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# Tired telomeres: Poor global sleep quality, perceived stress, and telomere length in immune cell subsets in obese men and women

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ABSTRACT

Poor sleep quality and short sleep duration are associated with increased incidence and progression of a number of chronic health conditions observed at greater frequency among the obese and those experiencing high levels of stress. Accelerated cellular aging, as indexed by telomere attrition in immune cells, is a plausible pathway linking sleep and disease risk. Prior studies linking sleep and telomere length are mixed. One factor may be reliance on leukocytes, which are composed of varied immune cell types, as the sole measure of telomere length. To better clarify these associations, we investigated the relationships of global sleep quality, measured by the Pittsburgh Sleep Quality Index (PSQI), and diary-reported sleep duration with telomere length in different immune cell subsets, including granulocytes, peripheral blood mononuclear cells (PBMCs), CD8+ and CD4+ T lymphocytes, and B lymphocytes in a sample of 87 obese men and women (BMI mean = 35.4, SD = 3.6; 81.6% women; 62.8% Caucasian). Multiple linear regression analyses were performed adjusting for age, gender, race, education, BMI, sleep apnea risk, and perceived stress. Poorer PSQI global sleep quality was associated with statistically significantly shorter telomere length in lymphocytes but not granulocytes and in particular CD8+ T cells ($b = -56.8$ base pairs per one point increase in PSQI, SE = 20.4, $p = 0.007$) and CD4+ T cells ($b = -37.2$, SE = 15.9, $p = 0.022$). Among separate aspects of global sleep quality, low perceived sleep quality and decrements in daytime function were most related to shorter telomeres. In addition, perceived stress moderated the sleep-CD8+ telomere association. Poorer global sleep quality predicted shorter telomere length in CD8+ T cells among those with high perceived stress but not in low stress participants. These findings provide preliminary evidence that poorer global sleep quality is related to telomere length in several immune cell types, which may serve as a pathway linking sleep and disease risk in obese individuals.



## 1. Introduction

Growing epidemiologic evidence links short sleep (i.e., sleeping 6 or fewer hours per night) and poor sleep quality with increased incidence and progression of several chronic medical conditions observed at greater prevalence among overweight and obese individuals, including type 2 diabetes, coronary heart disease, and metabolic syndrome (Ayas et al., 2003; Cappuccio et al., 2010a; Gangwisch et al., 2007; Grandner et al., 2012, 2013; Hall et al., 2008; Jennings et al., 2007). Sleep complaints and clinical sleep disorders such as obstructive sleep apnea (OSA) are common among the overweight and the obese (Beccuti and Pannain, 2011). However, the underlying biological mechanisms linking sleep and disease risk remain to be elucidated. In this regard, researchers have turned their attention to the role of accelerated cellular aging, as indexed by immune cell telomere length, as a plausible pathway.

Telomeres are DNA–protein complexes at the ends of eukaryotic chromosomes that protect the DNA that encodes genetic information from loss or instability (Blackburn, 1991; Lin et al., 2010). In adult human mitotic cells telomeres shorten with successive cell

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divisions. Critically short telomeres can send cells into replicative senescence, causing cell cycle arrest and malfunction as well as potential genomic instability. The contributors to accelerated cellular aging are complex and multifaceted; however, there is growing acceptance that telomere shortening in immune cells is a marker, and possibly a mechanism (Codd et al., 2013; Guo et al., 2011; Sahin et al., 2011), underlying premature morbidity and mortality in humans. Numerous clinical studies link short telomere length, measured primarily in leukocytes, with increased rates and risks of age-related diseases (Epel et al., 2009; Fitzpatrick et al., 2007; Franceschi and Campisi, 2014; Haycock et al., 2014; McElhaney and Effros, 2009; Zee et al., 2010; Zhao et al., 2014). Investigations of pathways through which short telomeres contribute to disease pathogenesis are topics of active scientific inquiry. Senescent cells show increased secretion of proinflammatory cytokines and extra cellular matrix-degrading enzymes, which may, in turn, drive accelerated disease progression (Blackburn, 2005; Effros et al., 2005). With respect to psychosocial predictors, short telomere length has been associated with a variety of psychological and behavioral factors, including psychological stress, depression, tobacco use, sedentary behavior, and obesity (Prather et al., 2013a; Puterman and Epel, 2012; Shalev et al., 2013; Starkweather et al., 2014; Verhoeven et al., 2013). Investigation of the relationship between telomere length and sleep, however, has been limited.

A handful of studies have investigated associations between sleep and telomere length (Cribbet et al., 2014; Jackowska et al., 2012; Lee et al., 2014; Liang et al., 2011; Prather et al., 2011). Overall, these studies support associations of short sleep duration and poor subjective sleep quality with shorter leukocyte telomere length. One limitation of this literature, however, has been the reliance on measures of telomere length in heterogeneous leukocyte populations, which include granulocytes and peripheral mononuclear cells (PBMCs). PBMCs can be further subdivided into lymphocytes (e.g., T cells, B cells and natural killer cells) and monocytes. With advancing age, the shortening of telomeres occurs primarily in CD8+ cells (Effros et al., 2005; McElhaney and Effros, 2009; Posnett et al., 1999), particularly those that have lost CD28 expression. CD28 is co-stimulatory molecule important for proliferative capacity. CD8+CD28− T lymphocytes are terminally differentiated effector CD8+ T lymphocytes that lose telomerase activity and secrete excess proinflammatory cytokines (Effros et al., 2005; McElhaney and Effros, 2009). An increased proportion of CD8+CD28− T cells predicts poorer antibody response (Effros et al., 1994), increased susceptibility to the common cold (Cohen et al., 2013), and early mortality in elderly adults (Wikby et al., 2002). To date, it remains unclear whether poor sleep is differentially associated with telomere length across different cell types. Given the importance of CD8+CD28− T cells in the aging immune system, an association of poor sleep with shortened telomeres in CD8+ T lymphocytes might be particularly significant.

Another limitation of the existing sleep-telomere literature is that there has been little investigation of the role stress plays in the associations between sleep and telomere length. Individuals experiencing elevated levels of stress regularly also experience poor sleep. Existing literature also suggests that sleep modulates the stress response. In this regard, experimental studies employing sleep deprivation demonstrate that sleep loss lowers one's threshold for what is perceived as stressful (Minkel et al., 2012) and leads to enhanced amygdala activation, a brain region critical to processing emotion and regulating stress physiology, in response to threatening stimuli (Yoo et al., 2007).

The aims of the present study were to investigate the associations between self-reported sleep duration, measured via daily diary reports, and subjective global sleep quality, assessed using the Pittsburgh Sleep Quality Index, with telomere length in granulocytes, PBMCs and sorted cells (CD4+ and CD8+ T lymphocytes, and B lymphocytes) in a sample of obese men and women. As a secondary exploratory analysis, we examined whether levels of perceived stress moderated associations between sleep and telomere length. Based on the existing literature, we hypothesized that shorter sleep duration and poorer global sleep quality would be associated with shorter telomere length, particularly in CD8+ T lymphocytes. Further, we hypothesized that these sleep-telomere relationships would be stronger in participants experiencing higher levels of perceived stress.

## 2. Methods

### 2.1. Participants

Study participants from the San Francisco Bay Area were recruited for a randomized controlled trial comparing a standard diet and exercise weight loss program to an enhanced program incorporating mindfulness-based eating and stress management techniques. Data for the present analysis come from the baseline assessment of this trial prior to randomization. To be eligible to participate in the study, individuals had to have a body mass index (BMI) score between 30 and 45, be 18 years old or older, and non-diabetic. The latter criterion was verified by fasting glucose (<126 mg/dl) and hemoglobin A1c (<6.0%, or $\geqslant$6.0% but <6.5% with a normal oral glucose tolerance test). Additional exclusion criteria included untreated hypothyroidism, use of immunomodulatory medications in the past 6 months (e.g., corticosteroids), being pregnant or planning to become pregnant in the next 12 months, presence of a psychiatric or medical condition that would preclude participation in the group intervention, current bulimia and/or weight loss of 15lbs or more in the past 3 months, and participation in mindfulness-based therapies in the past 2 months prior to enrollment. Informed consent was obtained from each participant prior to carrying out the study protocols. This study was approved by the Institutional Review Board of the University of California, San Francisco.

### 2.2. Study procedures

Participants completed sociodemographic and psychological questionnaires at home using a web-based platform and at their clinic assessment, which also included a blood draw and anthropometric measurement. Blood was drawn under 12-h fasting conditions between 8 AM and 12 PM into acid citrate dextrose venous vacuum collection tubes. PBMCs were isolated from whole blood by Ficoll–Hypaque density gradient centrifugation within 6 h of blood drawing, cryopreserved in liquid nitrogen, and stored at the UCSF Biological Specimen Bank until testing. Height and weight measures were obtained and BMI was calculated as weight (in kilograms) divided by height squared (in meters$^2$).

### 2.3. Sleep measures

Participants completed the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). The PSQI is a widely used and well-validated measure of global sleep quality. This 19-item measure yields seven component scores that reflect sleep difficulties in subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medication, and daytime dysfunction. A higher PSQI global score is indicative of poorer overall sleep quality. Participants also completed a daily activity diary over three consecutive days. As part of this diary, participants were asked "Around what time did you fall asleep last night?" and "What time did you wake up today?" This information was used



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