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Three-year changes of prothrombotic factors in a cohort of South African teachers with a high clinical suspicion of obstructive sleep apnea

Running head: Prothrombotic factors in sleep apnea

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SUMMARY

A hypercoagulable state might be one important mechanism linking obstructive sleep apnea (OSA) with incident myocardial infarction and stroke. However, previous studies on prothrombotic factors in OSA are not uniform and cross-sectional. We longitudinally studied prothrombotic factors in relation to OSA risk, adjusting for baseline levels of prothrombotic factors, demographics, metabolic parameters, aspirin use, and life style factors. The Berlin Questionnaire and/or neck circumference were used to define high OSA risk in 329 South African teachers (48.0% male, 44.6% black) at baseline and 3-year follow-up. Von Willebrand factor (VWF), fibrinogen, D-dimer, plasminogen activator inhibitor-1, clot lysis time (CLT), and soluble urokinase-type plasminogen activator receptor (suPAR) were measured in plasma. At baseline 35.7% of participants had a high risk of OSA. At follow-up, persistently high OSA risk, persistently low OSA risk, OSA risk remission, and new-onset OSA risk were present in 26.1%, 53.2%, 9.3%, and 11.3% of participants, respectively. New-onset OSA risk was associated with a significant and longitudinal increase in VWF, fibrinogen, CLT, and suPAR relative to persistently low OSA risk; in VWF, fibrinogen, and suPAR relative to remitted OSA risk; and in VWF relative to persistently high OSA risk. Persistently high OSA risk was associated with an increase in CLT and suPAR relative to persistently low OSA risk and in D-dimer relative to remitted OSA risk. Remitted OSA risk was associated with D-dimer decrease relative to persistently low OSA risk. In OSA, hypercoagulability is a dynamic process with a most prominent three-year increase in individuals with new-onset OSA risk.

Keywords: Blood coagulation; cardiovascular disease; fibrinolysis; longitudinal study; risk factor; sleep disordered breathing

INTRODUCTION

Obstructive sleep apnea (OSA) is a common sleep disorder caused by repeated upper airway obstruction during sleep and is characterized through symptoms of snoring, breathing cessations while sleeping, and daytime sleepiness and fatigue (1). The prevalence of OSA is higher in men than women and increases with age (1). Also, OSA is associated with metabolic factors, including obesity, hypertension, dyslipidemia and hyperglycemia (1). Meta-analyses have shown that OSA and habitual snoring both predict incident atherothrombotic events such as acute coronary syndromes and stroke, whereby length of follow-up had no influence on risk estimates (2-4). In addition to increasing the risk of arterial thrombosis, there is accumulating evidence, including from prospective population-based studies, to suggest that OSA may also be an independent risk factor for venous thromboembolism (5). Subclinical atherosclerosis, such as measured by carotid intima-media thickness and plaque, has also been shown to develop in patients with OSA, independent of other risk factors (6) and to be associated with snoring severity (7).

Besides an ongoing inflammatory response, sympathetic hyperactivity, and oxidative stress, possibly linking OSA with CVD morbidity and mortality (8, 9), numerous studies also have shown a hypercoagulable state in OSA (10, 11). Circulating levels of prothrombotic factors previously associated with incident cardiovascular disease (CVD), like fibrinogen (12), the coagulation activation marker D-Dimer (13) and von Willebrand factor (VWF) (14), were found to be increased in OSA. Increased platelet activity has also been shown in OSA (11). Regarding impaired fibrinolysis in OSA, studies show increased levels of antifibrinolytic plasminogen activator inhibitor (PAI)-1 (15, 16) and, compatible with reduced overall plasma fibrinolytic potential, prolonged fibrin clot lysis time (CLT) (17). Excess levels of soluble urokinase plasminogen activator receptor (suPAR) are also associated with CVD risk (18). OSA-related hypercoagulability may also play an important

role in venous thromboembolism. For instance, in patients with pulmonary embolism, the prevalence of OSA was 33%; moreover, compared with non-OSA patients, those with OSA had a lower international normalized ratio (INR) on admission and required, on average, a 39% higher dose of warfarin to achieve therapeutic INR (19). However, there remains some uncertainty about the uniqueness of the hypercoagulable state in OSA, as reports on unchanged prothrombotic markers do also exist (10, 11). One explanation might be the cross-sectional nature of previous studies. Investigating prothrombotic changes over time thus might provide important insight in the dynamics of the hypercoagulable state in OSA. In addition, cross-sectional data cannot rule out the possibility of reverse causation in that prothrombotic factors might also play a role in the pathophysiology of OSA.

The sympathetic nervous system (SNS) is continuously hyperactive in OSA as evidenced by increased norepinephrine (NEPI) excretion and muscle sympathetic nerve activity, and obesity further augments SNS activity in OSA (20, 21). Likely as a function of the duration of the ongoing SNS activation, β_2 -adrenergic receptor sensitivity is decreased in OSA (22). The SNS also regulates hemostatic function (23); for instance, during SNS activation, β_2 -adrenergic receptor sensitivity directly correlates with the formation of thrombin (24). It follows from this literature that prothrombotic changes could be greater in recent-onset than in long-standing OSA. Moreover, population-based prospective studies show waxing and waning of a diagnosis of OSA (25), so it is crucial to investigate the accompanying hypercoagulability longitudinally and not simply at one point in time.

The primary aim of this study was to investigate the relation of OSA risk with 3-year changes in prothrombotic factors. For this purpose, we longitudinally observed a bi-ethnic cohort of South Africans who were free of atherothrombotic disease. The secondary aim was to examine the extent to which OSA-dependent (NEPI excretion) and OSA-independent (obesity) SNS activation (21) account for prothrombotic changes related to OSA risk.

MATERIALS AND METHODS

Study participants

The participants of this study were enrolled in the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) cohort study between 2/2008 and 5/2009 (SABPA I) and undertook a follow-up investigation between 2/2011 and 5/2012 (SABPA II). In brief, the data collected in SABPA I included demographic factors (age, sex, ethnicity), a medical history and related questionnaires, chronic medications (including aspirin and oral anticoagulants), lifestyle factors, a psychosocial battery, cardiometabolic factors, biochemical and genome assessments, and renal function (26). All 2,170 black and white teachers, 20-65 years, working in the 43 schools of the Dr. Kenneth Kaunda Education district in the North West Province of South Africa were originally invited to participate, so yielding a homogenous sample in terms of socioeconomic status (26).

Exclusion criteria were lack of informed consent, pregnancy and lactation, use of alpha- and beta-blockers, psychotropic substance abuse, blood donation or vaccination in the previous 3 months, and tympanum temperature above 37.5 °C. For the purpose of this study, we additionally excluded teachers with a positive history of myocardial infarction (n=4) and stroke (n=2), as both these atherothrombotic diseases are associated with hypercoagulability in their own right.

For SABPA I, 409 teachers were recruited of which 359 took part in the 3-year follow-up visit. The Berlin Questionnaire (BQ) was used to help identify patients at risk of OSA (cf. below) and was completed at both visits by 329 teachers, who, during the observation period, were also apparently free of myocardial infarction or stroke. All subjects provided written informed consent to participate in the study, which was approved by the Ethics Review Board of the North-West University, Potchefstroom Campus.

Study design

All participants were equipped with a 24-h ambulatory BP measurement device at their respective schools between 7:00 and 8:00 AM. On the same day (Monday through Thursday), they were admitted to the multi-bedroom Metabolic Unit Research Facility of the North-West University at 17:00 PM, when demographic data were noted, the medical history was obtained, and patients completed the BQ. Participants received a standardized dinner at 6:30 PM and had a last snack with beverages at 8:30 PM followed by recreational activities until lights out at 10:00 PM. They had no access to alcoholic and caffeinated beverages and were requested to refrain from smoking and physical exercise for at least 12 h before data sampling the next morning. After waking at 6:30 AM, anthropometric measurement were done by level II anthropometrists, the ambulatory BP monitor was removed at 7:30 AM, followed by blood collection. Thereafter, participants had a standardized breakfast, were given feedback on their health status and received R100 (US\$6) as a travelling fee.

Definition of obstructive sleep apnea risk

To identify teachers with a high clinical suspicion of OSA, we used the self-administered BQ (27), which has been validated in English (28), augmented by information on neck circumference (NC), an independent predictor of OSA severity (29). The BQ asks about characteristic features across three categories referring to 1) snoring behaviors and witnessed apneas (5 items), 2) daytime fatigue/sleepiness (4 items), and 3) high BP/obesity (2 items). Typical and validated items of the BQ are “How often do you snore?”; “Has anyone noticed that you quit breathing during your sleep?” and “Have you ever nodded off or fallen asleep while driving a vehicle?” In SABPA, answers to category 3 were assessed objectively based on ambulatory BP monitoring and a calculated body mass index (BMI) ≥ 30 kg/m². Following the original BQ, family members were not asked to confirm category 1 items.

Following the BQ a high risk of OSA is indicated with a positive score in two or more categories, whereas low risk of OSA is defined by scoring positively in fewer than two categories. The BQ estimates the probability of OSA with 88% sensitivity and 77% specificity compared with the gold standard polysomnography (PSG) to diagnose OSA (27), and, more recently, was judged to be moderately accurate (sensitivity and specificity <90%) to screen for OSA (30). A NC ≥ 40 cm diagnosed subjects with OSA (apnea hypopnea index ≥ 5 /h by PSG) with 49% sensitivity and 88% specificity (31).

For the present study, we defined a participant to have a *high risk of OSA* if he or she met the BQ criteria for high OSA risk as delineated above and/or he or she met the sex specific cut point for NC indicating a high risk of OSA (men: 43.2 cm, women: 40.6 cm) (29). In contrast, participants were defined to have a *low risk of OSA* in the present study if they met criteria for low OSA risk stipulated by the BQ.

A doctor referral was advised in the case of a high risk of OSA per the BQ. However, only one white male participant had attempted to use continuous positive airway pressure (CPAP) after baseline that was terminated after two weeks, as it was deemed uncomfortable.

Coagulation assays

The details for the measurement of hemostatic factors in SABPA have previously been published (32, 33). A registered nurse collected morning fasting blood samples with a sterile winged infusion set from the participants' antebrachial vein branches into Vacutainer tubes (Becton Dickinson) containing either citrate or ethylenediaminetetraacetic acid (EDTA). The citrate and EDTA samples were centrifuged at 2000xg for 15 minutes at room temperature. Plasma aliquots were stored in Eppendorf tubes at -80°C until analysis. Citrate plasma was used for the analyses of VWF antigen (VWF:Ag), fibrinogen, fibrin D-dimer, PAI-1 antigen (PAI-1:Ag), and CLT, and EDTA plasma was used for the measurement of

suPAR. We determined fibrinogen levels with a viscosity-based method and D-dimer levels with the STA-Liatest D-Di immunoturbidimetric assay (STA Compact, STAGO Diagnostic, Roche, France). An enzyme-linked immunosorbent assay (ELISA) was used to determine levels of PAI-1:Ag (TriniLIZE PAI-1 Antigen, Trinity Biotech, Bray, Ireland), suPAR (suPARnostic, ViroGates, Copenhagen, Denmark), and VWF:Ag (antibodies purchased from DAKO, South Africa). Plasma fibrinolytic potential of tissue factor induced clots, lysed by exogenous t-PA, was analyzed using a turbidimetric method and tissue factor and t-PA concentrations to obtain CLT of about 60 min (32, 34). The coefficient of variance for all hemostatic assays was <10%. Normal ranges are 75-125% for VWF:Ag, 1.5-4.0 g/L for fibrinogen, 0-500 ng/mL for D-dimer, 4.0-43.0 ng/mL for PAI-1:Ag, 39-135 minutes for CLT, and 2.7-3.7 ng/mL for suPAR (32, 34, 35).

D-dimer levels were undetectable (<220 ng/ml) in 126 participants and substituted with the maximum likelihood method using R 3.1.0 software (Vienna, Austria). Because of occasional assay problems, baseline/follow-up values for VWF, fibrinogen, D-dimer, PAI-1, CLT, and suPAR were missing in respectively 7/3, 5/1, 5/2, 2/2, 12/5, and 21/3 participants, and replaced using the expectation maximization algorithm.

Assessment of metabolic parameters and life style factors

To calculate the BMI (kg/m^2), we measured height and weight to the nearest 0.1 cm and 0.1 kg with calibrated instruments (Precision Health Scale, A & D Company, Japan; Invicta Stadiometer, IP 1465, UK). With the participant in a standing position, NC was measured three times and averaged using a non-extensible and flexible anthropometric tape superior to the thyroid cartilage perpendicular to the long axis of the neck. We monitored ambulatory blood pressure (BP) with the validated Cardiotens® apparatus (CE0120, Meditech, Budapest, Hungary) over 24 h with 30-min intervals during the day (8:00 AM-

10:00 PM) and 60-min intervals during the night. Participants continued with their normal daily activities. The mean successful inflation rate was 89%. We calculated the mean arterial pressure (MAP, mmHg) by the formula $[2/3(\text{diastolic BP}) + 1/3(\text{systolic BP})]$ to be used as a covariate. Hemoglobin A1c (HbA1c, %), an index of a person's average blood glucose levels over the previous three months, was determined from an EDTA whole blood sample by means of a turbidimetric inhibition immunoassay using the Roche Integra 400 (Roche, Basel, Switzerland). High-sensitivity C-reactive protein (CRP, mg/L), γ -glutamyl transferase (GGT, U/L), an index of alcohol consumption, as well as total cholesterol (T-C, mmol/L) and high-density lipoprotein cholesterol (HDL-C, mmol/L), were measured in serum with two sequential multiple analysers (Konelab 20i™, Thermo Scientific, Vantaa, Finland; Unicel DXC 800, Beckman and Coulter, Munich, Germany). We calculated the T-C/HDL-C ratio as an indicator of dyslipidemia to be used as a covariate. Serum cotinine (ng/mL), a marker of smoking incidence, was analyzed by an accredited pathology lab (AMPATH, Pretoria, Gauteng, SA) with the Modular Roche Automated Analyzer (Basel, Switzerland). Urinary NEPI excretion (nmol/L) was determined over 8 h in SABPA I and over 24 h in SABPA II with the 3-Cat Urine ELISA Fast Track kit (LDN, Nordhorn, Germany). For the baseline and follow-up visit combined, missing values for HbA1c (n=10), T-C (n=3), HDL-C (n=3), CRP (n=4), GGT (n=5), cotinine (n=5), and NEPI (n=33) were replaced with expectation maximization algorithm. Even when missing completely at random, imputation of missing data is the preferred method over complete case analysis (36).

Statistical analysis

Data were analyzed using PASW statistics, version 18.0 with significance level at $p < 0.05$ (two-tailed). Except age, all continuously scaled variables were not normally distributed and thus logarithmically transformed before analysis. Univariate analysis of

covariance (ANCOVA) was used to examine the difference in prothrombotic factors between participants with high and low OSA risk at baseline and in prothrombotic changes between the baseline and follow-up visit, while adjusting for baseline values, between four groups of participants with differing exposure to OSA risk over three years: 1) "low OSA risk" (i.e., low risk at both visits); 2) "high OSA risk" (i.e., high risk at both visits); 3) "remitted OSA risk" (i.e., high risk at baseline and low risk at follow-up); and 4) "new-onset OSA risk" (low risk at baseline and high risk at follow-up). Covariates were selected a priori based on associations with the hypercoagulable state and/or OSA (1, 10, 31, 37, 38): age, sex, ethnicity, use of aspirin/anticoagulants, BMI, MAP, HbA1c, T-C/HDL-C ratio, CRP, GGT, cotinine, NEPI. Effect sizes to determine clinical significance of the observed associations in fully-adjusted ANCOVAs were expressed as partial eta-squared (η^2), with values of 0.01, 0.06, and 0.14 indicating small, medium, and large effects, respectively (39).

RESULTS

Participant characteristics

The median age of the 329 study participants was 46 years (range 20-63), 158 (48.0%) were male and 147 (44.7%) were black. At baseline, one participant used oral anticoagulants and was lumped with 12 aspirin users into one category. High OSA risk was present in 108 participants based on the BQ and in an additional 9 participants based on cut point values for NC, yielding a prevalence of 35.6% (n=117) for a high clinical suspicion of OSA in the whole sample. Table 1 shows participant characteristics per high vs. low risk of OSA at baseline. High OSA risk was more prevalent in men than women and cardiometabolic risk factors, including BMI, MAP, HbA1c, T-C/HDL-C ratio, CRP, as well as GGT and NEPI excretion, were also higher in participants with high vs. low OSA risk.

Relation between OSA risk and prothrombotic factors at baseline

Without adjustment for covariates (Table 1), fibrinogen, PAI-1 and CLT levels were significantly higher in participants with high vs. low OSA risk. After controlling for age, sex, ethnicity, MAP, HbA1c, T-C/HDL-C ratio, CRP, GGT, cotinine levels, and aspirin/anticoagulant use, CLT ($p=0.034$) remained significantly higher in high vs. low OSA risk participants and further adjustment for NEPI excretion did not change this result ($p=0.037$). However, when BMI was also controlled for in the final adjusted model (Table 2), this association was attenuated to non-significance ($p=0.75$), suggesting that BMI accounted for a substantial amount of variance of the relation between high OSA risk and reduced fibrinolytic activity at baseline. In agreement with this reasoning, the direct relationship of CLT with BMI, but also with HbA1c, T-C/HDL-C and GGT, showed a medium effect size in the fully adjusted model ($\eta^2>0.06$). In addition, there were large effect sizes for higher levels of VWF and PAI-1 in blacks compared with whites ($\eta^2>0.14$).

Course of obstructive sleep apnea risk over time

At the follow-up visit, 123 (37.4%) participants were defined to have high OSA risk, 106 based on the BQ, and an additional 17 based on the cut point for NC. There was no change in either low or high OSA risk over time in 261 (79.3%) participants with 175 (53.2%) having low OSA risk and 86 (26.1%) having high OSA risk at both visits. A change in OSA risk occurred in 68 (20.7%) participants; whereas 31 (9.4%) experienced remission of OSA risk and 37 (11.3%) had new-onset OSA risk.

Dynamics in obstructive sleep apnea risk and changes in covariates over time

There were significant differences in three-year changes of BMI ($p=0.025$, $\eta^2=0.029$) and MAP ($p=0.001$, $\eta^2=0.051$) between the four groups with differing dynamics in OSA risk

with adjustment for demographic factors, baseline level of the respective covariate, anytime aspirin/anticoagulant use (aspirin at baseline and/or at follow-up: n=44, oral anticoagulant at baseline: n=1), and changes over time in all other covariates. The mean $\pm$ SEM increase in BMI over time was greater in participants with new-onset OSA risk (1.47 ± 0.34 kg/m², $p=0.011$) and in those with high OSA risk (1.34 ± 0.25 kg/m², $p=0.016$) relative to the group with low OSA risk (0.60 ± 0.17 kg/m²). An increase in MAP over time was observed in the high vs. low OSA risk group (2.49 ± 0.88 vs. -1.95 ± 0.60 mmHg, $p<0.001$). No significant group differences were observed for changes over time in T-C/HDL-C ratio ($p=0.98$), HbA1c ($p=0.32$), GGT ($p>0.08$), cotinine ($p=0.23$), CRP ($p=0.11$), and NEPI ($p=0.55$).

Dynamics in OSA risk and changes in prothrombotic factors over time

There were statistically significant and small-to-medium effects for three-year changes between the four groups with differing dynamics in OSA risk in VWF ($p=0.005$, $\eta^2=0.040$), fibrinogen ($p=0.008$, $\eta^2=0.037$), D-dimer ($p=0.007$, $\eta^2=0.037$), CLT ($p=0.021$, $\eta^2=0.031$), and suPAR ($p=0.007$, $\eta^2=0.038$), but not PAI-1 ($p=0.69$) with adjustment for all covariates (including changes in BMI and NEPI over time). Table 3 shows the relationships between covariates and three-year changes in prothrombotic factors in the fully adjusted models with P-values and effect sizes. Lower baseline levels of prothrombotic factors showed large effects for an association with a greater increase in prothrombotic factors over time. Large effects were also seen for a direct relationship between changes in CRP and fibrinogen levels and for a greater increase in PAI-1 levels in blacks than whites. Fibrinogen levels also showed a greater increase in blacks than whites with a medium effect size. Additional statistically significant relationships were of small-to-medium effect size and thus similar with those seen for the relationships between dynamics in OSA risk and prothrombotic factors over time.

Figure 1 presents the comparisons of the covariate-adjusted three-year changes in prothrombotic factors between the four groups with differing dynamics in OSA risk. In participants with new-onset OSA risk, there was an increase over time in VWF ($p<0.001$), fibrinogen ($p=0.004$), CLT ($p=0.026$), and suPAR ($p=0.006$) levels relative to the group with low OSA risk. The group with new-onset OSA risk also showed an increase over time in VWF ($p=0.024$), fibrinogen ($p=0.004$), and suPAR ($p=0.041$) levels relative to the group with remitted OSA risk. There was a greater increase in VWF levels over time in the new-onset OSA risk group compared with the high OSA risk group ($p=0.033$). In turn, the latter showed an increase over time in CLT ($p=0.010$), and suPAR levels ($p=0.009$) compared with the low OSA risk group and in D-dimer ($p=0.001$) levels relative to those with remitted OSA risk. Except for a greater decrease in D-dimer levels over time in the group with remitted OSA risk than in the group with low OSA risk ($p=0.003$), prothrombotic changes did not differ between these two groups.

DISCUSSION

In our three-year longitudinal cohort study of black and white teachers from South Africa, we found the prevalence of a high clinical suspicion of OSA to be 35.6% at baseline. This prevalence is lower than the 43% observed in patients with stroke (40), but higher than the 24.3% in a large population-based study from Norway (41), both having used the BQ. However, in addition to the BQ, we considered NC to define OSA risk and objectively assessed hypertension instead of self-reporting, bearing in mind that 6.4% of adults in the general population are not aware of their hypertension (42). Furthermore, we found considerable variation of OSA risk between visits, with 20.7% of study participants showing a change in their OSA risk status over time. This observation concurs with earlier community-based studies showing that sleep disordered breathing measured at one single

point in time is rather weakly predictive of the severity of a breathing disorder several years later (25). Therefore, the natural course of OSA risk seems to be dynamic, making it crucial to also longitudinally investigate the accompanying physiology.

In agreement with several previous PSG studies (9, 10), we found cross-sectional relationships between a high risk of OSA and increased prothrombotic activity at baseline. Unadjusted levels of fibrinogen, PAI-1, and CLT were significantly higher in participants with high than low OSA risk and group differences in CLT were maintained after adjustment for covariates except BMI. Associations of CLT with BMI, but also with HbA1c, the T-C/HDL-C ratio, and GGT, were all of medium effect size. This observation concurs with previous PSG studies showing that metabolic factors partially account for impaired fibrinolysis in OSA (43). To the extent that the SNS is an important regulator of hemostatic function (23, 24) and obesity adds to SNS activation in OSA (21), the role of BMI as a mediator of prothrombotic activity (i.e., reduced fibrinolysis) in participants with high OSA risk could partially be explained by a mechanism increasing SNS activation. In contrast, adjustment for urinary NEPI excretion did not affect the significant association of OSA risk with CLT, suggesting that OSA-dependent SNS activation (20, 21) did not substantially account for fibrinolytic activity. However, we were unable to test for a mediating role of hypoxemia and hypercapnia during apnea, and arousals at apnea termination, key mechanisms in SNS overactivity in OSA. As previously reported in this study sample (32, 33), blacks had significantly higher levels in most prothrombotic factors when compared with whites. Large ethnic differences were seen for VWF and PAI-1, whereas small-to-medium effects were revealed for fibrinogen, D-dimer, and suPAR.

The main and novel finding of the present study is a change in prothrombotic factors over an observation period of three years in relation to OSA risk independent of important covariates of both hemostatic function and OSA, including metabolic factors and low-grade

systemic inflammation. The relationships between dynamics of OSA risk and changes in prothrombotic factors were of small-to-moderate effect size and in the range of several important covariates of hemostatic function, so indicating clinical significance. Most likely reflecting regression to the mean, lower levels of prothrombotic factors at baseline were independently associated with a greater increase in prothrombotic factors over time showing a large effect. Again, black ethnicity but also CRP changes, were associated with medium-to-large effects with an increase in fibrinogen and PAI-1 levels over time.

Post-hoc group comparisons showed that compared to participants with low OSA risk at both visits, CLT and suPAR levels had all increased between baseline and follow-up in participants with new-onset OSA risk and also in those with high OSA risk at both visits. In addition, the new-onset OSA risk group showed higher fibrinogen and VWF levels than the group with low OSA risk and also higher VWF levels than the group with high OSA risk, so three-year increases in prothrombotic factors were most prominent in relation to new-onset OSA risk. Increases in BMI and MAP over time were also greater in participants with new-onset OSA risk and high OSA risk, respectively, compared with the low OSA risk group. Therefore, dynamics in OSA may affect cardiovascular risk through several pathways.

Longer standing OSA with the accompanying SNS activation may result in decreased sensitivity of the β 2-adrenergic receptor (22), being involved in VWF release from endothelial cells upon adrenergic stimulation (23, 24, 44); therefore, still more sensitive β 2-adrenergic receptors could have elicited greater VWF increase in the new-onset compared to the high OSA risk group. However, VWF findings were independent of three-year changes in NEPI excretion, which, therefore appeared not to be an accurate marker of SNS activity to confirm such a hypothesized mechanism. We also found no evidence for OSA-independent SNS activation as a mechanistic explanation, as adjustment for BMI changes did not alter

results either. Nonetheless, in PSG studies, VWF levels were shown to directly correlate with total arousal index (45), and arousals are a potent trigger of SNS activation during sleep.

Participants with remitted OSA risk differed little in changes of prothrombotic factors from those with low OSA risk, with the former even showing a relatively greater decrease over time in D-dimer levels, an index of fibrin turnover. The reasons for this are speculative whereby temporary overshooting of antithrombotic activity during recovery from OSA might be one explanation. In our sample, only one participant had undergone CPAP treatment for a limited time. It thus might be of clinical interest to investigate whether "natural" remission of OSA with the accompanying decrease in prothrombotic activity lowers CVD risk comparable to CPAP therapy (46), which has also been shown to reduce prothrombotic activity, including VWF levels, in OSA patients (47). However, there are also placebo-controlled studies showing that, for instance, two months of CPAP treatment did not attenuate fibrin generation in patients with severe OSA (48).

The longitudinal design, selection of a comprehensive set of prothrombotic factors, and broad adjustment for important covariates of OSA and hemostatic function are strengths of our study. There are also limitations. Clearly, defining OSA status with full-night PSG would have additionally strengthened the confidence in our findings, although a systematic expert review has concluded that the BQ is moderately accurate to screen for OSA against the gold standard PSG (30). Therefore, future studies should aim to confirm our findings applying PSG to make a diagnosis of OSA. We did not assess prothrombin time and activated partial thromboplastin time, both of which had provided additional insight in hemostatic alterations related to dynamics in OSA risk over time. We investigated black and white individuals in the workforce from South Africa and free of a history of atherothrombotic disease. Therefore, our findings should not be generalized to clinical populations, elderly individuals, and other ethnicities. We did not assess genetic variants of prothrombotic factors,

while a previous study found no association between the PAI-1 (–675 4G/5G) polymorphism and OSA (50).

Taken together, we found that prothrombotic factors increased in participants with persistently high OSA risk, and even to a greater extent, in those with new-onset OSA risk, over an observation period of three years compared to participants with persistently low OSA risk or remitted OSA risk. Whether dynamics in prothrombotic factors will ultimately translate into the risk of incident atherothrombotic CVD in OSA remains to be explored.

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LEGENDS TO FIGURES AND TABLES

Legend to Figure 1

Panels A-E show mean $\pm$ SEM changes in levels of prothrombotic factors between baseline and the three-year follow-up for the four groups with differing dynamics in risk of obstructive sleep apnea (OSA). Original values are given for clarity. All values were adjusted for baseline age, sex, ethnicity, baseline level of the respective prothrombotic factor, and changes in levels of body mass index, mean arterial pressure, hemoglobin A1c, total cholesterol/high-density-lipoprotein cholesterol ratio, γ -glutamyl transferase, cotinine, C-reactive protein, anytime aspirin/anticoagulant use, and norepinephrine. suPAR, urokinase plasminogen activator receptor.

The numbers above error bars indicate significant post hoc comparisons of changes in levels of prothrombotic factors over time between individual OSA risk groups:

- ¹⁾ level increased in the new-onset OSA risk group relative to the low OSA risk group
- ²⁾ level increased in the new-onset OSA risk group relative to the remitted OSA risk group
- ³⁾ level increased in the new-onset OSA risk group relative to the high OSA risk group
- ⁴⁾ level increased in the high OSA risk group relative to the low OSA risk group
- ⁵⁾ level increased in the high OSA risk group relative to the remitted OSA risk group
- ⁶⁾ level increased in the low OSA risk group relative to the remitted OSA risk group

Legend to Table 1

Values shown are median (interquartile range) or absolute numbers (percentages). Group comparisons were calculated with independent-samples t-test or chi-square test. HDL, high-density lipoprotein; PAI-1:Ag, plasminogen activator inhibitor-1 antigen; uPAR, urokinase-type plasminogen activator receptor; VWF:Ag, von Willebrand factor antigen.

Legend to Table 2

All variables were entered in one block. All values of continuously scaled variables were logarithmically transformed except age. Significant unstandardized B coefficients with standard error (s.e.) are given in bold type. Effects sizes are expressed as partial eta squared (η^2). OAC, oral anticoagulant; OSA, obstructive sleep apnea; PAI, plasminogen activator inhibitor; uPAR, urokinase plasminogen activator receptor; T-C/HDL-C, total/high-density lipoprotein cholesterol ratio.

Legend to Table 3

All variables were entered in one block. All values of continuously scaled variables were logarithmically transformed except age. Significant unstandardized B coefficients with standard error (s.e.) are given in bold type. Effects sizes are expressed as partial eta squared (η^2). OAC, oral anticoagulant; OSA, obstructive sleep apnea; PAI, plasminogen activator inhibitor; uPAR, urokinase plasminogen activator receptor; T-C/HDL-C, total/high-density lipoprotein cholesterol ratio; the sign " Δ " denotes a change over time in the respective variable. The fully adjusted differences in the changes of the levels of prothrombotic factors over time between the four groups with differing dynamics in OSA risk from these univariate models are shown in Figure 1.

Table 1. Participant characteristics per baseline risk of obstructive sleep apnea

Variables	High risk (n=117)	Low risk (n=212)	P
Age, yrs	47.0 (41.0, 54.0)	45.0 (39.0, 52.0)	0.083
Male, n (%)	68 (58.1)	90 (42.5)	0.006
Black ethnicity, n (%)	55 (47.0)	92 (43.4)	0.528
Neck circumference, cm	39.0 (35.1, 42.1)	34.3 (32.5, 38.2)	<0.001
Body mass index, kg/m ²	32.0 (28.8, 36.4)	25.9 (22.8, 28.9)	<0.001
Systolic blood pressure, mmHg	132 (126, 144)	123 (116, 131)	<0.001
Diastolic blood pressure, mmHg	83 (78, 90)	77 (72, 82)	<0.001
Mean arterial pressure, mmHg	99 (95, 107)	92 (87, 97)	<0.001
Hemoglobin A1c, %	5.70 (5.45, 6.15)	5.50 (5.30, 5.80)	<0.001
Total cholesterol, mmol/L	5.30 (4.34, 6.25)	4.99 (4.09, 5.90)	0.039
HDL cholesterol, mmol/L	1.06 (0.83, 1.27)	1.19 (0.96, 1.49)	0.001
Total/HDL cholesterol, ratio	5.22 (3.98, 6.44)	4.02 (3.21, 5.10)	<0.001
γ-glutamyl transferase, U/L	35.7 (21.9, 59.9)	23.0 (14.0, 38.9)	<0.001
Cotinine, ng/mL	0 (0, 7.5)	0 (0-0)	0.062
Urinary norepinephrine, nmol/L	259 (121, 397)	191 (109, 289)	0.013
C-reactive protein, mg/L	4.30 (1.60, 8.20)	1.85 (0.99, 5.18)	<0.001
Aspirin/anticoagulants, n (%)	6 (5.1)	7 (3.3)	0.416
VWF:Ag, %	75.5 (59.6, 91.6)	69.9 (57.2, 87.9)	0.591
Fibrinogen, g/L	3.29 (2.88, 3.92)	3.10 (2.69, 3.52)	0.001
D-dimer, ng/ml	270 (190, 415)	245 (140, 398)	0.092
PAI-1:Ag, ng/ml	28.2 (23.6, 34.0)	25.6 (18.5, 33.6)	0.007
Clot lysis time, min	84.2 (75.9, 94.8)	73.9 (67.7, 82.1)	<0.001
Soluble uPAR, ng/ml	2.53 (2.09, 3.01)	2.33 (2.02, 2.84)	0.061

Table 2. Relationship between a high risk of obstructive sleep apnea and baseline levels of prothrombotic factors (fully adjusted univariate analyses of covariance)

Entered variables	von Willebrand factor baseline			Fibrinogen baseline			D-dimer baseline			PAI-1 baseline			Clot lysis time baseline			Soluble uPAR baseline		
	B, s.e.	P	η^2	B, s.e.	P	η^2	B, s.e.	P	η^2	B, s.e.	P	η^2	B, s.e.	P	η^2	B, s.e.	P	η^2
Age	.002, .001	.019	.017	.001, .000	.041	.013	.002, .002	.429	.002	.000, .001	.648	.001	.000, .000	.470	.002	.000, .001	.797	.000
Male sex	.000, .016	.982	.000	-.022, .011	.039	.013	-.169, .052	.001	.032	.036, .020	.068	.011	-.010, .009	.296	.003	-.034, .018	.058	.011
Black ethnicity	.187, .017	<.001	.287	.029, .011	.007	.023	.161, .053	.002	.029	.210, .020	<.001	.263	.002, .010	.852	.000	.066, .018	<.001	.041
Body mass index	.064, .089	.477	.002	.247, .058	<.001	.055	-.196, .284	.490	.002	.231, .107	.032	.015	.251, .051	<.001	.071	-.064, .098	.514	.001
Mean arterial pressure	-.209, .154	.176	.006	-.168, .100	.094	.009	1.073, .491	.030	.015	-.106, .185	.567	.001	-.055, .088	.531	.001	.215, .169	.203	.005
Hemoglobin A1c	-.042, .131	.750	.000	-.049, .084	.560	.001	-.426, .416	.306	.003	.024, .156	.876	.000	.390, .075	<.001	.079	-.060, .143	.675	.001
T-C/HDL-C	.018, .050	.725	.000	-.012, .032	.701	.000	.322, .159	.044	.013	.114, .060	.058	.011	.147, .029	<.001	.077	.072, .055	.186	.006
C-reactive protein	.037, .016	.022	.017	.088, .010	<.001	.184	.070, .051	.177	.006	-.026, .019	.182	.006	-.007, .009	.439	.002	-.011, .018	.526	.001
γ -glutamyl transferase	-.016, .022	.475	.002	-.016, .014	.250	.004	-.070, .070	.320	.003	.062, .026	.020	.017	.066, .013	<.001	.079	-.009, .024	.713	.000
Cotinine	-.007, .008	.363	.003	.015, .005	.003	.027	.031, .024	.205	.005	.002, .009	.835	.000	-.003, .004	.475	.002	-.003, .008	.730	.000
Aspirin/OAC use	-.043, .030	.155	.006	.010, .019	.610	.001	-.125, .096	.192	.005	.040, .036	.269	.004	-.018, .017	.289	.004	.068, .033	.040	.013
Norepinephrine	.034, .017	.046	.013	.019, .011	.083	.009	-.109, .055	.047	.012	.027, .021	.183	.006	-.001, .010	.928	.000	.008, .019	.655	.001
High OSA risk	-.008, .014	.580	.001	.001, .009	.922	.000	.053, .046	.247	.004	-.001, .017	.950	.000	.003, .008	.753	.000	.027, .016	.092	.009

Table 3. Relationships between covariates and changes in levels of prothrombotic factors over time from the fully adjusted univariate analyses of covariance

Entered variables	Δ von Willebrand factor			Δ Fibrinogen			Δ D-dimer			Δ PAI-1			Δ Clot lysis time			Δ Soluble uPAR		
	B, s.e.	P	η ²	B, s.e.	P	η ²	B, s.e.	P	η ²	B, s.e.	P	η ²	B, s.e.	P	η ²	B, s.e.	P	η ²
Baseline level of prothrombotic factor	-.690, .057	<.001	.320	-.291, .050	<.001	.098	-.693, .055	<.001	.336	-1.026, .078	<.001	.357	-.535, .038	<.001	.389	-.877, .056	<.001	.436
Age	.001, .001	.286	.004	.001, .000	.108	.008	.005, .002	.016	.018	.003, .001	.006	.024	.000, .000	.247	.004	.003, .001	<.001	.044
Male sex	.014, .013	.256	.004	-.002, .009	.847	.000	-.081, .040	.042	.013	.019, .022	.369	.003	.009, .006	.146	.007	-.044, .014	.001	.033
Black ethnicity	-.059, .018	.001	.032	.064, .010	<.001	.110	-.006, .048	.897	.000	.334, .030	<.001	.283	-.002, .007	.816	.000	.044, .017	.009	.022
Δ Body mass index	.038, .207	.853	.000	-.060, .135	.659	.001	-.081, .645	.901	.000	.131, .344	.704	.000	.316, .096	.001	.034	-.031, .222	.887	.000
Δ Mean arterial pressure	.095, .172	.582	.001	.036, .112	.746	.000	.802, .529	.130	.007	-.085, .283	.765	.000	.085, .079	.283	.004	-.096, .182	.599	.001
Δ Hemoglobin A1c	-.186, .149	.212	.005	.105, .097	.278	.004	.334, .461	.469	.002	.429, .247	.083	.010	.186, .069	.007	.023	.021, .160	.896	.000
Δ T-C/HDL-C	-.067, .073	.357	.003	-.014, .048	.767	.000	.157, .227	.491	.002	-.065, .122	.594	.001	.079, .034	.019	.017	.012, .079	.881	.000
Δ C-reactive protein	.050, .015	.001	.036	.088, .010	<.001	.211	.102, .046	.027	.016	-.029, .025	.248	.004	-.004, .007	.587	.001	.063, .016	<.001	.048
Δ γ-glutamyl transferase	.072, .032	.024	.016	-.036, .021	.076	.010	-.120, .098	.224	.005	.066, .053	.210	.005	.028, .015	.053	.012	.027, .034	.424	.002
Δ Cotinine	.019, .013	.133	.007	.014, .008	.091	.009	-.057, .039	.147	.007	.013, .021	.523	.001	-.007, .006	.203	.005	-.015, .013	.277	.004
Aspirin/OAC anytime	.002, .018	.896	.000	.002, .011	.870	.000	.036, .055	.516	.001	-.028, .029	.334	.003	.011, .008	.185	.006	.024, .019	.198	.005
Δ Norepinephrine	.006, .014	.668	.001	.015, .009	.086	.009	-.037, .042	.380	.002	.000, .023	.993	.000	-.010, .006	.110	.008	-.001, .015	.962	.000

Extra Table

What is known about this topic?
• Obstructive sleep apnea (OSA) is a common sleep disordered breathing that is associated with an increased risk of cardiovascular disease. • A hypercoagulable state may be one important mechanism linking OSA to atherothrombotic events like myocardial infarction and stroke. • Previous studies on hypercoagulability in OSA are inconsistent and cross-sectional.
What this paper adds
• During an observation period of 3 years, individuals with a persistently high OSA risk, and, even more so, those with new-onset OSA risk, had an increase in prothrombotic factors relative to those with a persistently low OSA risk or OSA remission. • Hypercoagulability in OSA is a dynamic process that partially relates to changes in OSA status over time.

Figure 1. Three-year changes in prothrombotic factors





