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# Partial sleep deprivation activates the DNA damage response (DDR) and the senescence-associated secretory phenotype (SASP) in aged adult humans

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ABSTRACT

Age-related disease risk has been linked to short sleep duration and sleep disturbances; however, the specific molecular pathways linking sleep loss with diseases of aging are poorly defined. Key cellular events seen with aging, which are thought to contribute to disease, may be particularly sensitive to sleep loss. We tested whether one night of partial sleep deprivation (PSD) would increase leukocyte gene expression indicative of DNA damage responses (DDR), the senescence-associated secretory phenotype (SASP), and senescence indicator $p16^{INK4a}$ in older adult humans, who are at increased risk for cellular senescence. Community-dwelling older adults aged 61–86 years ($n = 29$; 48% male) underwent an experimental partial sleep deprivation (PSD) protocol over 4 nights, including adaptation, an uninterrupted night of sleep, partial sleep deprivation (sleep restricted 3–7 AM), and a subsequent full night of sleep. Blood samples were obtained each morning to assess peripheral blood mononuclear cell (PBMC) gene expression using Illumina HT-12 arrays. Analyses of microarray results revealed that SASP ($p < .05$) and DDR ($p = .08$) gene expression were elevated from baseline to PSD nights. Gene expression changes were also observed from baseline to PSD in *NFKB2*, *NBS1* and *CHK2* (all $p$'s $< .05$). The senescence marker $p16^{INK4a}$ (*CDKN2A*) was increased 1 day after PSD compared to baseline ($p < .01$), however confirmatory RT-PCR did not replicate this finding. One night of partial sleep deprivation activates PBMC gene expression patterns consistent with biological aging in this older adult sample. PSD enhanced the SASP and increased the accumulation of damage that initiates cell cycle arrest and promotes cellular senescence. These findings causally link sleep deprivation to the molecular processes associated with biological aging.


## 1. Introduction

Aged adults experience more sleep complaints than younger individuals, with estimates that as much as 50% of adults aged 65 years or more experience insomnia symptoms (Ohayon, 2002) and other sleep difficulties (Petit et al., 2003; Foley et al., 1995). Moreover, sleep loss is thought to interact with numerous regulatory systems to influence health and chronic disease risk (McEwen, 2006; Robles and Carroll, 2011; Chen et al., 2014; Mullington et al., 2009; Van Cauter et al., 2007; Ju and Choi, 2013; Palesh et al., 2014), findings that are particularly salient for older adults with sleep difficulties. Disease morbidity and mortality risk are elevated among those with short sleep duration and poor sleep quality (Ohayon, 2002; Center for Disease Control, 2011; Colten and Altevogt, 2006; Motivala, 2011). However, the specific molecular pathways altered by sleep loss, which impact human disease, are poorly defined. Potential pathways include an increase in unrepaired cellular stress and accumulation of damage (Everson et al., 2014; Naidoo, 2009, 2012; Naidoo et al., 2008; Brown and Naidoo, 2010), which is thought to contribute to biological aging (Cribbet et al., 2014; Jackowska et al., 2012; Prather et al., 2011, 2015; Liang et al., 2011; Chen et al., 2014). Initial evidence has linked poor sleep quality and short sleep duration with greater cellular aging, as indicated by shorter leukocyte telomere length

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(Cribbet et al., 2014; Jackowska et al., 2012; Prather et al., 2011, 2015; Liang et al., 2011; Chen et al., 2014).

The accumulation of damage is part of the pathology of common chronic diseases of aging (López-Otín et al., 2013), and is suspected to be a mechanism by which aging itself promotes disease (Kennedy et al., 2014). Accumulated damage from chronic low grade inflammation (Franceschi and Campisi, 2014; Jurk et al., 2014), for example, leads to imbalance in the redox system, mitochondrial dysfunction, and reduced ability to repair (López-Otín et al., 2013). Driven in part by these processes, aging is also associated with an accrual of senescent cells (Campisi, 2005; Liu et al., 2009; Effros, 2005). Cellular senescence is a state of cell cycle arrest, commonly reached by replicative (e.g., critically short telomeres) or cell stress (e.g., DNA damage) pathways, and preceded by signaling of DNA damage response (DDR) elements derived from telomeric or non-telomeric damage (Campisi, 2005; Effros, 2005; Blackburn, 2005, 2000; Campisi and d'Adda di Fagagna, 2007).

Accrual of senescent cells is thought to effect the aging process, as removal of senescent cells in mice slows aging (Baker et al., 2011). These senescent cells have a unique secretory pattern, termed the senescence associated secretory phenotype (SASP), which is characterized by increased release of inflammatory cytokines and chemokines (e.g., IL-6, IL-8, monocyte chemotactic protein (MCP)-2, MCP-4, chemokine C-X-C motif ligand (CXCL)-1, CXCL-2, CXCL-3, granulocyte macrophage colony-stimulating factor (GM-CSF), intracellular adhesion molecule (ICAM)-1) that promote disease (Effros, 2005; Campisi and d'Adda di Fagagna, 2007; Erusalimsky, 2009; Coppé et al., 2010; Freund et al., 2010). Removing the senescent cells may reduce the development of the aging phenotype in mice by eliminating the inflammatory secretory patterns that contribute to the pathology. Indeed, genetically modified mice (*NFKB1−/−*) that have an enhanced inflammatory signal show accelerated aging through accumulation of senescent cells and enhancement of the SASP (Jurk et al., 2014). Furthermore, accumulation of senescence predicts shortened lifespan (Jurk et al., 2014).

Given these findings that aging is associated with an increase in DNA damage, accumulation of senescent cells, and enhancement of the inflammatory secretome, it is possible that these pathways serve as molecular links between short sleep duration and increases in age-related chronic disease burden in late life. In this experimental study, we test the hypothesis that sleep loss alters these molecular pathways by examining whether partial night sleep deprivation (PSD) in aged humans increases peripheral blood mononuclear cell (PBMC) expression of genes indicative of rises in DNA damage responses (DDR), increases in proinflammatory senescent associated secretory phenotype (SASP), and increases in cellular senescent signal expression ($p16^{INK4a}$). PSD replicates the kind of sleep loss that is ubiquitous in the general population and also resembles the reduction of sleep duration that is often found in persons with age-related medical disorders, however, its effects on physiological process has been examined almost exclusively in adult (Irwin et al., 2008; Irwin and Ziegler, 2005; Irwin et al., 2006, 1996, 2002, 2015), as opposed to older adult populations (Carroll et al., 2015).

## 2. Materials and methods

### 2.1. Participants

All subjects gave informed consent and the University of California, Los Angeles (UCLA) institutional review board approved the protocols. Initial medical interview, physical examination, and screening laboratory tests determined eligibility for the PSD. Subjects were invited to participate in the experimental session if they were deemed physically healthy including no past history of cancer or inflammatory disorders, were non-smokers, and had a body mass index (BMI) < 40 (calculated as weight (kg) divided by the square of height (m)). Participants completed a 2 week sleep diary, and were excluded if their normal sleep pattern was less than 7 h nightly and or they showed signs of circadian phase shifting (early or delayed sleep onset by more than 2 h); had atypical sleeping hours not occurring between 11 PM and 7 AM. Additional exclusion criteria included: current diagnosis of mental illness based on the Diagnostic and Statistical Manual of Mental Disorders (Editions IV (revised) or V); sleep apnea, restless leg syndrome, or other sleep disorders as identified during the night of adaptation to the sleep laboratory; chronic or acute (<2 weeks) infection; and comorbid uncontrolled chronic disease. The present analysis includes 29 participants who underwent the PSD protocol and had samples collected for RNA analyses.

### 2.2. Procedures

After eligibility and medical evaluation, subjects were invited for a four night stay at the UCLA Clinical Translational Research Center (CTRC) where they underwent experimental procedures. Following the first night, which served as an adaptation night, subjects had an uninterrupted night of sleep from 11 PM to 7 AM (baseline). On the third night, the partial sleep deprivation night was assigned. On this night, subjects were not allowed to sleep from 11 PM to 3 AM, sleep occurred between 3 AM and 7 AM (PSD), and awakening occurred regardless of sleep stage. The fourth night (1 day after the PSD) subjects were allowed to sleep uninterrupted from 11 PM to 7 AM. Blood samples were obtained each morning prior to eating and following baseline, PSD, and recovery nights for the assessment of cellular gene expression. Sleep patterns were monitored using polysomnography (PSG) recordings of sleep each night, and are reported separately (Carroll et al., 2015).

### 2.3. Measures

#### 2.3.1. PBMC gene expression patterns

Samples of quality-verified RNA extracted (RNeasy; Qiagen) from approximately 2 million PBMC were assayed by Illumina HT-12 arrays (Illumina Inc., San Diego, CA) in the UCLA Neuroscience Genomics Core Laboratory as previously described (Fredrickson et al., 2013). Briefly, RNA was tested for integrity and converted to fluorescent cRNA for hybridization to Illumina Human HT-12 v4 BeadArrays, following the manufacturer's standard protocol. Quintile-normalized gene expression values were generated for more than 35,000 probes derived from the National Center for Biotechnology Information Reference Sequence (NCBI) and other sources, and provides genome-wide transcriptional coverage of well-characterized genes, gene candidates, and splice variants.

#### 2.3.2. Senescence associated secretory phenotype (SASP)

Genes representative of key components of the SASP were selected a priori based on prior work showing differential gene expression patterns, with confirmatory protein quantification, in replicative and damage induced senescent cells (Coppé et al., 2008; Laberge et al., 2012). These included the following genes: *IL6*, *CSF2*, *CCL8*, *IL8*, *CCL13*, *ICAM1*, *CXCL1*, *CXCL2*, *CXCL3*, which were z-transformed and then summed to create a composite measure of SASP. Signals known to be upregulated during the **DNA damage response (DDR)** Jackson and Bartek, 2009 were selected to create a composite index by z-transforming and summing the following genes: *GADD45GIP1*, *TP53BP1*, *CHEK1*, *TP53*, *TERF2*, *SIRT1*, *TERT*,

*GADD45A*, *CDK7*, *DDB1*, *HUS1*, *NTHL1*, *MRE11A*, *ERCC2*, *PCNA*, *ERCC1*, *RAD51*, *OGG1*, *BRCA1*, *H2AFX*, *NBN*, *DDB2*, *RAD50*, *XPC*, *RPA1*, *MLH1*, *LIG1*, *MDC1*, *GADD45B*, *XPA*. Further selection was made of additional individual genes important in signaling senescence and initiating the inflammatory cytokine response after DDR: *ATM*, *NBS1* (*NBN*), and *CHK2* Coppé et al., 2008; Rodier et al., 2009. We also selected key genes that play a vital role in our pathways of interest: $p16^{INK4a}$ (*CDKN2A*) was selected as an established senescent cell signal (Baker et al., 2011; Krishnamurthy et al., 2004), and *NFKB1* and *NFKB2* gene expression were selected as the key transcriptional elements of both inflammation and SASP (Salminen et al., 2012). A complete list of gene names, along with correlation tables of each gene within the DDR and SASP composites can be found in the Supplementary material, Tables S1–S3.

### 2.4. Analytic strategy

SPSS statistical software v.22 was used to perform all statistical analyses. To test for the effect of PSD on gene expression, general linear model analyses were performed, controlling for baseline differences in gene expression and batch effects, along with BMI and sex. Adjusting for multiple comparisons, the Least Significant Difference (LSD) test was used for planned pairwise comparisons that examined estimated marginal mean differences between baseline and PSD, baseline and recovery nights, and PSD and recovery nights.

## 3. Results

A total of 29 older adults (48.3% male) aged 61–86, M(SD) = 71 (7.5) years, and average body mass index (BMI) of M(SD) = 27.3 (4.4) kg/$m^2$ completed the 4 night study and had blood collected for the examination of gene expression profiles upon waking.

As compared to baseline, PSD induced increases in SASP expression (Fig. 1; $p = .01$), *NFKB2* ($p = .008$), and *NFKB1* ($p = .07$). As compared to baseline, expression of SASP and *NFKB1* were similar after a night of recovery sleep, however NFKB2 remained slightly elevated at recovery ($p = .06$). The composite DNA damage response (DDR) showed a similar pattern, with increases in DDR gene expression after PSD compared to baseline (Fig. 2; $p = .08$), which remained elevated after a night of recovery sleep, however this was not significantly different from baseline ($p = .14$). Finally, PSD altered genes involved in signaling senescence. As compared to baseline, PSD induced increases in *NBS1* ($p = .004$), *CHK2* ($p < .001$) and *ATM* ($p = .09$). In contrast, increases in the senescent signal marker $p16^{INK4a}$ (*CDKN2A*) on microarray were delayed and found after a night of recovery sleep ($p < .01$). PSD did not significantly alter *MRE11* or *RAD50* gene expression. Confirmatory RT-PCR verified significant PSD-induced differences in gene expression for CXCL1, CXCL2, and IL8 (all $p < .001$) and a trend toward differential expression for NFKB2 ($p = .07$). RT-PCR failed to verify any significant PSD-induced difference in ICAM1, IL6, or CDKN2A (all $p > .18$).

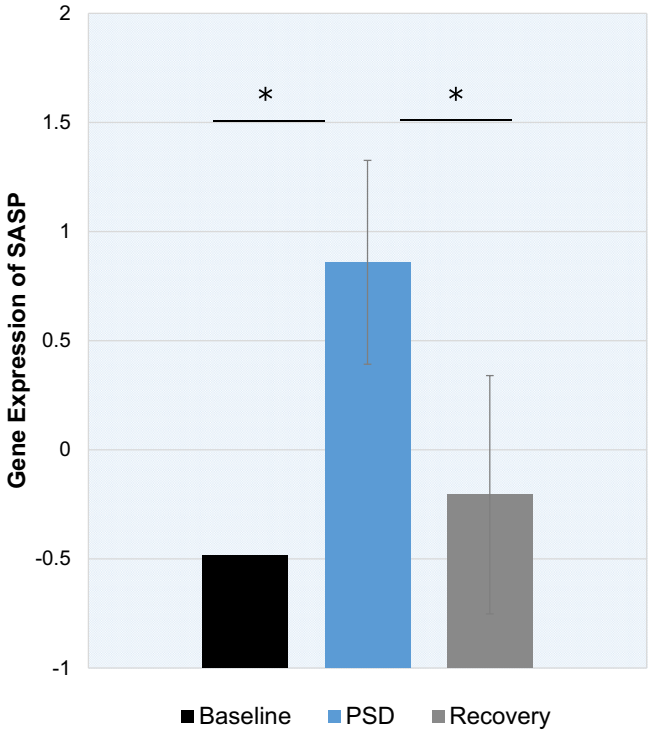


**Fig. 1.** Gene expression of the senescence associated secretory phenotype (SASP) at baseline, partial sleep deprivation (PSD), and 1 day after PSD (recovery) in older adults. SASP is a composite score created from a sum of 9 z-transformed genes. Error bars represent standard error of estimated marginal mean, adjusting for baseline, BMI and sex. $^{*}p < .05$.

## 4. Discussion

One night of partial sleep deprivation activated gene expression patterns in PBMCs consistent with the hypothesis that sleep loss promotes biological aging in older adults. We observed increases in expression of the NF-κB related gene (*NFKB2*) and an enhanced expression of representative genes that give rise to the senescent associated secretome. Moreover, we observed an increase in DNA damage response signals, along with the expression of *NBS1-CHK2* genes implicated in DDR initiated cell cycle arrest that promotes senescent and pre-senescent increases in inflammatory cytokines (Coppé et al., 2008; Rodier et al., 2009). PSD altered genes involved in signaling senescence including *NBS1* ($p = .004$), *CHK2* ($p < .001$) and *ATM* ($p = .09$). And 1 day following sleep deprivation we observed on microarray an increase in the gene expression of the senescent marker $p16^{INK4a}$, however confirmatory RT-PCR did not replicate this finding. Together, these findings indicate that sleep deprivation increases the DNA damage response, increases senescence associated secretory phenotypic expression pattern, and promotes pathways involved in the initiation of cellular senescence in aged adults. Future work should consider whether this effect is more pronounced after repeated days of sleep restriction or in aged individuals with chronic sleep disturbances such as insomnia.

The enhanced expression of SASP may occur in pre-existing senescent cells, which are prevalent in aged adults, and which is enhanced through NF-κB signaling. However, it also is possible that pre-senescent cells exhibit gene expression patterns similar to senescent cells after a sustained DDR has been initiated (Rodier et al., 2009), which in the present sample occurred after sleep deprivation. These proposed pathways are characterized in Fig. 3. Further work is necessary to distinguish the cell source for these responses after sleep deprivation.

Consistent with our results, prior work has reported that sleep deprivation induces increases in NF-κB signaling within immune cells of young adults (Irwin et al., 2008). Although the current study was unable to assess the extracellular signal that might initiate the intracellular changes, several proposed mechanisms may work together to drive this effect. The sympathetic nervous system is highly responsive to sustaining wakefulness during periods of high demand for sleep (Irwin and Ziegler, 2005; Irwin et al., 1999, 2003; Irwin and Cole, 2011). The release of norepinephrine stimulates β-adrenergic receptors on immune cells to activate

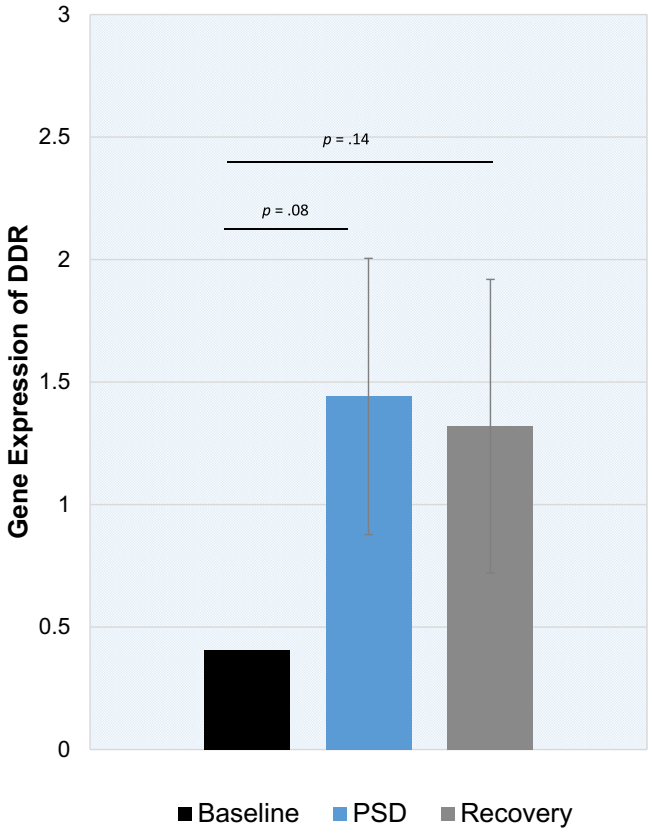


**Fig. 2.** Gene expression of the DNA damage response (DDR) at baseline, partial sleep deprivation (PSD), and 1 day after PSD (recovery) in older adults. DDR is a composite score created from a sum of 30 z-transformed genes. Error bars represent standard error of estimated marginal mean, adjusting for baseline, BMI and sex.

transcriptional elements, including NF-κB (Irwin and Cole, 2011; Cole et al., 2010). Although this transcription factor is known to regulate proinflammatory immune response genes (Irwin and Cole, 2011; Salminen et al., 2008), recent evidence has also implicated NF-κB as a major regulator of the senescent associated secretory phenotype within cells expressing senescent markers (Salminen et al., 2012; Rovillain et al., 2011; Chien et al., 2011). Our previous research has demonstrated PSD increases release of catecholamines (Irwin et al., 1999), as well as activation of these same proinflammatory NF-κB transcriptional control pathways within immune cells in young adults (Irwin et al., 2006). Knowing this, we speculate that sleep deprivation may enhance SASP in existing senescent cells of aged adults via norepinephrine ligation of β-adrenergic receptors on the cell surface, which signals transcriptional regulation via NF-κB. Future work is necessary to identify whether norepinephrine is a key neurotransmitter that can activate the SASP in senescent cells, with implications for both sleep loss as well as other social adversities known to activate the sympathetic nervous system response.

Our findings showing rises in DDR after sleep loss are consistent with animal research showing extended periods of sleep deprivation induce cellular stress and oxidative damage to DNA (Everson et al., 2014; Naidoo, 2009; Brown and Naidoo, 2010). We demonstrated that in humans a partial night of sleep loss increases the cellular stress – DNA damage response (DDR) pathway, as well as specific signaling molecules responsive to the DDR: *ATM* which phosphorylates *CHK2* (checkpoint kinase-2) to promote growth arrest, and *NBS1*, which can participate in DNA repair, especially in the telomeric region, when part of the *MRE11-RAD50-NBS1* (MRN) complex Campisi and d'Adda di Fagagna, 2007. In light of the increase in *NBS1*, we examined individually *MRE11* and *RAD50* gene expression. We did not observe a significant increase in *MRE11* or *RAD50*, suggesting the cells were likely not in growth arrest in order to repair damage, and then exit growth arrest. Rather we hypothesize that, in response to PSD, these cells were on a trajectory towards a senescent state. In addition to growth arrest, *NBS1*, *ATM*, and *CHK2* are thought to combine to stimulate proinflammatory activity associated with the SASP in damaged and/or senescent cells (Campisi and d'Adda di Fagagna, 2007; Rodier et al., 2009). These results support our conclusion that DDR signaling was induced by sleep deprivation and sufficient to signal growth arrest, which can drive senescence.

### 4.1. Strengths and limitations

Limitations to the current study design should be noted. First, the analysis of molecular signaling patterns using gene expression

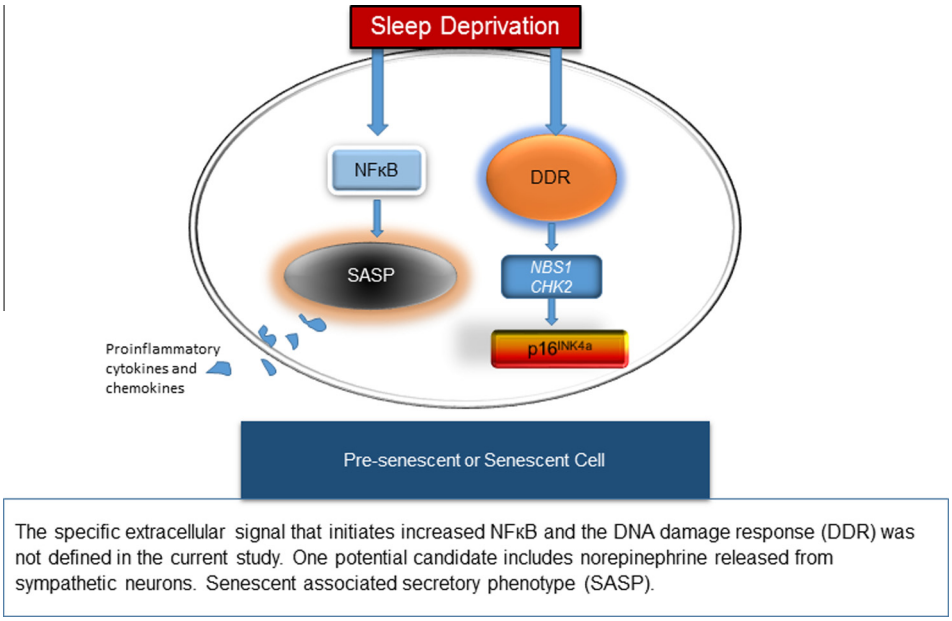


**Fig. 3.** Proposed molecular pathways promoting aging after sleep deprivation.

analysis within human peripheral blood mononuclear cells is an upstream measure of protein levels and cellular function. Future research should identify senescent cells to confirm the SASP is originating from this cell source after PSD, and that the molecular products (i.e., cytokines, chemokines) of gene expression are also noticeably increased. The present analysis does not account for any potential redistribution of leukocyte subsets that might be induced by PSD. Although we have previously reported that PSD does not induce significant change in the percentage of monocytes, it does appear to influence lymphocyte populations (Irwin et al., 1996). Observed patterns suggest a need for future work using models where cells are isolated, specific genes experimentally manipulated (e.g., silencing using siRNA, enhancing with viral vectors), and signaling pathways pharmacologically manipulated to definitively identify the causal mediators involved. Secondly, although we speculate that $\beta$-adrenergic signaling and related cascade effects on NF-κB pathways were involved in the observed changes after a night of partial sleep deprivation, and previous work has reported this (Irwin et al., 1999), we did not directly test $\beta$-adrenergic signaling activity in the present study. Future research should consider whether $\beta$-adrenergic blockade modifies biological aging dynamics induced by sleep deprivation. An alternative explanation for the current findings is that one night of PSD alters gene expression by delaying onset of the sleep-wake pattern, and the observed differences are a result of capturing gene expression at a different point in the sleep cycle rather than a PSD-induced alteration in daytime DDR and SASP. However, prior work has not found that one night of PSD alters the onset or amplitude of circadian hormones, cortisol and melatonin (Redwine, 2000). Several nights of chronic sleep restriction can induce gene expression consistent with increases in cellular stress and inflammation (Moller-Levet et al., 2013), suggesting the findings may not be due to a shift in amplitude timing. These results are concordant with findings in an animal model of chronic sleep loss (Everson et al., 2014). Moreover, it is important to note that the findings in the current study are in an older adult sample, which may have a different response to sleep loss than younger subjects, particularly given the reduced capacity of an aging system to respond to perturbations (e.g., drug clearance, tissue healing, recovery speed, vaccine antibody responses) (Sahin et al., 2011; Taffett, 2003), and existing evidence that sleep loss impacts older adults more strongly than younger individuals (e.g., increased circulating inflammation) (Vgontzas et al., 2003). Future work should determine whether the present results generalize to a younger population.

Several strengths of the current research exist. First, the methods employed include a well-controlled experimental design lasting 4 nights at a clinical translational research center where patients were regularly monitored by trained nursing staff. Participants were carefully screened for exclusion criteria to eliminate influences of excessive chronic disease burden, mental health problems, substance abuse, and sleep problems (i.e., short sleep duration, sleep apnea, or phase shifting). The present research applied gene expression analyses to identify key molecular changes induced by sleep deprivation that are implicated in aging biology, representing a novel approach to assessing biological aging processes in humans.

## 5. Conclusions

One night of partial sleep loss in older adults induced gene expression changes consistent with an increase in the DNA damage response and the promotion of the senescent associated secretory phenotype characterized by a proinflammatory profile, initiated via NF-κB transcriptional regulation. One night of sleep deprivation was also followed by a subsequent increase in gene expression suggesting the promotion of cellular senescence and an indication of cellular aging (Krishnamurthy et al., 2004). Our data support the hypothesis that sleep loss in aged adults activates these biological pathways. Hence, loss of sleep in late life may promote biological aging due to increased accumulation of damage that initiates cell cycle arrest and enhanced expression of the senescence associated secretory phenotype, which together increase susceptibility to cellular senescence. These findings causally link sleep deprivation to the biological processes intrinsic to aging, and further support the hypothesis that insufficient sleep may contribute to chronic disease risk through the activation of molecular pathways that drive biological aging.

## Conflict of interest

The authors declare no competing financial interests.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2015.08.024.

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