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Research Article

Fibrinolytic Activity and Platelet Function in Subjects with Obstructive Sleep Apnoea and a Patent Foramen Ovale: Is There an Option for Prevention of Ischaemic Stroke?

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Obstructive sleep apnoea (OSA) carries an increased risk of ischaemic stroke, but the underlying mechanism is not clear. As right-to-left shunting can occur through a patent foramen ovale (PFO) during periods of apnoea, we investigated nocturnal changes in fibrinolytic activity and platelet function in subjects who had OSA with or without PFO and in controls. We determined plasminogen activator inhibitor 1 (PAI-1) activity and antigen and platelet activation parameters. The severity of OSA was verified by polygraphy and PFO was detected by ear oximetry. We found a higher PAI-1 activity and antigen and a lower ratio of 2,3-dinor-$PGF_{1\alpha}$ to 2,3-dinor-TXB_2 in the subjects with OSA than in the controls. Linear regression analysis showed the apnoea-hypopnoea index (β-coefficient, 0.499; $P = 0.032$) and PFO (β-coefficient, 0.594; $P = 0.015$) to be associated independently with PAI-1 activity in the morning, while the increment in PAI-1 : Ag from evening to morning was significantly associated with the presence of PFO ($r_s = 0.563$, $P = 0.002$). Both OSA and PFO reduce fibrinolytic activity during nocturnal sleep. We hypothesize that subjects having both OSA and PFO may develop a more severe prothrombotic state during sleep than those having either OSA or PFO alone.

1. Introduction

Obstructive sleep apnoea (OSA) carries increased risks of hypertension, ischaemic stroke, myocardial infarction, and atrial fibrillation [1–6]. OSA occurs in up to 5% of the adult population in western countries [1, 2], and affected subjects show increased platelet and reduced fibrinolytic activity relative to controls [7]. OSA thus seems to be linked to cardiovascular diseases via a hypercoagulable state. Other factors may also contribute to this hypercoagulable state, but their role is unclear. One possible factor is a patent foramen ovale (PFO).

Many, although not all, studies have suggested an association between PFO, which is present in about one-quarter of adults, and ischaemic stroke [8–10]. The mechanism of stroke in patients with PFO is still unclear. Paradoxical embolism is considered the most likely mechanism, but it has rarely been proved, and it has been suggested that a hypercoagulable state may also be involved [10]. Transient atrial arrhythmias do occur in the presence of PFO [11], and, accordingly, OSA and PFO may act in concert to precipitate thrombus formation in the left atrium involving a hypercoagulable state together with atrial fibrillation.

The present study was designed to show whether OSA with PFO can produce a more severe hypercoagulable state during nocturnal sleep than OSA or PFO alone. We measured some key parameters of fibrinolysis and platelet function before and after nocturnal sleep in subjects who had OSA with or without PFO and in controls. These parameters were plasminogen activator inhibitor 1 (PAI-1) activity and antigen, shear-induced platelet aggregation (SIPA) and urinary excretion of the major thromboxane and prostacyclin metabolites. PAI-1, continuously produced in platelets [12], is the main physiological plasminogen activator-inhibitor with proatherogenic effects [13], and it has been reported to be induced by hypoxia [14]. SIPA was chosen as a measure for platelet aggregation because it may reflect more closely than other techniques the situation encountered by platelets in stenosed arteries or arterioles undergoing vasospasm [15]. The key metabolites of thromboxane (a potent vasoconstrictor and platelet aggregator) and prostacyclin (a potent vasodilator and inhibitor of platelet aggregation), 2,3-dinor-TXB_2 and 2,3-dinor-$PGF_{1\alpha}$ were chosen because they reflect platelet and endothelial functions *in vivo*.

2. Subjects and Methods

The protocol was approved by the Ethics Committee of the Medical Faculty, University of Oulu. Informed consent was obtained from all the subjects. Fifty-three subjects admitted for overnight sleep recording because of suspected sleep apnoea were recruited. Forty-eight subjects were initially investigated at our in-patient department by in-laboratory polysomnography and 5 had portable monitoring at home. After sleep recording 24 of them were diagnosed to have OSA. The rest ($n = 29$) did not have OSA and served as controls for those with OSA. Information on the subject's use of medication, previous diseases, height, and weight was obtained. Although all the subjects had been asked to stop daily intake of anti-inflammatory drugs for two weeks preceding blood sampling, 14 of them, 7 in the OSA group and 7 in the control group, had taken nonsteroidal anti-inflammatory drugs (NSAIDs). Subjects were considered to be hypertensive if their blood pressure readings had exceeded 160/90 mm Hg (as proposed in the WHO/ISH statement) [16] at least twice, or if they were taking antihypertensive medication. Previous strokes, diabetes, and cardiac diseases including atrial fibrillation were recorded. The subjects were asked to avoid exercise for one day before admittance. Blood samples were taken at 6 p.m., 10 p.m., 2 a.m., and 6 a.m., but on a separate day apart from sleep recording (usually one day after the day of sleep recording). Urine samples were gathered for a period immediately before (from 10 a.m. to 6 p.m.) and during sleep recording (from 10 p.m. to 6 a.m.). All the tests listed below were performed blindly.

2.1. Sleep Test. The in-laboratory polygraph examination was performed using a six-channel computerized polygraph with leads for a pulse oximeter, thoracoabdominal strain gauge, ECG, position transducer, nasal cannula pressure transducer, and leg EMG. A static charge mattress was also used for additional information. The home recordings were made using a Somnologica (Flaga^{hf}, Reykjavik, Iceland) 8-channel polygraph, which also included a nasal cannula pressure transducer. The methods used at hospital an at home were equally accurate. Apnoea was defined as the absence of breathing for more than 10 sec, and an obstructive apnoeic episode was identified as a complete cessation of oronasal airflow as detected by the nasal pressure sensor in the presence of continuous breathing efforts revealed by the thoracoabdominal strain gauges. Hypopnoea was defined as a reduction of at least 50% in the airflow signal. Mixed apnoeas and hypopnoeas were combined with obstructive ones to form an apnoea-hypopnoea index (AHI). OSA was diagnosed here if the AHI was ≥ 5/h (17).

2.2. Detection of a Patent Foramen Ovale. PFO was detected by ear oximetry, which is a noninvasive method with high sensitivity (85%) and specificity (100%) [9]. The oximetric recordings were obtained by continuous measurement of arterial oxygen saturation with a two-wavelength earpiece oximeter connected to a computer. The signals obtained from the optical sensors, corresponding to two photoplethysmograms, were digitized by an analogue-to-digital converter inside the oximeter and then transferred to the computer. The signals were further processed and the relative oxygen saturation value was continuously displayed as a curve on the screen. The signals were stored on the hard disc for later processing and off-line analysis. Special software was used for data processing and for the elimination of artefacts caused by the large, rapid changes in the blood content of the ear. To optimize the circulation of blood in the ear, an arterializing warm-up period of 10 minutes was allowed before the measurements were performed.

The subjects were first trained to perform both Valsalva (VM) and Müller's manoeuvre (MM). Then three measurements were performed with a VM and three with a combined VM and MM. VM consisted of blowing into a mouthpiece attached to a standard blood-pressure apparatus. The subjects were able to read the pressure scale of the apparatus during their test blowing, and they were asked to generate and hold a pressure of 40 mmHg for 15 seconds. The combination of VM and MM was accomplished by asking the subject to inspire rapidly through the closed mouthpiece immediately after VM. The subjects were always in a supine position with a pillow under their head during the recordings. PFO was considered to be present when a transient drop in the oxygen saturation curve after release of the provocation was seen in at least two recordings. The SaO_2 drop in the oxygen saturation curve was evaluated visually using off-line computer analysis. A typical drop lasts two to three seconds.

2.3. Shear-Induced Platelet Aggregation. SIPA was measured at 4-hour intervals between 6 p.m. and 6 a.m. Blood was collected without stasis via a plastic cannula into tubes containing 1/10 volume of 3.8% trisodium citrate. To obtain platelet-rich (PRP) and platelet-poor plasma (PPP), parts of

the blood samples were centrifuged at 330 g for 10 minutes and at 1500 g for 5 minutes. Aggregation was induced by applying a high shear force, 108 dynes/cm^2, for 5 minutes by a turbidometric technique that uses a thermostatic cone-plate streaming chamber. The method is described in detail elsewhere [17, 18]. SIPA was expressed as the maximum percentage change in light transmittance during the 5-minute period.

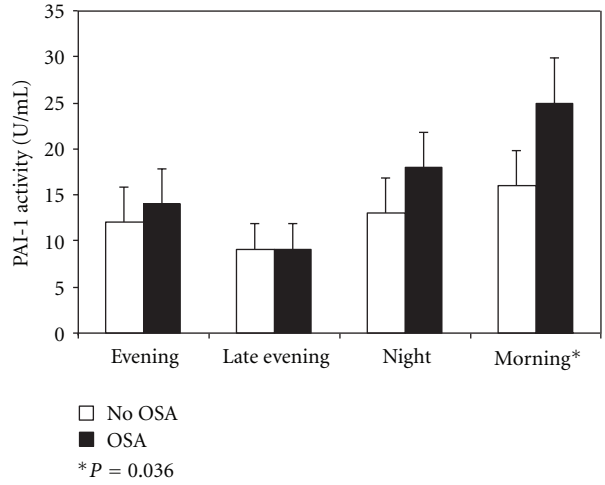
2.4. Determination of Prostanoids. Urine was collected after voiding the bladder from 10 a.m. till 6 p.m. (evening sample) and from 10 p.m. till 6 a.m. (morning sample). Samples for the determination of 2,3-dinor-6-ketoprostaglandin$F_{1\alpha}$ (2,3-dinor-$PGF_{1\alpha}$), 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2), and 2,3-dinor-thromboxane B_2 (2,3-dinor-TXB_2) were frozen immediately after collection and stored at −70°C until extraction. The prostanoids were determined by radioimmunoassay as previously described [19].

2.5. Determination of Fibrinolytic Factors. Blood samples were drawn into Biopool Stabilyte tubes and the plasma separated out within an hour by centrifugation at +4°C (1 800 g for 30 min). The samples were then immediately stored at −70°C and thawed once at +37°C for 30 minutes before use. PAI-1 : Ag and PAI-1 activity were determined according to the manufacturer's instructions using a TintElize PAI-1 kit and a Chromolize PAI-1 kit (Biopool International, CA, USA), respectively. The TintElize PAI-1 assay measures active, latent and tPA or uPA-complexed forms of PAI-1.

2.6. Statistics. Fisher's exact two-tailed test, Pearson's χ^2 test, Student's t-test, an analysis of variance (ANOVA), and Spearman's rank correlation coefficients (r_s) were used as appropriate. Multiple linear regression analyses were performed to establish independent associations between risk factors and levels of fibrinolytic and haemostatic factors and their nocturnal increments. Logarithmic transformation was used for variables which were not normally distributed (AHI, prostanoid metabolites). The variables taken into account in these analyses were age, sex, history of hypertension (or mean BP), diabetes, current smoking, use of anti-inflammatory drugs, and AHI (or body mass index, BMI). A P value < 0.05 was considered significant. All the statistical calculations were performed with SPSS 14.0 for Windows software (SPSS, Inc., Chicago, IL).

3. Results

The subjects' baseline characteristics are shown in Table 1. Twenty-nine subjects (controls) did not have OSA (AHI < 5/h), whereas 24 did. The subjects with OSA had a higher BMI ($P = 0.012$) and more frequently had hypertension ($P = 0.030$) and diabetes ($P = 0.036$) than those without OSA. PFO was detected in 3 subjects with OSA, who also had a significantly higher mean blood pressure than the OSA subjects without PFO ($P = 0.041$) or the control subjects with ($P = 0.018$) or without PFO ($P = 0.006$). BMI was significantly correlated with AHI ($r_s = 0.343$, $P = 0.012$), whereas AHI was correlated with a history of hypertension ($r_s = 0.324$, $P = 0.018$) and with age ($r_s = 0.304$, $P = 0.027$). Two of the OSA patients had a severe condition (AHI > 40), whereas the rest (including the 3 with PFO) had mild-to-moderate OSA (AHI 5–40). One subject had a history of paroxysmal atrial fibrillation, but he had neither OSA nor PFO. None of the subjects had venous thrombosis or embolism in their history.



Blood samples were drawn at 6 p.m. (evening), 10 p.m. (late evening), 2 a.m. (night) and 6 a.m. (morning).

Figure 1: PAI-1 activity (95% CI) in subjects with OSA and controls.

Changes in fibrinolytic activity and platelet function from evening to morning are shown in Table 2. All the subjects showed higher levels of PAI-1 : Ag and PAI-1 activity in the morning than in the evening (circadian variation). Figure 1 demonstrates that PAI-1 activity in the morning was significantly higher in the subjects with OSA than in the controls. Findings were similar if the subjects with PFO were excluded (data not shown). The subjects with PFO also showed high PAI-1 activities in the morning. We observed an increment in PAI-1 : Ag from evening to morning that correlated significantly with the presence of PFO ($r_s = 0.563$, $P = 0.002$).

SIPA tended to increase from evening to morning, but the increments did not reach statistical significance in any of the groups. SIPA in the subjects with OSA was higher than that in the controls at all time points, but the difference reached statistical significance only at 10 p.m. ($P = 0.049$). SIPA was significantly lower in the subjects with PFO (none of them had used NSAIDs) than in those without PFO, but the difference attenuated during the night (Figure 2). The morning ratio of 2,3-dinor-TXB_2 to 2,3-dinor-$PGF_{1\alpha}$ was significantly higher in the subjects with OSA than in the controls ($P = 0.006$). The ratio was on the same level in the subjects with PFO as in the controls.

To see whether OSA influences the urinary excretion of the thromboxane and prostacyclin metabolites differently we excluded the subjects with PFO and looked at the metabolite levels separately during day and night (Table 3).

Table 1: Baseline characteristics of 53 subjects investigated for OSA and PFO.

Variable	OSA with PFO ($n = 3$)	OSA without PFO ($n = 21$)	Control with PFO ($n = 6$)	Control without PFO ($n = 23$)	Total ($n = 53$)
Sex (female/male)	0/3	1/20	1/5	3/20	5/48
Mean (SD) age, years	51 (3)	46 (8)	51 (15)	42 (7)	45 (9)
Mean (SD) BP, mmHg	121 (2)	115 (11)	106 (11)	112 (12)	113 (12)
Mean (SD) BMI kg/m^2	31 (6)	32 (6)	27 (4)	28 (5)	30 (6)
Median (25 and 75%) AHI	19 (5, 30)	11 (8, 16)	1 (0, 2)	1 (0, 3)	4 (1, 11)
Previous history					
Ischaemic vascular disease	1*	1†	0	1‡	3
Atrial fibrillation	0	0	0	1	1
Diabetes mellitus	1	3	0	0	4
Hypertension	1	9	1	3	14
Current smoking	0	5	0	3	8

BP: blood pressure, BMI: body mass index (normal weight 18.5–24.9; overweight 25–29.9; obesity > 30), RDI: respiratory disturbance index.
*Myocardial infarction.
†Myocardial infarction and ischaemic brain infarction.
‡Ischaemic brain infarction.

Table 2: Changes in fibrinolytic and platelet parameters from evening to morning.

Variable	Control*		OSA*		PFO†	
	Evening	Morning	Evening	Morning	Evening	Morning
PAI-1: Ag, ng/mL	8 (6–10)	18 (12–24)	15 (10–20)	26 (17–35)	8 (4–13)	27 (9–45)
PAI-1 activity, U/mL	13 (9–16)	16 (12–20)	14 (9–18)	23 (17–29)	21 (17–25)	27 (10–43)
SIPA, %	47 (41–52)	50 (46–53)	52 (47–58)	54 (48–59)	36 (28–43)	44 (33–55)
2,3-dinor-TXB_2/2,3-dinor-$PGF_{1\alpha}$	1.2 (0.9–1.4)	1.2 (1.0–1.4)	1.6 (1.0–2.2)	1.7 (1.4–2.1)	0.9 (0.5–1.3)	1.1 (0.8–1.5)

Values are means (95% CI).
Blood samples were taken at 6 p.m. (evening) and at 6 a.m. (morning).
*Subjects with PFO are included.
†Subjects with OSA are included.

It appeared that the urinary excretion of 2,3-dinor-TXB_2 did not significantly differ between the subjects with OSA and the controls, whereas the excretion of 2,3-dinor-$PGF_{1\alpha}$ was significantly lower during night in the subjects with OSA. Thromboxane metabolite levels in urine were found to be influenced by the use of NSAIDs, as shown by a negative association between use of these drugs and urinary excretion of 2,3-dinor-TXB_2 ($\beta = -0.568$; $P = 0.026$). We did not observe any significant effects of use of NSAIDs on the other parameters of platelet function.

Linear regression analysis showed the presence of PFO to be associated independently with the PAI-1: Ag increment during the night (standardized regression coefficient, $\beta = 0.611$; $P = 0.015$), an association that was not significantly influenced by age, sex, hypertension, diabetes, current smoking, and use of anti-inflammatory drugs or AHI. Replacing AHI with BMI and/or hypertension with mean BP did not alter the result. In a similar model, PFO ($\beta = 0.594$; $P = 0.015$) and AHI ($\beta = 0.499$; $P = 0.032$) showed significant associations with PAI-1 activity in the morning. Linear regression analysis also showed hypertension to be associated significantly and independently with the increment in SIPA during the night ($\beta = 0.536$; $P = 0.020$).

4. Discussion

We observed decreased fibrinolytic activity and increased platelet activity in subjects with OSA after overnight sleep. PFO was associated with an increment in PAI-1: Ag during the night, and PFO and AHI were associated independently with PAI-1 activity in the morning. These findings indicate a prothrombotic state in subjects with OSA and PFO. The cooccurrence of OSA and PFO may result in a greater decrease of fibrinolytic activity than with either one alone.

Few investigations have been made into PAI-1 activity or PAI-1 : Ag levels in subjects with OSA [20, 21]. Rångemark et al. 21] found PAI-1 activity levels in patients to be more than twice those in controls, a relation that retained its significance after correction for age, BMI, and diastolic blood pressure. Von Känel et al. [21] found a positive correlation between AHI and PAI-1: Ag in OSA patients and showed a fall in PAI-1: Ag after continuous positive airway pressure (CPAP) treatment, suggesting that this treatment could reverse the decreased fibrinolytic activity found in OSA patients.

PFO seems to cause decreased fibrinolytic activity during nocturnal sleep, but it is not known whether this is due to transient opening of a right-to-left shunt during apnoeic

Table 3: Urinary excretion of thromboxane and prostacyclin in subjects without PFO.

Variable	Control		OSA	
	Day	Night	Day	Night
11 DH-TXB_2, pg/μmol creatinine	24 (8–41)	18 (9–26)	23 (19–28)	24 (16–31)
2,3-dinor-TXB_2, pg/μmol creatinine	18 (11–26)	15 (11–19)	16 (12–19)	15 (12–18)
2,3-dinor-$PGF_{1\alpha}$, pg/μmol creatinine	15 (10–20)	14 (9–19)	11 (8–14)	9 (6–12)*
2,3-dinor-TXB_2/2,3-dinor-$PGF_{1\alpha}$	1.2 (0.9–1.5)	1.2 (1.0–1.5)	1.7 (1.0–2.3)	1.8 (1.5–2.1)

*$P = 0.049$ for difference between the subjects with OSA and the controls during night.

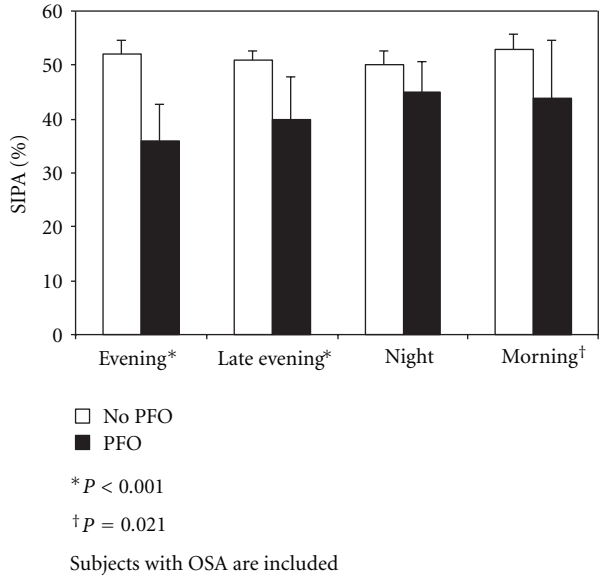


Figure 2: SIPA in subjects with and without PFO.

periods or to some other mechanism. Our finding that the presence of PFO predicts a PAI-1: Ag increment during sleep is a novel one and needs confirmation. Previous data on the relationship between PFO and coagulation factors do not include PAI-1 (activity or antigen) [10]. SIPA was significantly lower in the subjects with PFO than in subjects without PFO, but it tended to increase during the night. The reason for this remained unclear.

Low fibrinolytic activity promotes thrombogenesis during night and may predispose the subject to brain and myocardial infarction. PAI-1 is implicated in both thrombus stabilization [21] and atherosclerotic disease [22], and hence it could be a link between OSA and these diseases. Elevated PAI-1 [23] and fibrinogen levels [24] have been observed during the acute phase of ischaemic stroke, but it is not clear whether they precede the stroke or are caused by it. One study has nevertheless indicated that high levels of PAI-1 preceded the onset of a first myocardial infarction [25]. It has been demonstrated that OSA entails an excess risk of stroke and death from any cause after adjustment for all major confounders, including hypertension [3].

Prostacyclin and thromboxane play a fundamental role in the processes of platelet aggregation, vasoconstriction/relaxation, and inflammation. We determined the urinary excretion of their metabolites 2,3-dinor-$PGF_{1\alpha}$, 2,3-dinor-TXB_2 and 11-dehydro-TXB_2 and found the ratio between 2,3-dinor-TXB_2 and 2,3-dinor-$PGF_{1\alpha}$ to be higher in subjects with OSA than in controls. This effect seemed to be due to the lower urinary excretion of 2,3-dinor-$PGF_{1\alpha}$ in subjects with OSA. Similar findings have been reported in drug-free OSA patients [20, 26] of the same age, whereas the opposite effect has been reported in younger subjects with OSA [27].

Platelet aggregation measured by SIPA tended to be higher in subjects with OSA than in controls, but the difference was not significant. Several studies using different methodologies have indicated increased platelet activation and aggregation in OSA [28–31], whereas others have found only a slight effect [32] or none at all [20, 27, 33]. Some studies have also demonstrated that the increased platelet activity seen in OSA patients can be normalized by continuous positive airway pressure (CPAP) treatment [28, 31, 32]. There may be a relationship between platelet activity and OSA severity [29, 31]. Perhaps we were unable to demonstrate a significant difference in platelet aggregation because we had very few severe OSA cases in our material. Platelets contain a large amount of PAI-1 and translational active mRNA for PAI-1, and synthesize active PAI-1 after thrombin stimulation [12]. Could the higher levels of PAI-1: Ag and activity that we found be indirect markers of higher platelet activation?

The untoward effects of PFO and OSA on haemocoagulation may act in concert to promote thrombus formation, particularly if atrial fibrillation is present. PFO has been thought to be a risk factor for ischaemic stroke via paradoxical embolism, even though venous thrombosis is seldom detected as preceding acute brain infarction in subjects with cryptogenic stroke and PFO. Indeed, a population-based study has suggested that PFO itself is not a risk factor for cryptogenic ischaemic stroke [8]. The real reason for the high prevalence of PFO in young subjects with cryptogenic ischaemic stroke is still unclear. If such strokes are not mediated by paradoxical embolism, could some other mechanism be responsible, for example, *in situ* formation of a thrombus in the left atrium? As right-to-left shunt occurs during apnoeas in subjects with PFO [34], OSA in such subjects could result in more severe hypoxia and sympathetic activation during nocturnal sleep [35]. Hypoxia has been postulated as inducing increased production of reactive oxygen species and adhesion molecules in leukocytes and the endothelium and decreased NO availability, leading to endothelial dysfunction [36]. The consequence could be

promotion of thrombus formation in the left atrium in the presence of atrial fibrillation. Associations between atrial fibrillation and OSA [6] and PFO [11] have been observed, and both OSA and PFO may provoke atrial fibrillation, so that they could both promote nocturnal thrombus formation in the left atrial appendage. This could explain why OSA is an independent risk factor for stroke and why the risk is not mediated by hypertension [3]. The prevalence of PFO among subjects with OSA is high [37].

The major limitation of this study is the small number of subjects. The findings may be spurious and need confirmation in a larger body of material. The most important findings were nevertheless consistent and allowed us to put forward new hypotheses for further study. A significant activation of coagulation markers in subjects with PFO and OSA does not necessarily imply that this is indeed the mechanism that leads to stroke in such subjects. The observed elevated markers of coagulation activation could also result from confounding factors. Cardiac function was not monitored in all the subjects. Therefore, we cannot exclude an effect of undetected atrial fibrillation on coagulation activation. Another limitation is the fact that some subjects had been taking nonsteroidal anti-inflammatory drugs. We did not observe any effects of these drugs on any parameter other than the urinary excretion of thromboxane, however. It is difficult to collect a sufficient number of subjects with OSA who have been free of any drug treatment. Finally, obesity may also influence blood coagulation without OSA. Thus, lack of a control group consisting of obese subjects without OSA is also a weakness in our study.

The strengths of the study include the fact that we were able to take several confounding factors, such as hypertension, diabetes, smoking, and previous cardiovascular diseases, into account when interpreting the results and that all the measurements were performed blindly.

In conclusion, we were able to demonstrate higher levels of PAI-1 activity and a higher ratio of 2,3-dinor-TXB_2 to 2,3-dinor-$PGF_{1\alpha}$ in subjects with OSA early in the morning after overnight sleep than in controls. These observations suggest a prothrombotic state. We also demonstrated for the first time an increment in PAI-1 : Ag from evening to morning that correlated significantly with the presence of PFO. These observations suggest that subjects with both OSA and PFO may develop a more severe prothrombotic state during sleep than subjects having either OSA or PFO alone. A prospective study is urgently needed to demonstrate whether subjects having both OSA and PFO will run a greater risk for ischaemic strokes than those without this combination. If so, treatment of OSA may be an important option for prevention of ischeamic strokes among such subjects.

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