CHEST Online Supplement

Effect of Continuous Positive Airway Pressure on Cardiovascular Biomarkers

The Sleep Apnea Stress Randomized Controlled Trial

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e-Appendix 1. Study protocol

Eligibility Criteria

Eligibility for recruitment was ascertained by screening polysomnogram studies performed in the sleep laboratories and also by pre-screening patients presenting for sleep clinic visits who met the criteria of moderate to severe OSA. Electronic medical records were then reviewed to further assess inclusion and exclusion criteria. Those that met eligibility criteria were then approached for study recruitment.

Inclusion Criteria:

- Ages 20 to 75 years
- Moderate to severe OSA (AHI>15)
- CPAP adherence after a 2-week run-in period (4 hours of use for >70% of sleep time during the treatment week)

Exclusion Criteria:

- Current or planned use (outside of the trial) of specific OSA treatments (CPAP, oral appliances) or anticipated upper airway surgery or gastric bypass surgery in the subsequent 6 months
- Supplemental oxygen use
- A primary sleep disorder other than OSA
- Unstable medical conditions:

o New onset or changing angina
o Myocardial infarction or congestive heart failure exacerbation documented within the previous 6 months
o High grade cardiac dysrhythmia/heart block
o Known unaddressed coronary artery disease by history
o Stroke
o Uncontrolled hypertension or diabetes mellitus, thyroid disorder

- Cirrhosis
- Non-skin cancer diagnosed within the last 2 years
- Psychiatric disorders inadequately treated or compromised competence
- Daytime sleepiness with reports of sleepiness while driving or otherwise in situations which would present a risk for the subject or public (e.g., operating heavy equipment)
- Alcohol abuse (currently drinks >5 alcoholic drinks/day)
- Pregnancy
- Inability to provide informed consent
- Use or anticipated use of oral corticosteroids or other potent anti-inflammatory medications (e.g. etanercept, mycophenolate mofetil, azathioprine, hydroxychloroquine, etc.).

Study Design

Data were presented by blinded arm to the DSMB during meetings involving review of summary data.

Procedures

Research visits occurred in the University Hospitals Case Medical Center Dahms Clinical Research Unit during which participants underwent overnight polysomnography, anthropometry, morning venipuncture, overnight urine collection, evening saliva collection, resting morning and evening blood pressure measurement and collection of measures of vascular stiffness at baseline and 8 weeks. A medical history was obtained, including co-morbidity assessment, Epworth sleepiness scale, [33] use of statins, anti-hypertensive, anti-inflammatory drugs, and smoking habits. Height was measured with a rigid stadiometer and weight was measured with a calibrated digital scale using standardized methods. Anthropometric

measurements were collected including neck and waist circumference. At each overnight examination (baseline and follow-up), blood pressure was measured in triplicate in the supine position at approximately 21:00 and 9:30.

Data Collection

Height was measured with a rigid stadiometer and weight was measured with a calibrated digital scale using standardized methods. Anthropometric measurements were collected included neck and waist circumference. At each overnight examination (baseline and follow-up), blood pressure was measured in triplicate in the supine position at approximately 21:00 and again at 9:30.

Overnight 14-channel polysomnography was performed at the baseline and follow-up visits using the Compumedics E-series monitoring system (Abbotsford, Australia). The recording montage consists of C_3/A_2 and C_4/A_1 electroencephalograms (EEG); bilateral electrooculograms; a bipolar submental electromyogram; thoracic and abdominal respiratory inductance plethysmography (Summit IP ®, a newly developed auto-calibrated sensor that provides excellent sum and abdominal band data); "airflow" (by nasal-oral thermocouple); oximetry (using highly sensitive finger pulse oximeter, sampling frequency 25Hz), electrocardiogram (ECG); body position (mercury switch sensor); bilateral leg movements (piezo sensors), and nasal (via cannula for the baseline exam) or mask pressure. EEGs were recorded at 125 Hz; ECG at 250 Hz. Data were scored according to standard criteria. Apneas were identified when the amplitude (peak to trough) of the airflow signal is flat or nearly flat) for 10 seconds or longer. Hypopneas were identified if the amplitude of any of the respiratory signals is reduced by 30% of the amplitude of baseline accompanied by a 3% or greater oxygen desaturation.

Objective adherence data for sham and CPAP was collected by exporting data stored in the CPAP units. All participants received education regarding sleep hygiene and healthy lifestyle habits utilizing a standardized approach.

Outcomes

Oxidative stress and inflammatory markers

Blood was drawn after overnight fasting (and prior to blood pressure monitoring) in the supine position between 7-8AM the morning after the polysomnogram. 24-hour urine samples were collected for F2-isoprostane levels. The samples were then centrifuged and aliquoted according to standard protocols and stored at -80 degrees Celsius until ready for analysis. Assays were performed at the Cleveland Clinic and University of Vermont Laboratory for Clinical Biochemistry Research, Burlington. Plasma myeloperoxidase levels were analyzed with an ELISA based assay (*CardioMPO* Enzyme Immunoassay Reagent Kit) that had a total and within run coefficient of variation of 8.2% and 5.5% respectively. Myeloperoxidase has biphasic kinetics with a short half-life of 2-3 h and longer half-life of 8-10h.[1] F2-isoprostanes levels were analyzed by using an established, stable isotope dilution mass spectrometry based approach using high performance liquid chromatography (HPLC) with an on-line electrospray ionization tandem mass spectrometry. Samples were analyzed on an AB SCIEX 5000 triple quadrupole mass spectrometer .The urinary F2-isoprostanes levels were corrected by urinary creatinine to account for differences in urine dilution.[2]

IL-6 and sIL-6R were measured by quantitative sandwich enzyme immunoassays (Quantikine HS Human IL-6 and sIL-6R Immunoassays; R&D Systems, Minneapolis, Minn) with intra-laboratory coefficients of 9.1% and 6.6% respectively. Oxidized LDL was measured by competitive ELISA (Mercodia Oxidized LDL

Competitive ELISA, Mercodia AB; Uppsala, Sweden) using a monoclonal antibody. The inter-assay coefficient of variation is less than 10%. Lp(a) was measured with the BNII nephelometer (N Latex Lp(a) Reagent; Siemens, Inc., Deerfield, IL) utilizing a particle enhanced immunonepholometric assay. The intra-assay coefficient of variation ranges from 1.8–4.1% and inter-assay coefficient of variation range from 2.0–5.3%. The PON1 activity was measured (Roche C3 analyzer) by quantifying serum paraoxonase activity using paraoxon as substrate and aryl esterase activity using phenylacetate as substrate, as previously described.[3] The intra and inter-day assay coefficients of variance for the paraoxonase activity were 1.9% and 3.3% and for aryl esterase activity coefficients of variation were 3.4% and 3.9%.

Pulse wave analysis

Arterial stiffness measures (pulse wave analysis and pulse wave velocity) were collected in duplicate and averaged. Measurements were conducted in the morning and in the evening under standardized conditions prior to intake of anti-hypertensive medications after at least 10 minutes of rest in the supine position. Participants were in a quiet room and abstained from food, caffeine, alcohol and smoking at least 8 hours prior to the assessments. Vascular measures were collected for 116 participants as initiation of this data collection occurred subsequent to the inception of the trial.

Pulse wave analysis of the radial artery was performed by applanation tonometry (version 7, SphygmoCor, Atcor Medical, Sydney Australia) to obtain the radial artery waveform after appropriate equipment calibration. A quality-grading index was used to evaluate the reproducibility of the readings and to include only the measures with acceptable quality, i.e. only those readings with <10% variability in pulse height or the diastolic waveform. The central aortic pressure waveform was generated using the device validated transfer function. The augmentation index (AIx) was determined from the aortic pressure waveform and was corrected to a heart rate of 75 beats per minute. The AIx is a measure of arterial stiffness and represents the additional pressure in the central circulation caused by the reflected wave; it is defined as the augmentation pressure expressed as a percentage of the pulse pressure.

Pulse wave velocity

Carotid-femoral PWV (m/s) was measured by applanation tonometry using the intersecting tangent foot-to-foot algorithm (SphygmoCor system, Atcor Medical, Sydney Australia). The transit time was calculated as the delay from the R-wave of the electrocardiogram to the foot of the carotid and femoral waveforms. The difference between the carotid and femoral propagation time represents the PWV. The distance between the sternal notch and the femoral artery tonometry site was measured and used for the calculations. Readings that did not meet the quality limits were excluded from the analysis.

Statistical Analyses

This study was designed with the intention to randomize 128 completers (i.e. n=64 per group) to achieve sufficient power to detect a half standard deviation difference between the two groups assuming 80% power based upon a minimum effect size for each outcome of 0.50.

Secondary analyses were performed with adjustment for adherence, i.e. sham, CPAP non-adherent and CPAP adherent groups were compared adjusted for baseline, in which adherence was defined by average use hour ≥ 4, or adherent days (≥ 4 hours use) ≥70%. Given data demonstrating differences in cardiometabolic outcomes according to OSA severity and obesity, [35] exploratory stratified analyses were performed to evaluate for differences in the effect of CPAP on outcomes in relation to OSA severity (dichotomized at an AHI$\geq$30, i.e. standard cut-off to delineate severe OSA) and obesity (dichotomized using the median sample body mass index, BMI of 36.3 kg/m^2). Additional stratified analyses were conducted to examine hypoxic exposure defined by the baseline oxygen desaturation index ($\geq$3% and alternatively $\geq$4%). Linear models adjusted for the baseline value and interaction between study arm and primary predictors (binary variable of the AHI and BMI), were performed to compare the change of

biomarkers between study arms and AHI and BMI groups. Least square means were used to present and compare the means of change.

Results

At baseline, there was no significant correlation of F2-isoprostane levels and AHI, however, there was a statistically significant correlation of myeloperoxidase and AHI (r^2=0.17, p=0.046).

Adverse Events

Of those consented, there were 14 serious adverse events (8 during washout, 3 during sham and 3 during active CPAP) involving hospital admissions or visits to the ED that were unrelated to the study interventions or participation. Of the overall adverse events, 91.5% were not study related and these were balanced across the randomization arms (31.9% in the CPAP arm versus 27.8% in the sham CPAP arm).

References

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e-Table 1 Spearman correlation between AHI and outcomes

Outcome	N	rho	95% CI	P value
Systolic Blood Pressure evening	149	0.09	(-0.07,0.25)	0.27
Systolic Blood Pressure morning	149	0.12	(-0.04,0.29)	0.13
Diastolic Blood Pressure evening	149	0.11	(-0.05,0.27)	0.18
Diastolic Blood Pressure morning	149	0.15	(-0.01,0.31)	0.070
Mean Arterial Blood Pressure evening	149	0.11	(-0.06,0.27)	0.20
Mean Arterial Blood Pressure morning	149	0.12	(-0.04,0.28)	0.14
Pulse Pressure evening	149	0.06	(-0.11,0.22)	0.50
Pulse Pressure morning	149	0.06	(-0.11,0.22)	0.49
Pulse Wave Velocity evening	116	0.21	(0.03,0.39)	0.024
Pulse Wave Velocity morning	108	0	(-0.19,0.19)	0.99
Augmentation Index evening	113	-0.27	(-0.45,-0.09)	0.004
Augmentation Index morning	113	-0.25	(-0.43,-0.06)	0.009
Augmentation Pressure evening	116	-0.18	(-0.36,0.00)	0.053
Augmentation Pressure morning	113	-0.19	(-0.37,-0.00)	0.047
F2-isoprostanes	139	-0.05	(-0.22,0.12)	0.58
Myeloperoxidase	147	0.17	(0.00,0.33)	0.046
Soluble Interleukin-6 Receptor	141	-0.04	(-0.21,0.13)	0.64
Interleukin-6	141	0.19	(0.02,0.35)	0.028
Plasminogen Activator Inhibitor-1	148	0.21	(0.05,0.37)	0.012
Lipoprotein (a)	147	-0.02	(-0.19,0.14)	0.80
Fibrinogen	147	0.19	(0.03,0.35)	0.020
Oxidized LDL	147	0.12	(-0.04,0.29)	0.14
Paraoxonase	142	0	(-0.17,0.16)	0.97
Aryl esterase	142	0.16	(-0.00,0.33)	0.052

e-Table 2. Adherence data, CPAP vs Sham-CPAP, entire cohort

	Overall (N=149)		Sham (N=74)		CPAP (N=75)	
	Summary	n	Summary	n	Summary	p-value
Adherence (follow-up)	43.6	66	30.3	67	56.7	0.002
Average hours (follow-up)	3.7[1.7,5.5]	66	2.5[0.95,4.7]	67	4.5[2.6,5.7]	0.003
Adherence Days (≥4h) ≥70%	33.1	66	22.7	67	43.3	0.012

e-Table 3. Subgroup analysis stratified by severe obstructive sleep apnea

Factor	Sham+AHI<30 (N =45)	Sham+AHI≥30 (N = 26)	CPAP+AHI<30 (N = 51)	CPAP+AHI≥30 (N = 21)
AIx morning‡§	0.27 (-3.20,3.75)	0.69 (-3.57,4.95)	-6.70 (-10.25,-3.15)	-6.07 (-11.02,-1.13)
sIL-6R* (ng/ml)†	1.02 (0.97,1.07)	1.01 (0.95,1.08)	0.95 (0.91,0.99)	0.97 (0.91,1.04)

AHI = Apnea Hypopnea Index, CPAP = Continuous Positive Airway Pressure, sIL-6R = Soluble interleukin-6 receptor, AIx = Augmentation index
*Data was log-transformed for analysis, and transformed back for presentation.
† sIL-6R: Sham+AHI<30 differed from CPAP+AHI<30, p = 0.029
‡ AIx morning: Sham+AHI<30 differed from CPAP+AHI<30, p = 0.006
§ AIx morning: Sham+AHI≥30 differed from CPAP+AHI≥30, p = 0.042

e-Table 4. Subgroup analysis stratified by median body mass index

Factor	Sham+BMI≤36 (N = 39)	Sham+BMI>36 (N = 32)	CPAP+BMI≤36 (N = 34)	CPAP+BMI>36 (N = 38)
AIx morning‡§	2.42 (-1.15,5.98)	-2.09 (-6.14,1.96)	-5.05 (-9.04,-1.07)	-7.91 (-11.89,3.93)
sIL-6R* (ng/ml)†	1.04 (0.99,1.10)	0.99 (0.93,1.05)	0.97 (0.92,1.02)	0.95 (0.90,1.00)

BMI = Body Mass Index, CPAP = Continuous Positive Airway Pressure, sIL-6R = Soluble interleukin-6 receptor, AIx = Augmentation index
*Data was log-transformed for analysis, and transformed back for presentation.
† sIL-6R: Sham+BMI≤36 differed from CPAP+BMI≤36, p = 0.040
‡ AIx morning: Sham+BMI≤36 differed from CPAP+BMI≤36, p = 0.006
§ AIx morning: Sham+BMI>36 differed from CPAP+BMI>36, p = 0.044

e-Table 5. Correlation of Baseline Apnea Hypopnea Index and Outcomes*

Outcome	N	rho	95% CI	P value
Systolic Blood Pressure (mmHg, evening)	149	0.16	(-0.01,0.31)	0.058
Systolic Blood Pressure (mmHg, morning)	149	0.13	(-0.03,0.29)	0.11
Diastolic Blood Pressure (mmHg, evening)	149	0.19	(0.03,0.34)	0.018
Diastolic Blood Pressure (mmHg, morning)	149	0.19	(0.03,0.34)	0.02
Mean Arterial Pressure (mmHg, evening)	149	0.2	(0.05,0.35)	0.012
Mean Arterial Pressure (mmHg, morning)	149	0.19	(0.03,0.34)	0.022
Pulse Wave Velocity (mmHg, evening)	108	-0.03	(-0.22,0.16)	0.76
Pulse Wave Velocity (mmHg, morning)	116	0.17	(-0.01,0.34)	0.063
Augmentation Index (evening)	113	-0.25	(-0.41,-0.06)	0.009
Augmentation Index (morning)	113	-0.18	(-0.36,0.00)	0.051
F2-Isoprostanes/Creatinine, ng/mg	148	-0.04	(-0.20,0.12)	0.64
Myeloperoxidase, pmol/L	147	0.15	(-0.01,0.31)	0.065
Soluble Interleukin-6 Receptor, ng/ml	141	-0.11	(-0.27,0.06)	0.2
Interleukin-6, pg/ml	141	0.18	(0.02,0.34)	0.03
Lipoprotein (a), mg/dl	147	-0.02	(-0.18,0.14)	0.79
Oxidized LDL, U/L	147	0.08	(-0.09,0.23)	0.36
Paraoxonase, U/mL	142	0.06	(-0.10,0.22)	0.47
Aryl esterase, nmol/min/mL	142	0.15	(-0.01,0.31)	0.067

*Pearson correlation coefficients