06.039.

Centers for Disease Control and Prevention. (2013). *The state of aging and health in America 2013*. Retrieved from: https://www.cdc.gov/aging/pdf/state-aging-health-in-america-2013.pdf.

Cho, H. J., Lavretsky, H., Olmstead, R., Levin, M. J., Oxman, M. N., & Irwin, M. R. (2008). Sleep disturbance and depression recurrence in community-dwelling older adults: A prospective study. *The American Journal of Psychiatry*, *165*, 1543–1550. doi: 10.1176/appi.ajp.2008.07121882.

Dahl, J., Ormstad, H., Aass, H. C., Malt, U. F., Bendz, L. T., Sandvik, L., … Andreassen, O. A. (2014). The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology*, *45*, 77–86. doi: 10.1016/j.psyneuen.2014.03.019.

Dantzer, R., O'Connor, J. C., Lawson, M. A., & Kelley, K. W. (2011). Inflammation-associated depression: From serotonin to kynurenine. *Psychoneuroendocrinology*, *36*, 426–436. doi: 10.1016/j.psyneuen.2010.09.012.

Dimopoulos, N., Piperi, C., Psarra, V., Lea, R. W., & Kalofoutis, A. (2008). Increased plasma levels of 8-iso-PGF2alpha and IL-6 in an elderly population with depression. *Psychiatry Research*, *161*, 59–66. doi: 10.1016/j.psychres.2007.07.019.

Dipietro, L., Caspersen, C. J., Ostfeld, A. M., & Nadel, E. R. (1993). A survey for assessing physical activity among older adults. *Medicine & Science in Sports & Exercise*, *25*, 628–642.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., & Lanctot, K. L. (2010). A meta-analysis of cytokines in major depression. *Biological Psychiatry*, *67*, 446–457. doi: 10.1016/j.biopsych.2009.09.033.

Doyle, T. A., de Groot, M., Harris, T., Schwartz, F., Strotmeyer, E. S., Johnson, K. C., & Kanaya, A. (2013). Diabetes, depressive symptoms, and

inflammation in older adults: Results from the health, aging, and body composition study. *The Journal of Psychosomatic Research*, 75, 419–424. doi: 10.1016/j.jpsychores.2013.08.006.

Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., & De Benedictis, G. (2000). Inflamm-aging. An evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences*, 908, 244–254.

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 69(Suppl. 1), S4–S9. doi: 10.1093/gerona/glu057.

Gimeno, D., Kivimäki, M., Brunner, E. J., Elovainio, M., De Vogli, R., Steptoe, A., … Ferrie, J. E. (2009). Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychological Medicine*, 39, 413–423. doi: 10.1017/s0033291708003723.

Glaser, R., Robles, T. F., Sheridan, J., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2003). Mild depressive symptoms are associated with amplified and prolonged inflammatory responses after influenza virus vaccination in older adults. *Archives of General Psychiatry*, 60, 1009–1014. doi: 10.1001/archpsyc.60.10.1009.

Grabovich, A., Lu, N., Tang, W., Tu, X., & Lyness, J. M. (2010). Outcomes of subsyndromal depression in older primary care patients. *The American Journal of Geriatric Psychiatry*, 18, 227–235. doi: 10.1097/JGP.0b013e3181cb87d6.

Hayes, A. F. (2017). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. New York, NY: Guilford Publications.

Huang, W. Y., Huang, C. C., Chang, C. C., Kor, C. T., Chen, T. Y., & Wu, H. M. (2017). Associations of self-reported sleep quality with circulating interferon gamma-inducible protein 10, interleukin 6, and high-sensitivity C-reactive protein in healthy menopausal women. *PLoS One*, 12, e0169216. doi: 10.1371/journal.pone.0169216.

Irwin, M. R. (2015). Why sleep is important for health: A psychoneuroimmunology perspective. *Annual Review of Psychology*, 66, 143–172. doi: 10.1146/annurev-psych-010213-115205.

Irwin, M. R., & Opp, M. R. (2017). Sleep health: Reciprocal regulation of sleep and innate immunity. *Neuropsychopharmacology*, 42, 129–155. doi: 10.1038/npp.2016.148.

Irwin, M. R., & Piber, D. (2018). Insomnia and inflammation: A two-hit model of depression risk and prevention. *World Psychiatry*, 17, 359–361. doi: 10.1002/wps.20556.

Irwin, M. R., Witarama, T., Caudill, M., Olmstead, R., & Breen, E. C. (2015). Sleep loss activates cellular inflammation and signal transducer and activator of transcription (STAT) family proteins in humans. *Brain, Behavior, and Immunity*, 47, 86–92. doi: 10.1016/j.bbi.2014.09.017.

Ivashkiv, L. B. (2018). IFNγ: Signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. *Nature Reviews Immunology*, 18, 545–558. doi: 10.1038/s41577-018-0029-z.

Jain, S. K., Kahlon, G., Morehead, L., Lieblong, B., Stapleton, T., Hoeldtke, R., … Levine, S. N. (2012). The effect of sleep apnea and insomnia on blood levels of leptin, insulin resistance, IP-10, and hydrogen sulfide in type 2 diabetic patients. *Metabolic Syndrome and Related Disorders*, 10, 331–336. doi: 10.1089/met.2012.0045.

Kahl, K. G., Kruse, N., Faller, H., Weiss, H., & Rieckmann, P. (2002). Expression of tumor necrosis factor-alpha and interferon-gamma mRNA in blood cells correlates with depression scores during an acute attack in patients with multiple sclerosis. *Psychoneuroendocrinology*, 27, 671–681. doi: 10.1016/s0306-4530(01)00068-3.

Kennedy, B. K., Berger, S. L., Brunet, A., Campisi, J., Cuervo, A. M., Epel, E. S., … Sierra, F. (2014). Geroscience: Linking aging to chronic disease. *Cell*, 159, 709–713. doi: 10.1016/j.cell.2014.10.039.

Köhler, C. A., Freitas, T. H., Maes, M., de Andrade, N. Q., Liu, C. S., Fernandes, B. S., … Carvalho, A. F. (2017). Peripheral cytokine and chemokine alterations in depression: A meta-analysis of 82 studies. *Acta Psychiatrica Scandinavica*, 135, 373–387. doi: 10.1111/acps.12698.

Kok, R. M., & Reynolds, C. F., 3rd. (2017). Management of depression in older adults: A review. *JAMA*, 317, 2114–2122. doi: 10.1001/jama.2017.5706.

Kruskall, L. J., Campbell, W. W., & Evans, W. J. (2004). The Yale Physical Activity Survey for older adults: Predictions in the energy expenditure due to physical activity. *Journal of the American Dietetic Association*, 104, 1251–1257. doi: 10.1016/j.jada.2004.05.207.

Kubera, M., Lin, A. H., Kenis, G., Bosmans, E., van Bockstaele, D., & Maes, M. (2001). Anti-inflammatory effects of antidepressants through suppression of the interferon-gamma/interleukin-10 production ratio. *Journal of Clinical Psychopharmacology*, 21, 199–206. doi: 10.1097/00004714-200104000-00012.

Kwant, A., & Sakic, B. (2004). Behavioral effects of infection with interferon-gamma adenovector. *Behavioural Brain Research*, 151, 73–82. doi: 10.1016/j.bbr.2003.08.008.

Lee, E., Cho, H. J., Olmstead, R., Levin, M. J., Oxman, M. N., & Irwin, M. R. (2013). Persistent sleep disturbance: A risk factor for recurrent depression in community-dwelling older adults. *Sleep*, 36, 1685–1691. doi: 10.5665/sleep.3128.

Li, J., Vitiello, M. V., & Gooneratne, N. S. (2018). Sleep in normal aging. *Sleep Medicine Clinics*, 13, 1–11. doi: 10.1016/j.jsmc.2017.09.001.

Litteljohn, D., Nelson, E., & Hayley, S. (2014). IFN-γ differentially modulates memory-related processes under basal and chronic stressor conditions. *Frontiers in Cellular Neuroscience*, 8, 391. doi: 10.3389/fncel.2014.00391.

Liu, Y., Ho, R. C., & Mak, A. (2012). Interleukin (IL)-6, tumour necrosis factor-alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression. *Journal of Affective Disorders*, 139, 230–239. doi: 10.1016/j.jad.2011.08.003.

Maes, M., Scharpé, S., Meltzer, H. Y., Okayli, G., Bosmans, E., D'Hondt, P., … Cosyns, P. (1994). Increased neopterin and interferon-gamma secretion and lower availability of L-tryptophan in major depression: Further evidence for an immune response. *Psychiatry Research*, 54, 143–160. doi: 10.1016/0165-1781(94)90003-5.

Maes, M., Song, C., Lin, A. H., Bonaccorso, S., Kenis, G., De Jongh, R., … Scharpé, S. (1999). Negative immunoregulatory effects of antidepressants: Inhibition of interferon-gamma and stimulation of interleukin-10 secretion. *Neuropsychopharmacology*, 20, 370–379. doi: 10.1016/s0893-133x(98)00088-8.

Maglione, J. E., Ancoli-Israel, S., Peters, K. W., Paudel, M. L., Yaffe, K., Ensrud, K. E., & Stone, K. L. (2012). Depressive symptoms and subjective and objective sleep in community-dwelling older women. *Journal of the American Geriatrics Society*, 60, 635–643. doi: 10.1111/j.1532-5415.2012.03908.x.

Maglione, J. E., Ancoli-Israel, S., Peters, K. W., Paudel, M. L., Yaffe, K., Ensrud, K. E., & Stone, K. L. (2014). Subjective and objective sleep disturbance and longitudinal risk of depression in a cohort of older women. *Sleep*, 37, 1179–1187. doi: 10.5665/sleep.3834.

Matheny, M. E., Miller, R. R., Shardell, M. D., Hawkes, W. G., Lenze, E. J., Magaziner, J., & Orwig, D. L. (2011). Inflammatory cytokine levels and depressive symptoms in older women in the year after hip fracture: Findings from the Baltimore hip studies. *Journal of the American Geriatrics Society*, 59, 2249–2255. doi: 10.1111/j.1532-5415.2011.03727.x.

Miller, C. H., Maher, S. G., & Young, H. A. (2009). Clinical use of interferon-gamma. *Annals of the New York Academy of Sciences*, 1182, 69–79. doi: 10.1111/j.1749-6632.2009.05069.x.

Mohr, D. C., Goodkin, D. E., Islar, J., Hauser, S. L., & Genain, C. P. (2001). Treatment of depression is associated with suppression of nonspecific and antigen-specific T(H)1 responses in multiple sclerosis. *Archives of Neurology*, 58, 1081–1086. doi: 10.1001/archneur.58.7.1081.

Mura, G., & Carta, M. G. (2013). Physical activity in depressed elderly. A systematic review. *Clinical Practice & Epidemiology in Mental Health*, 9, 125–135. doi: 10.2174/1745017901309010125.

Myint, A. M., Bondy, B., Baghai, T. C., Eser, D., Nothdurfter, C., Schüle, C., … Schwarz, M. J. (2013). Tryptophan metabolism and immunogenetics in major depression: A role for interferon-γ gene. *Brain, Behavior, and Immunity*, 31, 128–133. doi: 10.1016/j.bbi.2013.04.003.

National Sleep Foundation. (2003). Sleep in America Poll. Retrieved from https://www.sleepfoundation.org/professionals/sleep-americar-polls/2003-sleep-and-aging.

Niles, A. N., Smirnova, M., Lin, J., & O'Donovan, A. (2018). Gender differences in longitudinal relationships between depression and anxiety symptoms and

inflammation in the health and retirement study. *Psychoneuroendocrinology*, *95*, 149–157. doi: 10.1016/j.psyneuen.2018.05.035.

O'Connor, J. C., André, C., Wang, Y., Lawson, M. A., Szegedi, S. S., Lestage, J., … Dantzer, R. (2009). Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. *The Journal of Neuroscience*, *29*, 4200–4209. doi: 10.1523/jneurosci.5032-08.2009.

Ohayon, M. M., Carskadon, M. A., Guilleminault, C., & Vitiello, M. V. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: Developing normative sleep values across the human lifespan. *Sleep*, *27*, 1255–1273. doi: 10.1093/sleep/27.7.1255.

Oxenkrug, G., Perianayagam, M., Mikolich, D., Requintina, P., Shick, L., Ruthazer, R., … Summergrad, P. (2011). Interferon-gamma (+874) T/A genotypes and risk of IFN-alpha-induced depression. *Journal of Neural Transmission*, *118*, 271–274. doi: 10.1007/s00702-010-0525-1.

Penninx, B. W., Kritchevsky, S. B., Yaffe, K., Newman, A. B., Simonsick, E. M., Rubin, S., … Pahor, M. (2003). Inflammatory markers and depressed mood in older persons: Results from the health, aging and body composition study. *Biological Psychiatry*, *54*, 566–572. doi: 10.1016/s0006-3223(02)01811-5.

Piber, D., Olmstead, R., Cho, J. H., Witarama, T., Perez, C., Dietz, N., … Irwin, M. R. (2019). Inflammaging: Age and systemic, cellular, and nuclear inflammatory biology in older adults. *The Journal of Gerontology, A: Biological Sciences*, *74*, 1716–1724. doi: 10.1093/gerona/glz130.

Pollard, K. M., Cauvi, D. M., Toomey, C. B., Morris, K. V., & Kono, D. H. (2013). Interferon-*γ* and systemic autoimmunity. *Discovery Medicine*, *16*, 123–131.

Raitala, A., Pertovaara, M., Karjalainen, J., Oja, S. S., & Hurme, M. (2005). Association of interferon-gamma +874(T/A) single nucleotide polymorphism with the rate of tryptophan catabolism in healthy individuals. *Scandinavian Journal of Immunology*, *61*, 387–390. doi: 10.1111/j.1365-3083.2005.01586.x.

Schmidt, F. M., Lichtblau, N., Minkwitz, J., Chittka, T., Thormann, J., Kirkby, K. C., … Himmerich, H. (2014). Cytokine levels in depressed and non-depressed subjects, and masking effects of obesity. *Journal of Psychiatric Research*, *55*, 29–34. doi: 10.1016/j.jpsychires.2014.04.021.

Schulz, R., Beach, S. R., Ives, D. G., Martire, L. M., Ariyo, A. A., & Kop, W. J. (2000). Association between depression and mortality in older adults: The cardiovascular health study. *Archives of Internal Medicine*, *160*, 1761–1768. doi: 10.1001/archinte.160.12.1761.

Scott, J. P., McNaughton, L. R., & Polman, R. C. (2006). Effects of sleep deprivation and exercise on cognitive, motor performance and mood. *Physiology & Behavior*, *87*, 396–408. doi: 10.1016/j.physbeh.2005.11.009.

Seidel, A., Arolt, V., Hunstiger, M., Rink, L., Behnisch, A., & Kirchner, H. (1995). Cytokine production and serum proteins in depression.

Scandinavian Journal of Immunology, *41*, 534–538. doi: 10.1111/j.1365-3083.1995.tb03604.x.

Simon, N. M., McNamara, K., Chow, C. W., Maser, R. S., Papakostas, G. I., Pollack, M. H., … Wong, K. K. (2008). A detailed examination of cytokine abnormalities in major depressive disorder. *European Neuropsychopharmacology*, *18*, 230–233. doi: 10.1016/j.euroneuro.2007.06.004.

Simonsick, E. M., Wallace, R. B., Blazer, D. G., & Berkman, L. F. (1995). Depressive symptomatology and hypertension-associated morbidity and mortality in older adults. *Psychosomatic Medicine*, *57*, 427–435. doi: 10.1097/00006842-199509000-00003.

Stewart, J. C., Rand, K. L., Muldoon, M. F., & Kamarck, T. W. (2009). A prospective evaluation of the directionality of the depression-inflammation relationship. *Brain, Behavior, and Immunity*, *23*, 936–944. doi: 10.1016/j.bbi.2009.04.011.

Valkanova, V., Ebmeier, K. P., & Allan, C. L. (2013). CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. *Journal of Affective Disorders*, *150*, 736–744. doi: 10.1016/j.jad.2013.06.004.

Vancassel, S., Capuron, L., & Castanon, N. (2018). Brain kynurenine and BH4 pathways: Relevance to the pathophysiology and treatment of inflammation-driven depressive symptoms. *Frontiers in Neuroscience*, *12*, 499. doi: 10.3389/fnins.2018.00499.

van den Biggelaar, A. H., Gussekloo, J., de Craen, A. J., Frölich, M., Stek, M. L., van der Mast, R. C., & Westendorp, R. G. (2007). Inflammation and interleukin-1 signaling network contribute to depressive symptoms but not cognitive decline in old age. *Experimental Gerontology*, *42*, 693–701. doi: 10.1016/j.exger.2007.01.011.

Von Korff, M., Wagner, E. H., & Saunders, K. (1992). A chronic disease score from automated pharmacy data. *Journal of Clinical Epidemiology*, *45*, 197–203. doi: 10.1016/0895-4356(92)90016-g.

White, J., Kivimäki, M., Jokela, M., & Batty, G. D. (2017). Association of inflammation with specific symptoms of depression in a general population of older people: The english longitudinal study of ageing. *Brain, Behavior, and Immunity*, *61*, 27–30. doi: 10.1016/j.bbi.2016.08.012.

Whooley, M. A., & Browner, W. S. (1998). Association between depressive symptoms and mortality in older women. Study of Osteoporotic Fractures Research Group. *Archives of Internal Medicine*, *158*, 2129–2135. doi: 10.1001/archinte.158.19.2129.

World Health Organization. (2017). Mental health of older adults. Retrieved from https://www.who.int/news-room/fact-sheets/detail/mental-health-of-older-adults.

Zalli, A., Jovanova, O., Hoogendijk, W. J., Tiemeier, H., & Carvalho, L. A. (2016). Low-grade inflammation predicts persistence of depressive symptoms. *Psychopharmacology*, *233*, 1669–1678. doi: 10.1007/s00213-015-3919-9.

Zhang, J. (2007). Yin and yang interplay of IFN-gamma in inflammation and autoimmune disease. *Journal of Clinical Investigation*, *117*, 871–873. doi: 10.1172/jci31860.