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Title

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Permalink

<https://escholarship.org/uc/item/9zp1c5kx>

Journal

The journals of gerontology. Series A, Biological sciences and medical sciences, 77(1)

ISSN

1079-5006

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Publication Date

2022

DOI

10.1093/gerona/9lab095

Peer reviewed

Original Article

Cross-Sectional and Prospective Associations of Rest-Activity Rhythms With Circulating Inflammatory Markers in Older Men

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Received: October 23, 2020; Editorial Decision Date: March 2, 2021

Decision Editor: David Le Couteur, MBBS, FRACP, PhD

Abstract

Chronic increases in pro-inflammatory cytokines in older adults, known as inflammaging, are an important risk factor for morbidity and mortality in the aging population. It has been suggested that circadian disruption may play a role in chronic inflammation, but there has been limited study that investigated the overall profile of 24-hour rest-activity rhythms in relation to inflammation using longitudinal data. In the Outcomes of Sleep Disorders in Older Men Study, we applied the extended cosine model to derive multiple rest-activity rhythm characteristics using multiday actigraphy, and examined their associations with 6 inflammatory markers (ie, C-reactive protein [CRP], interleukin 6 [IL-6], tumor necrosis factor alpha [TNF- α], tumor necrosis factor alpha soluble receptor II [TNF- α -sRII], interleukin-1 β [IL-1 β], interferon gamma [IFN- γ]) measured from fasting blood. We assessed both the cross-sectional association between rest-activity rhythms and inflammatory markers measured at baseline, and the prospective association between baseline rest-activity rhythms and changes in inflammatory markers over 3.5 years of follow-up. We found that multiple rest-activity characteristics, including lower amplitude and relative amplitude, and decreased overall rhythmicity, were associated with higher levels of CRP, IL-6, TNF- α , and TNF- α -sRII, but not IL-1 β and IFN- γ at baseline. Moreover, the lowest quartile of these 3 rest-activity characteristics was associated with an approximately 2-fold increase in the odds of having elevated inflammation (ie, having 3 or more markers in the highest quartile) at baseline. However, we found little evidence supporting a relationship between rest-activity rhythm characteristics and changes in inflammatory markers. Future studies should clarify the dynamic relationship between rest-activity rhythms and inflammation in different populations, and evaluate the effects of improving rest-activity profiles on inflammation and related disease outcomes.

Keywords: Circadian rhythms, Inflammation, Older men, Rest-activity characteristics

Aging is accompanied by important changes in the immune system. One such change is referred to as “inflammaging,” a phenomenon characterized by increased levels of pro-inflammatory cytokines that lead to

chronic low-grade inflammation in cells and tissues even in the absence of infections (1,2). Growing evidence has suggested that inflammaging is a strong risk factor for a wide range of aging-related adverse health

outcomes, including cardiovascular disease (CVD), cancer, cognitive decline, and all-cause mortality. For example, higher C-reactive protein (CRP) concentration has long been recognized as a reliable predictor of incident CVD and CVD mortality (3,4). Elevated levels of CRP, interleukin 6 (IL-6), tumor necrosis factor- α (TNF- α), and soluble TNF- α receptor 2 (TNF- α -sRII) have been linked to higher risks of certain cancers, including lung, breast, and ovarian cancer (5,6). Moreover, a meta-analysis showed that when compared to healthy controls, patients with Alzheimer's disease have higher concentrations of IL-6, TNF- α , and IL-1 β in peripheral blood (7). Given the wide range of health implications associated with inflammation in older adults, it is important to identify factors that could potentially influence inflammation levels in this population.

The circadian system plays an important role in immune function and inflammatory responses, and prior research has linked circadian disruption with elevated inflammation (8,9). For example, an inverted sleep-wake cycle that mimicked night work led to higher levels of multiple inflammatory markers under laboratory conditions (10–13), and observational studies showed increased markers of systemic inflammation among shift workers when compared to nonshift workers (14). The process of aging is associated with weakened circadian function (eg, decreased circadian amplitude and shifted phase), which is often manifested as nighttime sleep disturbances and altered sleep timing, reduced daytime activity levels, and overall impairment in the 24-hour rest-activity rhythms (15–17). Although lack of physical activity and sleep deficiencies have been consistently linked with elevated inflammatory markers in older adults (13,18–21), little research has focused on the overall rhythmic profiles of the diurnal rest-activity cycle, which may serve as unique predictors of inflammation and its related health outcomes in older adults.

In a large cohort of older men, we investigated various characteristics of rest-activity rhythms in relation to multiple inflammatory markers at baseline, as well as changes in inflammatory markers over 3.5 years of follow-up. We hypothesized that characteristics that indicate weakened rest-activity rhythms (ie, lower amplitude, reduced average levels of 24-hour activity, impaired overall rhythmicity, and extreme activity timing) are associated with higher baseline inflammation and greater increase in inflammation over time.

Method

Study Population and Analytic Samples

This analysis included participants from the Outcomes of Sleep Disorders in Older Men Study (MrOS Sleep), an ancillary study of the Osteoporotic Fractures in Men Study (MrOS, <http://mrosdata.sfccc-cpmc.net>) (22). The parent MrOS study enrolled 5994 community-dwelling, ambulatory men 65 years or older from 6 clinical centers in the United States in 2000–2002 (23). Of the original MrOS cohort, 3135 participants took part in the MrOS Sleep study, which collected objectively measures rest-activity data between 2003 and 2005 (baseline of the current analysis) (24). Fasting blood samples were obtained at baseline and again during a follow-up visit in 2007–2009 for a subset of the baseline MrOS Sleep participants. Both the parent MrOS and ancillary studies were approved by the Institutional Review Boards at each of the participating field sites (University of Alabama at Birmingham; University of Minnesota; Stanford University; University of California, San Diego; Oregon Health and Science University; University of Pittsburgh; Case Western Reserve University). Written informed consent was obtained from each participant prior to enrollment.

Assessment of Rest-Activity Rhythms

At baseline, MrOS Sleep participants wore the Sleep-watch-O (Ambulatory Monitoring, Inc.) on the nondominant wrist for 5 consecutive 24-hour periods. This actigraph contains a piezoelectric linear accelerometer, which generated a voltage each time the acceleration surpassed 0.003 g. Activity data were collected using the proportional integration mode and stored in 1-minute epochs. The orientation and sensitivity of the accelerometer were optimized for detecting sleep and wake status using wrist activity (25,26). To characterize the 24-hour rest-activity rhythms, we applied the extended cosine model. Compared to the traditional cosine models, this approach applies an antilogistic transformation and allows for greater flexibility in fitting the data, and therefore is more suitable for studying daily activity rhythms in the older population, whose diurnal patterns tend to deviate from a cosine shape (27).

Based on the extended cosine model, we calculated 5 rest-activity parameters as our primary independent variables (27): (i) Amplitude, measured as the difference between the highest and lowest point of the fitted curve, is an indicator of the strength of the rest-activity rhythms. (ii) Mesor, measured as minimum + 1/2 amplitude, is an indicator of average 24-hour activity levels. (iii) Amplitude:mesor ratio or relative amplitude is a normalized measure of rhythm strength accounting for average activity levels. (iv) Pseudo- F statistics, a model goodness-of-fit measure, is an indicator of the robustness of rest-activity rhythms. (v) Acrophase, measured as the time of peak activity, represents the timing of the rest-activity rhythm. In addition, we also derived 4 secondary rest-activity parameters, alpha (a larger value indicating narrower peaks and wider troughs), beta (a larger value indicating steeper rises and falls of the activity peak), t-left (a larger value indicating a later rise in activity), t-right (a larger value indicating a later decline in activity). Amplitude, mesor, amplitude:mesor ratio, pseudo- F statistics, alpha, and beta were categorized into quartiles, while acrophase, t-left, and t-right were categorized into 3 groups: low/early (mean – 1 standard deviation [SD]), normal (mean $\pm$ 1 SD), and high/late (mean + 1 SD). All categories of rest-activity variables were derived using the entire sample of 3058 men with valid actigraphy data.

Inflammatory Markers

Inflammatory markers examined in our study were measured in an ancillary study of MrOS that focused on the relationship between sleep apnea and changes in inflammation. Although the markers were originally chosen for assays due to their purported associations with apnea, we hypothesized that they may also be associated with circadian disruption based on findings from multiple earlier studies (11,14,28–30). Inflammatory markers were measured from fasting morning serum samples collected both at baseline and follow-up. The samples were processed on the same day and stored at -70°C . Baseline samples were collected on the same day when actigraphy recording started. All assays were performed at Johns Hopkins University Clinical Research Unit Core Laboratory. CRP was measured using an enzyme immune assay kit from ALPCO (Salem, NH) with interassay CVs ranging from 11.6% to 13.8%. Interferon gamma (IFN- γ), IL-6, IL-1 β , and TNF- α were measured using the Human ProInflammatory I 4-Plex Ultra-Sensitive Kit by Meso Scale Discovery (Rockville, MD). Interassay CVs ranged from 1.8% to 4.5% for IFN- γ , 2.0% to 9.9% for IL-6, 1.5% to 14.7% for IL-1, and 2.1% to 6.0% for TNF- α . TNF- α -sRII was measured using ELISA from R&D Systems (Minneapolis, MN), with interassay CVs ranging from 3.5% to 5.1%.

The distributions of baseline levels of all inflammatory markers were skewed right. For CRP, IL-6, TNF- α , and TNF- α sRII, we performed log transformation to approximate a normal distribution. For IFN- γ and IL-1, because their distributions remained highly skewed even after log transformation did not adequately improve the normality of the distribution, we created a dichotomous outcome variable (0, 1), where value 1 indicated the marker value in the upper quartile of the study population. We calculated changes in all markers between baseline and follow-up among those with repeated measures, with positive and negative values indicating increased and decreased marker levels over follow-up, respectively. The distributions of changes for all 6 inflammatory markers were approximately normal and no transformation was performed. Finally, we defined elevated inflammation, either at baseline or follow-up, as having 3 or more inflammatory markers in the upper quartile of the analytic sample.

Analytic Samples

Of the 3135 participants enrolled in the MrOS Sleep study, we excluded 86 with invalid or missing actigraphy data, 491 with missing information for one or more inflammatory markers, and 38 with extreme values of inflammatory markers. Extreme value is defined as above the top 1%, and this cutoff point was chosen based on visual inspection of the distribution of the marker values to remove outlier observations that were most likely due to measurement errors. The analytic sample was 2420 for cross-sectional analysis.

For prospective analysis, there were 951 men with measurements of all 6 inflammatory markers both at baseline and follow-up. Of these, we excluded 47 with extreme values (above top 1%) for changes in at least one inflammatory marker between baseline and follow-up, resulting in an analytic sample of 904. The distributions of changes in marker levels were approximately normal and no transformation was performed. In a separate analysis that focused on elevated inflammation as a binary outcome, we also excluded 122 men with elevated inflammation at baseline to examine the risk of developing incident elevated inflammation over follow-up among those without such a condition at baseline. [Supplementary Figure 1](#) shows a flowchart for deriving analytic samples in our study.

Covariates

At the enrollment of the parent MrOS study, information was collected on sociodemographic factors (age, education, race/ethnicity, marital status). Diet was measured using the Block 98 semiquantitative food frequency questionnaire (31), and a healthy diet score was calculated using factor analysis (32). At the baseline of the MrOS Sleep study, participants reported information on smoking, alcohol use, physical activity (assessed by the Physical Activity Scale for the Elderly (33)), self-rated health, and medical history of chronic diseases including CVD and depression (Geriatric Depression Scale Score ≥ 6 (34)). Body mass index (BMI; weight (kg)/height (m)²) was calculated using height and weight measured at baseline.

Statistical Analysis

Descriptive statistics were presented as means and SDs for continuous variables, and percentages for categorical variables. Group comparisons among quartiles of amplitude and categories of acrophase were conducted using analysis of variance for normally distributed variables, Kruskal-Wallis test for non-normally distributed continuous variables, and chi-square test for categorical variables. Variables in

descriptive analyses included both a priori specified covariates that we included in regression models (see below) as well as additional variables that were not included in the models but that provide important information about lifestyle and medical conditions of the study participants.

To investigate the associations between rest-activity rhythm characteristics and inflammatory markers, we used multiple linear regression models to estimate multivariable-adjusted geometric means for the concentrations of inflammatory markers in the cross-sectional analysis and multivariable-adjusted beta coefficients for changes in inflammatory markers between baseline and follow-up. For the binary outcome of elevated inflammation, we used multiple logistic regression to estimate odds ratios (ORs). For all regression analyses, we considered a series of models. Model 1 is a base model adjusted for age alone as a continuous variable. Model 2, which we consider as our main model, was adjusted for covariates determined a priori as potential confounders because they may influence both the rest-activity patterns and inflammation. Covariates in Model 2 include age (continuous), study site (Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Pittsburgh, PA; Portland, OR; San Diego, CA), education (less than high school, high school, some college, college, more than college), marital status (married, not married), race (White, non-White), smoking status (current, past, never), alcohol consumption (<1 , 1–13, 14+ drink/wk), and season of data collection (December–February, March–May, June–August, September–November). In Model 3, we included covariates in Model 2 and several additional lifestyle variables that are associated with rest-activity rhythms and may have an impact on inflammation, including physical activity (continuous), total sleep time (quartiles), sleep efficiency (continuous), and BMI (continuous). Physical activity and sleep are essential components of the rest-activity rhythms and they have been previously associated with inflammatory markers. BMI has a bidirectional relationship with the rest-activity rhythms and/or its underlying circadian clock, and is also a risk factor for inflammation. The main purpose of Model 3 is to examine whether the association of rest-activity rhythms and inflammation are independent of the well-established effects of sleep, physical activity, and obesity. We did not include medical conditions, because both weakened rest-activity rhythms and elevated inflammation are potential causes of many chronic diseases, and adjusting for common outcomes of both the dependent and independent variables may induce spurious associations (35). However, we did perform sensitivity analysis excluding people with several common chronic diseases (ie, CVD, depression, and cancer) to assess their potential impact on our results. For the analysis of changes in biomarkers, we also considered the role of baseline marker levels. On one hand, baseline markers may confound the association between baseline rest-activity rhythms and changes in marker levels over follow-up; on the other hand, adjusting for baseline levels when studying change as the outcome can also lead to biased results (36). We included baseline marker values as a sensitivity analysis but considered results obtained without adjusting for baseline values as our main results.

To test for trend, we modeled categorical variables as ordinal and evaluated the significance of coefficients using the Wald test. We avoided overemphasizing statistical significance using a single criterion, such as a p value threshold. Instead, we followed the guidelines by Greenland et al to provide a more balanced and comprehensive interpretation of our results by presenting effect estimates, 95% confidence intervals (CIs), and p values for all the associations, and evaluating our findings based on effect sizes, measures of statistical significance, dose-response patterns, and possible biological mechanisms (37).

Results

In Table 1, we present baseline characteristics according to quartiles of amplitude and categories of acrophase. When compared to the highest quartile of amplitude, lower quartiles were associated with an older age, lower alcohol intake, higher BMI, poorer self-rated health, lower levels of physical activity, lower sleep efficiency (Q1 only), a later sleep midpoint (Q1 only), and a higher prevalence of CVD, depression, and cancer. When compared to the normal category of acrophase, both the early and late categories were associated with a smaller percentage of college-educated participants, lower likelihood to be married, lower alcohol intake, higher BMI, poorer self-rated health, and shorter total sleep time, and the late category alone was additionally associated with lower levels of physical activity, lower sleep efficiency, and a higher prevalence of CVD and depression.

In cross-sectional analysis, we found that multiple inflammatory markers were associated with rest-activity characteristics in age-adjusted models (Supplementary Table 1) and after adjusting for additional potential confounders (Table 2). Of the 6 inflammatory markers, CRP, IL-6, TNF- α , and TNF- α -sRII showed consistent associations with multiple rest-activity parameters, and these associations were significant with amplitude, mesor, amplitude:mesor ratio, and pseudo- F statistics. Overall, the patterns support a relationship between weaker rest-activity rhythms and higher inflammation: a lower amplitude and relative amplitude (amplitude:mesor ratio), lower average activity level (mesor), and weaker robustness of rhythmicity (pseudo- F statistics) were all associated with higher circulating levels of CRP, IL-6, TNF- α , and TNF- α -sRII. For example, when compared to the highest quartile, the lowest quartile of amplitude:mesor ratio was associated with 31.8%, 23.3%, 6.0%, and 6.4% higher marker levels measured as geometric means for CRP, IL-6, TNF- α , and TNF- α -sRII, respectively (adjusted difference [p -for-trend], 0.47 μ g/mL [$<.0001$], 0.28 pg/mL [$<.0001$], 0.29 pg/mL [.0008], 0.26 pg/L [$<.0001$], respectively), while the lowest quartile of pseudo- F statistics was associated with 32.6%, 23.3%, and 8.9% higher levels of CRP, IL-6, and TNF- α -sRII (0.51 μ g/mL [$<.0001$], 0.24 pg/mL [$<.0001$], 0.31 [$<.0001$], respectively). Neither IL-1 β or IFN- γ were associated with any of the rest-activity markers. In addition, acrophase was generally not associated with any inflammatory marker, except for IL-6, which was higher among people with a late acrophase (adjusted geometric mean, 1.23 pg/mL) than that for the normal acrophase group (1.12 pg/mL). The results for alpha, beta, t-left, and t-right were generally weaker than those for the main rest-activity parameters, although we found several suggestive associations, including relationships between a higher alpha and higher levels of CRP and IL-6, a later t-left and higher IL-6, and an earlier t-right and higher CRP (Supplementary Table 1, Model 2). After adjusting for sleep duration and efficiency, physical activity level, and BMI, the effect sizes were slightly attenuated for IL-6, TNF- α , and TNF- α -sRII, but most of the observed patterns remained similar (Supplementary Table 1, comparing Model 3 with Model 2). Finally, removing participants with CVD, depression, and cancer reduced sample size by half and led to slight attenuation in results ($<10\%$ for most effect estimates), but the pattern of overall findings remained unchanged (data not shown).

When using elevated inflammation (ie, having 3 or more inflammatory markers in the upper quartile of the analytic sample) as a binary outcome in cross-sectional analysis, we found that a lower amplitude, amplitude:mesor ratio, and pseudo- F were all associated with higher odds of having elevated inflammation at baseline (Table

3). Specifically, when compared to participants in the highest quartile of these 3 rest-activity parameters, those in the lowest quartiles were about twice more likely to have elevated inflammation (OR [95% CI]_{Q1 vs Q4}, 2.22 [1.63, 3.02] for amplitude, 2.48 [1.82, 3.37] for amplitude:mesor ratio, and 1.81 [1.34, 2.46] for pseudo- F statistics) after multivariate adjustment (Table 3, Model 2). Additionally adjusting for sleep, physical activity, and BMI led to attenuated associations (1.75 [1.27, 2.41] for amplitude, 2.04 [1.47, 2.83] for amplitude:mesor ratio, and 1.41 [1.02, 1.95] for pseudo- F statistics), although the trends remained similar (Table 3, Model 3).

In prospective analysis, we found little evidence supporting a relationship between rest-activity rhythm characteristics and changes in inflammatory markers (results from Model 2 and for primary rest-activity parameters are presented in Table 4, and Supplementary Table 2 presents results from all models, as well as for all primary and secondary rest-activity parameters). Although a few results showed borderline statistical significance based on p value $< .05$, they did not exhibit a clear dose-response pattern and we cannot exclude chance as an explanation for these findings. Likewise, results from the analysis using incident elevated inflammation as the outcome were also largely null (Table 5), although there appeared to be suggestive evidence for a relationship of lower mesor and pseudo- F with elevated inflammation (OR [95% CI]_{Q1 vs Q4}, 1.80 [0.91, 3.57] for mesor, and 1.83 [0.98, 3.41] for pseudo- F). Adjusting for sleep, physical activity, and BMI further attenuated the associations (Table 5, Model 3). Adjusting for baseline marker values did not have a substantial impact on results (data not shown).

Discussion

In this analysis of older men, we observed a cross-sectional relationship between weakened rest-activity rhythms, characterized by lower amplitude and relative amplitude, and decreased overall rhythmicity, and higher levels of inflammatory markers in blood. However, we did not find a prospective relationship between baseline rest-activity characteristics and changes in inflammation, or incident elevated inflammation over an average of 3.5 years of follow-up.

The cross-sectional relationship between weakened rest-activity rhythms and elevated inflammatory markers is consistent with earlier studies that showed a critical role of circadian rhythms in inflammatory responses. Almost all of these studies focused on severe circadian misalignment such as shift work and/or disturbances in individual behavioral components such as sleep. For example, among healthy adults, Morris et al showed that a multiday lab protocol to induce circadian misalignment similar to night shift work led to significant increases in IL-6, CRP, and TNF- α by 15%, 7%, and 3%, respectively (10). In a large cross-sectional study of almost 2000 middle-aged adults in Finland, working the night shift was associated with significantly higher levels of CRP in men after adjusting for infection and multiple lifestyle factors (14). Sleep deficiency, which can both be a cause and consequence of impaired circadian function, has also been consistently linked to inflammation. In the MrOS study, an earlier analysis reported that short sleep duration (<5 hours) and longer wake after sleep onset (≥ 90 minutes) were both associated with higher inflammatory burden defined as the total number of inflammatory markers in the upper quartile (20). Moreover, in animal studies, experimentally induced circadian disruption by manipulating light-dark cycles led to elevated inflammatory responses, including heightened IL-6 release, following lipopolysaccharide exposure (29,38). Although these earlier studies did

Amplitude

Notes: MrOS = Osteoporotic Fractures in Men Study; SD = standard deviation.

t-*p* Values were derived from analysis of variance.

^b Values were derived from Kruskal-Wallis test.

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We found that among the rest-activity rhythm parameters, amplitude and relative amplitude, as well as pseudo-*F* statistics showed the most consistent associations with inflammation. The amplitude of rest-activity rhythms is determined by the difference between nighttime and daytime activities, and a smaller amplitude can be resulted from low physical activity levels during the day, or high levels of nighttime activity which often indicated sleep disturbances, or both. Moreover, amplitude is correlated with mesor (ie, mean activity level based on the fitted cosine curve), and a higher mesor is often an indicator of an overall active lifestyle. However, additionally adjusting for physical activity and sleep in the model only had a minimal impact on the results (ie, almost none of the effect sizes had an attenuation of 5% or more). Moreover, accounting for mesor by using relative amplitude as the exposure variable also showed robust associations with inflammatory markers. In addition, pseudo-*F* statistics, another characteristic that showed consistent associations with inflammatory markers, is a measure of model fitness and an indicator of the robustness of rest-activity rhythms, and cannot be easily derived by measuring physical activity and sleep alone. Taken together, these findings suggest that the observed associations between rest-activity rhythms and inflammation was not fully explained by conventional measures of individual behavioral components (ie, physical activity levels, sleep duration, and sleep efficiency), but instead may be indicative of a unique role of the overall rhythmic profile in inflammation. It is worth noting that several previous studies also pointed out an important role of overall rhythmicity in health and disease outcomes. For example, in an earlier analysis in the MrOS, participants in the lowest quartile of pseudo-*F* statistics showed 57% and 132% increases in total and CVD-specific mortality, respectively (17). In a more recent study, Wallace et al ranked multiple sociodemographic, medical, and lifestyle factors based on their predictive values for total mortality among MrOS participants, and pseudo-*F* statistics emerged among the strongest predictors for mortality, ranking higher than any sleep variable (39). These studies, combined with our results, further support a need to examine overall rest-activity patterns to identify novel behavioral phenotypes that may play a role in aging-related disease risks.

In contrast to the robust associations observed in cross-sectional analysis, we found little evidence supporting a prospective relationship between rest–activity rhythms and *changes* in inflammatory markers. Several factors may explain these discrepancies. First, the cross-sectional results may be primarily driven by the acute and bidirectional relationship between rest–activity rhythms and inflammation: Previous studies have shown that perturbations of the rest–activity rhythm such as total or partial sleep deprivation and circadian misalignment lead to acute (ie, within days) changes in inflammatory markers (10,40), while elevated inflammation due to infection and other disease processes can promote sleep and thus has an acute impact on patterns of rest and activity (41). This dynamic relationship between rest–activity rhythms and inflammation is driven by a complex network of transcription factors, enzymes, hormones, and neurotransmitters (9,42). On the other hand, while it is less clear whether and how weakened rest–activity rhythms may lead to the development of chronic inflammation and contribute to inflammaging over time, this long-term process may involve

Table 2. Cross-Sectional Associations[†] Between Rest–Activity Rhythm Characteristics and Inflammatory Markers

	Adjusted Geometric Mean (95% CI) [‡]				Adjusted OR (95% CI) [§]	
	CRP (μg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)	TNF-α-sRII (pg/L)	IFN-γ	IL-1β
Amplitude						
Q1	1.78 (1.64, 1.93)***	1.31 (1.24, 1.37)***	5.18 (5.05, 5.31)**	3.78 (3.68, 3.88)***	0.88 (0.67, 1.16)	1.34 (1.02, 1.76)*
Q2	1.56 (1.44, 1.69)**	1.17 (1.11, 1.23)***	5.08 (4.96, 5.20)*	3.70 (3.61, 3.79)***	1.00 (0.77, 1.30)	0.91 (0.69, 1.21)
Q3	1.28 (1.18, 1.38)	1.07 (1.02, 1.12)	5.03 (4.91, 5.15)	3.56 (3.47, 3.66)	0.83 (0.63, 1.08)	0.99 (0.75, 1.30)
Q4 (ref)	1.31 (1.21, 1.42)	1.03 (0.98, 1.08)	4.89 (4.78, 5.01)	3.52 (3.43, 3.61)	ref	ref
<i>p-trend</i>	<.0001	<.0001	0.001	<.0001	0.64	0.07
Mesor						
Q1	1.64 (1.51, 1.78)**	1.20 (1.15, 1.27)**	5.04 (4.92, 5.16)	3.71 (3.61, 3.81)	0.85 (0.64, 1.12)	1.18 (0.90, 1.56)
Q2	1.52 (1.41, 1.65)	1.17 (1.11, 1.23)*	5.16 (5.04, 5.28)	3.65 (3.56, 3.75)	1.05 (0.80, 1.36)	0.98 (0.75, 1.29)
Q3	1.33 (1.22, 1.44)	1.10 (1.05, 1.16)	4.99 (4.87, 5.11)	3.60 (3.51, 3.70)	1.07 (0.82, 1.39)	1.11 (0.84, 1.45)
Q4 (ref)	1.40 (1.29, 1.51)	1.08 (1.02, 1.13)	5.00 (4.88, 5.12)	3.59 (3.50, 3.68)	ref	ref
<i>p-trend</i>	0.001	0.0004	0.28	0.05	0.27	0.38
Amplitude:mesor ratio						
Q1	1.75 (1.61, 1.90)***	1.31 (1.25, 1.38)***	5.16 (5.04, 5.29)***	3.73 (3.63, 3.83)**	1.26 (0.96, 1.66)	1.31 (0.99, 1.74)
Q2	1.50 (1.38, 1.62)*	1.14 (1.09, 1.20)*	5.10 (4.98, 5.23)**	3.75 (3.65, 3.84)***	1.12 (0.85, 1.47)	1.09 (0.83, 1.45)
Q3	1.35 (1.24, 1.46)	1.06 (1.01, 1.11)	5.04 (4.92, 5.16)*	3.57 (3.48, 3.66)	1.22 (0.93, 1.59)	1.40 (1.07, 1.84)*
Q4 (ref)	1.32 (1.22, 1.43)	1.06 (1.01, 1.11)	4.87 (4.76, 4.99)	3.51 (3.42, 3.60)	ref	ref
<i>p-trend</i>	<.0001	<.0001	0.0008	<.0001	0.17	0.22
Pseudo-F						
Q1	1.79 (1.65, 1.95)***	1.27 (1.21, 1.33)***	5.17 (5.04, 5.30)	3.81 (3.71, 3.91)***	0.90 (0.68, 1.20)	1.00 (0.76, 1.32)
Q2	1.48 (1.37, 1.61)*	1.19 (1.14, 1.25)***	5.02 (4.90, 5.14)	3.64 (3.55, 3.74)*	0.88 (0.67, 1.16)	1.11 (0.85, 1.45)
Q3	1.36 (1.26, 1.48)	1.08 (1.03, 1.14)	5.00 (4.88, 5.12)	3.61 (3.52, 3.71)	1.10 (0.85, 1.44)	0.88 (0.67, 1.16)
Q4 (ref)	1.28 (1.19, 1.39)	1.03 (0.98, 1.08)	5.00 (4.88, 5.12)	3.50 (3.41, 3.59)	ref	ref
<i>p-trend</i>	<.0001	<.0001	0.07	<.0001	0.23	0.59
Acrophase						
Early (<13:04)	1.57 (1.42, 1.75)	1.16 (1.09, 1.24)	5.16 (4.99, 5.32)	3.67 (3.55, 3.80)	0.98 (0.74, 1.30)	1.08 (0.82, 1.43)
Normal (13:04–15:29, ref)	1.43 (1.36, 1.50)	1.12 (1.08, 1.15)	5.01 (4.94, 5.08)	3.62 (3.57, 3.68)	ref	ref
Late (>15:29)	1.57 (1.40, 1.75)	1.23 (1.15, 1.32)**	5.13 (4.96, 5.31)	3.68 (3.55, 3.81)	1.29 (0.98, 1.71)	0.86 (0.64, 1.17)

Notes: CI = confidence interval; CRP = C-reactive protein; IFN-γ = interferon gamma; IL = interleukin; TNF-α = tumor necrosis factor alpha; TNF-α-sRII = tumor necrosis factor alpha soluble receptor II.

[†]Models were adjusted for age, study site, education, marital status, race, smoking, alcohol, and season of data collection.

[‡]Raw marker levels were log-transformed and adjusted least squares means were backtransformed.

[§]Outcome variables were defined based on whether the participant's marker level was in the upper quartile (1) or not (0).

p* Value < .05 comparing to the reference group. *p* Value < .01 comparing to the reference group. ****p* Value < .001 comparing to the reference group.

pathways and mechanisms that are different from those driving the acute relationship. For example, multiple mechanisms have been proposed to cause chronic inflammation and inflammaging, including alterations in the glucocorticoids pathways, accumulation of cell damage and impaired cell repair machinery, overnutrition

and obesity, chronic infection, and changes to microbiota composition (43–45). The differences in biological pathways that are involved in acute and chronic inflammatory responses may explain the different results we observed in cross-sectional and prospective analyses. Another possibility is that at least some participants might

Table 3. Cross-Sectional Associations Between Rest–Activity Rhythm Characteristics and Elevated Inflammation[†]

		OR (95% CI)		
	No. (%)	Model 1 [‡]	Model 2 [§]	Model 3
Amplitude				
Q1	169 (28.7)	2.30 (1.70, 3.11) ^{***}	2.22 (1.63, 3.02) ^{***}	1.75 (1.27, 2.41) ^{***}
Q2	127 (20.9)	1.61 (1.18, 2.19) ^{**}	1.60 (1.17, 2.19) ^{**}	1.43 (1.04, 1.97) [*]
Q3	107 (17.7)	1.38 (1.00, 1.89) [*]	1.37 (1.00, 1.89) [*]	1.33 (0.97, 1.84)
Q4	79 (12.8)	ref	ref	ref
<i>p-trend</i>		<.0001	<.0001	.0008
Mesor				
Q1	135 (22.5)	1.19 (0.89, 1.60)	1.15 (0.85, 1.54)	1.01 (0.74, 1.40)
Q2	129 (21.4)	1.22 (0.91, 1.63)	1.23 (0.92, 1.65)	1.19 (0.88, 1.62)
Q3	113 (18.7)	1.05 (0.78, 1.41)	1.03 (0.76, 1.39)	1.02 (0.75, 1.39)
Q4	105 (17.2)	ref	ref	ref
<i>p-trend</i>		.15	.22	.71
Amplitude:mesor ratio				
Q1	169 (28.9)	2.49 (1.84, 3.37) ^{***}	2.48 (1.82, 3.37) ^{***}	2.04 (1.47, 2.83) ^{***}
Q2	127 (20.8)	1.65 (1.21, 2.26) ^{**}	1.64 (1.19, 2.24) ^{**}	1.56 (1.13, 2.15) ^{**}
Q3	110 (17.8)	1.47 (1.07, 2.03) [*]	1.51 (1.10, 2.09) [*]	1.57 (1.13, 2.17) ^{**}
Q4	76 (12.5)	ref	ref	ref
<i>p-trend</i>		<.0001	<.0001	<.0001
Pseudo-F				
Q1	156 (26.9)	1.88 (1.39, 2.52) ^{***}	1.81 (1.34, 2.46) ^{***}	1.41 (1.02, 1.95) [*]
Q2	123 (20.1)	1.35 (1.00, 1.83) [*]	1.34 (0.99, 1.83)	1.15 (0.84, 1.58)
Q3	115 (18.5)	1.28 (0.94, 1.74)	1.29 (0.95, 1.76)	1.21 (0.89, 1.66)
Q4	88 (14.5)	ref	ref	ref
<i>p-trend</i>		<.0001	.0002	.06
Acrophase [¶]				
Early	76 (21.7)	1.17 (0.88, 1.56)	1.12 (0.84, 1.50)	1.11 (0.83, 1.50)
Normal	334 (19.0)	ref	ref	ref
Late	72 (23.1)	1.24 (0.93, 1.67)	1.17 (0.86, 1.58)	1.07 (0.79, 1.45)

Notes: CI = confidence interval; OR = odds ratio.

[†]Defined as having 3 or more inflammatory markers in the highest quartile.

[‡]Model 1 was adjusted for age alone.

[§]Model 2 was adjusted for age, study site, education, marital status, race, smoking, alcohol, and season of data collection.

^{||}Model 3 was adjusted physical activity level, sleep duration, sleep efficiency, and body mass index as well as covariates in Model 2.

[¶]Acrophase was categorized as early (mean – 1 SD, <13:04), normal (mean ± 1 SD, 13:04–15:29), and late (mean + 1 SD, >15:29).

p* < .05. *p* < .01. ****p* < .001.

have had infections and/or other diseases at baseline that contributed to high levels of inflammation. Their inflammatory responses would have decreased after they recovered and they would exhibit lower inflammatory marker levels at follow-up, which may have attenuated the associations in the overall population. In support of this hypothesis, we found that when people with elevated inflammation at baseline were excluded, we observed a trend consistent with a relationship between weakened rest–activity rhythms and incident elevated inflammation at follow-up (Table 5). Finally, it is also possible that the null results resulted from a relatively short follow-up (3.5 years) and a more limited sample size, especially for the analysis focusing on incident elevated inflammation as a dichotomous variable. Although several of the effect estimates were modest to large (ORs ranged from 1.3 to 1.9, Table 5), none of them reached *p* < .05 due to large CIs. Future studies are needed to clarify the temporal relationship between rest–activity rhythms and inflammation and elucidate the underlying biological mechanisms associated with both acute and chronic responses.

Our study has several strengths. First, we used wrist actigraphy to objectively measure several different aspects of the rest–activity rhythm, and some of these rest–activity parameters have been previously linked to important clinical outcomes such as CVD and

mortality (17). Second, we measured multiple markers from different inflammatory pathways, which allowed us to examine the overall inflammatory burden that cannot be captured by a single marker due to the complex interrelationship of cytokines. Third, with repeated blood samples, we were able to examine both cross-sectional and prospective relationships between rest–activity rhythms and inflammation and compare their similarities and differences, which have not been previously described.

Our study also has several limitations. First, our study participants were all men, and most were White and had a high level of education. Therefore, the results cannot be generalized to women, racial/ethnic minority groups, and people with a lower socioeconomic status. It is well documented that sleep patterns and physical activity levels differ by sex, race and ethnicity, and socioeconomic conditions, and future studies should examine how differences in rest–activity rhythms contribute to health and disease outcomes in more diverse populations (46–49). Second, although we examined multiple pro-inflammatory markers, we did not have a complete assessment of inflammation due to the fact that this is a secondary analysis focusing on markers measured in an earlier study. In particular, we did not have measures on important anti-inflammatory markers, such as

Table 4. Prospective Associations[†] Between Rest–Activity Rhythm Characteristics and Changes in Metabolic Markers Between Baseline (2003–2005) and Follow-up (2007–2009)

		Multivariable-Adjusted [†] Beta Coefficient (95% CI)					
		CRP (μg/mL)	IFN-γ (pg/mL)	IL-1β (ng/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)	TNF-α-sRII (pg/L)
Amplitude							
Q1	–0.01 (–0.58, 0.57)	–0.13 (–0.55, 0.30)	–5.37 (–24.26, 13.52)	–0.20 (–0.46, 0.05)	0.01 (–0.18, 0.19)	0.04 (–0.11, 0.19)	
Q2	–0.28 (–0.83, 0.28)	0.07 (–0.34, 0.48)	3.77 (–14.56, 22.09)	–0.10 (–0.34, 0.15)	–0.08 (–0.26, 0.10)	–0.02 (–0.17, 0.12)	
Q3	–0.09 (–0.62, 0.44)	–0.06 (–0.45, 0.33)	–1.21 (–18.62, 16.21)	–0.04 (–0.27, 0.20)	–0.04 (–0.22, 0.13)	–0.02 (–0.16, 0.12)	
Q4	ref	ref	ref	ref	ref	ref	
<i>p-trend</i>	.78	.74	.74	.11	.94	.69	
Mesor							
Q1	0.63 (0.07, 1.20)*	0.11 (–0.31, 0.53)	–2.14 (–20.94, 16.65)	–0.01 (–0.26, 0.25)	0.09 (–0.09, 0.27)	0.04 (–0.11, 0.19)	
Q2	0.11 (–0.44, 0.66)	–0.12 (–0.52, 0.29)	1.91 (–16.23, 16.04)	–0.06 (–0.30, 0.19)	–0.15 (–0.33, 0.03)	–0.10 (–0.25, 0.04)	
Q3	0.19 (–0.35, 0.73)	–0.20 (–0.60, 0.20)	2.99 (–14.87, 20.86)	–0.07 (–0.32, 0.17)	–0.03 (–0.20, 0.15)	–0.01 (–0.16, 0.13)	
Q4	ref	ref	ref	ref	ref	ref	
<i>p-trend</i>	.05	.55	.81	1.00	.68	.93	
Amplitude:mesor ratio							
Q1	–0.36 (–0.92, 0.20)	–0.21 (–0.63, 0.21)	–8.46 (–27.11, 10.19)	–0.25 (–0.50, 0)	–0.04 (–0.22, 0.15)	–0.01 (–0.16, 0.13)	
Q2	–0.61 (–1.16, –0.06)*	–0.21 (–0.62, 0.20)	9.60 (–8.56, 27.75)	–0.18 (–0.43, 0.07)	–0.06 (–0.24, 0.11)	0.07 (–0.08, 0.21)	
Q3	–0.60 (–1.12, –0.08)*	–0.29 (–0.68, 0.10)	6.84 (–10.43, 24.11)	–0.21 (–0.44, 0.03)	–0.05 (–0.21, 0.12)	–0.08 (–0.22, 0.06)	
Q4	ref	ref	ref	ref	ref	ref	
<i>p-trend</i>	.20	.39	.51	.07	.64	.64	
Pseudo-F							
Q1	0.19 (–0.37, 0.75)	–0.11 (–0.52, 0.30)	–4.59 (–22.92, 13.74)	–0.01 (–0.25, 0.24)	–0.02 (–0.20, 0.16)	0.03 (–0.12, 0.17)	
Q2	0.01 (–0.54, 0.56)	–0.22 (–0.63, 0.18)	0.83 (–17.24, 18.90)	0.04 (–0.21, 0.28)	–0.08 (–0.26, 0.09)	0.01 (–0.13, 0.15)	
Q3	0.13 (–0.41, 0.66)	–0.50 (–0.90, –0.11)*	19.41 (1.82, 37.00)*	0.03 (–0.21, 0.27)	–0.17 (–0.34, 0.01)	–0.11 (–0.24, 0.03)	
Q4	ref	ref	ref	ref	ref	ref	
<i>p-trend</i>	.62	.90	.30	.99	.98	.42	
Acrophase [‡]							
Early	–0.43 (–0.99, 0.13)	–0.13 (–0.55, 0.29)	–5.31 (–23.95, 13.34)	–0.12 (–0.38, 0.13)	–0.003 (–0.19, 0.18)	–0.002 (–0.15, 0.15)	
Normal	ref	ref	ref	ref	ref	ref	
Late	0.43 (–0.14, 1.01)	–0.43 (–0.86, –0.01)*	–12.47 (–31.53, 6.58)	0.08 (–0.18, 0.34)	–0.09 (–0.28, 0.10)	0.06 (–0.09, 0.21)	

Notes: CI = confidence interval; CRP = C-reactive protein; IFN-γ = interferon gamma; IL = interleukin; SD = standard deviation; TNF-α = tumor necrosis factor alpha; TNF-α-sRII = tumor necrosis factor alpha soluble receptor II.

[†]Models were adjusted for age, study site, education, marital status, race, smoking, alcohol, and season of data collection.

[‡]Acrophase was categorized as early (mean – 1 SD, <13:04), normal (mean ± 1 SD, 13:04–15:29), and late (mean + 1 SD, >15:29).

**p* < .05.

IL-10, IL-12, and transforming growth factor-β. Recently, it has been proposed that the key to healthy aging is a well-balanced state between inflammatory and anti-inflammatory responses (44). More studies are needed to comprehensively assess the effects of altered rest–activity rhythms on different components of the immune system. Third, as noted before, we had limited power for prospective analysis. Finally, an observational study like ours is not designed to determine causality, and the relationship between rest–activity rhythms and inflammation may

likely involve bidirectional pathways and feedback mechanisms. Well-designed intervention studies are crucial to achieve a better understanding of the nature of the observed association and provide evidence for designing disease prevention and treatment strategies.

In summary, our study suggested that rest–activity rhythm characteristics are associated with low-grade inflammation in older population. Impairment in rest–activity rhythms is common among older adults and has been previously linked to various

Table 5. Prospective Associations Between Rest–Activity Rhythm Characteristics and Developing Elevated Inflammation Among Participants Without Elevated Inflammation[†] at Baseline

		OR (95% CI)		
	No. (%)	Model 1 [‡]	Model 2 [§]	Model 3
Amplitude				
Q1	26 (17.0)	1.56 (0.83, 2.95)	1.55 (0.81, 2.99)	1.32 (0.66, 2.66)
Q2	25 (13.8)	1.30 (0.70, 2.44)	1.30 (0.69, 2.47)	1.23 (0.64, 2.36)
Q3	32 (13.6)	1.30 (0.72, 2.35)	1.19 (0.65, 2.18)	1.16 (0.63, 2.14)
Q4	21 (9.9)	ref	ref	ref
<i>p-trend</i>		.20	.18	.43
Mesor				
Q1	28 (15.3)	1.71 (0.88, 3.31)	1.80 (0.91, 3.57)	1.70 (0.82, 3.52)
Q2	28 (14.5)	1.72 (0.89, 3.30)	2.05 (1.04, 4.01)*	2.05 (1.03, 4.10)*
Q3	32 (14.8)	1.86 (0.98, 3.51)	1.97 (1.02, 3.79)*	1.86 (0.95, 3.65)
Q4	16 (8.5)	ref	ref	ref
<i>p-trend</i>		.19	.13	.18
Amplitude/mesor				
Q1	25 (16.3)	1.35 (0.74, 2.46)	1.49 (0.80, 2.76)	1.27 (0.65, 2.45)
Q2	28 (15.8)	1.28 (0.71, 2.29)	1.35 (0.74, 2.46)	1.36 (0.74, 2.51)
Q3	25 (10.8)	0.90 (0.50, 1.61)	0.94 (0.51, 1.71)	0.95 (0.51, 1.74)
Q4	26 (11.8)	ref	ref	ref
<i>p-trend</i>		.2	.12	.32
Pseudo-F				
Q1	31 (17.9)	1.62 (0.89, 2.93)	1.83 (0.98, 3.41)	1.54 (0.79, 3.01)
Q2	24 (12.9)	1.17 (0.63, 2.16)	1.16 (0.61, 2.20)	1.08 (0.56, 2.08)
Q3	26 (12.9)	1.23 (0.68, 2.24)	1.34 (0.72, 2.49)	1.29 (0.69, 2.42)
Q4	23 (10.4)	ref	ref	ref
<i>p-trend</i>		.15	.10	.32
Acrophase [¶]				
Early	18 (16.8)	1.30 (0.74, 2.29)	1.17 (0.65, 2.13)	1.11 (0.60, 2.06)
Normal	74 (13.0)	ref	ref	ref
Late	12 (11.5)	0.82 (0.42, 1.57)	0.83 (0.42, 1.65)	0.78 (0.39, 1.58)

Notes: CI = confidence interval; OR = odds ratio.

[†]Defined as having 3 or more inflammatory markers in the highest quartile.

[‡]Model 1 was adjusted for age alone.

[§]Model 2 was adjusted for age, study site, education, marital status, race, smoking, alcohol, and season of data collection.

^{||}Model 3 was adjusted physical activity level, sleep duration, sleep efficiency, and body mass index as well as covariates in Model 2.

[¶]Acrophase was categorized as early (mean – 1 SD, <13:04), normal (mean ± 1 SD, 13:04–15:29), and late (mean + 1 SD, >15:29).

**p* < .05.

aging-related diseases. Given the well-established role of inflammation in morbidity and mortality in the aging population, elevated inflammation may serve as the mechanistic link driving the adverse effects of weakened rest–activity rhythms. Future studies should clarify the dynamic relationship between rest–activity rhythms and inflammation in different populations, and evaluate the effects of improving rest–activity profiles on inflammation and related disease outcomes.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Funding

The Osteoporotic Fractures in Men (MrOS) Study is supported by National Institutes of Health funding. The following institutes provide support: the National Institute on Aging (NIA), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Center for Advancing Translational Sciences (NCATS), and NIH Roadmap for Medical Research under the following grant numbers: U01 AG027810, U01 AG042124, U01 AG042139, U01 AG042140, U01 AG042143, U01 AG042145, U01

AG042168, U01 AR066160, and UL1 TR000128. The National Heart, Lung, and Blood Institute (NHLBI) provides funding for the MrOS Sleep ancillary study “Outcomes of Sleep Disorders in Older Men” under the following grant numbers: R01 HL071194, R01 HL070848, R01 HL070847, R01 HL070842, R01 HL070841, R01 HL070837, R01 HL070838, and R01 HL070839.

Conflict of Interest

Dr. F.A.J.L.S. has received lecture fees from Bayer HealthCare (2016), Sentara HealthCare (2017), Philips (2017), Vanda Pharmaceuticals (2018), and Pfizer Pharmaceuticals (2018). The other authors declare no conflicts of interest.

Author Contributions

Study concept and design: Q.X. and K.S. Statistical analysis: Q.X. Interpretation of data: Q.X., J.Q., F.A.J.L.S., and K.S. Critical revision of the manuscript for important intellectual content: Q.X., J.Q., D.S.E., S.R., N.E.L., S.A.-I., F.A.J.L.S., and K.S.

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