Relationship between sleep characteristics and markers of inflammation in Swedish women from the general population

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Abstract
Systemic inflammation is thought to mediate the link between sleep and cardiovascular outcomes, but previous studies on sleep habits and inflammation markers have found inconsistent results. This study investigated the relationship between sleep characteristics and C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor α (TNFα). A representative sample of 319 Swedish women was randomly selected from the general population for in-home polysomnography, sleep questionnaire and blood samples. As variables were highly correlated, principal component analysis was used to reduce the number of original variables. Linear regression with log-transformation of the outcomes (lnCRP, lnIL-6 and lnTNFα) and quantile regression were fitted to estimate cross-sectional relationships. Multivariable linear regression models suggested a significant association of insomnia symptoms (self-reported) with higher lnCRP levels (β = 0.11; 95% confidence interval [CI] = 0.02; 0.21), but not with lnIL-6 and lnTNFα. From quantile regression analysis we found that a high non-restorative index (subjective) and insomnia symptoms (self-reported) were associated with higher values of CRP, especially in the highest quantiles of the CRP distribution (90th percentile: β = 0.71; 95% CI = 0.17; 1.24, β = 1.23; 95% CI = 0.44; 2.02, respectively). Additionally, higher amounts of rapid eye movement (REM) sleep were associated with lower CRP values (90th percentile: β = −0.80; 95% CI = −0.14; −1.46). In conclusion, sleep disturbances (self-reported), specifically difficulties maintaining sleep and early morning awakenings, but not sleep duration (neither subjective nor objective), were associated with higher CRP levels. No association was found with IL-6 or TNFα. Elevated REM sleep was associated with lower CRP levels. The results suggest that inflammation might be an intermediate mechanism linking sleep and health in women.

KEYWORDS
Community-based, C-reactive protein, cross-sectional, polysomnography, sleep disturbance, women
Humans spend around one-third of their lives sleeping, and sleep has long been acknowledged as an essential component of human health (Grandner, 2017). Recently, the awareness of the substantial economic costs of insufficient sleep has increased (Hafner, Steponek, Taylor, Troxel, & van Stolk, 2017). Insufficient sleep has been recognized by the Centers for Disease Control and Prevention in the United States as a public health epidemic, with nearly 35% of US adults not getting enough sleep (<7 hr) (CDC, 2014). A similar picture has been drawn in countries across Africa and Asia, where 17% of adults aged over 50 years reported severe sleep problems (Stranges, Tigege, Gomez-Olive, Thorogood, & Kandalu, 2012). Among sleep disorders, insomnia is the most common, with 24.6% of Swedish adults reporting insomnia symptoms and 10.5% having chronic insomnia (Mallon, Broman, Akerstedt, & Hetta, 2014). Sleep disturbances have been linked to several health outcomes, including all-cause mortality and cardiovascular diseases (Grandner, 2017). It has been suggested that the link between sleep and cardiovascular outcomes is mediated by systemic inflammation: disturbed sleep might alter levels of cytokines known to have a fundamental role in inflammatory regulation, which in turn has an impact on cardiovascular risk (Grandner, Sands-Lincoln, Pak, & Garland, 2013; Irwin, 2015).

Previous studies on sleep habits and inflammation markers have found inconsistent results. For example, large epidemiological studies have found increases in C-reactive protein (CRP) levels in men with extreme (short and/or long) self-reported sleep durations, but not in women (Grandner, Buxton, et al., 2013; Richardson & Churilla, 2017). In contrast, other studies found associations only in women (Miller et al., 2009) or negative results, both with subjective and objective measures of sleep (Taheri et al., 2007). A recent meta-analysis of 72 studies found that sleep disturbances are related to CRP and interleukin-6 (IL-6), but not to tumor necrosis factor α (TNFα) (Irwin, Olmstead, & Carroll, 2016). However, there has not been an exploration of what feature of sleep disturbance plays a role in this association. Short sleep duration was not found to be associated with inflammation markers, either in the observational or in the experimental studies, whereas long sleep duration was associated with increases in CRP and IL-6 levels (Irwin et al., 2016). Many studies included in this meta-analysis, however, did not report adjusted estimates and the majority of them used sleep questionnaires instead of objective measures such as polysomnography (PSG).

Studies published so far rely mostly on self-reported sleep, and those based on PSG are usually characterized by a small sample size. The aim of the present study was to investigate the role of sleep (assessed with both questionnaires and polysomnography) in markers of inflammation (CRP, IL-6 and TNFα), using a sample of 319 Swedish women from the general population. The availability of objectively measured parameters related to the sleep microarchitecture might enable us to take a step forward in the understanding of the mechanisms through which sleep affects our health (Irwin, 2019). Given the modifiable nature of sleep behaviour, gaining new insights into its association with inflammatory markers might have important public health implications.

### 2 | METHODS

#### 2.1 | Study population

The population-based study “Sleep and Health in Women” started in 2000 when, in Phase I, a postal questionnaire with a detailed section on sleep disturbances was sent to a random sample of 10,000 women in the municipality of Uppsala, Sweden. The response rate in this first phase was 71.6%. Details of this study sample have been reported previously (Theorell-Haglow, Lindberg, & Janson, 2006).

Between 2002 and 2004, a second phase took place. A random sample of 400 non-pregnant women aged 20–70 years was drawn from the responders in Phase I for overnight in-home PSG, a second questionnaire and blood samples (Sahlin, Franklin, Stenlund, & Lindberg, 2009). Habitual snorers were intentionally oversampled, based on a proportion of 57.5% snorers versus 32.5% non-snorers, to guarantee enough variation in the index measuring obstructive sleep apnea (OSA) severity. After excluding women with incomplete PSG (recordings without full sleep period due to having started later or ended earlier than the participants’ actual sleep onset or wake up time), the study sample consisted of 319 women. No further exclusion criteria were applied. A flowchart showing how the population was obtained is presented in Figure 1. Written informed consent was obtained from all participants and the study was approved by the ethical committee of the University of Uppsala (Dnr 01–238).

#### 2.2 | Self-reported sleep

The day before the PSG recording, participants completed the Uppsala Sleep Inventory (Hetta, Broman, & Mallon, 1999) for self-reported habitual sleep. The items included difficulties initiating sleep, difficulties maintaining sleep, early morning awakenings, feeling sleepy during the daytime, and feeling tired during the day on a scale ranging from 1 to 5 (the higher the worst). Additionally, the Epworth Sleepiness Scale (ESS) (Johns, 1991) was used to assess daytime sleepiness. The morning after the PSG recording, participants were asked to self-report the total sleep time in minutes and to rate their sleep quality on a scale of 0–100, where 0 = very good sleep and 100 = very poor sleep. For a more intuitive interpretation, sleep quality has been reversed so that 100 reflects very good sleep and 0 reflects very poor sleep. Insomnia symptoms were defined as scoring 4–5 on at least one of the questions on difficulty initiating sleep, difficulty maintaining sleep or early morning awakening.
2.3 | Polysomnography

On the evening of the PSG recording, study participants picked up the PSG equipment at the Sleep Laboratory. A research nurse applied the electrodes, connected the sensors to the recorder and gave instructions before the participants returned to their homes to sleep. The PSG was conducted using a solid state, portable sleep recorder (Embla, Flaga hf, Iceland). Overnight PSG included the following recordings: continuous electroencephalogram (C3-A2, C4-A1), electrocardiogram, electrooculograms, electromyograms (submental, left and right anterior tibialis muscles), airflow (oronasal thermistor and nasal flow pressure sensor), respiratory effort from piezoelectric belts (thoracic and abdominal), finger oximetry, pharyngeal sounds from a piezo vibration sensor for snoring, and body position from a body position sensor.

Analyses were performed according to the classification criteria of the American Academy of Sleep Medicine (Iber & Iber, 2007) using the computer-assisted sleep classification system Somnolyzer 24 x 7 (Anderer et al., 2005). All scorings were also checked by a licensed sleep technician. Sleep stages 1–3 were denoted as N1, N2 and N3 and rapid eye movement sleep as REM. Changes between any of wake, N1, N2, N3 or REM were referred as stage shifts and expressed per hour. Shifts to wake from any of the sleep stages were expressed as awakenings, whereas arousals were defined as a change from any of the sleep stages to wake for a period of 3–15 s. Lights off and lights on, retrieved from sleep diaries, were used to establish time in bed and sleep efficiency, the latter defined as total sleep time (TST) divided by time in bed. Following the American Academy of Sleep Medicine guidelines (Iber & Iber, 2007), obstructive apneas were defined as the cessation of the oronasal airflow for at least 10 s with continuing abdominal and thoracic movements, whereas an obstructive hypopnea was defined as a 50% reduction in respiratory volume for at least 10 s, accompanied by abdominal and thoracic movements in combination with a desaturation of ≥3% or an arousal. The apnea–hypopnea index (AHI) was defined as the mean number of apneas and hypopneas per hour of sleep.

2.4 | Blood samples and anthropometric measures

In the morning after the PSG, participants returned to the hospital while fasting and blood samples were drawn (between 07:00am and 09:00am) for plasma CRP, IL-6 and TNFα. According to the manufacturer’s instructions, the minimum detectable levels for CRP were <0.2 mg/L, and for IL-6 <0.5 ng/L. In the statistical analyses, values of 0.1 mg/L and 0.25 ng/L were used for CRP and IL-6, respectively, when the minimum detectable level was found. Additionally, blood pressure was taken after 15 min of rest and height and weight were measured by a research nurse to calculate body mass index (BMI, kg/m²).

2.5 | Statistical analysis

Baseline characteristics and sleep variables of women participating in this study were reported. Categorical variables were summarized using absolute numbers and percentages, whereas continuous variables were summarized using means and standard deviations (SDs). We examined Pearson correlations (ρ) within sleep variables from the questionnaire and within sleep variables from PSG and, as variables were highly correlated, we reduced the number of original variables using principal component analysis (PCA). The number of principal components to retain, identified as PC(σ) for subjective variables and PC(ο) for objective variables, was decided according to the scree plot elbow rule (Cattell, 1966). Rotated factor loadings with orthogonal rotation (Varimax) are shown to allow the interpretation of PCA findings.

Associations between each principal component and inflammatory markers were analysed using linear regression, first adjusting for age, BMI and waist circumference (Model 1), and then also adjusting for physical activity (low, medium or high), smoking status (current smoker or not), alcohol consumption (total grams of alcohol per week) and depression (yes/no based on a score greater than 7 on the Hospital Anxiety and Depression Scale) (Model 2). In a third model, we also added subjective health (excellent/very good, good or fair/poor), AHI and medication use (use of medications that affect
sleep and/or inflammation) (Model 3). Medication use was based on diary records of use of medication the day before PSG and then coded according to the Anatomical Therapeutic Chemical (ATC) classification system. Opioids (N02A), antiepileptics (N03A), anxiolytics (N05B), hypnotics and sedatives (N05C), antidepressants (N06A) and antihistamines (R06A) were considered to be products that affect sleep, whereas insulin (A10A, A10B), cardiovascular medication (C01A, C02D, C03A, C03B, C03C, C03D, C03X, C07A, C07X, C08C, C08D, C09C, C10A), steroids (H02A, H03A, H03B), anti-inflammatory drugs (M01A), adrenergics (R03A, R03B, R03D) and cold medicines (R05C, R05D, R05X) were considered to be products that affect inflammation (Matthews et al., 2010). All outcome variables were log transformed (lnCRP, lnIL-6 and lnTNFα) due to their skewed distributions (Figure 2).

To further examine the relationship between principal components and inflammatory markers we also performed quantile regression analysis (Koenker & Hallock, 2001). Quantile regression makes no assumptions about the distribution of the residuals and it allowed us to evaluate the association of interest at different points in the distribution of the outcomes (10th, 25th, 50th, 75th and 90th percentiles) and not only at the mean, allowing for different patterns of association at different points in the distribution. The 95% pointwise confidence bands were obtained using 200 bootstrap replications.

Finally, subgroup analyses were conducted according to OSA severity (minimal, AHl < 5; mild, AHl 5–15; moderate, AHl 15–30; severe, AHl ≥ 30). In sensitivity analyses, we used the sleep questionnaire data only, therefore also including women with incomplete PSG. Stata version 15.1 (StataCorp LP, College Station, TX) and R version 3.5.3 were used to perform the statistical analyses. All statistical tests were two-sided and p-values less than 0.05 were considered statistically significant.

3 | RESULTS

Baseline and sleep characteristics of women included in the study are shown in Table 1. Women were on average 50 years old and they reported mostly a good or very good health status. Most of them reported a medium level of physical activity and the majority was not currently smoking. More than 50% of the women included were overweight or obese, they slept an average of 6 hr and 30% of them were complaining about at least one insomnia symptom (Table 1).

As expected, the estimated correlation between sleep variables from the polysomnography was very high (Figure 3). For example, we found $r = 0.78$ between objective total sleep time and sleep efficiency, $r = 0.77$ between awakenings and stage shifts and $r = -0.59$ between sleep efficiency and sleep latency. As for the answers in the sleep questionnaire, the estimated correlation among self-reported sleep variables was also high (Figure 3). For instance, we found $r = 0.77$ between feeling sleepy and not being rested by sleep. As a first step, we used the PCA as a dimension-reduction technique to create indices that characterize the main features of sleep. Using the scree plot elbow rule (Fig. S1), three principal components were identified for self-reported variables from the sleep questionnaire (variance explained was 34%, 19% and 13% for PC1(s), PC2(s) and PC3(s) respectively) and four principal components were identified for objective variables from PSG (PC1(o) explained 39% of the variance, followed by PC2(o) with 16%, PC3(o) with 12% and PC4(o) with 9%). Rotated factor loadings of retrieved principal components are shown in Supplementary Tables S1 and S2. According to the loading score, PC1(o) from the sleep questionnaire can be viewed as a non-restorative index (the higher the worse), PC2(o) as a measure of insomnia symptoms and PC3(o) as a measure of sleep duration and sleep quality. Principal components retrieved from PSG can be viewed as a measure of sleep continuity (PC1(o)) REM sleep (PC2(o)), sleep duration (PC3(o)) and slow-wave sleep (PC4(o)) (Figure 3).

Results from linear regression models adjusted for age, BMI and waist circumference suggested a significant association of insomnia symptoms (PC2(o)) with higher CRP levels, but not with IL-6 and TNFα. The association with CRP remained after adjustment for the other confounders (Table 2). Specifically, for each unit increase in PC2(o), the logarithm of CRP increased by 0.11 ($p = 0.11$; 95% CI = 0.02; 0.21). No association was found between the non-restorative index or sleep duration/sleep quality and

![Figure 2](image_url) Frequency distribution of C-reactive protein (CRP) (a), interleukin-6 (IL-6) (b) and tumor necrosis factor alpha (TNFα) (c) levels...
### TABLE 1 Baseline and sleep characteristics of the 319 women included in the study

| Variables | Data presented as means (SD) or as n (%) | Variables | Data presented as means (SD) or as n (%) |
|-----------|----------------------------------------|-----------|----------------------------------------|
| Number of participants | 319 | Polysomnography |  |
| Age, years | 50.2 (11.3) | Total sleep time, min | 375.8 (70.7) |
| BMI |  | Sleep efficiency, % | 82.9 (12.5) |
| BMI < 25 kg/m² | 136 (43%) | Sleep latency, min | 20.0 (20.8) |
| 25 ≤ BMI <30 kg/m² | 110 (34%) | REM latency, min | 123.2 (69.3) |
| BMI ≥ 30 kg/m² | 72 (23%) | N3, % | 13.5 (8.1) |
| Civil status |  | Sleep latency, min |  |
| Married | 229 (75%) | REM, % | 17.3 (6.5) |
| Divorced/single | 77 (25%) | REM, min | 67.1 (30.5) |
| Current smoker (yes) | 59 (19%) | AHIL, /hr | 10.2 (13.9) |
| Alcohol, g/week | 56.6 (60.9) | OSA |  |
| Physical activity |  | None/Minimal | 154 (49%) |
| Low | 38 (12%) | Mild | 96 (30%) |
| Medium | 227 (73%) | Moderate | 44 (14%) |
| High | 47 (15%) | Severe | 22 (7%) |
| Depression* (yes) | 48 (16%) | Arousal index, /hr | 32.8 (16.0) |
| Subjective health |  | Awakenings index, /hr | 3.0 (2.6) |
| Excellent/ very good | 134 (43%) | Stage changes index, /hr | 26.8 (8.7) |
| Good | 123 (40%) |  |  |
| Fair/poor | 54 (17%) | Sleep Questionnaire |  |
| Diabetes | 10 (3%) | Total sleep time, min | 381.7 (79.7) |
| Lung disease | 31 (10%) | Sleep quality, 0−100 | 43.0 (26.6) |
| Heart failure | 3 (1%) | Epworth Sleepiness Scale | 8.4 (4.1) |
| High blood pressure | 43 (20%) | Difficulties initiating sleep | 27 (9%) |
| Ever had heart attack | 3 (1%) | Difficulties maintaining sleep | 65 (21%) |
| Stroke | 2 (0.6%) | Early morning awakening | 47 (15%) |
| Medication (yes)b | 101 (32%) | Feeling tired | 61 (20%) |
| Hormone replacement therapy (yes) | 77 (25%) | Feeling sleepy | 66 (21%) |
| Systolic blood pressure, mmHg | 127 (21) | Non-resting sleep | 84 (27%) |
| Diastolic blood pressure, mmHg | 80 (10) | Difficulties in memory | 39 (13%) |
| Heart rate, beats/min | 64 (8) | Difficulties in concentrating | 34 (11%) |
| Fasting plasma glucose, mmol/L | 5.5 (1.1) | Insomnia symptoms | 96 (30%) |
| Fasting plasma HDL, mmol/L | 1.6 (0.4) |  |  |
| Fasting plasma LDL, mmol/L | 3.3 (0.9) |  |  |
| CRP | 2.7 (3.4) |  |  |
| IL-6 | 1.7 (2.1) |  |  |
| TNFα | 2.7 (2.8) |  |  |

Note: Abbreviations: AHI, apnea–hypopnea index; BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein cholesterol; IL-6, interleukin-6; LDL, low-density lipoprotein cholesterol; OSA, obstructive sleep apnea; REM, rapid eye movement sleep; SD, standard deviation; TNFα, tumor necrosis factor alpha.

*Depression = yes if depression score from Hospital Anxiety and Depression (HAD) Scale > 7.

bMedications that affect sleep and/or inflammation.

*D = Very good sleep.

†Answers were given on a scale ranging from 1 to 5 (the higher the worst). The table shows percentages of women who scored 4−5 in these items.

‡At least one of the nocturnal insomnia symptoms with scoring 4−5.
inflammatory markers. Similar results were found in a sensitivity analysis on the whole sample of 400 women (results not shown). In the PSG analysis, only the REM component was (negatively) associated with CRP values in the multivariate model 2 ($\beta = -0.10; 95\% CI = -0.19; -0.02$). Results did not change when using other indices of OSA severity (e.g., oxygen desaturation index [ODI4%] or total sleep time with oxyhaemoglobin saturation below 90% [TST90]). Additionally, when stratifying results by OSA severity we found a significant association between insomnia symptoms and lnCRP in women with moderate OSA ($\beta = 0.24; 95\% CI = 0.02; 0.47$), whereas no association was seen in the other groups. The association between REM sleep and lnCRP retained its statistical significance only in women with mild OSA ($\beta = -0.14; 95\% CI = -0.27; -0.01$) (Table 2).

When further examining the relationship between insomnia symptoms and CRP levels, only two of three insomnia symptoms were associated with inflammation: CRP values of women with difficulties in maintaining sleep were 40% higher compared to women without such difficulties ($\beta = 0.34 [\exp(\beta) = 1.40]; 95\% CI = 0.04; 0.64$). The average CRP levels in women with early morning awakenings were 72% higher compared to women without such complaints ($\beta = 0.54 [\exp(\beta) = 1.72]; 95\% CI = 0.22; 0.87$), whereas no association was observed between difficulties initiating sleep and CRP levels ($\beta = -0.41; 95\% CI = -0.83; 0.01$).

A graphical summary of quantile regression results of the association between sleep dimensions and CRP, adjusted for age and BMI, is shown in Figure 4. A high non-restorative index (PC1(s)) and insomnia symptoms (PC2(s)) were associated with higher values of CRP, especially in the highest quantiles of the CRP distribution (90th percentile: $\beta = 0.71; 95\% CI = 0.17; 1.24; \beta = 1.23; 95\% CI = 0.44; 2.02$, for PC1(s) and PC2(s), respectively), whereas no association was observed with sleep duration and sleep quality (PC3(s)). According to the objective measurements, only the sleep component referring to REM sleep (PC2(o)) seemed to be associated with CRP, in a way that higher amounts of REM sleep were associated with lower CRP values, especially in the highest quantiles of the CRP distribution (90th percentile: $\beta = -0.80; 95\% CI = -0.14; -1.46$). No association was observed between principal components and IL-6 or TNFα (Supplementary Fig. S2 and S3). Fully adjusted models are shown in Supplementary Table S3. Results are consistent with those presented above (Figure 4).

### DISCUSSION

In this cross-sectional study in women, self-reported insomnia symptoms, specifically difficulty in maintaining sleep and early morning awakenings, were associated with higher levels of CRP. No associations were found between self-reported sleep characteristics and IL-6 or TNFα. In addition, to our knowledge, this is the first study in which a negative association between REM sleep and CRP levels has been shown.

Difficulties maintaining sleep and early morning awakenings were found to be associated with higher CRP levels, whereas problems in initiating sleep were not related to CRP levels in our
TABLE 2  Associations between principal components and inflammatory markers; results from linear regression analysis with log transformation of the outcomes

| Sleep Questionnaire | Ln (CRP) | Ln (IL-6) | Ln (TNFα) |
|---------------------|----------|-----------|-----------|
|                      | β        | 95% CI    | p-value   | β        | 95% CI    | p-value   | β        | 95% CI    | p-value   |
| PC1<sub>ω</sub> - Non-restorative index |          |           |           |          |           |           |          |           |           |
| Model 1              | 0.051    | -0.014; 0.115 | .123     | 0.030    | -0.039; 0.098 | .392     | -0.004    | -0.047; 0.039 | .845     |
| Model 2              | 0.046    | -0.023; 0.116 | .191     | 0.025    | -0.048; 0.098 | .499     | -0.006    | -0.053; 0.041 | .791     |
| Model 3              | 0.031    | -0.047; 0.110 | .432     | 0.020    | -0.063; 0.104 | .631     | -0.021    | -0.073; 0.032 | .442     |
| PC2<sub>ω</sub> - Insomnia symptoms |          |           |           |          |           |           |          |           |           |
| Model 1              | 0.136    | 0.051; 0.221 | .002     | 0.060    | -0.032; 0.152 | .198     | 0.038     | -0.020; 0.096 | .196     |
| Model 2              | 0.125    | 0.036; 0.214 | .006     | 0.069    | -0.026; 0.163 | .152     | 0.042     | -0.019; 0.102 | .175     |
| Model 3              | 0.111    | 0.016; 0.206 | .023     | 0.061    | -0.041; 0.163 | .242     | 0.036     | -0.029; 0.100 | .281     |
| PC3<sub>ω</sub> - Sleep duration and quality |          |           |           |          |           |           |          |           |           |
| Model 1              | -0.035   | -0.139; 0.069 | .506     | -0.017   | -0.126; 0.093 | .765     | -0.007    | -0.077; 0.062 | .841     |
| Model 2              | -0.010   | -0.118; 0.097 | .849     | 0.001    | -0.111; 0.113 | .986     | -0.002    | -0.074; 0.070 | .953     |
| Model 3              | -0.005   | -0.114; 0.104 | .928     | 0.011    | -0.104; 0.126 | .856     | 0.008     | -0.066; 0.082 | .837     |

| Polysomnography | Ln (CRP) | Ln (IL-6) | Ln (TNFα) |
|-----------------|----------|-----------|-----------|
|                  | β        | 95% CI    | p-value   | β        | 95% CI    | p-value   | β        | 95% CI    | p-value   |
| PC1<sub>ω</sub> - Sleep continuity |          |           |           |          |           |           |          |           |           |
| Model 1          | 0.026    | -0.052; 0.104 | .515     | 0.017    | -0.066; 0.100 | .689     | 0.008     | -0.047; 0.062 | .776     |
| Model 2          | 0.020    | -0.060; 0.100 | .618     | 0.018    | -0.065; 0.102 | .665     | 0.009     | -0.047; 0.065 | .748     |
| Model 3          | 0.019    | -0.073; 0.110 | .684     | -0.006   | -0.102; 0.090 | .904     | -0.023    | -0.087; 0.041 | .482     |
| PC2<sub>ω</sub> - REM sleep |          |           |           |          |           |           |          |           |           |
| Model 1          | -0.099   | -0.182; -0.017 | .018     | -0.036   | -0.124; 0.052 | .425     | -0.019    | -0.076; 0.039 | .526     |
| Model 2          | -0.102   | -0.186; -0.018 | .018     | -0.029   | -0.118; 0.060 | .524     | -0.030    | -0.089; 0.030 | .327     |
| Model 3          | -0.082   | -0.170; 0.006 | .067     | -0.018   | -0.111; 0.074 | .697     | -0.014    | -0.075; 0.048 | .662     |
| PC3<sub>ω</sub> - Sleep time |          |           |           |          |           |           |          |           |           |
| Model 1          | -0.064   | -0.148; 0.019 | .130     | -0.003   | -0.091; 0.086 | .951     | -0.042    | -0.099; 0.016 | .155     |
| Model 2          | -0.057   | -0.142; 0.027 | .185     | 0.013    | -0.076; 0.102 | .776     | -0.043    | -0.101; 0.015 | .143     |
| Model 3          | -0.055   | -0.144; 0.034 | .229     | 0.027    | -0.067; 0.120 | .572     | -0.044    | -0.106; 0.017 | .157     |
| PC4<sub>ω</sub> - Slow-wave sleep |          |           |           |          |           |           |          |           |           |
| Model 1          | 0.030    | -0.055; 0.114 | .493     | 0.000    | -0.092; 0.092 | .995     | -0.009    | -0.067; 0.050 | .770     |
| Model 2          | 0.045    | -0.042; 0.133 | .310     | 0.021    | -0.073; 0.115 | .659     | 0.009     | -0.052; 0.070 | .774     |
| Model 3          | 0.055    | -0.034; 0.144 | .222     | 0.028    | -0.067; 0.124 | .561     | 0.021     | -0.041; 0.083 | .513     |

Note: Model 1 is adjusted for age, BMI and waist circumference; Model 2 is adjusted for age, BMI, waist circumference, physical activity, smoking, alcohol and depression; Model 3 is adjusted for age, BMI, waist circumference, physical activity, smoking, alcohol, depression, subjective health, AHI and medication use. Principal components are added in the model as continuous variables. AHI, apnea–hypopnea index; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; IL-6, interleukin-6; REM, rapid eye movement sleep; SD, standard deviation; TNFα, tumor necrosis factor alpha.

study. Accordingly, sleep initiation is of least importance. On the other hand, the inability to remain asleep seems to lead the association between insomnia symptoms and CRP in a way that, if individuals wake up frequently, they may have a more activated immune system.

Previous studies have found sex differences in relation to insomnia and inflammation (Dolsen, Crosswell, & Prather, 2019), with some evidence for the association in men, but not in women (Jackowska, Kumari, & Steptoe, 2013; Laugsand, Vatten, Bjorngaard, Hveem, & Janszky, 2012; Liukkonen et al., 2007). Our results differ from these earlier findings, but are in agreement with other observational studies in which sleep disturbances were associated with higher levels of CRP in women (Prather, Epel, Cohen, Neylan, & Whooley, 2013; Suarez, 2008). In contrast to some previous results (Miller et al., 2009; Prather, Vogelzangs, & Penninx, 2015), our study does not support the hypothesis that sleep duration is associated with markers of inflammation (CRP, IL-6 or TNFα). However, sleep duration itself may not be as important as the individual judgement about sleep. In quantile regression analysis we also found an association between the subjective non-restorative index and CRP levels in the highest quantiles of the
CRP distribution. Therefore, it is possible that CRP increases through the deprivation of restorative sleep. It is likely, however, that reverse causation plays a role here. A low restoration index might be a consequence of high CRP levels, as it is known that an increase in immune system activity might lead to tiredness, fatigue and daytime sleepiness (Bryant, Trinder, & Curtis, 2004).

We also found insights into an inverse association between REM sleep and CRP levels. The awareness of the importance of REM sleep in people’s health is increasing. Recently, it has been found that REM sleep is involved in the resetting of emotions and memory consolidation (Goldstein & Walker, 2014; Xia & Storm, 2017). The relationship between sleep and inflammation, however, has for long been known to be bidirectional: lack of sleep impairs the immune response, and responses to infections may promote sleep (Irwin & Opp, 2017). In addition, reduced REM sleep was found both after administration of IL-6 and throughout the course of an infection (Irwin, 2019). This raises the need for experimental studies of REM deprivation and inflammation to determine a direction of causality.

With respect to objective and self-reported measures of sleep, the correspondence was poor. The reason for the weak relation between sleep questionnaires and PSG could be that individuals have difficulties in judging their sleep. Additionally, whereas subjective sleep assessed through questionnaires is often related to the previous 3 months, PSG refers to a specific night. Temporary disturbances occurring that specific night may cause objective sleep to deviate from the long-term perception of overall sleep quality. A meta-analysis found a difference in sleep architecture between insomniacs and normal sleepers in a way that individuals with insomnia spend significantly less time in REM sleep (Baglioni et al., 2014). Our study is consistent with the results from this meta-analysis in the way that women with insomnia symptoms have a mean REM sleep duration 10 min shorter than normal sleepers. This shortened duration of REM sleep in individuals with insomnia symptoms, and the association between insomnia symptoms and CRP, might partially explain the increased CRP levels observed in our study in women with less REM sleep.

Some important limitations need to be taken into account when interpreting our findings. A cross-sectional study might bias the estimate of the relationship between sleep characteristics and inflammatory markers, complicating any causal assessment. Also, inflammatory markers were obtained through a single blood sample. CRP levels, however, are quite stable over 24 hr (Meier-Ewert et al., 2001), in contrast to IL-6, which follows a circadian pattern (Vgontzas et al., 2005), and when temporal variations are encountered, maximum levels are observed in the morning (Koc, Karaarslan, Abali, & Batur, 2010). Additionally, despite statistical significance, as we found small changes in CRP, it is hard to translate these findings into clinical practice. Moreover, approximately 20% of the initial sample was excluded from the analyses due to incomplete PSG. In sensitivity analysis, however, we found similar results when the total sample of 400 women was included to evaluate the association between self-reported sleep and inflammation. Finally, results on OSA stratification should be interpreted with caution, as we have limited power to draw solid conclusions on the associations within OSA subgroups.

The strengths of the study are as follows. Sleep was assessed both with a self-reported questionnaire and with in-home PSG.
Contrary to most epidemiologic studies with PSG measurements, our study includes a large sample, representative of Swedish women from the general population. In addition, multiple inflammatory markers were measured and we used high-sensitivity instruments to detect low levels of CRP. Thanks to the availability of objective measurement of breathing during sleep, we could adjust our analysis for AH1 as a measure of obstructive sleep apnea. Some previous studies have reported that sleep apnea is positively associated with CRP (Shamsuzzaman et al., 2002). Others have reported that individuals with sleep apnea often have difficulties in initiating or maintaining sleep and complain about early awakenings (Beneto, Gomez-Siurana, & Rubio-Sanchez, 2009). Besides AH1 and lifestyle factors, we also adjusted our analyses for health status and medication use. Concerning the statistical methods, we conducted a principal component analysis, which is an elegant and efficient way of handling correlated characteristics, such as sleep variables. Additionally, we applied different statistical techniques well-suited for skewed data and we obtained similar conclusions independently of the method being used. Besides the increased reliability of our findings, this is also a way to allow these models, especially quantile regression, to gain visibility in a field that could benefit from them.

In conclusion, the present findings suggest that self-reported sleep disturbances, but not sleep duration, are associated with inflammation. We, therefore, suggest that inflammation might be an intermediate mechanism linking sleep and health in women. Our findings on a negative association between REM sleep and inflammation need to be replicated in longitudinal studies to determine the direction of the association.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

EL and JT-H initiated the study, led the data collection and critically revised the paper. TA coordinated the data management, helped with the interpretation of the data and contributed to the writing of the paper. FG analysed the data, wrote the first draft of the paper and edited the paper according to the co-authors’ suggestions. RB contributed to the statistical analyses and to the writing of the paper. YTL and AT helped with interpretation of the data and critically revised the paper.

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