Sleep and Breathing (2019) 23:473–481
https://doi.org/10.1007/s11325-018-1703-x

SLEEP BREATHING PHYSIOLOGY AND DISORDERS • ORIGINAL ARTICLE

Catestatin serum levels are increased in male patients with obstructive sleep apnea

Josip A. Borovac[1] · Zoran Dogas[2,3] · Daniela Supe-Domic[4] · Tea Galic[2,5] · Josko Bozic[1,2]

Received: 25 April 2018 / Revised: 3 July 2018 / Accepted: 30 July 2018 / Published online: 7 August 2018

Abstract

Purpose Obstructive sleep apnea (OSA) is a complex sleep disorder associated with autonomic and sympathetic dysregulation. To the contrary, catestatin, an endogenous pleiotropic peptide cleaved from chromogranin A, is known for its inhibitory effects on catecholamine release and sympathetic activity. The aims of the study were to determine catestatin serum levels among male OSA patients compared to healthy control subjects and to explore associations of catestatin with anthropometric, polysomnographic, and lipid profile parameters.

Methods Seventy-eight male OSA patients aged 50.3 ± 8.8 years and 51 age/sex/BMI-matched control subjects aged 50.4 ± 7.8 years were enrolled in the study. Catestatin serum levels were determined by an enzyme-linked immunosorbent assay (ELISA).

Results Catestatin serum levels were significantly higher among OSA patients compared to control subjects (2.9 ± 1.2 vs. 1.5 ± 1.1 ng/mL, $p < 0.001$). Serum catestatin levels significantly correlated with apnea-hypopnea index (AHI) among non-obese OSA subjects ($r = 0.466$, $p = 0.016$; $\beta = 0.448$, $p = 0.026$), while in whole OSA population, catestatin levels significantly correlated with neck circumference ($r = 0.318$, $p < 0.001$; $\beta = 0.384$, $p < 0.001$) and high-density lipoprotein (HDL) cholesterol ($r = -0.320$, $p < 0.001$; $\beta = -0.344$, $p < 0.001$). In multivariate-adjusted regression model, serum catestatin was significant and independent predictor of OSA status (OR 4.98, 95% CI 2.17–11.47, $p < 0.001$).

Conclusions Catestatin serum levels are significantly increased in male OSA population and positively correlate with disease severity in non-obese patients. OSA status is independently predicted by catestatin levels; however, this finding is restricted to patients with moderate-to-severe disease. Further studies are necessary to elucidate the mechanistic role of catestatin in the complex pathophysiology of OSA.

Keywords Catestatin · Cholesterol · HDL · Chromogranin A · Polysomnography · Sleep apnea · Obstructive

Introduction

Obstructive sleep apnea (OSA) is a common sleep disorder marked by partial or complete obstruction of the upper airway causing detrimental physiological effects that are propagated by intermittent hypoxia and hypercapnia. An airway passage collapse thereby leads to recurrent arousals, sleep fragmentation and increased respiratory efforts during the night, overstimulation of sympathetic activation, and finally, oxidative stress and systemic inflammation causing multiple end-organ morbidities and excessive daytime sleepiness [1]. The general impact of OSA on physiological homeostasis is best reflected in the fact that it has been independently associated with cardiovascular comorbidities such as arterial hypertension, coronary artery disease, atrial fibrillation, stroke, and increased arterial stiffness [2, 3]. In terms of harmful metabolic effects,

✉ Josko Bozic
josko.bozic@mefst.hr

1 Department of Pathophysiology, University of Split School of Medicine, Soltanska 2, 21000 Split, Croatia

2 Department of Neuroscience, University of Split School of Medicine, Soltanska 2, 21000 Split, Croatia

3 Sleep Medicine Center, University of Split School of Medicine, Soltanska 2, 21000 Split and University Hospital of Split, Spinciceva 1, 21000 Split, Croatia

4 Department of Medical Laboratory Diagnostics, University Hospital of Split, Spinciceva 1, 21000 Split, Croatia

5 Study of Dental Medicine, University of Split School of Medicine, Soltanska 2, 21000 Split, Croatia

OSA has an independent role in insulin resistance, diabetes mellitus, dysregulation of cortisol secretion, and dyslipidemia, and affects even cognitive and psychomotor functions in humans [4–8].

Catestatin, on the other hand, is an endogenous functional protein in humans that has pleiotropic roles in regulation of various organ systems and is proteolytically cleaved from chromogranin A (ChgA) that acts as a precursor to several functional peptides including vasostatin-1, vasostatin-2, pancreastatin, beta-granin, prochromacin, proteins WE-14 and GE-25, serpinin, parastatin, and, finally, catestatin [9]. In 1997, Mahata et al. observed that catestatin exerted inhibitory effects on catecholamine release from adrenal chromaffin cells and adrenergic neurons through noncompetitive reversible antagonism of nicotinic acetylcholine receptors (nAchR) [10]. Even more, catestatin is secreted throughout the human body and is stored in secretory vesicles of nerve endings, endocrine tissues, cardiac muscle, skin, and sensory organs [11]. Recently, roles of catestatin in lipid metabolism and macrophage-driven atherosclerosis have been described [12–14]. The study of O'Connor et al. showed that catestatin plasma levels negatively correlated with body mass index (BMI) and plasma leptin concentrations in the population of normotensive and hypertensive humans at genetic risk of hypertension [15].

Finally, it has been shown that OSA and its hallmark mechanisms of intermittent hypoxia and sleep fragmentation are related to lipid metabolism dysregulation and cardiometabolic disease [16–19]. Due to previously implicated roles of catestatin in these interactions, we aimed to investigate catestatin serum levels in the adult male population of OSA patients. The role of catestatin in this population is currently unknown and has not been previously elaborated in the literature, except in children with OSA [20]. Therefore, a primary objective of the study was to determine catestatin serum levels in OSA patients and compare them to those in healthy control subjects. The secondary objectives of the study were to investigate potential associations of catestatin serum levels with anthropometric measurements, lipid metabolism parameters, and polysomnographic (PSG) indices of sleep in OSA patients.

Materials and methods

The Ethics Committee of the University of Split School of Medicine approved the study. All procedures performed in this study were in accordance with the ethical standards of the institutional Ethics Committee and with the 1964 Helsinki declaration and its revision from 2008. An informed written consent was obtained from all participants included in the study.

Subjects

In this cross-sectional clinical study, out of 120 screened subjects, 78 male subjects with a de novo diagnosis of obstructive sleep apnea (OSA) were consecutively enrolled at our Sleep Medicine Center, between December 2016 and February 2018. Relevant clinical practice guidelines by American Academy of Sleep Medicine (AASM) and European Sleep Research Society (ESRS) were followed in terms of diagnostic testing for OSA in adults [21, 22]. According to established criteria, male patients with apnea-hypopnea index (AHI) ≥ 15 during the full-night polysomnography, ≥ 18 and < 65 years of age, were included in the study. Patients that had a verified presence and documented medical history of following conditions were excluded from the study: (a) diabetes mellitus (both type I and II), significant cardiovascular, renal, respiratory, neurological and psychiatric disease, systemic inflammatory and/or autoimmune disease, acute or chronic immunocompromising state, or active malignant disease; (b) patients previously treated for OSA; (c) patients taking lipid-lowering drugs, and (d) female patients. No significant medication intake was recorded in the total sample of subjects included in this study.

Healthy male volunteers participated as matching controls in our sample, and in total, 60 subjects were screened of which 51 subjects were prospectively included in the study after fulfilling designated inclusion and exclusion criteria. The control group was matched for age and body mass index (BMI) to OSA group in order to minimize confounding. Due to the high prevalence of OSA in the general population and to ensure that control subjects have a low risk for OSA development prior to study inclusion, all healthy volunteers were screened for OSA risk with the *snoring*, *tiredness*, *observed apnea*, *high blood pressure-body mass index*, *age*, *neck circumference*, and *gender* (STOP-BANG) questionnaire and subjects that scored ≥ 3 points on this questionnaire were excluded from the study. Likewise, STOP-BANG questionnaire was administered to OSA patients to ensure that this population indeed has a high pre-test probability of OSA. The sensitivity of STOP-BANG questionnaire and obtained score of ≥ 3 points to detect moderate or severe OSA is 93 and 100%, respectively [23]. Furthermore, subjects that had an Epworth Sleepiness Scale (ESS) score > 9 were not enrolled in the control group, and for that purpose, we used ESS version validated in Croatian language [24]. The PSG sleep assessment was not performed among control subjects due to the obtained low pretest probability for OSA. Exclusion criteria and assessment protocols were identical for both studied groups.

All subjects (total $N = 129$) included in the study underwent detailed medical interview, physical examination, and baseline anthropometric measuring. For the body weight (kg) and height (cm) measurements, calibrated scale was used (Seca, Birmingham, UK), while BMI was calculated by the

following formula: body weight (kg) divided by height-squared (m^2). Neck circumference (cm) was measured midway of the neck, between the mid-cervical spine and mid-anterior neck, in the standing upright position. While the subjects were standing, waist circumference (cm) was measured at the mid-point between the inferior tip of the ribcage and the superior aspect of the iliac crest, while hip circumference (cm) was measured at the point yielding the maximum circumference over the buttocks using a tape measure. Waist-to-hip ratio (WHR) was calculated as waist (W) divided by hip (H) measurement.

Sleep assessment

All OSA patients underwent full-night attended PSG at the Sleep Medicine Center during which following measurements were continuously recorded: electrooculography, electroencephalography, mental and tibial electromyography, electrocardiography, nasal airflow, pulse oximetry, thoracic and abdominal movements, and snoring intensity (Alice 5LE, Philips Respironics, Eindhoven, the Netherlands). All data were stored on a computer system and manually scored in accordance with the published ESRS guidelines [25].

According to the established guidelines, apnea was defined as a complete cessation of airflow for at least 10 s, while in hypopnea airflow is decreased by more than 50% for at least 10 s, in combination with a reduction in hemoglobin oxygen saturation of at least 3% [26]. Full-night PSG measurements that lasted less than 6 h were not accepted, and in such cases, another sleep study was undertaken.

Blood sampling and laboratory analysis

Peripheral blood samples were drawn from each subject 7 to 14 days after sleep studies were performed. No treatment for OSA was administered to any subject during this time interval. Venous blood samples were obtained from antecubital vein in each subject after fasting for 12 h. All blood samples were analyzed in accordance with good standards of laboratory practice in the same biochemical laboratory and by the same specialist in medical biochemistry that was blinded to subject's assignment in the study groups. Lipid panel consisting of triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and total cholesterol were measured by routine laboratory methods. Non-HDL cholesterol fraction was calculated by the formula = [total cholesterol − HDL-c].

Catestatin (Cat. no. EK-053-27CE, EIA kit, Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) levels in serum were determined by an enzyme-linked immunosorbent assay (ELISA) with reported sensitivity for catestatin of 0.05 ng/mL with linear range of 0.05–0.92 ng/mL. Reported cross-reactivity with endogenous human catestatin peptide for the used assay kit was 100% with *intra-assay* coefficient of variability (CV) < 10% and *inter-assay* CV < 15%.

Statistical analysis

Statistical analysis of the acquired data was performed using SPSS Statistics for Windows® (version 25.0, IBM, Armonk, NY, USA) and Prism 6 for Windows® (version 6.01, GraphPad, La Jolla, CA, USA). Normality of distribution of continuous variables was assessed with the Kolmogorov-Smirnov test, and data were shown as mean (M) ± standard deviation (SD). Categorical variables were presented as whole numbers (N) and percentages (%). For the measurement of differences between the two studied groups, an independent samples *t* test and chi-squared (χ^2) test were used for continuous and categorical variables, respectively. Pearson's correlation analysis was used to determine an association of independent variables with serum catestatin levels with correlation coefficient (*r*) reported. A multiple linear regression with forward algorithm was performed to determine significant and independent predictors of catestatin serum levels with age, BMI, and anthropometric indices included as covariates in the regression model. For this analysis, standardized beta (β) values and *p* values were reported. Finally, a multivariate logistic regression analysis was performed to determine associations of serum catestatin and baseline lipid profile parameters for positive OSA status. The multivariate regression model was adjusted for age, BMI, and neck/waist/hip circumference. Multivariate-adjusted odds ratio (OR), 95% confidence intervals (95% CI), and significance level (*p*) were reported for the regression analysis. The statistical significance reported in all instances was two-tailed, set at $p < 0.05$ level.

Results

Baseline population characteristics

Control group and OSA group did not significantly differ in age, body height, body weight, and BMI. Anthropometric parameters including neck/waist/hip circumference and WHR were significantly higher in OSA group compared to controls. In all items that measured daily activities, no significant differences between two groups were observed (Table 1).

OSA patients had significantly higher triglycerides and non-HDL cholesterol fraction compared to control subjects (2.0 ± 0.8 vs. 1.5 ± 0.6 mmol/L, $p < 0.001$ and 5.0 ± 1.0 vs. 4.6 ± 1.1 mmol/L, $p = 0.048$, respectively). Furthermore, HDL-c fraction was significantly lower in OSA patients in comparison to control subjects, 1.2 ± 0.3 vs. 1.4 ± 0.4 mmol/L, $p < 0.001$, respectively. There were no significant differences observed between the two groups in respect to total cholesterol and LDL-c levels in peripheral blood (Table 1).

Table 1 Baseline characteristics of study population

	Control group ($N = 51$)	OSA group ($N = 78$)	p*
Baseline anthropometric characteristics			
Age (years)	50.4 ± 7.8	50.3 ± 8.8	0.921
Height (cm)	183.1 ± 6.3	184.3 ± 6.9	0.312
Weight (kg)	98.6 ± 12.7	102.7 ± 12.1	0.082
BMI (kg/m^2)	29.1 ± 3.4	30.2 ± 3.1	0.109
Neck circumference (cm)	38.2 ± 2.6	42.2 ± 3.5	< 0.001
Waist circumference (cm)	98.8 ± 11.7	109.7 ± 14.3	< 0.001
Hip circumference (cm)	104.4 ± 7.4	110.1 ± 10.5	0.001
WHR	0.94 ± 0.05	0.99 ± 0.06	< 0.001
Daily activities			
Cups of coffee/day	1.6 ± 1.2	1.34 ± 1.0	0.2
Number of cigarettes/day	6.6 ± 12.3	6.1 ± 10.8	0.835
Smoking (N, %)	15 (29.4)	25 (32.1)	0.751
Baseline lipid profile			
Triglycerides (mmol/L)	1.5 ± 0.6	2.0 ± 0.8	< 0.001
Total cholesterol (mmol/L)	6.0 ± 0.9	6.1 ± 0.9	0.447
LDL cholesterol (mmol/L)	4.1 ± 0.8	4.2 ± 0.8	0.692
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.2 ± 0.3	< 0.001
Non-HDL cholesterol (mmol/L)	4.6 ± 1.1	5.0 ± 1.0	0.048

Data are presented as mean ± SD (standard deviation) or as stated otherwise

BMI body mass index, *c* cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *WHR* waist-to-hip ratio

*Independent samples *t* test or chi-square test, significance level (p) set at < 0.05

Catestatin levels in control subjects and OSA patients

Catestatin serum levels were significantly higher in OSA patients, compared to control subjects, with mean 2.9 ± 1.2 vs. 1.5 ± 1.1 ng/mL, $p < 0.001$, respectively (Fig. 1).

Catestatin levels and polysomnographic (PSG) parameters of sleep

The mean AHI score in OSA population was 44.7 ± 18.7 events per hour. The average values of parameters obtained during the full-night PSG along with sleep-related questionnaire scores among patients with OSA are provided in Table 2.

No significant correlations were observed between serum catestatin levels and PSG parameters of sleep when the analysis was performed in the total population of OSA patients ($N = 78$; data not shown).

When the OSA patients were stratified according to the BMI in two groups, BMI ≤ 30 kg/m^2 ($N = 30$) and BMI > 30 kg/m^2 ($N = 48$), significant correlations were obtained in OSA subgroup with BMI ≤ 30 kg/m^2 (Table 3). Of note, AHI ($r = 0.466$, $p = 0.016$) and number of central apnea events ($r = 0.482$, $p = 0.013$) positively correlated with serum catestatin levels. A significant negative correlation was obtained between serum catestatin level and mean peripheral capillary oxygen saturation (SpO_2) ($r = -0.379$, $p = 0.047$). However, when these variables were entered in the multiple linear regression model (significant at $p < 0.001$), adjusted for age and anthropometric parameters, no significant associations with serum catestatin levels were retained by any polysomnographic variable, except AHI ($\beta = 0.448$, $p = 0.026$).

In OSA patients with BMI > 30 kg/m^2, no significant correlations between catestatin serum levels and any of the PSG sleep parameters were observed (Table 3). Furthermore, no significant differences in any of lipid profile parameters and mean serum catestatin levels were observed when two OSA subgroups (BMI ≤ 30 vs. BMI > 30 kg/m^2) were compared.

Correlation of catestatin levels with anthropometric and lipid profile parameters

Regarding the anthropometric parameters, significant association with catestatin was established only for neck circumference ($r = 0.318$, $p < 0.001$) and WHR ($r = 0.203$, $p = 0.029$); however, multiple linear regression model showed that only neck circumference was the significant independent correlate of serum catestatin levels ($\beta \pm$ SE, 0.384 ± 0.37, $p < 0.001$).

Catestatin serum level showed significant and negative correlation with HDL-c fraction ($r = -0.320$, $p < 0.001$). Moreover, HDL cholesterol retained significant interaction

with catestatin in the multiple linear regression analysis adjusted for age, BMI, and anthropometric indices with $\beta \pm$ SE, -0.344 ± 0.30, $p < 0.001$. No significant correlations were observed for catestatin in respect to triglycerides, total cholesterol, LDL cholesterol, and non-HDL cholesterol fractions both in bivariate and multiple linear regression analysis (Table 4).

Independent predictors of OSA status

The multivariate logistic regression analysis adjusted for age, BMI, and neck/waist/hip circumference showed that neck circumference (OR 1.86, $p < 0.001$), catestatin (OR 4.98, $p < 0.001$), triglycerides (OR 5.22, $p = 0.010$), and HDL-c (OR 0.35, $p = 0.014$) were independent and significant predictors of positive OSA status in the regression model (Table 5).

Discussion

The main finding in our study is that catestatin serum levels were significantly higher in male OSA patients, compared to healthy age- and BMI-matched control subjects with a low pre-test probability of OSA. This is the novel finding in the context of adult OSA population and, to our current knowledge, catestatin levels in OSA were thus far reported in a single study of Kim et al., conducted among prepubertal pediatric OSA patients [20]. In the study conducted by Kim et al., catestatin plasma levels were significantly lower in children with OSA compared to control subjects. Regarding this study, it should be emphasized that leukocyte telomere length was designated as the primary outcome of interest and that the study included both male and female children with the mean age of children being 7.7 ± 1.4 years. Additionally, this study included prepubertal children of both sexes, thus making the possible effects of sex hormones on catestatin levels absent. These fundamental differences in study design and enrolled patient population might have been responsible for the opposite results in terms of catestatin levels in OSA. It is also possible that catestatin has inherent biological dynamics in terms of secretion and systemic circulation dependent on age and maturation and perhaps low levels of catestatin in pediatric OSA population might reflect the low reserve of endogenous catestatin in these patients at that particular age. Finally, based on the discussed relevant differences, it should become obvious that these two studies should not be mutually compared when interpreting the role of catestatin in OSA.

Since OSA, among other dysfunctions, is marked by excessive sympathetic nervous activity and systemic vasoconstriction, it could be inferred that increased catestatin levels in OSA patients might reflect the degree of negative-loop feedback aimed to counteract chronic exposure to *pro-adrenergic* systemic effects that are sustained in OSA [27]. Since catestatin is known for its vasodilating and antihypertensive properties and antagonization of adrenergic activation through blockade of catecholamine secretion, it is plausible to suggest that increased circulating levels of catestatin in patients with OSA might be the indirect marker of the increased sympathetic and catecholamine activity [10, 28, 29]. Indeed, Schillaci et al. emphasized that catestatin might serve as an "important endogenous brake on the sympathetic nervous system" [30]. In parallel to this, another condition known for the sustained overactivation of sympathetic nervous system and increased

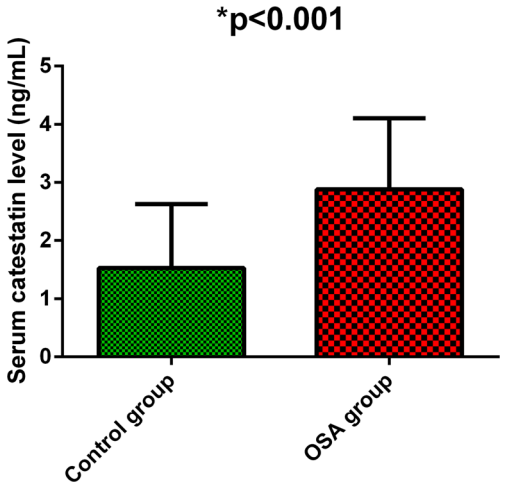

Fig. 1 Mean catestatin serum levels in control group ($N = 51$) and OSA group ($N = 78$). Data are presented as mean ± SD. *Tested with independent samples *t* test

Table 2 Polysomnographic parameters and average sleep-related questionnaire scores in population of patients with OSA ($N = 78$)

Parameter	Value
AHI (events/h)	44.7 ± 18.7
ODI (events/h)	41.9 ± 19.1
Mean SpO_2 (%)	92.9 ± 3.3
Minimum SpO_2 (%)	74.0 ± 11.3
Total snoring time (min)	99.2 ± 109.1
Total sleep time (h)	6.5 ± 1.1
Obstructive apnea[a]	136.8 ± 121.3
Central apnea[a]	35.6 ± 51.3
Hypopnea[a]	113.5 ± 76.9
Heart rate index[b]	63.1 ± 11.1
STOP score	3.0 ± 0.8
STOP-BANG score	5.4 ± 1.1
ESS score	8.6 ± 4.4

Data are presented as mean ± SD (standard deviation)

AHI apnea-hypopnea index; *ESS* Epworth Sleepiness Scale; *ODI* oxygen desaturation index; *SpO_2* arterial oxygen saturation; *STOP BANG* snoring, tired, observed, blood pressure, BMI, age, neck circumference, gender questionnaire

[a] Number of events per total sleep time

[b] Average heart rate measured during the sleep session

Table 3 A Pearson's correlation analysis of serum catestatin levels with polysomnographic (PSG) sleep parameters in OSA patients, stratified by BMI ≤ 30 ($N = 30$) and > 30 kg/m^2 ($N = 48$)

Parameter	Serum catestatin level (ng/mL)	
	r-value[b]	*p* value
OSA patients with BMI ≤ 30 kg/m^2 ($N = 30$)		
AHI (events/h)	0.466	0.016
ODI (events/h)	0.272	0.179
Mean SpO_2 (%)	− 0.379	0.047
Obstructive apnea events[a]	0.366	0.066
Central apnea events[a]	0.482	0.013
Hypopnea events[a]	− 0.256	0.208
OSA patients with BMI > 30 kg/m^2 ($N = 48$)		
AHI (events/h)	0.082	0.573
ODI (events/h)	0.076	0.601
Mean SpO_2 (%)	0.128	0.376
Obstructive apnea events[a]	0.132	0.360
Central apnea events[a]	0.064	0.657
Hypopnea events[a]	0.031	0.829

AHI apnea-hypopnea index, *ODI* oxygen desaturation index, *SpO_2* arterial oxygen saturation

[a] Number of events per total sleep time

[b] *r*-value denotes Pearson's correlation coefficient

Table 4 A Pearson's correlation analysis of serum catestatin levels with baseline lipid profile and anthropometric parameters in total population of patients with OSA ($N = 78$)

Parameter	Serum catestatin level (ng/mL)	
	r-value[a]	*p* value
Anthropometric parameters		
Age (years)	0.022	0.804
Body weight (kg)	0.059	0.510
Body height (cm)	0.048	0.587
BMI (kg/m^2)	0.063	0.475
Neck circumference (cm)	0.318	< 0.001
Waist circumference (cm)	0.121	0.172
Hip circumference (cm)	0.019	0.827
WHR	0.203	0.029
Lipid parameters		
Triglycerides (mmol/L)	0.165	0.061
Total cholesterol (mmol/L)	0.037	0.678
HDL cholesterol (mmol/L)	− 0.320	< 0.001
LDL cholesterol (mmol/L)	0.077	0.389
Non-HDL cholesterol (mmol/L)	0.158	0.074

BMI body mass index, *c* cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *WHR* waist-to-hip ratio

[a] *r*-value denotes Pearson's correlation coefficient

catecholamine activity, chronic heart failure, showed similar association with dynamics of circulating catestatin [31].

Furthermore, our OSA population had a substantial disease burden as evidenced by mean AHI score of 44.7 events/h, possibly implicating that sympathetic activity in this population is particularly increased, as reflected in nearly twofold increase in catestatin serum levels compared to control subjects. Although significant correlations of catestatin serum levels with AHI score and other polysomnographic indices were not observed when whole sample of OSA patients was analyzed, the subset analysis of non-obese OSA patients (BMI ≤ 30 kg/m^2) showed that catestatin exhibited significant and consistent correlation with AHI, in both bivariate and multiple linear regression analysis. This secondary finding in our study suggests that circulating catestatin levels positively correlate with disease severity in non-obese OSA subjects thus possibly reflecting the increasing neurohumoral and sympathetic activity triggered by high intermittent hypoxia burden. Since significant correlation of catestatin levels with AHI was abolished in obese OSA patients (BMI > 30 kg/m^2), it should come to attention that central obesity and visceral fat activity might play a blunting or *ceiling* role in the catestatin metabolism through an unknown mechanism. This is particularly reinforced by the fact that catestatin serum levels were virtually the same in both obese and non-obese OSA subjects. Importantly, obesity is an established risk factor for the development and progression of OSA that plays a complex role in disease pathophysiology and has been associated with multiple systemic inflammation pathways, energy homeostasis, and leptin signaling dysregulation, factors that were out of scope in this study [32]. Moreover, a study by Ernst et al. conducted among patients with OSA revealed that AHI and ODI proportionally increased according to the degree of obesity and this increase was associated with BMI in men [33]. Catestatin has an anti-obesity effect since preclinical study showed that catestatin exhibited functional role in mediating fat and adipose tissue metabolism by regulating adrenergic and leptin signaling [34]. However, these putative mechanisms should be investigated in future studies since the direct interaction between catestatin and obesity in the complex pathophysiology of OSA is not yet elucidated.

Moreover, catestatin showed a positive correlation with central apnea events and negative correlation with mean SpO_2 among non-obese subjects; however, these associations were abolished when adjusted for confounders. Of note, the role of catestatin in central cardiorespiratory control was evaluated in the preclinical study by Gaede et al. showing that catestatin exhibited sympathoinhibitory effects in caudal ventrolateral medulla thereby reducing phrenic nerve amplitude, decreasing phrenic inspiratory period and increasing phrenic expiratory period [35]. These effects might have been extended to pre-Bötzinger complex (preBötC) that is located in that anatomical region and has a central role in respiratory rhythm

Table 5 A multivariate logistic regression analysis performed in total population of enrolled participants (N=129) showing the predictive association of serum catestatin levels and other parameters with positive OSA status (set as the dependent outcome variable)

Variable	OR	95% CI	*p*
Age (years)	0.99	0.94–1.04	0.725
BMI (kg/m^2)	1.09	1.09–1.26	0.242
Waist circumference (cm)	1.04	0.91–1.20	0.578
Hip circumference (cm)	0.92	0.77–1.10	0.368
Neck circumference (cm)	1.86	1.35–2.54	< 0.001
Catestatin (ng/mL)	4.98	2.17–11.47	< 0.001
Triglycerides (mmol/L)	5.22	1.50–16.30	0.010
HDL cholesterol (mmol/L)	0.35	0.21–0.81	0.014

95% CI 95% confidence interval, *BMI* body mass index, *HDL* high-density lipoprotein, *OR* multivariate adjusted odds ratio obtained through multivariate logistic regression analysis

generation in mammals [36]. A previous study by the same group showed that catestatin had sympathoexcitatory effects in rostral ventrolateral medulla and increased phrenic nerve activity [37]. These findings altogether suggest that catestatin exerts neuromodulatory effects on central respiratory activity in mammals; however, how this mechanism might translate to human subjects with OSA remains unknown and warrants further investigation.

Regarding anthropometric parameters, in our sample of OSA patients, catestatin serum levels significantly and positively correlated with neck circumference ($r = 0.318$, $\beta = -0.384$; $p < 0.001$). No significant relationship was observed between any other anthropometric parameter and catestatin serum levels, when adjusted for multiple confounders.

Furthermore, in our study, we sought to explore potential interactions of catestatin with lipid profile parameters in OSA population. After multiple analyses were performed, significant association was retained only between catestatin and HDL-c levels and this was an inverse relationship ($r = -0.320$, $\beta = -0.344$; $p < 0.001$). Moreover, OSA subjects had significantly lower levels of HDL cholesterol compared to control subjects, accompanied with significantly higher triglyceride and non-HDL cholesterol levels. Notably, HDL-c exerts anti-atherogenic and antioxidant properties, and its function has been impaired in subjects with OSA, while LDL-to-HDL cholesterol ratio increased concomitantly with disease severity [38, 39].

Only one clinical study thus far examined relationship of catestatin with vascular and metabolic parameters in untreated subjects with arterial hypertension. In this study, catestatin plasma concentrations showed an independent positive correlation with HDL cholesterol ($\beta = 0.299$, $p = 0.002$) [13]. This finding is contrary to our results, and authors hypothesized that increased fatty acid oxidation in the liver and lipolysis in the adipose tissue, mediated by the previously reported catestatin-driven inhibition of α-adrenergic receptors and increased leptin receptor sensitivity, led to upregulated cholesterol efflux into HDL-c thus proposing a mechanistic link between HDL-c and catestatin [34, 40]. It is important to highlight that these opposite results in the context of HDL-c association with catestatin might be largely explained by the different populations, and two different disease entities that were investigated in our and aforementioned study further emphasized by the fact that we enrolled exclusively male patients, whereas the other study had a predominant enrollment of women.

From the mechanistic standpoint, since HDL function and levels are impaired in the OSA subjects and catestatin decreased hepatic/plasma lipid metabolism in diet-induced obesity mice, it is possible that low levels of HDL cholesterol in OSA subjects dictate higher catestatin secretion in the peripheral circulation through a negative feedback loop. However, since catestatin is an endogenous peptide with pleiotropic roles in body homeostasis and cardiovascular system, in particular, it is difficult to ascertain whether these interactions between catestatin and HDL cholesterol are indeed independent and not driven by other comorbidities and mediating factors (e.g., arterial hypertension, circulating norepinephrine, and epinephrine levels, etc.) that were not measured in our study.

Finally, our multivariate regression analysis showed that catestatin was independently associated with nearly fivefold increase in odds for OSA, along with neck circumference, triglycerides, and HDL cholesterol, when adjusted for multiple confounders in the model. This result reinforces the notion that catestatin is a robust independent predictor of OSA.

Our study has some limitations as it was a single-center study which did not include female subjects; therefore, our results might not be generalized to a whole population. Moreover, parameters of neurohumoral and adrenergic activity were not directly measured in this study. Finally, a full-night PSG was not performed among healthy volunteers that reached low score (< 3) on STOP-BANG questionnaire, and for this reason, it cannot be fully excluded that some of the 51 control subjects had a clinically relevant OSA. Moreover, pre-test probability calculators have inherent limitations in a sense that they are population- and center-dependent. However, a score < 3 obtained on STOP-BANG questionnaire has a high negative predictive value for OSA and thus is a well-established instrument for OSA screening in general population.

In conclusion, catestatin serum levels are significantly higher in male OSA patients compared to healthy control subjects and significantly correlate with disease severity, as measured by AHI, among non-obese OSA patients. Furthermore, serum catestatin levels negatively correlate with HDL cholesterol and can be evaluated as an independent predictor of OSA status; however, this association is restricted to patients with

moderate-to-severe OSA. Finally, increased circulating levels of catestatin in OSA population with moderate-to-high disease severity might reflect elevated sympathetic and adrenergic activity; however, more clinical studies are required to elucidate the complex role of catestatin in OSA in terms of disease mechanisms.

Acknowledgements We would like to thank Natalija Ivkovic, M.Sc. (Sleep Medicine Center), for her important contribution in conducting sleep assessment studies.

Funding The Croatian Science Foundation provided financial support in the form of grant to principal investigator Prof. Zoran Dogas (Project no. 5935). The sponsor had no role in the design or conduct of this research.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (name of institute/committee) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Lévy P, Kohler M, McNicholas WT, Barbé F, McEvoy RD, Somers VK, Lavie L, Pépin J-L (2015) Obstructive sleep apnoea syndrome. Nat Rev Dis Primers 1:15015. https://doi.org/10.1038/nrdp.2015.15
2. Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, Young T (2008, Circulation) Sleep Apnea and Cardiovascular Disease: an American Heart Association/American College of Cardiology Foundation Scientific Statement From the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council on Cardiovascular Nursing. In Collaboration With the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health). 118(10):1080–1111. https://doi.org/10.1161/CIRCULATIONAHA.107.189375
3. Galic T, Bozic J, Ivkovic N, Gunjaca G, Ticinovic TK, Dogas Z (2016) Effects of mandibular advancement device treatment on arterial stiffness and glucose metabolism in patients with mild to moderate obstructive sleep apnea: a prospective 1 year study. Sleep Breath 20(1):69–77. https://doi.org/10.1007/s11325-015-1186-y
4. Kent BD, McNicholas WT, Ryan S (2015) Insulin resistance, glucose intolerance and diabetes mellitus in obstructive sleep apnoea. J Thorac Dis 7(8):1343–1357. https://doi.org/10.3978/j.issn.2072-1439.2015.08.11
5. Bozic J, Galic T, Supe-Domic D, Ivkovic N, Ticinovic Kurir T, Valic Z, Lesko J, Dogas Z (2016) Morning cortisol levels and glucose metabolism parameters in moderate and severe obstructive sleep apnea patients. Endocrine 53(3):730–739. https://doi.org/10.1007/s12020-016-0925-6
6. Adedayo AM, Olafiranye O, Smith D, Hill A, Zizi F, Brown C, Jean-Louis G (2014) Obstructive sleep apnea and dyslipidemia: evidence and underlying mechanism. Sleep Breath 18(1):13–18. https://doi.org/10.1007/s11325-012-0760-9
7. Nadeem R, Singh M, Nida M, Waheed I, Khan A, Ahmed S, Naseem J, Champeau D (2014) Effect of obstructive sleep apnea hypopnea syndrome on lipid profile: a meta-regression analysis. J Clin Sleep Med 10(5):475–489. https://doi.org/10.5664/jcsm.3690
8. Galic T, Bozic J, Pecotic R, Ivkovic N, Valic M, Dogas Z (2016) Improvement of cognitive and psychomotor performance in patients with mild to moderate obstructive sleep apnea treated with mandibular advancement device: a prospective 1-year study. J Clin Sleep Med 12(2):177–186. https://doi.org/10.5664/jcsm.5480
9. Troger J, Theurl M, Kirchmair R, Pasqua T, Tota B, Angelone T, Cerra MC, Nowosielski Y, Matzler R, Troger J, Gayen JR, Trudeau V, Corti A, Helle KB (2017) Granin-derived peptides. Prog Neurobiol 154:37–61. https://doi.org/10.1016/j.pneurobio.2017.04.003
10. Mahata SK, O'Connor DT, Mahata M, Yoo SH, Taupenot L, Wu H, Gill BM, Parmer RJ (1997) Novel autocrine feedback control of catecholamine release. A discrete chromogranin a fragment is a noncompetitive nicotinic cholinergic antagonist. J Clin Invest 100(6):1623–1633. https://doi.org/10.1172/jci119686
11. Mahata SK, Mahata M, Fung MM, O'Connor DT (2010) Catestatin: a multifunctional peptide from chromogranin A. Regul Pept 162(1–3):33–43. https://doi.org/10.1016/j.regpep.2010.01.006
12. Kojima M, Ozawa N, Mori Y, Takahashi Y, Watanabe-Kominato K, Shirai R, Watanabe R, Sato K, Matsuyama TA, Ishibashi-Ueda H, Koba S, Kobayashi Y, Hirano T, Watanabe T (2018) Catestatin prevents macrophage-driven atherosclerosis but not arterial injury-induced neointimal hyperplasia. Thromb Haemost 118(1):182–194. https://doi.org/10.1160/th17-05-0349
13. Durakoglugil ME, Ayaz T, Kocaman SA, Kirbas A, Durakoglugil T, Erdogan T, Cetin M, Sahin OZ, Cicek Y (2015) The relationship of plasma catestatin concentrations with metabolic and vascular parameters in untreated hypertensive patients: influence on high-density lipoprotein cholesterol. Anatol J Cardiol 15(7):577–585. https://doi.org/10.5152/akd.2014.5536
14. Bandyopadhyay GK, Mahata SK (2017) Chromogranin A regulation of obesity and peripheral insulin sensitivity. Front Endocrinol (Lausanne) 8:20. https://doi.org/10.3389/fendo.2017.00020
15. O'Connor DT, Kailasam MT, Kennedy BP, Ziegler MG, Yanaihara N, Parmer RJ (2002) Early decline in the catecholamine release-inhibitory peptide catestatin in humans at genetic risk of hypertension. J Hypertens 20(7):1335–1345
16. Li J, Thorne LN, Punjabi NM, Sun CK, Schwartz AR, Smith PL, Marino RL, Rodriguez A, Hubbard WC, O'Donnell CP, Polotsky VY (2005) Intermittent hypoxia induces hyperlipidemia in lean mice. Circ Res 97(7):698–706. https://doi.org/10.1161/01.RES.0000183879.60089.a9
17. Li J, Grigoryev DN, Ye SQ, Thorne L, Schwartz AR, Smith PL, O'Donnell CP, Polotsky VY (2005) Chronic intermittent hypoxia upregulates genes of lipid biosynthesis in obese mice. J Appl Physiol (1985) 99(5):1643–1648. https://doi.org/10.1152/japplphysiol.00522.2005
18. Qian Y, Yi H, Zou J, Meng L, Tang X, Zhu H, Yu D, Zhou H, Su K, Guan J, Yin S (2016) Independent association between sleep fragmentation and dyslipidemia in patients with obstructive sleep apnea. Sci Rep 6:26089. https://doi.org/10.1038/srep26089

19. Drager LF, Togeiro SM, Polotsky VY, Lorenzi-Filho G (2013) Obstructive sleep apnea: a cardiometabolic risk in obesity and the metabolic syndrome. J Am Coll Cardiol 62(7):569–576. https://doi.org/10.1016/j.jacc.2013.05.045
20. Kim J, Lee S, Bhattacharjee R, Khalyfa A, Kheirandish-Gozal L, Gozal D (2010) Leukocyte telomere length and plasma catestatin and myeloid-related protein 8/14 concentrations in children with obstructive sleep apnea. Chest 138(1):91–99. https://doi.org/10.1378/chest.09-2832
21. Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, Harrod CG (2017) Clinical practice guideline for diagnostic testing for adult obstructive sleep apnea: an American Academy of sleep medicine clinical practice guideline. J Clin Sleep Med 13(3):479–504. https://doi.org/10.5664/jcsm.6506
22. Bassetti CL, Đogaš Z, Peigneux P (2014) European Sleep Research Society (ESRS) European sleep medicine textbook. European Sleep Research Society
23. Chung F, Abdullah HR, Liao P (2016) STOP-Bang questionnaire: a practical approach to screen for obstructive sleep apnea. Chest 149(3):631–638. https://doi.org/10.1378/chest.15-0903
24. Pecotic R, Dodig IP, Valic M, Ivkovic N, Dogas Z (2012) The evaluation of the Croatian version of the Epworth sleepiness scale and STOP questionnaire as screening tools for obstructive sleep apnea syndrome. Sleep Breath 16(3):793–802
25. Fischer J, Dogas Z, Bassetti CL, Berg S, Grote L, Jennum P, Levy P, Mihaicuta S, Nobili L, Riemann D, Puertas Cuesta FJ, Raschke F, Skene DJ, Stanley N, Pevernagie D (2012) Standard procedures for adults in accredited sleep medicine centres in Europe. J Sleep Res 21(4):357–368. https://doi.org/10.1111/j.1365-2869.2011.00987.x
26. Epstein L, Kristo D, Strollo P Jr, Friedman N, Malhotra A, Patil S, Ramar K, Rogers R, Schwab R, Weaver E (2009) Adult obstructive sleep apnea task force of the American Academy of sleep medicine. Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults. J Clin Sleep Med 5(3):263–276
27. Narkiewicz K, Somers VK (2003) Sympathetic nerve activity in obstructive sleep apnoea. Acta Physiol Scand 177(3):385–390. https://doi.org/10.1046/j.1365-201X.2003.01091.x
28. Mahata SK, Kiranmayi M, Mahapatra NR (2018) Catestatin: a master regulator of cardiovascular functions. Curr Med Chem 25(11):1352–1374. https://doi.org/10.2174/0929867324666170425100416
29. Meng L, Ye XJ, Ding WH, Yang Y, Di BB, Liu L, Huo Y (2011) Plasma catecholamine release-inhibitory peptide catestatin in patients with essential hypertension. J Cardiovasc Med (Hagerstown) 12(9):643–647. https://doi.org/10.2459/JCM.0b013e328346c142
30. Schillaci G, De Vuono S, Pucci G (2011) An endogenous brake on the sympathetic nervous system: the emerging role of catestatin in hypertension. J Cardiovasc Med (Hagerstown) 12(9):609–612. https://doi.org/10.2459/JCM.0b013e328348d925
31. Liu L, Ding W, Li R, Ye X, Zhao J, Jiang J, Meng L, Wang J, Chu S, Han X, Peng F (2013) Plasma levels and diagnostic value of catestatin in patients with heart failure. Peptides 46:20–25. https://doi.org/10.1016/j.peptides.2013.05.003
32. Romero-Corral A, Caples SM, Lopez-Jimenez F, Somers VK (2010) Interactions between obesity and obstructive sleep apnea: implications for treatment. Chest 137(3):711–719. https://doi.org/10.1378/chest.09-0360
33. Ernst G, Bosio M, Salvado A, Dibur E, Nigro C, Borsini E (2016) Difference between apnea-hypopnea index (AHI) and oxygen desaturation index (ODI): proportional increase associated with degree of obesity. Sleep Breath 20(4):1175–1183. https://doi.org/10.1007/s11325-016-1330-3
34. Bandyopadhyay GK, Vu CU, Gentile S, Lee H, Biswas N, Chi NW, O'Connor DT, Mahata SK (2012) Catestatin (chromogranin A(352-372)) and novel effects on mobilization of fat from adipose tissue through regulation of adrenergic and leptin signaling. J Biol Chem 287(27):23141–23151. https://doi.org/10.1074/jbc.M111.335877
35. Gaede AH, Pilowsky PM (2012) Catestatin, a chromogranin A-derived peptide, is sympathoinhibitory and attenuates sympathetic barosensitivity and the chemoreflex in rat CVLM. Am J Physiol Regul Integr Comp Physiol 302(3):R365–R372. https://doi.org/10.1152/ajpregu.00409.2011
36. Smith JC, Ellenberger HH, Ballanyi K, Richter DW, Feldman JL (1991) Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science 254(5032):726–729
37. Gaede AH, Pilowsky PM (2010) Catestatin in rat RVLM is sympathoexcitatory, increases barosensitivity, and attenuates chemosensitivity and the somatosympathetic reflex. Am J Physiol Regul Integr Comp Physiol 299(6):R1538–R1545. https://doi.org/10.1152/ajpregu.00335.2010
38. Tan KC, Chow WS, Lam JC, Lam B, Wong WK, Tam S, Ip MS (2006) HDL dysfunction in obstructive sleep apnea. Atherosclerosis 184(2):377–382. https://doi.org/10.1016/j.atherosclerosis.2005.04.024
39. Kawano Y, Tamura A, Kadota J (2012) Association between the severity of obstructive sleep apnea and the ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol. Metabolism 61(2):186–192. https://doi.org/10.1016/j.metabol.2011.06.004
40. Verghese PB, Arrese EL, Soulages JL (2007) Stimulation of lipolysis enhances the rate of cholesterol efflux to HDL in adipocytes. Mol Cell Biochem 302(1–2):241–248. https://doi.org/10.1007/s11010-007-9447-0