

## Resistin and Cardiac Remodeling in Patients with Obstructive Sleep Apnea

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### Abstract

**Background:** Resistin is an adipocytokine, associated with obesity and inflammation. Its exact role in insulin resistance and diabetes is still controversial, but there is now enough data, concerning its direct effects on myocardial cells. The relation between resistin plasma levels with risk of new onset heart failure in humans has been confirmed in several large studies.

**Materials and methods:** Resistin plasma levels were measured in 30 patients with obstructive sleep apnea and mild systolic dysfunction (ejection fraction 45, 7% ± 6, 17%), and compared to fifteen patients with obstructive sleep apnea and normal ejection fraction (ejection fraction 60, 3 ± 6, 3%). The effect of bilevel positive airway pressure therapy was evaluated during a three month follow-up in 19 patients. The dynamics of markers of haemodynamic stress – NT-pro-BNP was determined in addition. The association between resistin, obesity, insulin resistance and severity of obstructive sleep apnea was analysed.

**Results:** Resistin plasma levels were significantly higher in the group with mild systolic dysfunction in comparison to those with preserved ejection fraction (6, 92 ng/ml vs 2, 78 ng/ml). This difference lost significance after adjustment for confounders. In a linear regression analysis resistin levels were not associated with body mass index, obesity, homeostasis model assessment- index, systolic and diastolic blood pressure, or obstructive sleep apnea severity. Though not of statistical significance its plasma levels, decreased (8, 53 vs. 4, 16 ng/ml; p=0, 12) as a result of a three-month bilevel positive airway pressure therapy.

**Conclusions:** According to our data it is elusive to determine whether resistin plasma levels are associated with early myocardial damage. Its application for the monitoring of the effect of bilevel positive airway pressure therapy is tentative.

**Keywords:** Resistin; Early cardiac remodeling; Insulin resistance; Obstructive sleep apnea; Bilevel positive airway pressure therapy

### Background

Cross-sectional results from the Sleep Heart Health Study have shown a significant association between obstructive sleep apnea and chronic heart failure [1]. The prevalence of obstructive sleep apnea in a population with chronic heart failure has been shown to be as high as 30-40% [2-4]. Comorbidities, accompanying obstructive sleep apnea such as systemic hypertension and obesity [5], are also associated with the development of chronic heart failure [6]. Ciccone et al. [7] have established that the severity and duration of obstructive sleep apnea are independent contributors for early atherosclerotic lesions, as assessed by the intima media thickness [7]. Moreover emerging data suggest that obstructive sleep apnea is not only associated with, but also contributes to the progression of cardiac remodeling in heart failure through different mechanisms.

Resistin has been related to both increased risk of coronary heart disease and to increased inflammatory markers known to be associated with increased risk of left ventricular systolic dysfunction and heart failure [8-10]. Initially resistin has been reported to increase resistance to insulin in mice, where it is expressed by adipocytes [11]. In humans resistin is secreted to a greater extent by macrophages [12,13]. Its place in triggering insulin resistance is largely debated and still elusive [14,15]. In general however it is predominantly associated with markers of inflammation – C-reactive protein, tumor necrosis factor- $\alpha$  and interleukin-6, which are well accepted predictors of heart failure incidence [16,17].

Despite the controversies, regarding its role in the metabolic syndrome, increased resistin plasma levels have been reported in patients with prehypertension and masked hypertension [18,19]. Recently resistin has also been reported as an independent risk factor, responsible for the promotion of heart failure in middle age and elderly patients [20,21].

Although there is an increasing understanding of the physiologic consequences of obstructive sleep apnea, till now there is no established biomarker, related neither to the cardiac remodeling, nor to the clinical response to obstructive sleep apnea treatment in patients with systolic dysfunction.

Considering this the primary aim of our study was to measure resistin plasma levels in obstructive sleep apnea patients with mild left ventricular dysfunction and compare them to a group of patients with normal ejection fraction. Our secondary aim was to follow resistin plasma levels after three month bilevel positive airway pressure therapy. In addition we analysed the consequences on the echocardiographic characteristics of left ventricular dysfunction - left ventricular fraction shortening, ejection fraction, thickness of the septum and posterior wall, systemic blood pressure control and correlated them to resistin levels; we compared the intermediate-term effect of bilevel positive airway pressure on other biomarkers, related to the haemodynamic and oxidative stress.

Ciccione et al. [7] have demonstrated that in obstructive sleep apnea patients the proper application of positive airway pressure ventilation may reverse the early atherosclerotic lesions (intima-media thickness) [22]. Regarding cardiac functioning bilevel positive airway pressure decreases the amount of blood returning to the heart, thereby decreasing the work of heart. Bilevel positive airway pressure provides inspiratory and expiratory positive airway pressures. The inspiratory boost of pressure improves ventilation and decreases the work of breathing. It is generally applied to improve the blow off carbon dioxide and oxygenation. Expiratory positive airway pressure is the pressure during exhalation. It keeps air passages open, so that full exhalation is performed and the next breath comes in easier.

Based on this data we assumed that the application of bilevel positive airway pressure would be beneficial in respect to the pathophysiological consequences of obstructive sleep apnea (inflammation and oxidative stress) as well as the haemodynamic characteristics related with it.

## Materials and Methods

### Subjects

We performed a randomized study in which forty-five patients with newly diagnosed obstructive sleep apnea were included. The study was approved by the Ethics Committee of the Medical University, Sofia. Only patients who gave informed consent for participation were recruited. Patients were selected among those that have been referred to the Sleep Lab because of signs and symptoms associated with obstructive sleep apnea. The polysomnography study was carried out in the Sleep Lab of the University Hospital "Alexandrovska", Clinic of Internal Medicine and Division of Pulmonology during the period January –April, 2012. After polysomnography was performed, subjects that agreed to participate in the study went through an echocardiographic investigation. Echocardiographic measurements were performed independently by two observers. According to these measurements patients were divided into those with normal systolic function and mild systolic dysfunction (ejection fraction 45-55%).

To assess the role of resistin and isoprostanes in cardiac remodeling, these parameters were compared between patients with newly diagnosed obstructive sleep apnea with normal ejection fraction and those with newly diagnosed obstructive sleep apnea and mild systolic dysfunction. Fifteen patients with normal ejection fraction

served as a "control group". None of the participants was aware of having obstructive sleep apnea or has undergone any treatment modality associated with it.

As bilevel positive airway pressure is not reimbursed only nineteen patients of those with newly diagnosed obstructive sleep and systolic dysfunction went through this treatment modality. Eleven of the patients were on standard therapy (either ACE – inhibitor or  $\beta$ -blocker, depending on the etiology of the systolic dysfunction). To compare the effect of bilevel positive airway pressure therapy to standard therapy patients have been followed up for three months. The investigators have been blinded to the treatment modalities.

### Inclusion criteria

Inclusion criteria were: 1) newly diagnosed obstructive sleep apnea; 2) hypertension within less than three years of presentation. The definition of obstructive sleep apnea was based on a combination of clinical symptoms (i.e. daytime excessive sleepiness) and a standard polysomnography – Compumedics, E-series. Obstructive sleep apnea was diagnosed if there were complaints of sleepiness and the apnea-hypopnea index was more than 5 events per hour.

In the group of patients with mild systolic dysfunction the inclusion criteria was ejection fraction 45-55%.

### Exclusion criteria

Exclusion criteria were as follows: 1) long-term continuous positive airway pressure or bilevel positive airway pressure therapy; 2) central sleep apnea or Cheyne-Stokes respiration; 3) chronic obstructive pulmonary disease, chronic respiratory failure or need for supplemental oxygen; 4) ischemic episode or unstable angina; 5) recent episode of acute heart failure within the last 6 months; 6) concomitant large hemispheric or brainstem stroke; 7) renal failure; 8) endocrinopathology; 9) neoplasm.

### Study design

Patients with systolic dysfunction and obstructive sleep apnea were recruited after baseline polysomnography. Patients were provided with the same positive airway pressure device (BiPAP-ST, Devilbiss). The cardiologists performing echocardiograms were blinded to patient assignment. The use of the same machine provided against bias, regarding superiority of any device that participants may have had. The research co-ordinators contacted participants monthly and received their device card memory.

### Polysomnography

Respiratory measurements: Full polysomnography was performed in all the patients (Compumedics, E-series, Australia). Continuous recordings were taken with electrode positions C3/A2-C4/A1-Cz/01 of the international 10–20 Electrode Placement System. Eye movements, chin electromyogram and ECG modified V2 lead. Sleep was scored manually according to standard criteria [25]. Airflow was measured using nasal pressure associated with the sum of buccal and nasal thermistor signals. Respiratory efforts were monitored with abdominal and thoracic bands. Arterial oxygen saturation (SaO<sub>2</sub>) was measured using a pulse oximeter (Medair, Hudiskvall, Sweden). An apnea was defined as a complete cessation of airflow for >10s, and a hypopnea as a >50% reduction in the nasal pressure signal or a 30–50% decrease, associated with either oxygen desaturation of >3% or an arousal both

lasting for >10 s. Apnoeas were classified as obstructive, central or mixed according to the presence or absence of respiratory efforts. The classification of hypopnoeas as obstructive or central was based upon the shape of the inspiratory part of the nasal pressure curve. Scoring of respiratory events in the polysomnography was according to the definitions provided by the American Academy of Sleep Medicine [23]. Patients were divided into two groups – patients with mild-moderate apnea – AHI <30e/h; severe apnea AHI >30e/h. Continuous positive pressure was used for all patients on the titration night and the titration-targeted elimination of apnoeas. Once apnoeas were eliminated, the pressure was increased to eliminate hypopnoeas followed by snoring.

**24 Ambulatory Blood Pressure Monitoring:** Non-invasive 24-h ambulatory blood pressure monitoring was performed on the non-dominant arm using BOSO TM2420/TM 2480 Profilemanager (Bosh&Sohns, Germany). The device was programmed to obtain blood pressure readings at 20-min intervals during the day (07.00–22.00 hours) and at 30-min intervals during the night (22.00–07.00 hours). The ambulatory blood pressure monitoring was always performed during a working day. The recording was then analysed to obtain 24-h daytime and nighttime average systolic and diastolic blood pressure, average arterial blood pressure and heart rates [24].

**Echocardiogram:** All participants underwent resting two-dimensionally guided M-mode echocardiography at baseline. Left ventricular septal wall thickness, posterior left ventricular wall thickness, and left ventricular end-diastolic diameter were measured at end-diastole. Left ventricular end-systolic diameter and LAD were measured at end-systole. All measurements were obtained in accordance with the American Society of Echocardiography guidelines using a leading-edge-to-leading edge technique [25]. Left ventricular fraction of shortening was calculated as (left ventricular end-diastolic diameter – left ventricular end-systolic diameter) / (left ventricular end-diastolic diameter). Two cardiologists performed the investigation independently. The intra- and interobserver coefficients of variability were respectively 7% and 25%. The cardiologists were blinded to treatment modality regimens.

### Laboratory assays

**Clinical blood tests:** Routine blood examinations included: peripheral blood cell counts; basic biochemistry - fasting plasma glucose, fasting serum insulin, creatinine, fasting serum triglyceride, low density, very low density and high-density-lipoprotein cholesterol. Insulin resistance was calculated using the HOMA index: plasma glucose (mmol/l) x serum insulin (mU/l) / 22,5 [26].

**Resistin plasma levels measurement:** Fasting blood samples were drawn in the morning, after waking-up, before the ingestion of any medications. Samples were collected in 9ml tubes, containing EDTA as an anticoagulant. They were centrifuged instantly after collection and isolated plasma was stored in vials at –80 ° C until assayed. Resistin was determined by an ELISA kit following the producer's protocol (RayBio\_ Human Resistin, Cat#:ELH-Resistin-001) The intra- and interassay coefficients of variation in this assay kit ranged from 10 to 12%. Plasma resistin levels were measured in ng/ml.

**NT-pro-BNP:** Plasma levels of NT-pro-BNP were determined by electrochemiluminescent immunoassay for quantitative determination - (Elecys- pro-BNP II assay, Cobas, Roche). Functional sensitivity 0,6-4130 pmol/l. Coefficient of variation – 20%. NT-pro-BNP plasma levels were measured in pmol/l.

**HRAM determination of 8-isoprostane in urine samples:** The levels of 8-isoprostane were measured in urine samples. Overnight urine was taken immediately after waking – up before the ingestion of any medication or food. Urine was collected in 10 ml sterile plastic tubes and was stored at –80° C until analysis was performed. 8-isoprostanes were determined by high resolution accurate mass spectrometry on LTQ Orbitrap® Discovery (ThermoScientific Co, USA) mass spectrometer, equipped with Surveyor® Plus HPLC system and IonMax® electrospray ionization module. The analyses were carried out by stable isotope dilution method in negative ionization mode using HESI II (heated electrospray ionization) source type.

The concentration and purification of 8-isoprostane from urine samples was processed by affinity sorbent (Cayman Chemical, USA), following the producer's protocol. The urinary isoprostane levels were standardized to the levels of urinary creatinine. It was measured applying the enzyme method - Creatinine plus version 2 (CREP2) - Cobas Integra (Roche).

**Adherence to Treatment:** Compliance to bilevel positive pressure ventilation was determined by monthly download of device memory. It was mainly expressed as average hours of night use. Compliance was evaluated as good if ventilation was performed >4h per night. All the patients had more than four hours of use of the machine during the study period. The first three days of titration of the pressure regimen were not taken into account.

Compliance of the patients in the standard treatment modality was assessed by the number of tablets used per month. Patients on this modality were given medication by the investigator. The medication was prescribed in a required dose regimen and the exact number of pills was preliminary calculated and supplied by the researchers. Medications were given in a bottle to the patients. The number of tablets actually used divided by the total number of given tablets served as an index of compliance.

### Statistical Methods

Data are presented as mean ± standard deviation. Kolmogorov – Smirnov was used for detection of distribution of variables. Parametric correlation analysis (Pearson) was performed to establish the association between variables. The t-test was used for comparisons of baseline and three month clinical parameters at the end of follow-up. A linear regression was performed to study the associations between the analysed parameters. A p-value <0.05 was considered as statistically significant. Statistical analysis was performed with a standard statistical program package (SPSS version 14.0).

### Results

Anthropometric, echocardiographic, glucometabolic and haemodynamic characteristics of the patients

Table 1 details participants' characteristics. Nineteen participants (18 men; 1 woman) were enrolled in the bilevel positive airway pressure group, and 11 patients received only standard treatment modality (11 men). All patients had left ventricular ejection fraction < 50%. There were no apparent differences between the two groups, regarding their anthropometric characteristics – age, waist circumference. The systolic dysfunction, assessed by ejection fraction was almost similar. All patients were receiving optimal doses of angiotensin-converting enzyme inhibitors, diuretics and beta-blockers according to the guidelines for treatment of hypertension and early

systolic dysfunction – data presented in Table 1. The etiology of heart failure was similar in both groups (bilevel positive airway pressure / standard treatment). In the bilevel positive airway pressure group there were 5 patients with diabetes – 25%; 5 patients with impaired

glucose tolerance- 25%; nine patients with normal glucose metabolism – 50%. In the group that remained on standard treatment four were diabetics – 27%, and the rest did not have any impairments in their glucose metabolism – 63% -Table 2.

	BiPAP group (19)	Standard therapy (11)	Control group (15)
Anthropometric characteristics			
Age, years	55.4 ± 10.3	53.15 ± 7.68	49.75 ± 6.78 p-0.623
Men:Women	18/1	11/0	12/3
Weight, kg			144.3 ± 32.26 p-0.421
BMI, kg/m <sup>2</sup>	41.05 ± 5.95	39.06 ± 6.92	38.06 ± 7.57 p-0.485
Waist circumference, cm	134.6 ± 13.32	130.3 ± 16.29	132.16 ± 19.76 p-0.421
Smokers (current:former:nonsmoker)	12:6:1	5:4:2	7:2:1
Sleep study characteristics			
Mild-moderate OSA (AHI <30e/h)	3/19 (16%)	2/11(18%)	0
Severe OSA (AHI >30e/h)	16/19(84%)	9/11(82%)	15/15(100%)
AHI, events/hour	50.93 ± 25.4	49.14 ± 28.3	65.94 ± 30.58 p-0.321
Sleep duration, min	211 ± 21.6	209 ± 45.9	203.5 ± 44.3 p-0.087
Time SpO <sub>2</sub> <90%, %	79.43 ± 28.9	56.35 ± 37.8	50.43 ± 23.41 p-0.114
Lipid profiles			
HDL, mmol/l	1.20 ± 0.29	1.35 ± 0.38	1.31 ± 0.41 p-0.912
LDL, mmol/l	2.74 ± 0.75	3.38 ± 1.06	3.25 ± 1.05 p-0.428
VLDL, mmol/l	0.94 ± 0.53	0.83 ± 0.34	0.81 ± 0.25 p-0.781
Tot Chol, mmol/l	4.86 ± 1.03	5.57 ± 0.99	5.39 ± 1.28 p-0.613
Triglycerids, mmol/l	1.81 ± 0.64	1.83 ± 0.76	1.83 ± 0.56 p-0.345
Glucometabolic markers			
Fasting glucose, mmol/l	6.24 ± 2.71	4.87 ± 7.66	6.01 ± 2.1
Immunoreactive insulin, mUI/ml	18.47 ± 10.65 p*-0.316	15.3 ± 8.67 p**-0.229	19.46 ± 14.26 p***-0.151

HOMA index	4.18±2.37	3.43±2.48	6.01±7.21 p***-0.177
HbA1C, %	6.59 ± 1.31 p*-0.253	5.92 ± 0.63 p**-0.103	6.04 ± 0.82 p***-0.210
Biomarkers			
Isoprostanes pg/μml crn	0.164 ± 0.09	0.125 ± 0.05 p**-0.172	0.049 ± 0.02 p***-0.000
NT –pro-BNP pmol/ml	51.75 ± 46.3	39.12 ± 8.76 p**-0.453	-
Resistin,ng/ml	8.53 ± 7.96	4.45 ± 0.78 p**-0.375	2.51 ± 0.75 p***-0.078
FFA,mmol/l	0.189 ± 0.06	0.258 ± 0.11 p**-0.273	0.198 ± 0.10 p***-0.305

**Table 1:** Basic characteristics of patients - p-Kruskall –Wallis comparison between three groups.

p\* - Mann -Whithney comparison BiPAP vs standard; p\*\* - Mann - Whithney comparison standard vs controls ; p\*\*\*- Mann -Whithney comparison BiPAP vs controls.

Echocardiographic characteristics	BiPAP group (19)	Standard therapy -11	Control group (15)
LVFS%	45.64 ± 5.48	45.87 ± 6.87	60.37 ± 6.30 p-0.439
EF %	45.64 ± 5.48	45.87 ± 6.87	60.37 ± 6.30 p-0.439
Septum, mm	13.75 ± 2.1	11.82 ± 2.2	12.68 ± 1.53 p-0.658
BW,mm	13.29 ± 1.8	12.76 ± 1.9	12.66 ± 1.63 p-0.721
Blood pressure characteristics			
Diurnal Systolic Blood Pressure, mmHg	134.66 ± 8.17	142.3 ± 9.48	136.28 ± 13.81 p-0.092
Diurnal Diastolic Blood Pressure, mmHg	80.01 ± 9.31	81.3 ± 9.82	82.8 ± 8.89 p-0.794
Nocturnal Systolic Blood Pressure, mmHg	126.05 ± 9.63	109.2 ± 8.12	121.85 ±10.57 p-0.091
Nocturnal Diastolic Blood Pressure, mmHg	78.8 ± 9.41	67.3 ± 9.65	73.02 ± 7.33 p-0.459
Cardiomyopathy			
Ischaemic/Non-ishaemic	8/19 (42%)	6/11(55%)	1/15(6%)
Diabetics	5/19(26%)	4/11(36%)	2/15(13%)
Imapired glucose tolerance	5/19(26%)	0	8/15(53%)
Normal glucose metabolism	9/19(47%)	7/11(64%)	5/15(33%)



Treatment			
ACEI	18/19 (95%)	9/11(82%)	13/15(87%)
B-Blockers	12/19 (63%)	7/11(63%)	10/15(67%)
Diuretics	17/19(89%)	8/11(72%)	9/15(60%)

**Table 2:** Echocardiographic and haemodynamic characteristics of patients.

Comparing the groups of treatment modality according to their sleep study characteristics no large discrepancies could be observed. In the bilevel positive airway pressure group 84% (16/19) patients had severe apnea, almost the same is the percentage in the group on standard therapy -- 82% (9/11). The duration of sleep, apnea, hypopnea and apnea-hypopnea index were also comparable. The time of sleep under SpO<sub>2</sub><90% however was significantly longer in the bilevel positive airway pressure group – 79% vs. 56% - Table 1.

The anthropometric characteristics of the control group with preserved ejection fraction were almost similar to those in patients with mild systolic dysfunction. The mean age of the group was 49, 75 ± 6,78years. The average body mass index – 38, 06 ±7, 57. All of them had hypertension (less than three yaers from onset) that was treated. Only one had ischaemic heart disease. Two patients (13%) had diabetes; Eight (53%) had impaired glucose tolerance; five (34%) had normal glucose metabolism. The average apnea-hypopnea index was 65, 94 ± 30,58e/h. All patients had severe apnea.

Comparison between resistin plasma levels, biomarkers of oxidative stress and markers of glucose metabolism in patients with mild systolic

dysfunction and preserved systolic function and the association of resistin with them.

Resistin plasma levels were measured in the nineteen patients with mild systolic dysfunction that have been on bilevel positive airway pressure therapy and compared to those in the control group with preserved ejection fraction. As shown in (Table 1), resistin plasma levels were higher in the patients with systolic dysfunction. They did not correlate to the body mass index (p=0.52); immuno-reactive insulin (p=0.946); fasting blood glucose (p=0.396); free fatty acids (p=0.142) or to homeostasis model assessment index (p=0.759).

Comparison of the effects of bilevel positive airway pressure on Left Ventricular Systolic Function in patients with systolic dysfunction.

In patients with bilevel positive airway pressure the three month treatment period led to a slight, not statistically significant change in the echocardiographic parameters (Table 3).

During the follow-up none of the patients had any change in their current therapy.

	BiPAP group		Standard treatment	
	Baseline	3d month	Baseline	3d month
Echocardiographic characteristics				
LVFS%	26.26 ± 4.12	28 ± 3.74	25.14 ± 3.09	22.87 ± 5.27
EF %	45.64 ± 5.48	46.40 ± 6.68	45.87 ± 6.87	45.12 ± 9.27
Septum, mm	13.75 ± 2.1	13.09 ± 1.37	11.82 ± 2.2	11.45 ± 1.8
BLW,mm	13.29 ± 1.8	13.17 ± 1.38	12.76 ± 1.9	12.91 ± 1.8
24h blood pressure characteristics				
Diurnal Systolic blood pressure, mmHg	134.66 ± 8.17	127.8 ± 7.12	142.3 ± 9.48	133.92 ± 8.49
Diurnal Diastolic blood pressure, mmHg	80.01 ± 9.31	80.38 ± 8.29	81.3±9.82	81.1 ± 8.76
Nocturnal Systolic blood pressure, mmHg	126.05 ± 9.63	124.5 ± 7.41	109.2±8.12	115.24 ± 9.16
Nocturnal Diastolic blood pressure, mmHg	78.8 ± 9.41	71.8 ± 8.03	67.3 ± 9.65	64 ± 8.83

**Table 3:** Baseline and third month echocardiographic characteristics in the two groups.

The effects of bilevel positive airway pressure on 24h blood pressure profiles in patients with systolic dysfunction.

To assess whether the effects of bilevel positive airway pressure are primarily due to improving the haemodynamic characteristics of blood pressure control in five patients with bilevel positive airway pressure and 5 patients without bilevel positive airway pressure - 24h RR monitoring was performed at baseline and at the end of the follow-

up. Data is presented in (Table 3). No statistically significant changes in blood pressure profiles have occurred during the period.

The effects of bilevel positive airway pressure on biomarkers of haemodynamic, oxidative stress, resistin and free fatty acids in patients with systolic dysfunction

The oxidative stress, accompanying the apneas and hypopneas, as well as the haemodynamic changes, related to the swingings of the

intrathoracic pressure are discussed as the main triggers of the adverse effects of obstructive sleep apnea on the cardiovascular system. To assess the role of bilevel positive airway pressure on them we used the isoprostanes - a validated biomarker of oxidative stress and NT-Pro-BNP a marker of haemodynamic burden.

All patients with systolic dysfunction were followed up and their urinary levels of isoprostanes were measured at baseline and at the third month. In the BiPAP group there was a statistically significant change of urinary isoprostanes - (Table 4) The plasma levels NT-pro-BNP were followed up in 17/19 (89%) in the bilevel positive airway pressure and 8/11 (72%) in the other group. In both groups its plasma levels decreased, not reaching a statistical significance. In the bilevel positive airway pressure group the plasma levels of resistin decreased almost twice - from 8,53 to 4,16ng/ml, but this was not of statistical significance.

	BiPAP group		Standard treatment	
	Baseline	3d month	Baseline	3d month
NT-pro-BNP pmol/l	51.75 ± 46.3	7.45 ± 6.12 p-0.066	39.12 ± 8.76	11.4 ± 7.12 p-0.235
Isoprostanes	0.164 ± 0.09	0.098 ± 0.05 p-0.011	0.125 ± 0.05	0.097 ± 0.03 p-0.262
Resistin ng/ml	8.53 ± 7.96	4.16 ± 3.37 p-0.12	4.45 ± 0.78	3.82 ± 2.08 p-0.768
FFA, mmol/l	0.189 ± 0.06	0.197 ± 0.09 p-0.798	0.258 ± 0.11	0.299 ± 0.13 p-0.610

**Table 4:** Baseline and third month isoprostanes and NT-pro-BNP.

## Discussion

Obesity in obstructive sleep apnea patients may contribute to heart failure by various mechanisms including neurohormonal activation and increased oxidative stress [27], infiltration of myocytes with free fatty acids [28] and B-type natriuretic peptide depletion [29]. The intermittent hypoxia in obstructive sleep apnea may aggravate abnormal adipose tissue functioning in obese and may lead to dysregulated secretion of molecules, called adipocytokines that influence the function and structural integrity of various tissues.

The major findings of our study are that in obstructive sleep apnea patients with mild systolic dysfunction plasma levels of the adipokine - resistin were increased in comparison to the control group of obstructive sleep apnea patients with preserved ejection fraction. None of the other markers for oxidative stress (isoprostanes), insulin resistance (free fatty acids) or haemodynamic burden (NT-pro-BNP) showed any difference between the two groups. After adjustment for systolic and diastolic blood pressure, however, the difference, regarding resistin plasma levels lost significance. Moreover resistin levels could not be associated to none of the parameters explored - body mass index, insulin resistance, severity of sleep apnea, systolic and diastolic blood pressure. During the three month follow -up of bilevel positive airway pressure therapy only the plasma levels of resistin and urinary isoprostanes decreased. The change of resistin plasma levels, however, was not of statistical significance (large number of outliers and interindividual variation).

Until now the the exact role of resistin in the pathogenesis of coronary heart disease, arterial hypertension and cardiac remodeling in obstructive sleep apnea patients has not been investigated. Neither is the link between resistin and insulin resistance and the subsequent cardiovascular damage.

Resistin is a newly discovered adipocyte-secreted polypeptide that has been implicated in the development of insulin resistance. It belongs to a family of cysteine-rich secretory proteins collectively termed the resistin-like molecules family and is also described as “adipose tissue secretory factor” or “FIZZ” found in inflammatory zones protein [11]. Resistin expression and serum concentrations are related to obesity, insulin resistance, and inflammation in humans [13,30]. These mechanisms are directly involved in the pathogenesis of coronary heart disease [30,31] and the development and progression of both ischaemic and nonischaemic cardiomyopathy [32,33]. Beyond the indirect effects, overexpression of resistin in cardiomyocytes has been associated with altered response to ischemia-reperfusion injury [34], depressed contractility and hypertrophy [35]. All these observations made our association between resistin and cardiac remodeling in obstructive sleep apnea patients theoretically plausible.

Insulin resistance is among the novel risk factors for heart failure. Its prevalence in obstructive sleep apnea patients has been reported to reach the levels of 47% [37]. Early reports suggested that resistin is associated with obesity and insulin resistance in rodents. Deletion of the resistin gene reduces the impact of obesity on glucose homeostasis [38]. Conversely, acute administration of resistin impairs glucose tolerance and insulin action [39]. Mice with chronic hyperresistinemia exhibit modest fasting hyperglycemia and glucose intolerance, associated with increased hepatic glucose production in the setting of hyperinsulinemia. Though the preliminary data from animal studies suggest a possible link between resistin and insulin resistance, in humans this is largely debated. Furuhashi and associates [40] found that resistin levels are not related to insulin resistance, at least not in patients with essential hypertension, while Zhang and colleagues [41] pointed out a relationship between fasting serum resistin and blood glucose values in patients with essential hypertension and abnormal glucose tolerance.

In our study we could not observe a significant correlation between resistin plasma levels and the insulin resistance, as calculated by the homeostasis model assessment-index (p-0,759). Other markers of insulin resistance - free fatty acids (p-0,142), fasting blood glucose (p-0,396) and oxidative stress (p-0,180) also showed no association to this adipocytokine. The lack of a relation between resistin and insulin resistance markers was observed not only in obstructive sleep apnea patients with early cardiac remodeling but also in the control group. Regarding the association between resistin and body mass index, no correlation could also be detected.

We found however that patients with mild systolic dysfunction had higher plasma levels of resistin in comparison to the control group of obstructive sleep apnea patients with preserved ejection fraction.

Similar are the results in the Framingham offspring study. Frankel et al. [20], describe that resistin was associated and appeared to add to the risk of developing heart failure, even after adjustment for insulin resistance and inflammatory markers. They established that higher concentrations of resistin were associated with greater degrees of insulin resistance and higher concentrations of C-reactive protein. The authors however suggest that resistin may promote to heart failure via mechanisms independent of insulin resistance, inflammation or B-

type natriuretic peptides. In their study they found that elevated plasma concentrations of resistin are associated with subsequent development of heart failure even after accounting for obesity, insulin resistance, inflammation and concurrent and incident coronary heart disease. This is confirmed by Butler et al. [21]. The investigators describe that serum resistin concentrations are associated with risk for heart failure in elderly patients that was comparable among prevalent and incident coronary heart disease and diabetes mellitus subgroups. Analysing our own results it remains elusive whether resistin is potential biomarker of early cardiac remodeling in obstructive sleep apnea patients. The co-existence of many comorbidities and the interplay between pathophysiological pathways complicates the analysis of its potential role.

In addition to insulin resistance, arterial hypertension is among the triggering mechanism in heart failure pathogenesis. Increased resistin plasma levels have been reported in patients with prehypertension and masked hypertension [18,19]. All of our patients received antihypertensive therapy, so that we could not analyse the presence of any association between the adipokine and blood pressure control. In patients on bilevel positive airway pressure therapy who had ambulatory blood pressure monitoring there was a slight not significant decrease of blood pressure at the end of the third month. The decrease of resistin plasma levels, however did not correlate to that in blood pressure. According to our results it is unlikely that resistin could promote cardiac remodeling by means of haemodynamic loading. This is confirmed by the fact that resistin plasma levels decrease before those of NT – pro BNP (51,47 vs.7,93, p-0,066) - a well validated marker of hemodynamic stress and cardiac and vascular remodeling.

Recent studies show that recombinant resistin increases the expression of inflammatory and adhesion molecules - vascular cell adhesion molecule-1 and intracellular adhesion molecule-1, up-regulates the monocyte chemoattractant chemokine-1, and promotes endothelial cell activation via endothelin-1 release on human endothelial cells, suggesting a potential role in atherosclerosis and inflammation [9].

In obstructive sleep apnea patients Yamamoto et al. [42] found a relation between resistin plasma levels and severity of obstructive sleep apnea. The distinction in the levels of resistin between the groups in this study is well remarked in comparison to ours. A reason for this can be that our patients were extremely obese – the average body mass index >40. In the study of Yamamoto et al. [42] the average body mass index is 28. The adipose tissue dysfunction in extremely obese patients and the complex interplay in the regulation of adipokine secretion can therefore obscure the causation link. We should consider the larger number of current and former smokers in our study since the persistent low grade inflammation in smokers could also complicate the analysis of the data. Another fact that obscures analysis is that we had mainly patients with severe obstructive sleep apnea. Only 20% of OSA patients with mild systolic dysfunction and 33% of the control group had moderate apnea.

We followed up the effect of bilevel positive airway pressure in patients with mild systolic dysfunction [19] and obstructive sleep apnea and compared them to those in obstructive sleep apnea patients remaining on standard treatment [11]. There were no apparent imbalances in the baseline characteristics between the two groups. The echocardiographic characteristics that were monitored during the study did not change much neither in the group on bilevel positive airway pressure therapy, nor in that on standard therapy.

In contrast to the standard clinical parameters where no distinctive dynamics was detected the adipokines and markers of oxidative stress lowered as a result of the bilevel positive airway pressure treatment.

We found a decrease in resistin (8,53 vs. 4,16 ng/ml, p-0.12) and isoprostane levels (0,164 vs. 0,098,p-0.011) in patients with bilevel positive airway pressure. However, only the decrease of isoprostanes remained of clinical significance. The haemodynamic burden, evaluated by the NT-pro-BNP showed no significant decrease of plasma NT-pro-BNP in the bilevel positive airway pressure group. Despite that recent experimental data [43], determine mechanical stretch as an enhancer of resistin expression, the results of our can not confirm this.

### Limitations

Limitations of our study are: 1) it was predominantly performed in patients with extreme obesity - more than 90% had body mass index >35; 2) most of our patients suffered from severe apnea, thus the influence of obstructive sleep apnea patients severity was also depraved; 3) we did not explore a marker of inflammation neither to observe any correlations between resistin plasma levels, nor to explore its dynamics as a result of therapy; 4) additionally, our study demonstrated higher levels of resistin in patients with cardiac remodeling but does not establish causality; 5) the ability to speculate about the potential mechanisms linking resistin and heart failure is limited by the small number of participants, short follow-up and limited range of relevant biomarkers.

### Conclusions

According to our data resistin plasma levels are independent of insulin resistance in obstructive sleep apnea patients. Their role as a marker of early myocardial damage and their significance for the monitoring of the effect of bilevel positive airway pressure therapy remains elusive.

### Abbreviations

OSA: Obstructive Sleep Apnea; AHI: Apnea/Hypopnea Index; BiPAP: Bilevel Positive Airway Pressure; BMI: Body Mass Index; HOMA-I: Homeostasis Model Assessment Index; IRI: Immuno Reactive Insulin; EF: Ejection Fraction; LVFS: Left Ventricular Fraction Shortening; RR: Riva Rocchi; FFA: Free Fatty Acids; CHF: Chronic Heart Failure; HF: Heart Failure; LV: Left Ventricular; BP: Blood Pressure; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; ECG: Electrocardiogram; EEG: Electroencephalography.

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### Authors' contributions

RCh, OG, JP, RB and DP participated in the design of the study and in writing the manuscript. RCh recruited the patients and collected the data. SSR and VM performed the HRAM analysis and free fatty acids



measurements. EM performed the echocardiography. AC performed the ELISA for resistin and the enzymatic determination of urinary creatinine levels as well as the measurement of NT-pro-BNP. All reviewed and approved the final version of the manuscript.

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