Respiratory Medicine (2008) **102**, 1399–1405

available at www.sciencedirect.com

journal homepage: www.elsevier.com/locate/rmed

Airway inflammation in obstructive sleep apnea: Is leptin the missing link?☆

Sofia Antonopoulou [a], Stelios Loukides [a,*], Georgios Papatheodorou [b], Charis Roussos [a], Manos Alchanatis [a]

[a] *Sleep Laboratory, 1st Respiratory Medicine Dept, Medical School, University of Mesogeion 152, 15669, Athens, Greece*
[b] *Clinical Research Unit, Athens Army General Hospital, Athens, Greece*

Received 29 January 2008; accepted 24 April 2008
Available online 7 July 2008

KEYWORDS
Exhaled breath condensate;
Inflammation;
Leptin;
Obstructive sleep apnea;
Severity;
Smoking;
BMI

Summary
Background: Local and systemic inflammation is implicated in the pathophysiology of Obstructive Sleep Apnea (OSA). Exhaled breath condensate (EBC) is a non-invasive sampling method for the lower airways. However, it is important to consider the potential effect of the systemic origin whereas systemic inflammation is significantly elevated. This prospective study was designed to investigate whether airway inflammation is significantly related to plasma leptin levels in OSA patients. Simultaneously, it was designed to investigate whether inflammatory variables predict parameters expressing disease severity and finally whether smoking habit affect the above measurements.
Patients & Methods: About 45 OSA patients (mean AHI 40 ± 25, 28 smokers) and 25 healthy controls (AHI < 5, 15 smokers) were studied and underwent overnight diagnostic polysomnography. We measured pH, 8-isoprostane, TNF-α and IL-6 in EBC and leptin in plasma. Plausible associations between leptin and inflammatory parameters were analyzed after adjustment for proper variables. Similar associations between inflammatory variables and parameters of disease severity were also performed.
Results: An increased level of leptin and respective increase of inflammatory variables was found. No significant association was observed between parameters of EBC and plasma leptin levels. A part of the parameters of disease severity is significantly associated with pH and 8-isoprostane. Smoking did not seem to be a critical confounding factor for evaluation of the above measurements.
Conclusions: Increased levels of leptin were not associated with the observed airway inflammation in OSA. The observed airway inflammation seemed to be independent of smoking habit with limited association with disease severity.

☆ Institution at which the work was performed: 1st Respiratory Medicine Dept, Medical School, University of Athens, Athens.

* Corresponding author. Smolika 2 16673, Athens, Greece. Tel.: +302108954603; fax: +302107770423.
E-mail addresses: santonod@hotmail.com (S. Antonopoulou), ssat@hol.gr (S. Loukides), gepapatheodorou@otenet.gr (G. Papatheodorou), croussos@med.uoa.gr (C. Roussos), nalxanat@med.uoa.gr (M. Alchanatis).

0954-6111/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2008.04.021

Introduction

Obstructive sleep apnea (OSA) is characterized by repetitive episodes of upper airway closure during sleep associated with excessive daytime sleepiness and abnormalities in cardiopulmonary and metabolic function. Several studies have implicated airway[1,2] and systemic[3,4] inflammation in the pathophysiology of this seemingly all-mechanical problem. These inflammatory changes are mainly attributed to biomarkers expressing oxidative stress or cytokines related to obesity.[5,6]

Leptin, a 16 kD adipokine, regulates weight centrally and it is also a part of a cytokine network which governs the inflammatory-immune response by releasing proinflammatory cytokines.[7] The already reported association between OSA and hyperleptinemia may be explained by the common link to obesity.[8,9] However, several authors have reported elevated leptin levels in patients with OSA even after controlling for obesity measures, possibly through mechanisms which involve the effects of hypoxemia, sleep fragmentation or heightened sympathetic activity.[10,11]

Exhaled breath condensate (EBC) is a non-invasive sampling method, easily repeatable, safe and useful for sampling the airways and monitoring airway inflammation.[12,13] Despite the traditional theory that most of the mediators are coming from the lower airways (involving bronchi and alveoli), it is important to consider the potential effect of the systemic origin where systemic inflammation is significantly elevated.[12,14]

Since leptin is associated with systemic inflammation, we hypothesized that the possible increase of leptin levels might also be related to a respective activity in airway inflammation. We therefore assessed airway inflammation by measuring the levels of 8-isoprostane, interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α) and pH in EBC and studied their plausible relation with plasma levels of leptin. In order to eliminate confounding factors which affect the above measurements, all associations were analyzed after taking all the proper adjustments into consideration. As a secondary outcome, we investigated whether values of inflammatory variables and particularly EBC pH — a parameter measurable in the field — are affected by smoking habit and predict the severity indexes of the disease.

Methods

Study subjects

We studied 112 consecutive patients referred to Sleep Laboratory of the 1st Respiratory Department of Athens University Medical School with symptoms suggestive of OSA, for further diagnostic investigation. After a full night diagnostic polysomnography, evaluation of inclusion and exclusion criteria of the study, 45 OSA patients (37 males, age 52 ± 12 years, BMI 33.5 ± 7, 28 smokers) finally formed our patients group. Patients with Apnea/Hypopnea Index (AHI) $\geq$10 were included in the study. Exclusion criteria were respiratory infection in the last 4 weeks, history of chronic liver and renal failure, metabolic syndrome, malignancy, daily hypercapnia as assessed by arterial carbon dioxide tension ($PaCO_2$), diabetes, clinical apparent cardiovascular disease, non well-controlled hypertension, increased lipid profile, atopy, self-reported asthma or reversibility >12% of airway obstruction after administration of a β_2-agonist and finally Chronic Obstructive Pulmonary Disease (COPD) as assessed by the current guidelines.[15] Twenty-five healthy subjects non-randomly selected, matched for age, gender and BMI, (18 males, age 51 ± 7 years, BMI 31 ± 3, 15 smokers) were used as control group. In all control subjects, OSA was excluded by using an overnight diagnostic polysomnography. They were mainly recruited from a population used as healthy subjects in other studies of our group. Control subjects had AHI <5, no significantly reported symptoms of daytime sleepiness as assessed by Epworth Sleepiness Scale (ESS), no history of medical diseases, were non-atopic, and were not receiving any daily medication. None of them had a history of respiratory infection in the last 4 weeks before enrollment. Written informed consent was obtained from all subjects, and the study was approved by the institutional ethics committee.

OSA variables

Polysomnography

ESS was used in all subjects for assessment of subjective daytime sleepiness.[16] Simultaneously, body weight and height were evaluated in order to calculate the BMI (weight/height^2 in Kg/m^2). Neck circumference and hip to waist ratio were also measured.

Overnight polysomnography (Alice 4, Respironics, Inc, Murryville, PA, USA) was performed between 11 pm to 6 am according to standard procedure.[17] Definitions for respiratory events were the following: (1) Obstructive apnea: complete cessation of airflow with continued paradoxical chest and abdominal movement for >10 s, (2) Hypopnea: reduction of airflow of >50% from baseline for >10 s in association with a 4% desaturation or EEG arousal. The number of events per hour of sleep was obtained by dividing the total number of events by the total sleep time (TST) and was defined as the AHI. OSA severity was assessed by number of AHI, mean duration of AH in seconds, lowest and mean oxygen saturation (SaO_2) %, total time in minutes with saturation <90%, and the number of 4% drops in oxygen saturation during TST defined as ''Oxygen Desaturation Index'' (ODI).

Lung function

Pulmonary function tests were measured by a dry spirometer (Vica-test, Model VEP2; Mijnhardt; Rotterdam, Holland). Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were measured according to the American Thoracic Society guidelines.[18] Reversibility test was performed according to the GOLD guidelines.[15]

Exhaled breath condensate

EBC was collected and processed according to recently published recommendations[12] by using a condenser (EcoScreen; Jaeger, Wurzburg, Germany). The condensate (at least 1 ml) was collected and immediately transferred to Eppendorf tubes and was stored at $-70\,^{\circ}C$. All variables with exception of pH were measured within 3 months from the initial storage.

Measurement of inflammatory mediators

Leptin was assayed using an enzyme immunoassay (ELISA) (Biosource Europe, Nivelles, Belgium). The detection limit of the assay was 3.5 ng/ml. 8-Isoprostane concentration was determined by a specific enzyme immunoassay kit (Cayman Chemicals, Ann Arbor, MI, USA), as previously described.[19] The detection limit of the assay was 5 pg/ml. TNF-α was measured by ELISA using an equivalent kit with detection limit of the assay 0.09 pg/ml (Biosource Europe, Nivelles, Belgium). IL-6 was measured by ELISA using a relevant kit with detection limit of the assay 0.1 pg/ml (Ultra sensitive Biocource, Europe, Nivelles, Belgium). pH was measured, as previously described, using a pHmeter (Consort P-903).[20]

Study protocol

On day 1, all subjects underwent a medical history and medical examination by an experienced pneumonologist, spirometry for measuring FEV_1, FEV_1/FVC before and after bronchodilation, biochemical blood tests for electrolytes, lipid profile, renal and liver function and blood gases for $PaCO_2$ assessment. Patients eligible for the study underwent one night diagnostic polysomnography. In eligible subjects, BMI, neck circumference, hip to waist ratio and Epworth Sleepiness Scale were also assessed. EBC collection and venous blood samples for leptin measurement were obtained at 6 am immediately after the end of the overnight diagnostic polysomnography procedure. Smoking was not permitted for the whole night until time of samples drawing.

Statistical analysis

Data is presented as mean $\pm$ SD. Statistical comparisons between two groups were estimated with unpaired *t*-test except from non-normally distributed values (mean SaO_2 %, $TSaO_2 < 90$ % [min]), where Mann-Whitney test was used. To assess the independent association between leptin and inflammatory mediators, a linear regression model was used. Leptin was considered to be the dependent variable after adjusting for BMI, age, neck circumference, hip to waist ratio and smoking habit and inflammatory variables were the independent ones. To further explore the relationship between leptin, inflammatory mediators and parameters of disease severity, a multivariate linear regression model was used with similar adjustments for leptin and additional adjustments for inflammatory variables for smoking, age and BMI. In this model, dependent variables were leptin and inflammatory variables and independent parameters of disease severity. Data for regression analysis is presented with R^2 for the whole model and standardized β coefficient. $p < 0.05$ was considered significant. SPSS 12.0 (Chicago IL, USA) was used for analysis.

Results

Flow chart for subjects' recruitment is presented in Fig. 1. Subjects' characteristics are summarized in Tables 1 (all) and 2 (smokers and non-smokers).

Variables of interest

Leptin

Leptin values are presented in Fig. 2. Leptin levels were significantly higher in OSA patients compared to normal subjects (24 ± 16 ng/ml vs. 8 ± 3 ng/ml, $p < 0.0001$). Smoking OSA patients did not significantly differ from non-smoking ones (25 ± 15 ng/ml vs. 23 ± 17 ng/ml, $p = 0.8$). Both smoking and non-smoking OSA patients had significantly higher levels compared to groups of normal subjects.

EBC variables

8-Isoprostane, TNF-α, IL-6 and pH values are presented in Fig. 3A, B, C, and D, respectively. 8-Isoprostane levels in EBC were significantly higher in OSA patients compared to normal subjects (30.5 ± 19 pg/ml vs. 12 ± 3 pg/ml, $p < 0.0001$). Smoking OSA patients did not significantly differ from non-smoking ones (30 ± 19 pg/ml vs. 31 ± 19 pg/ml, $p = 0.7$). TNF-α levels in EBC were significantly higher in OSA patients compared to normal subjects (1.4 ± 0.9 pg/ml vs. 0.64 ± 0.3 pg/ml, $p = 0.0002$). Smoking OSA patients did not significantly differ from non-smoking ones

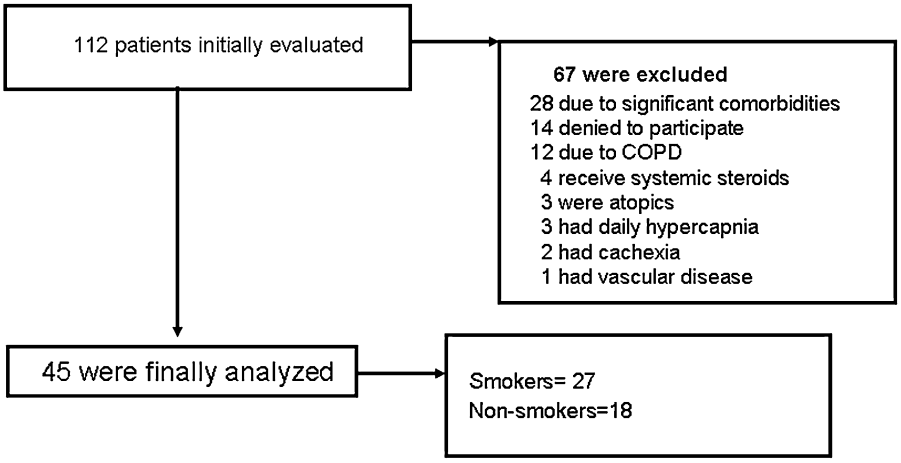

Figure 1 Flow chart for subjects' recruitment.

Table 1 Subjects' characteristics

Variables	Normals $n = 25$	OSA patients $n = 45$	p Value
Age, yr	51 ± 7	52 ± 12	0.67
Smoking (pack-years)	44 ± 21	47 ± 34	0.82
BMI (kg/m^2)	31 ± 3	33.5 ± 7	0.06
ESS	0.6 ± 0.9 (0–3)	8 ± 4 (2–19)	NA
TST (min)	272 ± 39	282 ± 53	0.9
AHI	<5	39 ± 25	<0.0001
Mean duration of AH in sec min sec	NA	40 ± 7	NA
Minimum SaO_2%	91 ± 3	78 ± 12	<0.0001
Mean SaO_2%	95 ± 3	88 ± 7	<0.0001
ODI	1.4 ± 0.8	41 ± 28	<0.0001
$TSaO_2$ <90 %(min)	1.7 ± 0.6	48 ± 67	<0.0001
FEV_1% pred	99 ± 10	107 ± 25	0.12

Data is presented as mean ± SD except that of ESS where ranges are also provided.
Abbreviations: OSA: Obstructive Sleep Apnoea; BMI: Body Mass Index; ESS: Epworth Sleepiness Scale; AHI: Apnea/Hypopnea index; SaO_2: Oxygen saturation; ODI: Oxygen Desaturation Index; $TSaO_2$ <90 (min): Total time in minutes during which SaO_2 was lower than 90%; TST: Total Sleep Time in minutes; FEV_1: Forced Expiratory Volume in 1 s; NA: Not Applicable.

(1.4 ± 1 pg/ml vs. 1.5 ± 0.9 pg/ml, $p = 0.7$). IL-6 levels in EBC were significantly higher in OSA patients compared to normal subjects (0.53 ± 0.3 pg/ml vs. 0.21 ± 0.2 pg/ml, $p = 0.03$). Smoking OSA patients did not significantly differ from non-smoking ones (0.54 ± 0.3 pg/ml vs. 0.48 ± 0.3 pg/ml, $p = 0.4$). pH levels in EBC were significantly lower in OSA patients compared to normal subjects (7.44 ± 0.2 vs. 7.64 ± 0.1, $p = 0.0009$). pH levels in EBC did not significantly differ between OSA smokers and non-smokers (7.43 ± 0.2 vs. 7.45 ± 0.2, $p = 0.7$). Both smoking and non-smoking OSA patients had significantly lower levels compared to the related groups of normal subjects.

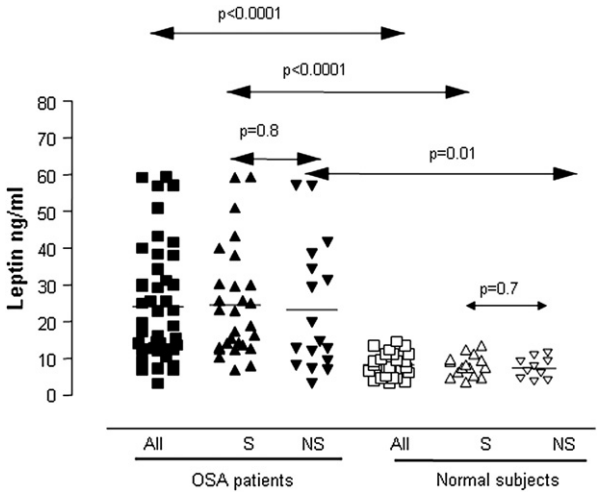

Figure 2 Plasma leptin concentration in control subjects [$n = 25$□, smokers $n = 15$△, non-smokers $n = 10$▽] and patients with Obstructive Sleep Apnoea (OSA) [$n = 57$■, smokers $n = 28$▲, non-smokers $n = 17$▼]. Each symbol represents one individual. Horizontal bars represent mean values. See text for details.

Linear regression data

Linear regression data between leptin (dependent variable) after adjustment for age, BMI, neck circumference, hip to

Table 2 Subjects' characteristics after subdivided according to smoking habit

Variables	OSA		Normals		p Value*		p Value**	
	Smokers $n = 28$ 5F–23M	Non-smokers $n = 17$ 3F–14M	Smokers $n = 15$ 4F–11M	Non-smokers $n = 10$ 3F–7M			#	^
Age, yr	51 ± 10	54 ± 14	52 ± 7	50 ± 7	0.5	0.5	0.8	0.43
Smoking (pack-years)	47 ± 34	0	44 ± 21	0	NA	NA	0.7	NA
BMI (kg/m^2)	35 ± 7	32 ± 6	32 ± 3	29 ± 3	0.13	0.07	0.1	0.3
ESS	8 ± 5 (2–19)	8 ± 4 (3–16)	0.7 ± 0.9 (0–3)	0.5 ± 0.8 (0–3)	0.9	0.9	NA	NA
TST (min)	290 ± 46	271 ± 62	289 ± 86	271 ± 62	0.3	0.6	0.8	
AHI	44 ± 28	31 ± 19	<5	<5	0.09	NA	**<0.0001**	**<0.0001**
Mean duration of AH in seconds	37 ± 6	38 ± 7	NA	NA	0.28	NA	NA	NA
Minimum SaO_2%	77 ± 14	80 ± 8	92 ± 2	91 ± 3	0.4	0.7	**<0.0001**	**<0.0001**
Mean SaO_2%	87 ± 8	90 ± 5	95 ± 3	96 ± 4	0.2	0.8	**<0.0001**	**<0.0001**
ODI	44 ± 30	35 ± 24	1.4 ± 0.7	1.3 ± 0.8	0.4	0.7	**<0.0001**	**<0.0001**
$TSaO_2$ <90% (min)	58 ± 77	33 ± 44	1.8 ± 0.7	1.7 ± 0.6	0.2	0.7	**<0.0001**	**<0.0001**
FEV_1% pred	104 ± 26	112 ± 21	97 ± 8	101 ± 13	0.3	0.38	0.3	0.2

Data is presented as mean ± SD except that of ESS where ranges are also provided.
Bold letters indicate statistical significant difference.
* within each group ** between OSA and normals [#smokers and ^non-smokers, respectively].
Abbreviations: F: Female; M: Male; OSA: Obstructive Sleep Apnoea; BMI: Body Mass Index; ESS: Epworth Sleepiness Scale; AHI: Apnea/Hypopnea Index; SaO_2: Oxygen saturation; ODI: Oxygen Desaturation Index; $TSaO_2$ <90 (min): Total time in minutes during which SaO_2 was lower than 90%; TST: Total Sleep Time in minutes; FEV_1: Forced Expiratory Volume in 1 s; NA: Not Applicable.

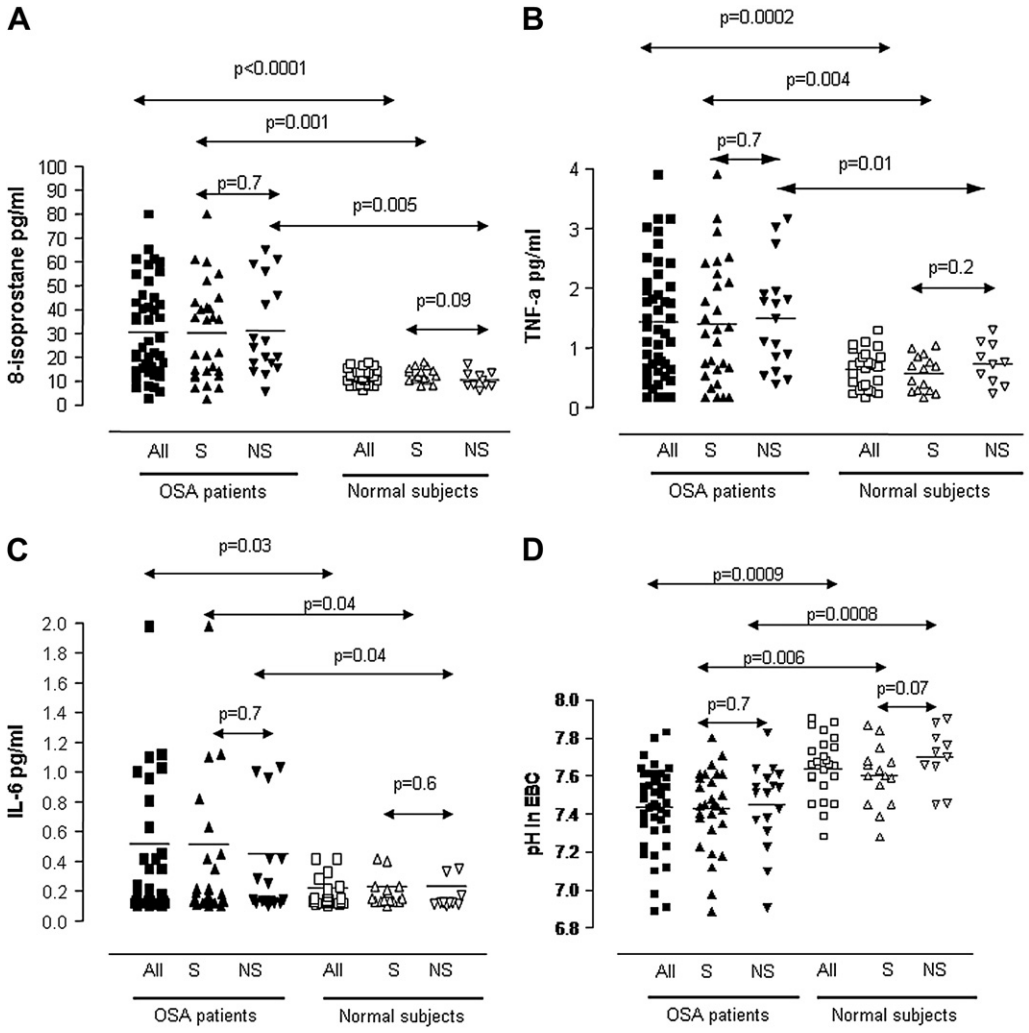

Figure 3 A, B, C, D: 8-Isoprostane, TNF-α, IL-6 and pH values, respectively in Exhaled Breath Condensate (EBC) in control subjects [$n = 25$□, smokers $n = 15$△, non-smokers $n = 10$▽] and patients with Obstructive Sleep Apnoea (OSA) [$n = 57$■, smokers $n = 28$▲, non-smokers $n = 17$▼]. Each symbol represents one individual. Horizontal bars represent mean values. See text for details. Detection limits for 8-isoprostane, TNF-α and IL-6 are 5 pg/ml, 0.09 pg/ml and 0.1 pg/ml, respectively.

waist ratio, smoking (pack-years) and parameters of inflammation in EBC (independent variables) did not show any significant association (standardized coefficient were -0.2, 0.08, 0.15, -0.24 with p value 0.14, 0.6, 0.3, 0.1 for IL-6, pH, 8-isoprostane and TNF-α, respectively). Adjusted R^2 for the whole model was 0.06. Similar results were also observed for normal subjects (data for normals is not shown).

Linear regression data between leptin (dependent variable) after adjustment for age, BMI, neck circumference, hip to waist ratio and smoking (pack-years), inflammatory parameters of EBC (dependent variables) adjusted for age, smoking habit and BMI and variables expressing disease severity (independent variables) revealed the following significant findings: pH was significantly associated with mean AH duration in seconds and ODI (adjusted R^2 -0.2 for the whole model, β coefficient 0.17, $p = 0.02$ and β coefficient 0.22, $p = 0.03$, respectively). 8-Isoprostane was significantly associated with AH duration (adjusted R^2 0.25 for the whole model, β coefficient 0.3, $p = 0.03$). For the rest non-mentioned data, all plausible associations either in OSA patients or normal subjects revealed no significance.

Discussion

The important and novel finding of this study is the absence of significant relationship between the increased levels of leptin and increased airway inflammation in OSA. Moreover, a parameter measurable in the field, pH, seems to predict limited but interesting part of disease severity.

Our study confirms previous ones regarding the presence of hyperleptinemia and local airway inflammation in OSA.[2,8,10] The different values for EBC mediators compared to previous studies might be attributed to the larger numbers of patients selected in our study compared to previous ones, as well as to different standardization procedures and assays which mainly affect measurements in different laboratories.

The critical step of studies involving OSA patients and investigating the role of hormones of adipose tissue and the underlying systemic and airway inflammation, is to select a homogenous study population without significant comorbidities in order to eliminate any plausible bias on the evaluation process. Another critical issue for leptin evaluation is the proper adjustments for variables which might affect its values.[8,9,21,22,23,24]

In our study, we tried to eliminate all the above dependent variables by excluding patients with hypercapnia, draw blood samples in a specific time and adjust leptin in regression analysis for all the remaining confounding factors. After this detailed protocol and the proper adjustments, we did not find any association between leptin and airway variables in EBC. The recently published recommendations of EBC consider a potential effect of systemic inflammation on EBC mediators as important, but with no study to be existed in order to confirm the above theory.[12] Previous data showed that leptin stimulates airway inflammatory cells' chemotaxis and enhances their functional capacity by leading to oxidative burst and cytokine secretion.[25,26] Based on this data, we expected to find a good association between hyperleptinemia and airway inflammatory process either in the direction of oxidative stress (8-isoprostane) or cytokine secretion (TNF-α). However, our study failed to show a close relation between systemically evaluated leptin and airway inflammation. This observation might mean that EBC is not influenced by the leptin induced systemic inflammation but simultaneously might represent some study limitations mainly attributing to the mediators studied and the absence of any data relating to leptin airway expression. Regarding the first limitation, we strongly believe that airway inflammation was evaluated by three important components which include both oxidative stress and cytokine secretion. As for the second one, this interesting theory needs further studies in order to be supported, as no relevant data exists so far.

By subdividing patients according to smoking habits, we present some new data in this direction since all studies dealing with EBC and OSA did not take smoking into account.[2,27,28] Smoking is considered as one of the risk factors for OSA with recent data to support a synergistic effect between cigarette smoking and OSA on some of the biochemical cardiovascular risk markers.[29,30] The adjustment of the EBC variables in relation to smoking eliminates any plausible bias in the statistical procedure in this direction. Surprisingly, and in contrast to already known data,[12] smoking seems not to influence any of the airway parameters studied. This might be attributed to the time of EBC collection [at 6 am immediately after an overnight polysomnography] in combination with the fact that all smoking subjects were free from smoking at least 8–10 h before the time that samples were drawn. Alternatively by the fact that EBC collection technique (nasal clip) might eliminate the consequence of smoking related nasal mucosal inflammation[12] and the plausible explanation that the overnight mechanical model of ''resistive breathing''[31] which leads to generation of oxidative stress products as well as in local induction of cytokines, overcomes any effect of smoking in this process.

Another interesting finding of this study is the introduction of a new parameter in disease assessment, the pH. EBC pH is easy to be performed, measurable in the field, reproducible, it has established normal values and may be measured with portable equipment.[12,32] The associations of pH – after taking proper adjustments into consideration – with mean duration of AH and ODI might be a coincidence. Alternatively, if pH is considered, as a plausible parameter of oxidative stress, it might be related either with the model of resistive breathing or with that of hypoxia induced-inflammation. The absence of any other significant associations is not surprising, since in such a detailed mechanical model it is not easy for a parameter to express the whole underlying severity variables. The significant association between 8-isoprostane and AH duration partially confirms previous findings and the already reported association between oxidative stress and resistive breathing.[31] Finally, we do not believe that the absence of significantly daytime sleepiness as assessed by ESS might influence the above results since its not always related with the underlying severity of the disease.[17]

In conclusion, our study confirms previous ones regarding the presence of hyperleptinemia and local airway inflammation in OSA. The new findings of the study are the absence of any relationship between the above, the absence of any influence of smoking habit and the introduction of a new variable, pH, which might express the mechanical model of hypoxia and resistive breathing.

Conflict of interest statement

None of the authors have any conflicts to disclose.

References

1. Hatipoğlu U, Rubinstein I. Inflammation and obstructive sleep apnea syndrome. How many ways do I look at thee? *Chest* 2004;**126**:1–2.
2. Carpagnano GE, Kharitonov SA, Resta O, Foschino-Barbaro MP, Gramiccioni E, Barnes PJ. 8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy. *Chest* 2003;**124**: 1386–92.
3. Shamsuzzaman AS, Winnicki M, Lanfranchi P, et al. Elevated C-reactive protein in patients with obstructive sleep apnea. *Circulation* 2002;**105**:2462–4.
4. Minoguchi K, Yokoe T, Tazaki T, et al. Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea. *Am J Respir Crit Care Med* 2005;**172**:625–30.
5. Schulz R, Mahmoudi S, Hattar K, et al. Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Impact of continuous positive airway pressure therapy. *Am J Respir Crit Care Med* 2000;**162**:566–70.
6. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab* 1997;**82**:1313–6.
7. Otero M, Lago R, Gomez R, et al. Towards a proinflammatory and immunomodulatory emerging role of leptin. *Rheumatology (Oxford)* 2006;**45**(8):944–50.
8. Marik P. Leptin, obesity and obstructive sleep apnea. *Chest* 2000;**118**:569–71.
9. Barcelo A, Barbe F, Llompart E, et al. Neuropeptide Y and Leptin in patients with obstructive sleep apnoea syndrome: role of obesity. *Am J Respir Crit Care Med* 2005;**171**:183–7.

10. Phillips BG, Kato M, Narkiewicz K, Choe I, Somers VK. Increases in leptin levels, sympathetic drive and weight gain in obstructive sleep apnea. *Am J Physiol Heart Circ Physiol* 2000;**279**:H234—7.
11. Tatsumi K, Kasahara Y, Kurosu K, Tanabe N, Takiguchi Y, Kuriyama T. Sleep oxygen desaturation and circulating leptin in obstructive sleep apnea-hypopnea syndrome. *Chest* 2005; **127**:716—21.
12. Horváth I, Hunt J, Barnes PJ. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005;**26**:523—48.
13. Kharitonov SA. Exhaled markers of inflammatory lung diseases: ready for routine monitoring? *Swiss Med Wkly* 2004;**134**:175—92.
14. Rysz J, Kasielski M, Apanasiewicz J, et al. Increased hydrogen peroxide in the exhaled breath of uraemic patients unaffected by haemodialysis. *Nephrol Dial Transplant* 2004;**19**:158—63.
15. Global Initiative for Chronic Obstructive Lung Disease. Global Strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. NHLBI/WHO workshop report. Bethesda, National Heart, Lung and Blood Institute, April 2001; update of the Management Sections, GOLD website (www.goldcopd.com). Date updated: July 2006.
16. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991;**14**:540—5.
17. Malhotra A, White DP. Obstructive sleep apnoea. *Lancet* 2002; **360**:237—45.
18. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;**152**:1107—36.
19. Psathakis K, Papatheodorou G, Plataki M, et al. 8-Isoprostane, a marker of oxidative stress, is increased in the expired breath condensate of patients with pulmonary sarcoidosis. *Chest* 2004;**125**:1005—11.
20. Kostikas K, Papatheodorou G, Ganas K, Psathakis K, Panagou P, Loukides S. pH in expired breath condensate of patients with inflammatory airway diseases. *Am J Respir Crit Care Med* 2002;**165**:1364—70.
21. Reseland JE, Mundal HH, Hollung K, et al. Cigarette smoking may reduce plasma leptin concentration via catecholamines. *Prostaglandins Leukot Essent Fatty Acids* 2005;**73**(1):43—9.
22. Nicklas BJ, Tomoyasu N, Muir J, Goldberg AP. Effects of cigarette smoking and its cessation on body weight and plasma leptin levels. *Metabolism* 1999;**48**:804—8.
23. Shimura R, Tatsumi K, Nakamura A, et al. Fat accumulation, leptin, and hypercapnia in obstructive sleep apnea-hypopnea syndrome. *Chest* 2005;**127**:543—9.
24. Patel SR, Palmer LJ, Larkin EK, Jenny NS, White DP, Redline S. Relationship between obstructive sleep apnea and diurnal leptin rhythms. *Sleep* 2004;**27**:235—9.
25. Galdefie-Chezet F, Poulin A, Vasson MP. Leptin regulates functional capacities of polymorphonuclear neutrophils. *Free Radic Res* 2003;**37**:809—14.
26. Juge-Aubry CE, Henrichot E, Meier CA. Adipose tissue: a regulator of inflammation. *Best Pract Res Clin Endocrinol Metab* 2005;**19**:547—66.
27. Goldbart AD, Krishna J, Li RC, Serpero LD, Gozal D. Inflammatory mediators in exhaled breath condensate of children with obstructive sleep apnoea syndrome. *Chest* 2006;**130**:143—8.
28. Carpagnano GE, Kharitonov SA, Resta O, Foschino-Barbaro MP, Gramiccioni E, Barnes PJ. Increased 8-isoprostane and interleukin-6 in breath condensate of obstructive sleep apnea patients. *Chest* 2002;**122**:1162—7.
29. Wetter DW, Young TB, Bidwell TR, Badr MS, Palta M. Smoking as a risk factor for sleep disordered breathing. *Arch Intern Med* 1994;**154**:2219—24.
30. Lavie L, Lavie P. Smoking interacts with sleep apnea to increase cardiovascular risk. *Sleep Med* 2008;**9**:247—53.
31. Vassilakopoulos T, Roussos C, Zakynthinos S. The immune response to resistive breathing. *Eur Respir J* 2004;**24**:1033—43.
32. Paget-Brown AO, Ngamtrakulpanit L, Smith A, et al. Normative data for pH of exhaled breath condensate. *Chest* 2006;**129**: 426—30.