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Effect of continuous positive airway pressure on day/night rhythm of prothrombotic markers in obstructive sleep apnea

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Abstract

Objective—Obstructive sleep apnea (OSA) has been associated with an increased risk of atherothrombotic events. A prothrombotic state might partially explain this link. This study investigated OSA patients' day/night rhythm of several prothrombotic markers and their potential changes with therapeutic continuous positive airway pressure (CPAP).

Methods—The study included 51 OSA patients [apnea hypopnea index (AHI) ≥ 10] and 24 non-OSA controls (AHI < 10). Of the 51 OSA patients, 25 were randomized to CPAP and 26 to placebo-CPAP. Twelve blood samples were collected over a 24 h period to measure prothrombotic markers. For the apneic patients these samples were collected before treatment and after 3 weeks of treatment with either CPAP or placebo-CPAP. Day/night variation in prothrombotic markers was examined using a cosinor analysis.

Results—Compared with controls, OSA patients showed lower mesor (mean) and amplitude (difference between maximum and minimum activity) of D-dimer. In unadjusted (but not in adjusted) analysis, patients showed higher mesor of plasminogen activator inhibitor-1 ($p < 0.05$ in all cases). No significant group differences were seen in mesor and amplitude for either soluble tissue factor or von Willebrand factor, or the acrophase (time of the peak) and periodic pattern for any prothrombotic markers. There were no significant differences in changes of periodic pattern and in day/night rhythm parameters of prothrombotic markers pre- to post-treatment between the CPAP and placebo condition.

Conclusions—There may be altered day/night rhythm of some prothrombotic markers in OSA patients compared with controls. CPAP treatment for 3 weeks did not affect day/night rhythm of prothrombotic markers in OSA patients differently from placebo-CPAP.

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Conflicts of interest

This was not an industry-supported study. Dr Ancoli-Israel has consulted or advised for Johnson & Johnson, Merck, NeuroVigil, Inc., Pfizer, Philips, Purdue Pharma LP, Sanofi-Aventis, and Somaxon. Drs Dimsdale, von Känel, Mills, Natarajan, Loredó, and Gamst as well as Ms Wolfson have indicated no financial conflicts of interest.

Keywords

Day/night rhythm; Continuous positive airway pressure; Hemostasis; Polysomnography; Sleep apnea; Treatment

1. Introduction

Obstructive sleep apnea (OSA) is a prevalent sleep disorder characterized by transient and repetitive upper airway collapse during sleep. There is increasing evidence both from epidemiologic and mechanistic studies for a link between OSA and cardiovascular disease (CVD) that seems independent of sociodemographic and established cardiovascular risk factors [1]. Prospective studies have found that OSA increases the risk of incident atherothrombotic events such as myocardial infarction (MI) and stroke [2–5]. There is also evidence that OSA is associated with an increased risk of venous thromboembolism, including deep vein thrombosis and pulmonary embolism [6,7].

Several mechanisms may contribute to the initiation and progression of atherosclerosis in OSA [1]. One of these is a prothrombotic state due to a hemostatic imbalance between the coagulation and fibrinolysis systems [8,9]. Continuous narrowing of sclerotic arteries through fibrin deposits in the vessel wall develops over decades; at the time of atherosclerotic plaque rupture, enhanced clotting may accelerate thrombus growth, thereby inducing critical myocardial or cerebral ischemia [10]. A vulnerable plaque may rupture when exposed to high shear forces during apnea-triggered blood pressure (BP) peaks. The risk of MI in OSA patients is elevated between midnight and 6:00 AM [11], possibly suggesting a triggering role of OSA physiology for MI onset.

Following up on this day/night variation in MI onset in patients with OSA and a day/night rhythm in hemostatic activity [12,13], we recently reported the first and only data on chronobiological variation in prothrombotic markers in OSA from a sample of 38 OSA patients and 22 non-OSA controls (who are also part of the present study) [14]. We found that compared with controls, OSA patients had greater circulating levels of plasminogen activator inhibitor (PAI)-1, a major endogenous inhibitor of fibrinolysis [15], over a 24 h period; however, adjustment for systolic blood pressure (BP), body mass index (BMI), and smoking made this group difference non-significant. Relatively lower levels of the fibrin degradation product D-dimer in OSA patients relative to controls further supported the notion of a decreased fibrinolytic capacity in OSA over a 24 h period. The first aim of this study was to confirm these previous findings for PAI-1 and D-dimer using a larger sample size of OSA patients and to additionally investigate the day/night variation of two other prothrombotic markers – soluble tissue factor (sTF) and von Willebrand factor (vWF) – between OSA patients and controls. Soluble TF plays a major role in atherothrombosis by virtue of exerting procoagulant activity and thrombus propagation after plaque rupture [16]. The VWF exerts procoagulant function through stabilizing clotting factor VIII and the firm attachment of platelets to the site of the atherosclerotic lesion, where exposure of subendothelial structures occurs during MI [17].

Continuous positive airway pressure (CPAP) may reduce prothrombotic activity in OSA patients, including levels of PAI-1 [18], fibrinogen [19], and platelet aggregability [20], but findings are mixed [21]. Previous studies assessed prothrombotic markers at only one or two time points. Therefore, a natural question is whether CPAP treatment might normalize alterations in the 24 h day/night rhythm of prothrombotic activity in OSA. The second aim of this study was to investigate the effect of three weeks of treatment with either CPAP or placebo-CPAP on the day/night rhythm of PAI-1, D-dimer, sTF, and VWF in OSA patients.

We hypothesized that, compared with placebo-CPAP, CPAP would favorably affect prothrombotic changes in OSA patients. We adjusted our analysis for important confounders of the apnea–hemostasis relationship, namely age, BMI, and BP [8].

2. Methods

2.1. Study participants

All participants provided written informed consent to the study protocol, which was approved by The University of California San Diego (UCSD) Human Research Protection Program. We recruited patients with untreated OSA and healthy non-OSA controls from the community by advertisement, word-of-mouth, or referral from local medical practitioners. Participants with a history of major medical illnesses other than OSA and hypertension, current psychiatric diagnoses, and intake of psychotropic medication were excluded from the study. Patients who were receiving antihypertensive medications had these completely tapered for 3 weeks before they underwent the study protocol. No participants received any other medication on a regular basis (including anticoagulant medication, aspirin or other non-steroidal anti-inflammatory drugs).

2.2. Assessment of covariates

Data were collected on age and gender. Subjects who currently smoked at least one cigarette per day were termed smokers. Smoking status was not recorded for five subjects. The BMI was computed as the ratio of body weight in kilograms divided by the square of height in meters (kg/m^2). BMI was not recorded for one subject. BP data were collected and averaged over three seated measurements after resting for 5 min. The mean arterial pressure (MAP) was computed by the formula: $2/3 \times \text{diastolic BP} + 1/3 \times \text{systolic BP}$.

2.3. Study protocol

All participants arrived at the UCSD General Clinical Research Center Gillin Laboratory of Sleep and Chronobiology at 5:00 PM for a 3-day admission. At this time a venous catheter was inserted. Starting at 6:00 PM, a blood sample was collected every 2 h for the next 24 h. The catheter was kept patent with normal saline. During the 24 h blood draws, participants avoided vigorous activities but were not required to remain at bed rest throughout the sampling interval. There was no direct instruction on posture, but participants were encouraged to be in a supine position while they slept. Food was offered from a standard menu list. Lighting and room temperature were not restricted. Smoking, alcoholic beverages, and caffeine consumption were not allowed during the 24 h blood draws.

Beginning at 8:00 PM the next day, participants were instrumented for standard polysomnography (PSG) with the Grass Heritage (model PSG 36-2, West Warwick, RI, USA). Lights out occurred at 10:00 PM, and PSG recording continued until 7:00 AM. Rechtschaffen and Kales' criteria were used to score sleep recordings [22]. We defined apneas as decrements in airflow of $\geq 90\%$ from baseline for ≥ 10 s and hypopneas as decrements in airflow of $\geq 50\%$ but $<90\%$ from baseline for ≥ 10 s with an accompanying desaturation of 4%. Arterial oxygen saturation (SaO_2) was monitored using a pulse oximeter (Biox 3740; Ohmeda; Louisville, CO, USA) and analyzed using software (Profox; Escondido, CA, USA). The numbers of apneas and hypopneas per hour of sleep were calculated to obtain the apnea–hypopnea index (AHI). A diagnosis of OSA was made if AHI ≥ 10 respiratory events per hour of sleep. Non-apnea controls were defined per an AHI <10 . We also assessed data on sleepiness with the 8-item Epworth Sleepiness Scale yielding a score between 0 and 24 [23].

The study involved a parallel group comparison of 3 weeks of treatment of OSA patients with either therapeutic CPAP or placebo-CPAP. Patients were randomized to either treatment group in a double-blind fashion (by necessity, PSG technicians were unblinded to the randomization). On the first night of admission, the appropriate CPAP mask (therapeutic or placebo) was fitted, and the patient was trained on the use of the equipment. Titrations took place on the second night of admission. Patients randomized to therapeutic CPAP underwent standard CPAP titration. CPAP was started at a pressure of 4 cmH₂O and was increased by 1–2 cmH₂O increments based on the presence of apneas, hypopneas, snoring, or respiratory effort-related arousals. Titration was considered successful when all significant respiratory events stopped and the patient had spent at least 15 min of sleep at the final CPAP level. Patients randomized to placebo-CPAP underwent a mock-titration night. We used a modified version of a previously described placebo-CPAP system [24]. In brief, the placebo-CPAP consisted of a CPAP mask with ten 1/4 inch drill holes for adequate room air exchange. To control for CPAP-blower noise, the pressure was set at 8 cmH₂O. A pressure reducer was placed in the tubing between the CPAP unit and the modified mask. With this system, the pressure at the mask was 0.5 cmH₂O at end expiration and 0 cmH₂O during inspiration, and the patients felt a gentle breeze at the nose. In both treatment conditions, the CPAP systems were the same (ResMed S7 Elite CPAP with HumidAire 2i™ integrated heated humidifier; ResMed Corp., San Diego, CA, USA).

On the third night of admission, subjects slept with their assigned treatment. The next morning, they were instructed and discharged with their assigned home treatment. Research staff conducted frequent phone calls with patients to answer questions about the equipment and to encourage compliance with the therapy. All CPAP units had a hidden compliance meter allowing measurement of the nightly time the unit was switched on at pressure. After 3 weeks of home treatment, subjects were readmitted for another 24 h sampling of prothrombotic markers and full overnight PSG with their assigned treatment (post-treatment AHI was not available from one OSA patient). All participants randomized to the placebo arm slept with placebo-CPAP for the entire sleep period.

2.4. Prothrombotic markers

Venous blood was drawn into plastic tubes containing 3.8% sodium citrate (ratio: 9:1). Samples were spun in a refrigerated centrifuge between 4°C and 8°C for 10 min at 3000 g. Plasma was immediately frozen in polypropylene tubes at –80°C until analyzed. Plasma levels of PAI-1 antigen, D-dimer, VWF antigen (Asserachrom; Diagnostica Stago; Asnières, France), and of sTF antigen (Imubind tissue factor; American Diagnostica; Stamford, CT, USA) were determined by enzyme-linked immunosorbent assay following the instructions of the manufacturer. The lower detection limits of these assays are 2% of VWF, 5 ng/mL of D-dimer, 1.0 ng/mL of PAI-1, and 10 pg/mL of sTF. To minimize intra-assay variance, all samples from each participant were analyzed in the same run. There were potentially 1512 total data points assessed for each factor (12 time points × 75 OSA patients and controls for pre-treatment plus 12 time points × 51 OSA patients for post-treatment). Because of sampling problems, 40 (2.6%), 34 (2.2%), 35 (2.3%), and 43 (2.8%) measurements were missing for D-dimer, PAI-1, VWF, and sTF, respectively. Inter- and intra-assay coefficients of variation were <10% for all prothrombotic factors. To ensure that sufficient data per subject to estimate the requisite rhythm parameters, we excluded 22 (of a total of 101) subjects, who had fewer than six repeats on one or more prothrombotic markers at pre-treatment and/or post-treatment. In addition, after excluding outliers to improve data quality, the final dataset comprised 51 OSA patients and 24 controls. Thresholds to define outliers (i.e. the upper 2.5% quantile of each marker's distribution) were 1412 ng/ml for D-dimer, 179 ng/ml for PAI-1, 706 pg/ml for sTF, and 329 IU/ml for VWF. In addition, very low (i.e. implausible) values were also omitted using lower bounds of 12.6 ng/ml for D-dimer, 0.08

ng/ml for PAI-1, 2.42 pg/ml for sTF and 1.73 IU/ml for vWF. Removal of high and low-end outliers resulted in about 45 values being omitted for each marker.

2.5. Statistical methods

Data were analyzed using SPSS 17.0 for Windows and the public domain software package R v. 2.7.1 (<http://cran.stat.ucla.edu/>). The level of significance was set at $p < 0.05$ (two-tailed). All values of the prothrombotic markers were log-transformed. One-way analysis of variance, Student's t -test, and Pearson chi-squared test were applied to test for group differences in continuous and categorical data, respectively. Data are expressed as percentage values, means $\pm$ SD, or for skewed values median with interquartile range (IQR).

A cosinor analysis was used to examine the day/night rhythmicity of prothrombotic markers with the model specified as $y = \text{mes} + \text{amp} * \cos(2\pi(t - \phi)/24)$ where y was the (log) marker value, t represented time-of-day, mes represented the mesor, amp the amplitude and ϕ the acrophase of the day/night rhythm (see Table 1 for full definitions of day/night variables). This cosinor curve was fitted using mixed-effects models allowing for subject-specific intercept and rhythm-slopes (i.e. slopes for the cos and sin terms in the model) for each individual [25–27]. Day/night rhythm parameters (i.e. mesor, amplitude, acrophase) and their 95% confidence intervals (CIs) were estimated. These parameters were derived from the output of the cosinor models. In particular, using the Law of Cosines, the model can be reparametrized as follows: $y = \text{mes} + \text{amp} * \cos(2\pi(t - \phi)/24) = \text{mes} + \text{amp} [\cos(2\pi t/24) * \cos(2\pi\phi/24) + \sin(2\pi t/24) * \sin(2\pi\phi/24)] = \text{mes} + \beta_1 * \cos(2\pi t/24) + \beta_2 * \sin(2\pi t/24)$, where $\beta_1 = \text{amp} * \cos(2\pi\phi/24)$ and $\beta_2 = \text{amp} * \sin(2\pi\phi/24)$. The mixed model was used to estimate β_1 and β_2 , and these coefficients were then used to derive the amplitude, $\text{amp} = \sqrt{\beta_1^2 + \beta_2^2}$ and acrophase $\phi = 24 * \arctan(\beta_2/\beta_1)/2\pi$. Non-parametric bootstrap resampling (with 1000 bootstrap samples) was used to obtain 95% bias-corrected accelerated CIs for the estimated mesor, amplitude and acrophase [28].

Two sets of models were fitted to investigate the two sets of hypotheses examining (i) differences between OSA patients and controls at baseline and (ii) effects of CPAP treatment on OSA patients by comparing pre- with post-treatment changes in day/night rhythm parameters between the placebo-CPAP and CPAP arms. In the first set of models, baseline data from all participants (i.e. those with and without OSA) were used. OSA status was included as a binary variable (OSA patients vs controls) to test for differences in mesor between patients with and without OSA. Interactions between OSA status and cos and sin terms were included to test whether rhythm patterns varied between OSA patients and controls. Unadjusted and adjusted cosinor models were fitted with the adjusted models controlling only for key covariates [i.e. age, BMI, and MAP, and $\text{SaO}_2 < 90\%$ of time in bed (TIB)] to guard against model overfitting [29] and because the small number of smokers and women in the sample would inflate convergence problems with the bootstrap simulation. Non-parametric bootstrap resampling-based 95% CIs [28] were computed for the mean difference between OSA patients and controls in day/night rhythm parameters.

The second set of models was restricted to patients with OSA. Again, a cosinor mixed effects model was fitted allowing for subject-specific intercept and rhythm-slopes (i.e. slopes for the cos and sin terms in the model for each individual [25–27]. Visit (pre- vs post-treatment), treatment group (CPAP vs placebo) were included as fixed effects. Interactions between visit, treatment arm, and/or the cos and sin terms were included in the models. A significant interaction would indicate that day/night rhythms differed between treatment groups. The mesor, amplitude, and acrophase were computed for each visit, and, as before, bootstrap resampling was used to compute bias-corrected 95% CIs. In particular, for each bootstrap sample, the cosinor model was fitted, and coefficients from this model were used to compute the mesor, amplitude, and acrophase for each treatment group and visit. This

process was repeated 1000 times, and the distribution of changes (from pre- to post-treatment) in day/night rhythm parameters within and between treatment arms was used to estimate means and 95% CIs for these changes. Unadjusted and adjusted cosinor models were fitted with the adjusted models controlling only for key covariates modeled as fixed effects (i.e. age, BMI, MAP, and $\text{SaO}_2 < 90\%$ of TIB).

3. Results

3.1. Subject characteristics

The demographic and medical characteristics of the 75 study participants per OSA status and treatment allocation are presented in Table 2. The proportion of men was greater ($p = 0.013$) and MAP was higher ($p = 0.032$) in both groups of OSA patients compared with controls; no significant differences across the three groups were seen in terms of age, BMI, smoking status, and sleepiness. Comparison on treatment allocation revealed similar gender distribution and BP, but higher AHI ($p = 0.006$) in patients receiving CPAP relative to those receiving placebo-CPAP. Daily use of CPAP (5.7 ± 1.3 h) vs placebo-CPAP (6.4 ± 1.5 h) did not differ between groups ($p > 0.08$). There was a significantly greater decrease in the number of respiratory events per hour (median, IQR) in the CPAP group ($-30.2, -65.6/-21.3$) compared with the placebo-CPAP group ($-4.8, -10.8/+4.1$) ($p < 0.001$).

3.2. Comparison in day/night rhythm parameters of prothrombotic markers between OSA patients and non-OSA controls before treatment

3.2.1. Cosinor model—Table 3 shows the unadjusted cosinor models of prothrombotic markers at baseline between OSA patients (i.e. before OSA patients were allocated to either treatment) and controls. The significant main effect for the ‘cos t ’ term in the D-dimer model and for the ‘sin t ’ term in the PAI-1 model indicated a periodic pattern. However, interactions between the ‘cos t ’ and ‘sin t ’ terms with OSA status were non-significant indicating that the shape of the day/night pattern in D-dimer and PAI-1 did not vary between OSA patients and controls. Soluble TF and VWF did not show a significant day/night pattern. Further adjustment for age, BMI, and MAP did not change the significance of the cosinor model parameters for PAI-1, sTF, and VWF (data not shown); however, the ‘sin t ’ term (0.10 ± 0.04) and the ‘sin $t * \text{OSA}$ ’ term (-0.10 ± 0.05) became significant for D-dimer ($p < 0.05$), suggesting that the day/night pattern in D-dimer differed between OSA patients and controls when covariates had been taken into account. There also emerged significant associations between BMI and PAI-1 (0.04 ± 0.01) and between MAP and PAI-1 (0.01 ± 0.01) ($p < 0.05$).

Table 4 shows the unadjusted mesor, amplitude, and acrophase of prothrombotic markers.

3.2.1. Mesor—As we previously reported in a subgroup of the participants studied here [12], the D-dimer mesor was significantly lower and the PAI-1 mesor was significantly higher in OSA patients compared with controls. By contrast, there were no significant group differences in the mesor of sTF and VWF. Adjustments for age, BMI, MAP, and $\text{SaO}_2 < 90\%$ of TIB did not substantially alter these results for D-dimer, sTF, or VWF (data not shown). However, in the adjusted model, PAI-1 mesor did not differ significantly between OSA patients and controls.

3.2.2. Amplitude—The amplitude of D-dimer was significantly lower in OSA patients than in controls; no significant group differences were seen in the amplitude of PAI-1, sTF, and VWF. Adjustments for age, BMI, MAP, and $\text{SaO}_2 < 90\%$ of TIB did not substantially alter these results (data not shown).

3.2.3. Acrophase—OSA patients and controls showed a similar acrophase of PAI-1, D-dimer, VWF, and sTF. Adjustments for age, BMI, MAP, and SaO₂ <90% of TIB did not substantially alter these results (data not shown).

3.3. Treatment effects on day/night rhythm parameters of prothrombotic markers

3.3.1. Cosinor model—The unadjusted cosinor models for the prothrombotic markers with the two treatment conditions is given in Table 5. For the placebo arm, there was a significant periodic pattern for D-dimer, PAI-1, and sTF before starting treatment, as well as significant changes in periodic pattern for PAI-1 and VWF with placebo-CPAP treatment. CPAP versus placebo arms did not differ in periodic pattern parameters prior to treatment. Similarly, changes in periodic pattern pre- to post-treatment did not differ between treatment groups. Further adjustments for age, BMI, MAP, and SaO₂ <90% of TIB did not substantially alter the significance of these results.

3.3.2. Mesor, amplitude, and acrophase—There were no significant differences in the changes from pre- to post-treatment in mesor, amplitude, and acrophase of all prothrombotic markers between the two treatment groups (Fig. 1).

Within-group comparisons revealed a significant increase in the mesor of PAI-1 from pre- to post-treatment in the group of OSA patients who were allocated to placebo-CPAP (Fig. 1A). Moreover, OSA patients who were allocated to CPAP treatment showed a significant decrease in the amplitude of VWF between visits (Fig. 1B). Further adjustment for age, BMI, MAP, and SaO₂ <90% of TIB yielded the within-placebo-group changes in mesor non-significant for PAI-1 (0.17; 95% CI: −0.003, 0.37) and non-significant for VWF amplitude in the CPAP group (−0.088; 95% CI: −0.21, 0.01). Acrophase of D-dimer, PAI-1, sTF, and VWF did not significantly change with either treatment condition (Fig. 1C).

4. Discussion

Consistent with previous studies on day/night variation in prothrombotic markers [12,30], there was a significant day/night variation over the 24 h period in D-dimer and PAI-1 for study participants as a whole. VWF did not show day/night variation over the 24 h period, a finding that is consistent with a previous smaller study [31], but not with a large population-based study [30], suggesting that large samples might be required to detect VWF variability across the 24 h cycle. Soluble TF also did not show day/night variation over the 24 h period. This result cannot be compared with results from the literature because, to the best of our knowledge, day/night variation in sTF has not previously been investigated. Day/night variation in prothrombotic markers over the 24 h period did not significantly differ between OSA patients and controls. However, corroborating our previous findings from a subgroup of subjects of the present sample [14], relative to controls, OSA patients had a significantly higher mean values over the 24 h period (mesor) for PAI-1 as well as significantly lower mesor and amplitude values for D-dimer. Moreover, controlling for covariates, including BMI and MAP both being related to insulin resistance and the metabolic syndrome, yielded the group difference in PAI-1 mesor as non-significant. Altogether, these findings add to the increasing evidence for a prothrombotic state in OSA, which in part might be accounted for by metabolic disturbances [32].

As we have discussed previously [14], the constellation of increased levels of antifibrinolytic PAI-1 in combination with lowered level of the fibrin degradation product D-dimer is compatible with the notion of reduced fibrinolytic capacity in OSA patients. PAI-1 is the major endogenous inhibitor of fibrinolysis (i.e. of tissue-type PAI in the blood) and hence determines net fibrinolytic activity [15]. Notably, the acrophase of PAI-1 was similar in OSA patients and controls. In OSA patients, one would expect increased peak

activity of PAI-1 during the night and early morning hours when the risk of MI in OSA patients is elevated [11]. However, in OSA, it is possible that PAI-1 does not contribute to the acute onset of MI but rather to the steady progression of atherosclerosis. D-Dimer is commonly understood as a marker of fibrin formation that contributes to atherothrombotic diseases [33]. However, D-dimer levels also reflect fibrin degradation that is reduced when fibrinolytic activity is attenuated through high PAI-1 activity [15]. Therefore, lowered levels of the fibrin-splitting product D-dimer have been proposed as a global screening measure of a decreased fibrinolytic potential [34]. We measured PAI-1 antigen that highly correlates with PAI-1 activity [35] and shows day/night changes similar to PAI-1 activity [12].

In addition to hyperactive platelets [8], elevated PAI-1 levels are probably the most consistently reported prothrombotic alteration in OSA patients so far [32,36–39]. We applied a threshold AHI of 10 to define OSA, but other studies showed that PAI-1 is already elevated with an AHI as low as 5 with a further increase per 5-unit AHI increments until an AHI of 15 [39]; a similar relationship was found for fibrinogen but not D-dimer levels [39]. In fact, differences in D-dimer between OSA patients and controls are less consistent [9,39,40]; they seem less related to apnea status but more to OSA characteristics such as hypertension status [41] and oxygen desaturations [42]. We further found that day/night rhythm parameters in sTF and VWF levels were both not significantly different between OSA patients and controls. This finding concurs with some previous studies [9,43], including another recently published day/night rhythm analysis across six time points over the 24 h period [44], but not with other investigations on sTF and VWF in OSA [45,46]. Differences between studies in terms of statistical power and AHI cut-offs to define OSA might partially explain this inconsistency. Another explanation might be that sTF and VWF are not only prothrombotic markers, but also indicate endothelial dysfunction and damage. Endothelial dysfunction in OSA relates to chronic intermittent hypoxia as well as sleep loss and fragmentation [47], all of which may incompletely be captured by the AHI alone. In agreement with this reasoning, it was shown in OSA patients that sTF and VWF correlated not with AHI but strongly so with apoptotic circulating endothelial cells. Moreover, 8 weeks of CPAP treatment resulted in a significant decrease in sTF that was correlated with a decrease in apoptotic endothelial cells [46].

We further investigated the effect of a 3-week treatment with therapeutic CPAP in comparison with placebo-CPAP on day/night rhythm of prothrombotic markers in patients with mild-to-moderate OSA. CPAP treatment reduced the risk of cardiovascular events and mortality in OSA patients in the long term [48] and, even within four months, also reduced carotid intima-media thickness, an early sign of atherosclerosis [49]. Therefore, normalization of day/night variation in prothrombotic changes with therapeutic CPAP might be one important mechanism to decrease the atherothrombotic risk in OSA patients. However, we did not observe a significant difference by treatment condition in the changes of periodic pattern, mesor, amplitude, and acrophase in prothrombotic markers from pre- to post-treatment. Adjustments for common covariates of prothrombotic markers and OSA did not substantially alter these findings.

This study's finding of no CPAP treatment effect contrasts with some previous studies, in which single point measurements of prothrombotic marker levels decreased with CPAP [8]. Some of these studies applied markedly shorter duration of CPAP treatment (e.g. some effects were already seen after one night of treatment) [19] and had poorer compliance with CPAP [20] than our study. However, only two previous studies applied a placebo-CPAP controlled design. They showed lowered PAI-1 levels, but unchanged D-dimer, sTF, and VWF levels, after 2 weeks of CPAP treatment [18], and also unchanged levels of several other prothrombotic markers after 1 month of CPAP treatment [21]. It is therefore possible

that 3 weeks of CPAP treatment was too short to achieve a significant decrease in prothrombotic measures when assessed against a placebo-CPAP effect.

There are several limitations of this study. Although prothrombotic measures were assessed at 12 time points over the 24 h cycle, identifying day/night variations over several days might have yielded more reliable day/night rhythm parameters. Additional prothrombotic measures were not assessed, including fibrinogen and platelet activity, both of which play important roles in atherothrombotic events and have been shown to be elevated in OSA as well as to respond to CPAP, although in uncontrolled studies [8]. Our findings might not generalize to OSA patients with cardiovascular diseases and those with more severe apnea. Sleepiness was similar in OSA patients and controls as some controls might have responded to the study advertisement because of increased sleepiness. This might have attenuated group differences in prothrombotic factors because subjective sleep complaints have also been associated with prothrombotic changes, including D-dimer and VWF [50]. We did not account for the menstrual cycle which may affect prothrombotic measures, including those investigated in our study [51,52]. Although the difference was not statistically significant, the OSA-CPAP group started out higher on apnea severity than the OSA-placebo group. Taken together, we found evidence that OSA patients exhibit reduced fibrinolytic activity over the 24 h period, which was not restored by three weeks of therapeutic CPAP. Whether longer-term treatment with CPAP reverses these changes remains to be established.

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Fig. 1.

Treatment effects on day/night rhythm parameters of prothrombotic markers. Values are given as unadjusted bootstrap-based mean changes with 95% confidence intervals in day/night rhythm parameters of (log)prothrombotic factors from pre- to post-treatment (visit 2 minus visit 1) in the groups of obstructive sleep apnea patients treated with CPAP (C) or placebo-CPAP (P). Changes in mesor (A), amplitude (B), and acrophase (C) of prothrombotic factors did not differ significantly between the two treatments. * $p < 0.05$: significant within group difference for an increase in (log)PAI-1 mesor with placebo-CPAP treatment (A) and for a decrease in (log)VWF amplitude with CPAP treatment (B). CPAP,

continuous positive airway pressure; PAI-1, plasminogen activator inhibitor-1; sTF, soluble tissue factors; VWF, von Willebrand factor.

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Table 1

Day/night rhythm variables

Variable	Definition	Interpretation
Mesor	Mean of the modeled rhythm over the 24 h period	Lower values indicate a lower mean level
Amplitude	Difference between the maximum and minimum activity	Lower values indicate a more blunted rhythm
Acrophase	Time of day of the maximum activity	Higher values indicate a more delayed rhythm; lower values indicate a more advanced rhythm

Table 2

Characteristics of participants per apnea status and treatment allocation

Variables	OSA-CPAP (n = 25)	OSA-placebo (n = 26)	Controls (n = 24)	p
Age (years)	48.4 ± 9.11	49.1 ± 9.2	47.0 ± 8.4	0.704
Men/women (%)	92/8	92/8	62/38 ^a	0.007
Body mass index (kg/m ²)	31.4 ± 6.2	29.1 ± 3.9	28.1 ± 4.5	0.059
Current smokers (%)	17	4	20	0.116
Systolic BP (mmHg)	134.6 ± 14.3	131.8 ± 18.0	120.1 ± 13.3 ^b	0.004
Diastolic BP (mmHg)	78.4 ± 8.5	81.5 ± 9.6	75.7 ± 9.3	0.091
MAP (mmHg)	97.2 ± 9.7	98.3 ± 12.0	90.5 ± 9.9 ^c	0.026
ESS score	10.4 ± 5.0	11.1 ± 5.7	9.5 ± 6.0	0.639
Mean SaO ₂ (%)	93.4 ± 4.1	93.9 ± 7.1	94.5 ± 2.3	0.693
Minimum SaO ₂ (%)	77.7 ± 12.3	79.1 ± 18.2	82.9 ± 6.2	0.377
SaO ₂ <90% of TIB (%)	1.7 (0–10.6)	0 (0–2.1)	0.3 (0–2.2)	0.154
Apnea–hypopnea index	32.6 (25.6–61.7) ^d	20.4 (16.9–44.5)	5.8 (2.0–7.4) ^e	<0.001

BP, blood pressure; CPAP, continuous positive airway pressure; ESS, Epworth Sleepiness Scale; MAP, mean arterial pressure; OSA, obstructive sleep apnea; SaO₂, arterial oxygen saturation; TIB, time in bed.

Data are percentages, mean ± SD, or median (IQR).

p-Values refer to the comparison between the three groups of study participants, using analysis of variance for continuous measures and Pearson chi-square test for categorical variables.

Post-hoc comparisons:

^aDifferent in controls from CPAP group (*p* = 0.013) and placebo-CPAP group (*p* = 0.011).

^bLower in controls than in CPAP group (*p* = 0.002) and placebo-CPAP group (*p* = 0.009).

^cLower in controls than in CPAP group (*p* = 0.032) and placebo-CPAP group (*p* = 0.012).

^dHigher in CPAP group than in placebo-CPAP group (*p* = 0.006).

^eLower in controls than in CPAP group (*p* < 0.001) and placebo-CPAP group (*p* = 0.001).

Table 3

Cosinor model for prothrombotic factors in OSA patients vs controls

Parameter	D-Dimer	PAI-1	sTF	VWF
Cos t	$-0.14 \pm 0.04^*$	0.16 ± 0.09	$<-0.01 \pm 0.04$	0.01 ± 0.06
Sin t	0.07 ± 0.04	$0.40 \pm 0.09^*$	0.05 ± 0.03	-0.03 ± 0.05
Cos $t \times \text{OSA}$	0.07 ± 0.05	-0.03 ± 0.10	-0.04 ± 0.05	-0.06 ± 0.07
Sin $t \times \text{OSA}$	-0.07 ± 0.05	0.10 ± 0.11	-0.02 ± 0.04	-0.06 ± 0.06

PAI-1, plasminogen activator inhibitor-1; sTF, soluble tissue factors; VWF, von Willebrand factor.

The columns show the unstandardized β -coefficient $\pm$ SE.

* $p < 0.05$: significant periodic pattern of prothrombotic marker.

Coding of parameters: obstructive sleep apnea (OSA) status: 1 = OSA patients, 0 = controls; gender: women = 1, men = 0.

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Table 4

Day/night rhythm parameters of prothrombotic factors between OSA patients and controls

Parameter	Group	D-Dimer (ng/ml)	PAI-1 (ng/ml)	sTF (pg/ml)	VWF (IU/ml)
Mesor	OSA	5.60 (5.48, 5.71) *	3.55 (3.40, 3.69) *	4.96 (4.86, 5.08)	4.62 (4.50, 4.73)
(factor concentration)	Control	5.83 (5.67, 5.99)	3.21 (3.05, 3.37)	5.01 (4.81, 5.19)	4.64 (4.46, 4.83)
Amplitude	OSA	0.07 (0.03, 0.11) *	0.51 (0.43, 0.60)	0.05 (0.02, 0.08)	0.10 (0.03, 0.15)
(factor concentration)	Control	0.16 (0.09, 0.22)	0.43 (0.28, 0.54)	0.05 (0.02, 0.06)	0.03 (0.00, 0.06)
Acrophase	OSA	12.0 (9.9, 15.5)	5.0 (4.4, 5.6)	9.8 (7.3, 12.6)	16.1 (14.0, 19.2)
(time)	Control	10.2 (8.3, 11.7)	4.5 (3.0, 5.7)	6.3 (2.7, 11.7)	19.5 (1.9, 23.7)

OSA, obstructive sleep apnea; PAI-1, plasminogen activator inhibitor-1; sTF, soluble tissue factors; VWF, von Willebrand factor.

The data columns show the unadjusted bootstrap estimates of the day/night rhythm parameters for each prothrombotic factor (95% confidence interval).

* $p < 0.05$: OSA patients significantly different from controls.

Table 5

Cosinor model for prothrombotic factors in obstructive sleep apnea patients for the two treatment arms

Parameter	D-dimer (ng/ml)	PAI-1 (ng/ml)	sTF (pg/ml)	VWF (IU/ml)
Cos t	$-0.07 \pm 0.04^*$	$0.15 \pm 0.06^*$	$-0.06 \pm 0.03^*$	-0.08 ± 0.05
Sin t	0.04 ± 0.04	$0.51 \pm 0.08^*$	0.04 ± 0.03	-0.04 ± 0.05
Cos $t \times$ post-treatment	-0.02 ± 0.05	$-0.14 \pm 0.07^*$	$<-0.01 \pm 0.04$	$0.15 \pm 0.07^*$
Cos $t \times$ CPAP	-0.01 ± 0.05	-0.04 ± 0.09	0.05 ± 0.04	0.07 ± 0.08
Cos $t \times$ post-treatment $\times$ CPAP	0.08 ± 0.07	0.10 ± 0.09	-0.04 ± 0.06	-0.11 ± 0.09
Sin $t \times$ post-treatment	-0.05 ± 0.05	-0.06 ± 0.07	-0.03 ± 0.04	-0.08 ± 0.07
Sin $t \times$ CPAP	-0.09 ± 0.05	-0.04 ± 0.11	-0.03 ± 0.04	-0.10 ± 0.07
Sin $t \times$ post-treatment $\times$ CPAP	0.04 ± 0.07	0.06 ± 0.09	0.02 ± 0.06	0.18 ± 0.09

CPAP, continuous positive airway pressure; PAI-1, plasminogen activator inhibitor-1; sTF, soluble tissue factors; VWF, von Willebrand factor.

The columns show the unadjusted unstandardized β -coefficient $\pm$ SE.*
 $p < 0.05$: significant periodic pattern of prothrombotic marker.