NEW RESEARCH

JCSM
Journal of Clinical Sleep Medicine

http://dx.doi.org/10.5664/jcsm.3522

Modulation of Inflammatory and Hemostatic Markers in Obstructive Sleep Apnea Patients Treated with Mandibular Advancement Splints: A Parallel, Controlled Trial

Agata Niżankowska-Jędrzejczyk, D.D.S., Ph.D.[1]; Fernanda R. Almeida, D.D.S., Ph.D.[2]; Alan A. Lowe, D.M.D., Ph.D.[2]; Aleksander Kania, M.D., Ph.D.[3]; Paweł Nastałek, M.D.[3]; Filip Mejza, M.D., Ph.D.[3]; Jonathan H. Foley, Ph.D.[4]; Ewa Niżankowska-Mogilnicka, M.D., Ph.D.[3]; Anetta Undas, M.D., Ph.D.[5]

[1]Department of Orthodontics, Jagiellonian University Medical College, Krakow, Poland; [2]Department of Oral Health Sciences, University of British Columbia, Vancouver, British Columbia, Canada; [3]Department of Pulmonology, Jagiellonian University Medical College, Krakow, Poland; [4]Department of Biochemistry, University of Vermont, Burlington, VT; [5]Institute of Cardiology, Jagiellonian University Medical College, Krakow, Poland

SCIENTIFIC INVESTIGATIONS

Study Objective: Obstructive sleep apnea (OSA) is associated with systemic inflammation and a hypercoagulable state. The current study aim was to investigate whether mandibular advancement splint (MAS) therapy affects inflammatory and hemostatic parameters in patients with mild-to-moderate OSA.

Methods: Twenty-two patients with mild-to-moderate OSA and 16 control subjects were studied. OSA subjects were treated with a titratable MAS for 6 months. Baseline plasma C-reactive protein, interleukin-1β, interleukin-10, interleukin-6, P-selectin, fibrinogen, D-dimer, plasminogen activator inhibitor-1 (PAI-1), thrombin-antithrombin complex, activated thrombin-activatable fibrinolysis inhibitor (TAFIa), 6-keto-PGF1α, glucose, and fibrin clot lysis time (CLT) were measured in all subjects. After 3 months of MAS therapy, measurements were repeated for the 22 patients, and after 6 months all measurements were repeated for all study subjects.

Results: MAS treatment reduced significantly AHI at 3 months (24 vs 13.1/h) and further improved it at 6 months (13.1 vs 7.05/h). Compared with controls, OSA subjects had a significant higher baseline mean levels of fibrinogen, TAFIa, 6-keto-PGF1α, and glucose. MAS treatment significantly improved levels of IL-1β, D-dimer, TAFIa, and CLT. Despite residual apneas, MAS treatment group presented similar measured homeostatic and inflammatory levels to controls except for glucose.

Conclusion: Treatment with MAS in mild-to-moderate OSA subjects improves the inflammatory profile and homeostatic markers.

Keywords: Obstructive sleep apnea, sleep apnea, oral appliance, mandibular advancement splint, snoring, inflammatory markers, homeostatic markers, TAFIa, fibrinolysis, interleukin-10

Citation: Niżankowska-Jędrzejczyk A; Almeida FR; Lowe AA; Kania A; Nastałek P; Mejza F; Foley JH; Niżankowska-Mogilnicka E; Undas A. Modulation of inflammatory and hemostatic markers in obstructive sleep apnea patients treated with mandibular advancement splints: a parallel, controlled trial. *J Clin Sleep Med* 2014;10(3):255-262.

Obstructive sleep apnea (OSA), the most prevalent entity in the spectrum of sleep breathing diseases, affects from 2% to 4% of the whole population.[1] It is caused by diminished patency of the upper airways despite the maintenance of the respiratory effort and is characterized by repetitive episodes of hypoxia during sleep. Untreated OSA increases the risk of cardiovascular diseases, including arterial hypertension, ischemic heart disease, and ischemic stroke.[2] Episodes of intermittent hypoxia and reoxygenation, and sleep fragmentation and its consequences in OSA lead to chronic systemic inflammation, which could accelerate the development of atherosclerosis.[3] Growing evidence indicates both a systemic inflammatory and prothrombotic state in OSA patients. Increased fibrinogen and blood viscosity and an increase in plasminogen activator inhibitor-1 (PAI-1), a major regulator of fibrinolysis in vivo, has been observed in OSA patients.[4,5] To our knowledge, activated thrombin-activatable fibrinolysis inhibitor (TAFIa), another potent inhibitor of plasminogen activation, has not yet been evaluated in OSA patients. Several studies reported increased or unaltered thrombin formation in OSA patients, as reflected by the thrombin-antithrombin complexes (TAT) levels.[6] Data on alterations in plasma levels of C-reactive protein (CRP),[7-9] interleukin-6 (IL-6),[10] interleukin-1β (IL-1β),[11] and interleukin-10 (IL-10)[11] in OSA are inconsistent.

The gold standard of treatment of severe OSA is the use of continuous positive airway pressure (CPAP). In mild and moderate OSA, other therapeutic options are also available,

BRIEF SUMMARY

Current Knowledge/Study Rationale: The aim of our study was to assess the efficiency of fibrinolysis and its determinants, in particular thrombin activatable fibrinolysis inhibitor (TAFI), in OSA patients, since until now plasminogen activator inhibitor-1 and D-dimer have been the only fibrinolytic parameters studied in this disease. This is the first study to investigate the effects of mandibular advancement splints on hemostasis in OSA patients.

Study Impact: The study demonstrates antifibrinolytic mechanisms in OSA patients, largely mediated by increased TAFI. MAS treatment in OSA patients is associated with favorable alterations in hemostasis, including improved fibrinolysis.

including mandibular advancement splints (MAS). MAS protrudes the mandible with the aim of increasing upper airway caliber, thereby preventing collapse of the upper airway during sleep.[12,13] It has been demonstrated that MAS treatment ameliorates the polysomnographic (PSG) variables in OSA patients, decreases symptoms during both the day and night,[14,15] and reduces arterial blood pressure.[16]

Unlike the data on CPAP treatment,[4,9] studies on the effect of MAS treatment on inflammatory and hemostatic parameters are scarce.[17,18] MAS treatment improved the endothelial function despite the presence of residual apneas.[17,18] To our knowledge, there have been no reports on blood coagulation, platelet, and fibrinolysis parameters in OSA patients treated with MAS. We hypothesized that MAS therapy produces antithrombotic and anti-inflammatory effects, though less pronounced, similar to those observed during CPAP treatment. The aim of the current study was to assess the influence of MAS on inflammatory and hemostatic markers in mild-to-moderate OSA patients.

METHODS

Subjects

This was an open-label interventional study, performed in consecutive, previously untreated mild-to-moderate OSA patients (OSA group) and asymptomatic, non-snoring healthy subjects (AHI < 5 h/sleep, Epworth Sleepiness Scale [ESS] < 9) who served as controls (hospital staff). All subjects were Caucasians. Inclusion criteria for the OSA group were 10 < AHI < 30 h/sleep (recorded during PSG), daily hypersomnolence (ESS > 10), and ≥ 8 healthy teeth in the arch. Exclusion criteria were: central or predominant mixed sleep apnea, cardiovascular diseases other than hypertension, liver and kidney disorders, diabetes mellitus, any diagnosed chronic lung diseases, cancer, acute infection (within 3 months prior to enrolment), and periodontopathy or severe tempomandibular joint disease. Subjects receiving anticoagulant therapy or with a history of deep vein thrombosis or pulmonary embolism were also excluded from the study. The control group was matched with the OSA group for age, gender, and body mass index. The study was approved by the Ethics Committee of the Jagiellonian University Medical College, and all subjects gave their written informed consent.

Protocol

Subjects were admitted to the hospital in the afternoon, and they were asked to abstain from consuming caffeine containing beverages after 15:00. In the OSA group, the PSG, routine laboratory tests, and blood sampling for measurement of inflammatory and hemostatic markers were performed at baseline and after 3 and 6 months of MAS treatment. In the control group, PSG was performed at baseline (to exclude OSA), and the same inflammatory and homeostatic measurements were performed at baseline and after 6 months.

Mandibular Advancement Splint

Klearway custom-made MAS for OSA patients were individually designed and produced in the Great Lakes Orthodontics Laboratories (NY, USA). The initial forward position of the lower jaw was 65% to 70% of maximal protrusion. After 1 month of continuous night use of MAS, patients were asked to start titration (twice a week -1 turn of the screw ([about 0.25 mm]) until the snoring intensity decreased to 2-3 on the visual scale (rating the intensity of snoring from 1 = silence to 10 = the loudest assessed by sleep partner). An additional 4 turns were performed, and objective assessment was done during PSG 3 months after beginning treatment. If at that time PSG results were not yet satisfactory, titration was further continued.

Sleep Assessment

Standard full-night, in-laboratory polysomnography (PSG) was performed using a computerized recording system SOMNOlab (Weinmann, Germany) and scored according to the AASM criteria[19] with the following channels: 2-channel electroencephalogram, 2-channel electrooculogram, 1-channel electromyogram, nasal and oral airflow (thermal probe), hemoglobin saturation (oximeter probe), electrocardiogram, chest and abdomen respiratory movements (belt sensors), leg movements (tibial surface electrodes), and with video recording.

The PSG recordings were scored manually for sleep stages and respiratory events, which were defined as follows: obstructive apnea was defined as complete cessation of airflow ≥ 10 s, accompanied by respiratory movements of chest and abdomen; obstructive hypopnea as ≥ 50% flow reduction accompanied by hemoglobin oxygen saturation (SpO_2) drop ≥ 3% and respiratory movements. Airflow was measured by thermistor, as preferred currently by our sleep laboratory. We used the "alternative" hypopnea definition (≥ 50% flow drop + ≥ 3% desaturation) as a more sensitive criterion than the recommended definition (≥ 30% flow drop + ≥ 4% desaturation).

AHI was defined as a number of apnea and hypopnea episodes per hour of sleep. Mild-to-moderate OSA was defined as 5-30 episodes of obstructive apnea and hypopnea per hour of sleep. Desaturation index was calculated as a number of significant SpO_2 drops of 3% per hour of sleep.

Inflammatory and Hemostatic Markers

Peripheral venous blood was collected between 06:00 and 07:00 after the PSG study. The supernatant was stored at -80°C until analysis. Fibrinogen was measured in citrated plasma using the von Clauss method. High sensitive plasma C-reactive protein was measured by nephelometry (Siemens, Marburg, Germany), while serum interleukin-1ß, interleukin-10, and interleukin-6 were measured using immunoenzymatic assays (R@D Systems, Abingdon, Great Britain). Plasma D-dimer was measured by the VIDAS system (Biomerieux, France). Commercially available ELISAs were used to determine plasma P-selectin (R@D Systems), PAI-1 (Hyphen, France), and TAT (Siemens, Marburg, Germany). Activated thrombin-activatable fibrinolysis inhibitor (TAFIa) was measured using a functional assay.[20] Fibrin clot lysis time (CLT) was measured as described elsewhere.[21] Briefly, citrated plasma was mixed with 15 mmol/L calcium chloride, 10,000-diluted human tissue factor (Innovin, Dade Behring), 12 µmol/L phospholipid vesicles, and 60 ng/mL recombinant tissue-type plasminogen activator (Boerhinger Ingelheim, Ingelheim, Germany). The turbidity of this mixture was

Table 1—Demographic data for patients with OSA (OSA group, n = 22) and control subjects (control group, n = 19).

Variables	OSA Group	Control Group	p value
Age (years)	52.50 ± 8.33	54.06 ± 12.09	0.65
Height (cm)	175.39 ± 6.03	178.75 ± 4.27	0.07
Weight (kg)	92.77 ± 9.28	89.65 ± 11.94	0.38
BMI (kg/m^2)	30.15 ± 2.77	28.02 ± 3.36	0.05
Neck circumference (cm)	42.17 ± 2.94	41.84 ± 2.50	0.73
Abdomen circumference (cm)	104.02 ± 8.24	99.72 ± 9.34	0.15
Hip circumference (cm)	103.73 ± 4.60	102.16 ± 5.77	0.37
Systolic pressure (mm Hg)	130.00 ± 12.15	124.69 ± 15.56	0.26
Diastolic pressure (mm Hg)	83.41 ± 7.89	80.00 ± 7.29	0.20
Epworth Sleepiness Scale	9.15 ± 4.90	8.25 ± 5.68	0.62
SpO_2 (%) during the day	96.47 ± 1.22	97.00 ± 1.32	0.23

Data presented as mean ± standard deviation.

measured at 405 nm at 37°C. The inter- and intraassay coefficients of variation for all assays were < 8%. The samples from the OSA group and controls were randomized and blinded during the laboratory analysis in order to avoid any systematic bias, and they were analyzed at the same time and with the same method for each substance.

Statistical Analysis

Statistical analysis was performed using SPSS 11.5 for Windows, Chicago, IL, USA. Data were expressed as median and quartiles (Q1 to Q3). The normality of data distribution was assessed by the Shapiro-Wilk test. Due to the non-normal distribution of most data, the Mann-Whitney U test was used for baseline comparisons. The Friedman test was used for comparison between repeated measurements during follow-up of the OSA group. The Wilcoxon test with the Bonferroni correction were used as post hoc comparisons ($\alpha = 0.05/3 = 0.016$). The Wilcoxon rank test was used for comparison between control baseline and control at 6 months ($p < 0.05$). The Mann-Whitney U test was used for comparison between control and OSA groups at 6 months ($p < 0.05$). The correlations between variables were estimated by using the Spearman rank-order correlation ($p < 0.05$).

RESULTS

Baseline Data

Of the 27 patients from the OSA group initially enrolled in the study, initially 4 subjects were excluded from the final analysis (side effects of MAS [n = 2], cancer diagnosed during the study [n = 1], and noncompliance [n = 1]). The sample then involved only one female patient, who was then excluded from the final analysis, resulting in 22 male patients in the OSA group. All 19 control subjects completed the study; for comparison purposes, the 3 female patients were excluded from the final analysis, resulting in 16 control male subjects. At the baseline, groups did not differ with regard to most clinical and anthropometric variables (**Table 1**). We observed, however, the increased prevalence of arterial hypertension in OSA (n = 14 [63.6%] vs. n = 5 [31.3%] in the control group) and a higher number of smokers (n = 3) and ex-smokers (n = 7) in the OSA group than the controls (13.6% vs. 6.2% and 31.8% vs. 6.2%, respectively).

All subjects with arterial hypertension received adequate treatment to control both systolic and diastolic blood pressure; this treatment was not changed throughout the 6-month study period. Similarly mean BMI of both the OSA group and control group, although lower in controls, did not change through the 6-month study period. MAS significantly improved symptomatic outcomes (ESS from 12.8 to 4.2) with only small and transient side effects at the beginning of the titration process, as shown in **Table 1**.

Polysomnographic Analysis

There were 5 patients who had an AHI < 5 at 3 months who did not agree to do the 6-month PSG but completed the rest of the protocol, and therefore were included in the analysis. As shown in **Table 2**, the AHI and measurements of oxygen desaturation were higher in the OSA group than the control group at baseline. There were significant improvements in these measurements after 6 months of MAS therapy when compared to baseline. At 3-month evaluation, the mean O_2, minimum O_2, and percentage of time below 85% O_2 did not show a significant improvement. There was a significant and increased improvement in the AHI at 6 months of therapy compared to 3 months. After 6 months of MAS therapy, the sleep apnea patients still presented significant abnormalities in the AHI and desaturation indices when compared to the control subjects, with the exception of the mean oxygen saturation.

Inflammatory and Hemostatic Markers

Controls

The control group showed normal values of inflammatory and hemostatic variables at the baseline evaluation. After 6 months, most variables were stable, with the exception of IL-1β which slightly decreased.

Sleep Apnea Patients versus Controls

Comparative analysis of baseline inflammatory and hemostatic markers are illustrated in **Table 3**. Our analysis demonstrated that patients with OSA showed higher median levels

Table 2—Polysomnographic data of controls and of patients with sleep apnea at baseline and at 3 and 6 months of MAS treatment.

	Control Group (n = 16)	OSA Group (n = 22)		
Variables	**Baseline**	**Baseline**	**3 months**	**6 months**
AHI (/h)	2.05** (1.13 to 3.55)	24.00 (15.70 to 31.25)	13.1‡‡ (4.98 to 21.40)	7.05††,α (4.30 to 11.65)
Supine AHI (/h)	4.0** (2.45 to 7.25)	36.50 (20.38 to 55.48)	15.35‡‡ (4.28 to 35.07)	12.5†† (5.45 to 21.00)
Desat. Index (/h)	2.0** (1.30 to 2.85)	20.75 (14.0 to 29.25)	13.5‡‡ (3.60 to 16.15)	8.85†† (4.15 to 14.50)
Mean SpO_2 (%)	95.6** (94.65 to 95.78)	94.05 (93.08 to 94.83)	94.45 (93.98 to 95.20)	94.65† (93.58 to 95.40)
Min SpO_2 (%)	86.0** (83.50 to 87.00)	78.00 (71.75 to 81.00)	81.00 (76.25 to 83.25)	81.5† (77.00 to 84.50)
SpO_2 < 90% (min)	0.51** (0.22 to 4.99)	37.80 (13.06 to 48.81)	7.4‡ (3.40 to 31.77)	5.87†† (1.97 to 20.38)
SpO_2 < 85% (min)	0.0** (0.00 to 0.09)	2.83 (0.52 to 10.38)	0.35 (0.06 to 4.90)	0.32† (0.07 to 1.90)

Data expressed as median (interquartile range Q1 to Q3). **p < 0.01, significant difference between baseline control and baseline OSA group. ‡p < 0.05, ‡‡p < 0.01, significant difference between baseline and 3 month treatment for OSA group. †p < 0.05, ††p < 0.01, significant difference between baseline and 6 months treatment for OSA group. αp < 0.05, significant difference between 3 months and 6 months treatment.

of fibrinogen, IL-1β, P-selectin, TAFIa, 6-keto-PGF1α, and glucose than controls. Furthermore, we observed markedly elevated plasma TAFIa levels in the OSA group compared with the controls.

MAS Treatment

After 3 months of treatment, MAS was associated with reduced levels of IL-1β (0.35 to 0.19 pg/mL) and increased levels of D-dimer (241.5 to 274 μg/L). As seen in **Figure 1**, CLT showed significant improvement at 3 months and further shortening of lysis time at 6 months (median, from 87.5 to 72 and to 66 min, respectively); the final value was then not different from control levels. After 6 months of treatment, TAFIa showed almost 50% reduction compared with the baseline concentration, which was a further improvement from the 3-month evaluation and was significantly different than baseline values (85.3 to 45.3 pmol/L; **Figure 2**). Some other variables showed an improvement but did not reach the level of significance.

MAS Treatment Versus Control

Despite residual apneas, at 6 month follow-up, the MAS treatment group presented no significant difference in hemostatic and inflammatory parameters when compared to controls with the exception of fibrinogen, P-selectin, and 6-keto-PGF1α. Glucose levels were still slightly high, but not significantly; it was higher at 6 months (5.45 mmol/L) when compared to controls (5.1 mmol/L).

Influence of PSG Changes on Inflammatory Markers

Despite residual apneas, the absolute amount of change in the AHI was correlated to the change in 11-dehydro-TXB2 ($r = 0.501$, $p = 0.018$). The change in the oxygen desaturation index was correlated to the change in 11-dehydro-TXB2 ($r = 0.544$, $p = 0.009$) and insulin ($r = 0.583$, $p = 0.004$). The mean oxygen desaturation change was correlated with the insulin level change ($r = 0.541$, $p = 0.009$), as seen in **Figure 3**.

DISCUSSION

The current study is the first to show that an effective 3- to 6-month treatment with MAS in patients with mild-to-moderate OSA alters the inflammatory cytokine profile by decreasing IL-1β and IL-10 as well as by producing a marked profibrinolytic effect. We have also demonstrated that mild-to-moderate OSA was characterized by hypofibrinolysis largely determined by TAFIa. Increased TAFIa levels in OSA have not been observed previously. The current study expands our knowledge on the hemostatic abnormalities observed in OSA and their modulation by MAS treatment. MAS was well tolerated in our study except two subjects had side effects (they were excluded from the final analysis). Thus we confirmed the effectiveness of MAS in mild-to-moderate OSA[12,13,21] and its good tolerance.

PSG Changes Measured during MAS Therapy

MAS treatment in our mild-to-moderate OSA group was effective and significantly improved all assessed PSG parameters. In the present study, AHI dropped by 70% to 7/hour of sleep. Assessment of AHI index is one of the most commonly used criteria of effectiveness of MAS therapy, with AHI values ≤ 10/hour of sleep used as a threshold for treatment efficacy. In our study treatment was effective in 15 (68.2%) out of 22 OSA subjects. In earlier studies with MAS therapy, about 50% patients have reached this AHI threshold.[21] AHI drop by at least 50% is considered to be the most liberal criterion. This threshold was met by 68.2% in our OSA group, which represents satisfactory results, given the fact that in other studies the mean proportion of patients with AHI drop ≥ 50% was comparable to our results (72.7% of subjects).[21] Another PSG parameter confirming MAS treatment efficacy was the rise of minimal

Table 3—Concentrations of inflammatory markers in controls and OSA patients at baseline and at 3 and 6 months of treatment.

	Control Group (n = 16)	OSA Group (n = 22)			Control Group (n = 16)
Variables	**Baseline**	**Baseline**	**3 Months**	**6 Months**	**6 Months**
Fibrinogen (g/L)	3.30 (2.63 to 3.48)	3.9* (3.30 to 5.00)	3.60 (3.05 to 3.90)	3.60 (3.35 to 4.40)	3.05[α] (2.50 to 3.78)
CRP (mg/L)	1.11 (0.60 to 1.53)	0.84 (0.54 to 2.44)	0.67 (0.43 to 1.49)	0.73 (0.55 to 1.93)	0.87 (0.61 to 1.22)
IL-6 (pg/mL)	1.06 (0.72 to 2.16)	1.06 (0.71 to 1.45)	1.19 (0.84 to 1.62)	1.23 (0.82 to 1.71)	1.13 (0.74 to 1.64)
IL-1β (pg/mL)	0.24 (0.21 to 0.27)	0.35* (0.21 to 0.39)	0.19[‡‡] (0.18 to 0.25)	0.22[†] (0.19 to 0.25)	0.2[β] (0.16 to 0.24)
IL-10 (pg/mL)	3.93 (3.13 to 4.86)	5.71 (2.83 to 7.88)	3.27 (2.26 to 5.12)	2.95 (2.09 to 4.06)	3.91 (2.88 to 4.99)
P-selectin (ng/mL)	79.18 (59.36 to 105.51)	104.18* (69.41 to 141.18)	108.94 (92.33 to 123.57)	116.27 (80.07 to 129.69)	73.94[α] (55.75 to 90.01)
D- dimer (µg/L)	242.75 (177.58 to 387.09)	241.59 (173.17 to 298.38)	274.03 (188.30 to 435.26)	297.84[†] (213.16 to 368.03)	325.83 (213.89 to 462.86)
PAI-1 (ng/mL)	19.95 (15.23 to 23.20)	28.16 (14.20 to 31.52)	19.67 (15.50 to 23.84)	19.07 (14.06 to 21.44)	17.09 (13.47 to 21.32)
TAT (µg/L)	3.82 (2.83 to 4.80)	3.28 (2.60 to 5.69)	3.44 (2.74 to 5.32)	3.62 (2.55 to 7.36)	2.73 (2.46 to 2.94)
TAFIa (pmol/L)	45.02 (28.32 to 80.47)	85.93* (65.69 to 92.18)	60.42 (40.06 to 85.52)	45.27[†] (22.41 to 79.88)	55.58 (24.75 to 76.65)
CLT (min)	73.00 (60.25 to 82.75)	88.0* (75.00 to 95.50)	72.0[‡] (65.25 to 79.75)	66.0[††] (62.25 to 75.00)	69.00 (62.00 to 84.00)
9α,11β-PGF2 (pg/mL)	3.50 (2.10 to 5.0)	2.75 (2.20 to 3.40)	3.20 (2.25 to 4.03)	3.60 (2.98 to 5.13)	3.90 (2.98 to 4.88)
6-keto-PGF1α (pg/mL)	10.55 (8.03 to 15.58)	22.2* (13.03 to 26.88)	22.35 (15.98 to 25.85)	17.80 (9.85 to 23.23)	10.5[α] (8.85 to 13.95)
11-dehydro-TXB2 (pg/mL)	10.40 (6.43 to 16.28)	12.70 (10.35 to 28.63)	8.30 (6.28 to 22.65)	9.65 (7.38 to 17.40)	11.05 (6.93 to 23.15)
Glucoze (mmol/L)	5.10 (4.57 to 5.34)	5.44* (4.90 to 5.88)	5.45 (4.88 to 5.90)	5.45 (4.95 to 5.90)	5.20 (4.90 to 5.28)
Insulin (µU/mL)	7.47 (5.20 to 12.89)	10.74 (7.97 to 15.60)	11.44 (8.02 to 18.71)	9.59 (6.62 to 13.63)	10.77 (6.47 to 14.10)

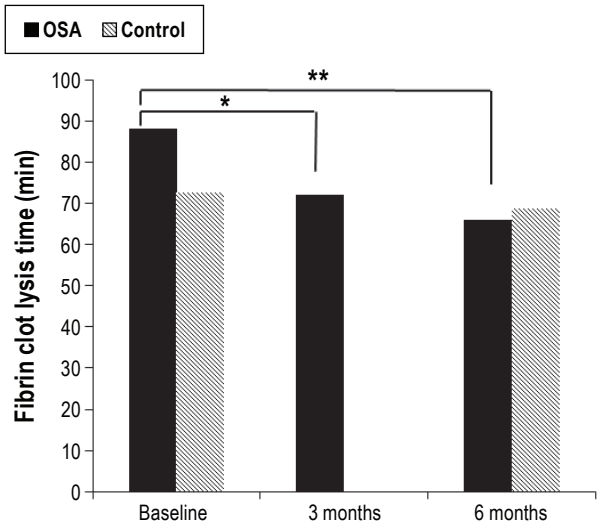
Data expressed as median (interquartile range Q1 to Q3). *$p < 0.05$, significant difference between baseline control and baseline OSA group. [‡]$p < 0.05$, [‡‡]$p < 0.01$, significant difference between baseline and 3 month treatment for OSA group. [†]$p < 0.05$, [††]$p < 0.01$, significant difference between baseline and 6 month treatment for OSA group. [α]$p < 0.05$, significant difference between control and OSA groups after 6 month trial. [β]$p < 0.05$, significant difference between control at baseline and control after 6 months.

hemoglobin saturation rate assessed by PSG. MAS therapy was associated with significant AHI reduction after the first 3 months of therapy and further improvement at the 6-month assessment. After the first PSG assessment at 3 months, some patients with residual apneas advanced their appliance further, but the amount was not assessed or controlled. Since mandibular advancement is directly related to the efficacy of MAS, the further improvement in inflammatory markers could also be related to additional efficacy of treatment.

Markers of Systemic Inflammation

In contrast to some studies,[9,22] we failed to observe elevated CRP and Il-6, which might result from less severe OSA and lower body weight in the current OSA patients. Indeed, it has been suggested that elevated CRP in OSA may be related to obesity and not OSA itself.[7,8] In mild-to-moderate OSA subjects, we have demonstrated elevated IL-1β, a marker of systemic inflammation and activated innate immunity,[23] as well as elevated fibrinogen, which is in line with most published reports.[4] A marked drop in serum IL-1β might indirectly reflect an attenuated systemic inflammatory state during MAS therapy, which agrees with the study by Tomiyama et al.,[10] who among other parameters measured IL-1β on CPAP treatment. We have also observed increased IL-10, an anti-inflammatory cytokine,[23] in OSA patients, thereby confirming the results published by Sahlman et al.[11] who studied interleukin-10 in mild OSA. This finding might represent a compensatory mechanism aiming to reduce the inflammatory response. Interestingly, we observed a 50% decrease in serum IL-10 for the following 6 months of MAS treatment. The precise mechanism underlying this change is not clear. It remains to be established whether lower IL-10 is a deleterious effect of MAS, or whether it could be perceived as normalization of this cytokine during MAS treatment.

Figure 1—The effect of 3 and 6 months of MAS treatment on mean CLT and the comparison of the mean TAFIa in the OSA group versus controls.



*p < 0.05, **p < 0.01

Fibrinolysis

Previous studies have reported that OSA can affect fibrinolysis as evidenced by an elevated D-dimer level that acts as a marker for increased fibrin formation and degradation in vivo.[5] However, the available data on D-dimer levels, both before and after CPAP treatment are inconsistent[24]; furthermore, we did not observe intergroup baseline differences in D-dimer levels without any changes in its level after MAS treatment. To determine the efficiency of fibrinolysis, a well-established assay introduced by Lisman et al.[25] was used, in which without the addition of exogenous thrombin, coagulation reactions are triggered by tissue factor (TF) in the presence of phospholipid vesicles. The CLT measurement has been successfully used to assess the fibrinolytic potential in patients with cardiovascular diseases.[26,27] Using this method, we have demonstrated for the first time that CLT is impaired in OSA. Importantly, prolonged CLT in OSA was associated largely with increased TAFIa concentrations. To our knowledge, TAFIa has not been determined in untreated or treated OSA patients, including those on CPAP treatment. TAFIa cleaves the C-terminal lysines from the plasmin-modified fibrin, which inhibits plasminogen activation and thus suppresses fibrin clot lysis. Moreover, TAFIa levels correlated positively with CLT as demonstrated, e.g., in patients with venous thrombosis.[28] Impaired CLT in OSA probably results from systemic intermittent hypoxemia and/or systemic inflammation. Altogether, our findings and other studies[2,5] indicate that hypofibrinolysis might contribute to an increased risk of thromboembolic events observed in OSA patients.[2] Moreover, it has been convincingly reported that arterial and venous thromboembolism are characterized by prolonged CLT,[26-28] which might suggest that hypofibrinolysis contributes to an increased risk of thrombotic events in OSA. We have also observed increased plasma fibrinogen levels in mild-to-moderate OSA which corroborated previous findings.[4] In our study, MAS treatment did not change plasma fibrinogen concentrations at 3 and 6 months. A decrease in fibrinogen levels following CPAP treatment was described by Chin et al.[4] Most likely, in less severe forms of OSA as studied by our group, the impact of therapy could be much less pronounced.

Significantly, MAS treatment has been shown to favourably modulate fibrinolytic activity in mild-to-moderate OSA by decreasing both plasma PAI-1 and TAFIa. The decrease of PAI-1 is in line with the results reported by von Känel et al.[5] in patients after CPAP treatment of more severe OSA. It might be hypothesized that suppressed inflammation during the effective MAS therapy reduces the synthesis and release of these two fibrinolysis inhibitors into the circulation, as suggested by their correlations with some inflammatory markers in the current study. TAFIa concentrations were influenced by the AHI value and time spent with SpO_2 below 90%; therefore, the reduction in TAFIa appears to be closely associated with improved markers of OSA severity. It should be highlighted that effective MAS therapy accelerated impaired CLT and thus could reduce the thrombotic risk in OSA.

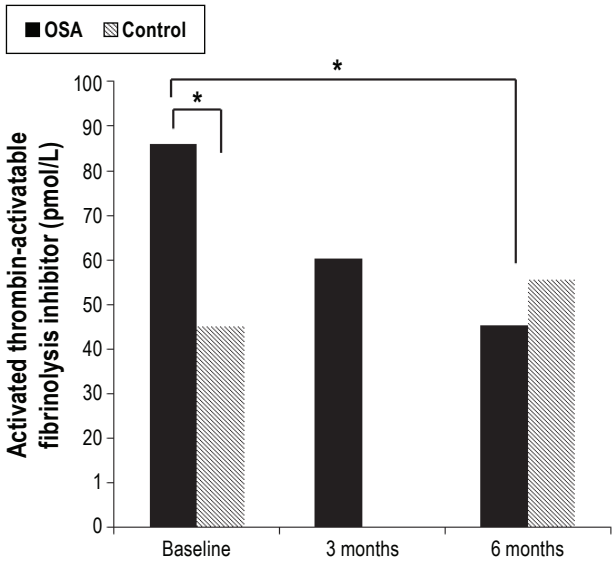
Coagulation and Platelet Activation

P-selectin and 6-keto-PGF1α, which both were elevated at baseline compared to controls, remained unaltered after 6 months in both groups; therefore, the baseline intergroup difference also was observed post treatment. Higher P-selectin in the mild-to-moderate OSA group might indicate increased platelet activation. Similarly increased serum P-selectin was found in Japanese patients with moderate and severe OSA.[29] In contrast to some previous reports,[6,30] we did not observe any difference in thrombin generation markers between OSA patients and the controls. A potential explanation is that heightened thrombin generation could be a marker of elevated blood pressure, but not OSA itself. Moreover, in the current study, mild-to-moderate OSA hypertensive patients were effectively treated, which resulted in no differences in systolic and diastolic pressure between the OSA and control subjects. The present study suggests that a hypercoagulable state in mild-to-moderate OSA patients is relatively resistant to modulation by MAS therapy despite the improved PSG parameters. This intriguing issue merits further investigation.

Study Limitations

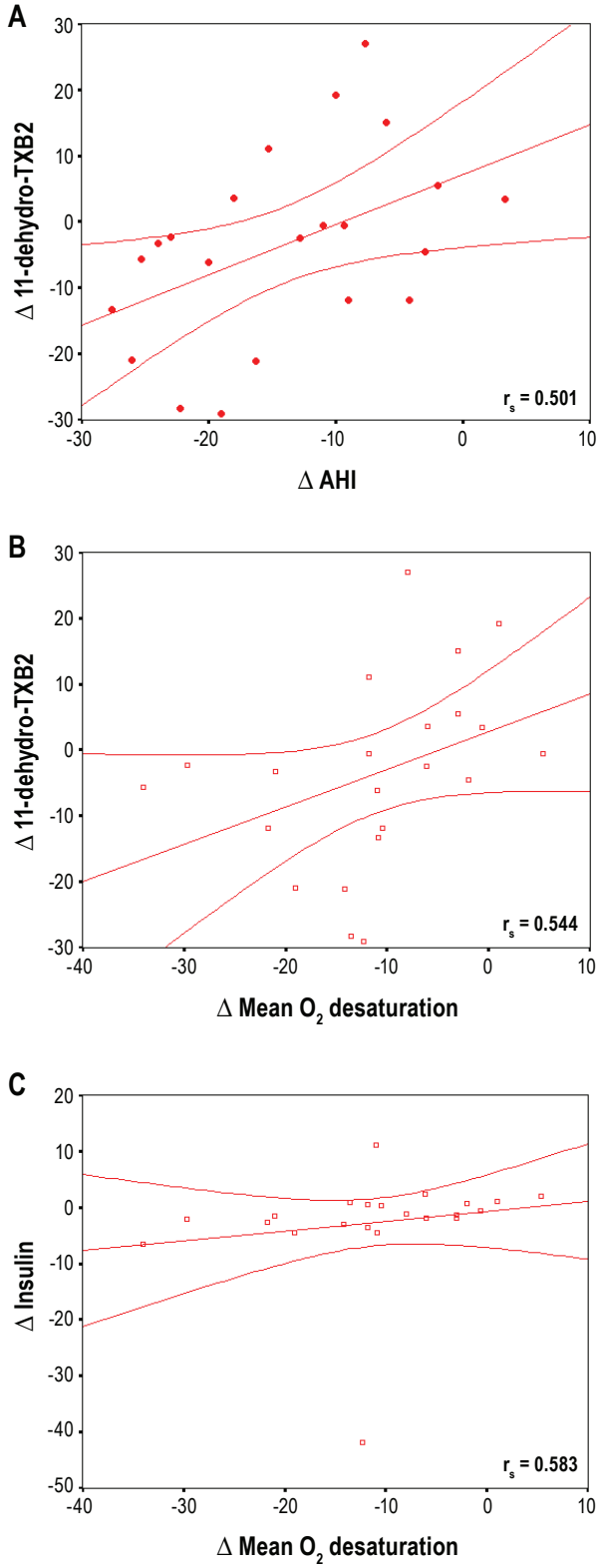
Firstly, the size of the current study was relatively low; however, the subjects were representative for mild-to-moderate OSA. Secondly, it was not a randomized controlled study, but to our knowledge sham MAS has never been used in human studies and our primary goal was not the comparison of the effect of MAS with CPAP therapy. The different effects of CPAP therapy have already been studied extensively. The current methodology, in particular an open-label study design, might thus confer a systemic bias. Different rates of current smoking, as observed in subjects and controls, could potentially influence some of our results. However, the absolute numbers of smoking subjects were too low (2 in OSA and 1 in control group) to significantly influence the results. Thirdly, a follow-up duration of longer than six months has not been planned, and the monitoring of clinical events was deemed

Figure 2—The effect of 3 and 6 months of MAS treatment on mean TAFIa and the comparison of the mean TAFIa in OSA versus control.



*p < 0.05

Figure 3—Scatter plots showing a significant association of: **(A)** the change in AHI and the change in 11-dehydro-TXB2; **(B)** the change in mean oxygen desaturation and the change in 11-dehydro-TXB2; and **(C)** the change in mean oxygen saturation and the change in the insulin levels.



All changes were calculated as values at 6 months minus the baseline values. Regression line and lines for 95% confidence interval are shown.

beyond the scope of the present study. Another potential limitation could be oligosymptomatic or asymptomatic undetected VTE episodes in the study group. However, based on the estimated prevalence of VTE in this age group, it is unlikely that more than one patient experienced VTE in the past, so this cannot confound the data interpretation in our study. We have excluded all females from the final analysis since our final study group had only one female, and there are inconsistent data indicating that there are sex associated differences in hemostatic markers, particularly fibrinolytic parameters, e.g., TAFI. Therefore our findings may not be extrapolated to the female population. Another limitation of this study is that controls were thinner than our OSA group, and one could state that the controls were healthier than the OSA group. Previous studies have shown a similar effectiveness of MAS compared to CPAP in the improvement of cardiovascular outcomes, despite residual apneas related to MAS treatment. This has been hypothesized to be related to a better compliance rate with MAS than CPAP. In the current study, compliance was not measured, and there were no specific questionnaires related to MAS usage, but patients stated that they used the appliance as recommended (entire night). This is a limitation of the current study, and we may speculate that some minor improvements of the inflammatory markers in a subset of patients could be related to poor compliance. Finally, statistically significant associations, especially in small groups, does not necessarily mean a cause-effect relationship. However, the current hypothesis generating study shows novel links between OSA and the prothrombotic/inflammatory state as well as the impact of MAS therapy.

In conclusion, we showed that OSA is characterized by impaired CLT associated with elevated PAI-1 and TAFIa, and this abnormality can be corrected as early as after 3-6 months

of MAS therapy. Further studies on larger patient populations with different severity of OSA are needed to elucidate mechanisms behind the observed changes in fibrinolytic and inflammatory mechanisms.

REFERENCES

1. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle aged adults. *N Engl J Med* 1993;328:1230-5.
2. Bradley TD, Floras JS. Obstructive sleep apnea and its cardiovascular consequences. *Lancet* 2009;373:82-93.
3. Ryan S, Taylor CT, McNicholas WT. Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnea syndrome? *Thorax* 2009;64:631-6.
4. Chin K, Ohi M, Kita H, et al. Effects of NCPAP therapy on fibrinogen levels in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 1996;153:1972-6.
5. von Känel R, Loredo JS, Ancoli-Israel S, Dimsdale JE. Association between sleep apnea severity and blood coagulability: treatment effects of nasal continuous positive airway pressure. *Sleep Breath* 2006;10:139-46.
6. Takagi T, Morser J, Gabazza EC, et al. The coagulation and protein C pathways in patients with sleep apnea. *Lung* 2009;187:209-13.
7. Sharma SK, Mishra HK, Sharma H, et al. Obesity, and not obstructive sleep apnea, is responsible for increased serum hs-CRP levels in patients with sleep disordered breathing in Delhi. *Sleep Med* 2008;9:149-56.
8. Ryan S, Nolan GM, Hannigan E, Cunningham S, Taylor C, McNicholas WT. Cardiovascular risk markers in obstructive sleep apnea syndrome and correlation with obesity. *Thorax* 2007;62:509-14.
9. Yokoe T, Minoguchi K, Matsuo H, et al. Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. *Circulation* 2003;8:1129-234.
10. Tomiyama H, Okazaki R, Inoue D, et al. Link between obstructive sleep apnea and increased bone resorption in men. *Osteoporos Int* 2008;19:1185-92.
11. Sahlman J, Miettinen K, Peuhkurinen K, et al; Kuopio Sleep Apnea Group. The activation of the inflammatory cytokines in overweight patients with mild obstructive sleep apnea. *J Sleep Res* 2010;19:341-8.
12. Cistulli PA, Gotsopoulos H, Marklund M, Lowe AA. Treatment of snoring and obstructive sleep apnea with mandibular repositioning appliances. *Sleep Med Rev* 2004;8:443-57.
13. Marklund M, Legrell PE. An orthodontic oral appliance. *Angle Orthod* 2010;80:1116-21.
14. Lam B, Sam K, Mok WY, et al. Randomised study of three non-surgical treatments in mild to moderate obstructive sleep apnea. *Thorax* 2007;62:354-9.
15. Gauthier L, Laberge L, Beaudry M, Laforte M, Rompré PH, Lavigne GJ. Efficacy of two mandibular advancement appliances in the management of snoring and mild-moderate sleep apnea: a cross-over randomized study. *Sleep Med* 2009;10:329-36.
16. Andrén A, Sjöquist M, Tegelberg A. An effect on blood pressure after treatment of obstructive sleep apnea with a mandibular advancement appliance- a three-year follow-up. *J Oral Rehabil* 2009;36:719-25.
17. Itzhaki S, Dorchin H, Clark G, Lavie L, Lavie P, Pillar G. The effects of 1-year treatment with a herbst mandibular advancement splint on obstructive sleep apnea, oxidative stress and endothelial function. *Chest* 2007;131:740-9.
18. Trzepizur W, Gagnadoux F, Abraham P, et al. Microvascular endothelial function in obstructive sleep apnea: Impact of continuous positive airway pressure and mandibular advancement. *Sleep Med* 2009;10:746-52.
19. Kushida CA, Littner MR, Morgenthaler T, et al. Practice parameters for the indications for polysomnography and related procedures: an update for 2005. *Sleep* 2005;28:499-521.
20. Kim PY, Foley J, Hsu G, Kim PY, Nesheim ME. An assay for measuring functional activated thrombin-activatable fibrinolysis inhibitor in plasma. *Anal Biochem* 2008;372:32-40.
21. Ferguson KA, Cartwright R, Rogers R, Schmidt-Nowara W. Oral appliances for snoring and obstructive sleep apnea: a review. *Sleep* 2006;29:244-62.
22. Punjabi N, Beamer B. C-reactive protein is associated with sleep disordered breathing independent of adiposity. *Sleep* 2007;30:29-34.
23. Akdis M, Burgler S, Crameri R, et al. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. *J Allergy Clin Immunol* 2011;127:701-21.
24. von Känel R, Natarajan L, Ancoli-Israel S, Mills PJ, Loredo JS, Dimsdale JE. Day/night rhythm of hemostatic factors in obstructive sleep apnea. *Sleep* 2010;33:371-7.
25. Lisman T, Leebeek FW, Mosnier LO, et al. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. *Gastroenterology* 2001;121:131-9.
26. Meltzer ME, Doggen CJ, de Groot PG, Rosendaal FR, Lisman T. Plasma levels of fibrinolytic proteins and the risk of myocardial infarction in men. *Blood* 2010;116:529-36.
27. Guimarães AH, de Bruijne EL, Lisman T, et al. Hypofibrinolysis is a risk factor for arterial thrombosis at young age. *Br Haematol J* 2009;145:115-20.
28. Meltzer ME, Lisman T, de Groot PG, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood* 2010;116:113-21.
29. Minoguchi K, Yokoe T, Tazaki T, et al. Silent brain infarction and platelet activation in obstructive sleep apnea. *Am J Respir Crit Care Med* 2007;175:612-7.
30. von Känel R, Loredo JS, Powell FL, Adler KA, Dimsdale JE. Short-term isocapnic hypoxia and coagulation activation in patients with sleep apnea. *Clin Hemorheol Microcirc* 2005;33:369-77.

ACKNOWLEDGMENTS

The authors greatly acknowledged Dr. Micheal E. Nesheim, Ph.D. (deceased in June 2011) for valuable comments. We thank Adam Ćmiel, Ph.D., for statistical analysis; Alicja Ziółkowska, R.N., for blood collection; Teresa Iwaniec, Ph.D., for performing laboratory investigations; and Ewa Figiel, M.S., for her help in preparing this manuscript.

SUBMISSION & CORRESPONDENCE INFORMATION

Submitted for publication May, 2013
Submitted in final revised form October, 2013
Accepted for publication November, 2013
Address correspondence to: Anetta Undas, M.D., Ph.D.; Institute of Cardiology Jagiellonian, University Medical College, 80 Pradnicka St., 31-202 Krakow, Poland; Tel: +48-12-6143004; Fax: +48-12-4233900; E-mail: mmundas@cyf-kr.edu.pl

DISCLOSURE STATEMENT

This was not an industry supported study. This study was supported by a grant of Polish Ministry of Science (to Dr. Undas) N402 179834. The authors have indicated no financial conflicts of interest.