



# Possible Role of Serum Leptin as Biomarker in COPD

Yanitsa A. Zhelyazkova<sup>1</sup>, Tanya T. Tacheva<sup>1</sup>, Dimo M. Dimov<sup>2</sup>, Denitsa G. Vlaykova<sup>1</sup>, Aneliya V. Bivolarska<sup>3</sup>, Tatyana I. Vlaykova<sup>1,3</sup>

<sup>1</sup> Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

<sup>2</sup> Department of Health Care, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

<sup>3</sup> Department of Biochemistry, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria

**Corresponding author:** Tatyana I. Vlaykova, Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, 11 Armeiska St., Stara Zagora, 6000, Bulgaria, E-mail: tvlaykova@abv.bg; Tel.: +359 888002438

**Received:** 04 Apr 2019 ♦ **Accepted:** 13 June 2019 ♦ **Published:** 31 Dec 2019

**Citation:** Zhelyazkova YA, Tacheva TT, Dimov DM, Vlaykova DG, Bivolarska AV, Vlaykova TI. Possible role of serum leptin as biomarker in COPD. Folia Med (Plovdiv) 2019;61(4):512-21. doi: 10.3897/folmed.61.e47745.

## Abstract

Leptin is one of the adipokines shown to exert a significant effect in respiratory diseases, including chronic obstructive pulmonary disease (COPD).

The aim of the present study was to evaluate the possible role of serum leptin as biomarker in COPD.

The serum leptin levels were assessed in 58 patients with stable COPD and 21 controls applying ELISA method.

The leptin levels were higher, although not significantly, in COPD patients than in controls ( $221.52 \pm 24.28$  (SE) vs.  $165.04 \pm 26.01$  pg/ml,  $p=0.197$ ). This tendency turned out significant when only females were compared ( $414.60 \pm 60.63$  vs.  $219.40 \pm 44.15$  pg/ml,  $p=0.038$ ). The levels of leptin were highly dependent on the BMI both in COPD patients ( $p<0.001$ ) and in controls ( $p=0.024$ ): they were the highest in obese individuals and decreased with reducing the BMI.

In the COPD group, women had significantly higher leptin levels than men ( $p<0.0001$ ) independent of the BMI. The non-smoking patients had significantly higher leptin levels than ex-smokers ( $p=0.007$ ) and current smokers ( $p=0.007$ ). In patients with BMI above 25, several associations were observed: patients with mild COPD had higher serum leptin level than those with severe or very severe COPD ( $p=0.038$ ); the leptin levels correlated positively with  $FEV_1\%$  ( $r=0.304$ ,  $p=0.045$ ) and  $FEV_1/FVC$  ratio ( $r=0.348$ ,  $p=0.021$ ), and tended to correlate negatively with ABCD GOLD groups ( $Rho=-0.300$ ,  $p=0.043$ ) and with the CAT points ( $Rho=-0.258$ ,  $p=0.091$ ); the leptin levels below 300 ng/ml determined 4.08-fold higher risk for more severe COPD.

The results of our study confirm that the serum leptin levels depend significantly on the BMI and are interfered by gender and smoking habits. However, this adipokine cannot be used as a serum biomarker for distinguishing COPD patients, but its decrease might be associated with aggravation of the disease.

## Keywords

biomarker, COPD, ELISA, leptin

## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable disease characterized by persistent respiratory symptoms and airflow limitation due to airways and/or alveolar anomalies commonly caused by significant exposure to harmful particles or gases.<sup>1</sup> COPD is caused by a combination of small airways disease (obstructive bronchiolitis) and parenchyma destruction (emphysema) and the relative proportion of them varies in individuals. Chronic inflammation causes structural changes and narrowing of the small airways.<sup>1</sup> Undoubtedly, COPD is an inflammatory disease. An abnormal inflammatory response has been observed not only at the pulmonary level in COPD patients, but also at systemic level. Recent studies suggest that COPD is not only a pulmonary disease, but in fact it might be the consequence of a systemic inflammatory process.<sup>2</sup> The origin of systemic inflammation is not clear. It is suggested that cytokines such as TNF- $\alpha$ , IL-6, and IL-1 $\beta$  produced by injured or activated lung cells can reach the systemic circulation and contribute to the activation of new cells in the lungs. Research on inflammation in COPD is promising. The hopes are related to the discovery of new treatment approaches targeting specific patient populations identified by the clinical phenotype or biomarkers.<sup>3</sup>

These biomarkers can be inflammatory mediators, proteases, or adipokines synthesized and secreted by adipose tissue, in particular leptin. Leptin is an adipocyte-derived hormone, and it is now known to have pleiotropic functions. Leptin is a pro-inflammatory adipokine that affects both innate and adaptive immune responses.<sup>4</sup> Besides in the adipocytes, it is found to be also expressed by the human lungs, mainly by bronchial epithelial cells and alveolar type II pneumocytes and macrophages.<sup>2</sup>

A growing number of studies have examined the potential role of leptin in the respiratory system.<sup>2,5-11</sup> Recent investigations have identified the lung as a leptin responsive and producing organ.<sup>12</sup> Studies further suggest a significant impact of leptin on specific respiratory diseases, including obstructive sleep apnea, hypopnea syndrome, asthma, COPD and lung cancer.<sup>2,5-11,13</sup> However, as new investigations are under way, the picture is becoming more complex.<sup>12</sup>

In this respect we aimed to explore the possible role of serum leptin levels as biomarkers in COPD.

## MATERIALS AND METHODS

### Patients and controls

Fifty-eight patients with COPD and 21 healthy subjects as controls were enrolled in the present cross-sectional study. The patients were recruited from the Clinic of Internal Medicine at the University Hospital of Trakia University, Stara Zagora, Bulgaria. The study was approved by the Eth-

ics Committee of the Medical Faculty in the Trakia University, Stara Zagora, Bulgaria. Informed consents were obtained from patients and controls before the study.

The inclusion criteria for COPD patients were as follows: women or men older than 40 years; forced expiratory volume in one second ( $FEV_1$ ) as a percentage of predicted value ( $FEV_1\%$  predicted) of  $< 80\%$ ; the ratio  $FEV_1$ /forced vital capacity ( $FEV_1/FVC$ ) of  $< 0.70$  (70%), reversibility value of  $< 0.12$  after inhalation of 400 mg salbutamol.

Demographic data (age, height, weight, BMI) were obtained for individuals in both groups. Smoking status was also noted, as well as the smoking habits, assessed as packs per year. The demographic and clinical data are presented in **Table 1**.

### Spirometric analyses

Patients and some controls underwent fast spirometry according to the method reported by Quinter using a spirometer (Pony FX, Cosmid). Lung function values for each participant were compared with the predicted normal values of Quinter et al. Lung function was defined by the  $FEV_1$  (forced expiratory volume in one second) as a percentage of predicted value and the ratio of  $FEV_1$  to the forced vital capacity (FVC) ( $FEV_1/FVC\%$ ). Normal values were considered as those between 80 and 120% for  $FEV_1\%$  predicted and more than 0.70 for  $FEV_1/FVC$ .

According to GOLD (Global strategy for the diagnosis, management, and prevention of COPD) 2017<sup>1</sup> the classification of airflow limitation severity in COPD based on post-bronchodilator  $FEV_1$  in patients with  $FEV_1/FVC < 0.70$  (70%) includes:

GOLD I: mild -  $FEV_1 \geq 80\%$  predicted

GOLD II: moderate -  $50\% \leq FEV_1 < 80\%$  predicted

GOLD III: severe -  $30\% \leq FEV_1 < 50\%$  predicted

GOLD IV: very severe -  $FEV_1 < 30\%$  predicted

Assessment of symptoms included a simple measure of breathlessness such as the Modified British Medical Research Council (mMRC) Questionnaire (relates well to other measures of health status and predicts future mortality risk) and CAT assessment.

An understanding of the impact of COPD on an individual patient combines the symptomatic assessment with the patient's spirometric classification and/or risk of exacerbations. The 'ABCD' assessment tool of the 2017 GOLD update was a major advancement from the simple spirometric grading system of the earlier versions of GOLD because it incorporated patient-reported outcomes and highlighted the importance of exacerbation prevention in the management of COPD.<sup>1</sup> In 2017, ABCD groups were derived exclusively from patient symptoms and their history of exacerbation, including prior hospitalizations. They remain vital for the diagnosis. The spirometry is not essential for the diagnosis and remains out of ABCD assessment. This classification may facilitate consideration of individual therapies and also help guide escalation and de-escalation therapeutic strategies for a specific patient.

**Table 1.** Demographic and clinical data of patients with COPD and control individuals

Characteristics	Controls (N) (%)	COPD patients (N) (%)
Sex	(21)	(58)
male	14 (66.7%)	46 (79.3%)
female	7 (33.3%)	12 (20.7%)
Age at inclusion in the study		
mean $\pm$ SD (years)	54.48 $\pm$ 8.00	68.05 $\pm$ 6.41
median (range) (years)	54.00 (42-70)	68.50 (50-79)
BMI		
normal (18.5-24.9)	8 (38.1%)	14 (24.1%)
overweight (25-29.9)	8 (38.1%)	24 (41.4%)
obese ( $\geq$ 30)	5 (23.8%)	20 (34.5%)
Smoking		
non-smokers	6 (28.6%)	5 (8.6%)
ex-smokers	4 (19.0%)	36 (62.1%)
current smokers	11 (52.4%)	17 (29.3%)
Smoking habits (packs/year)		
mean $\pm$ SD,	27.11 $\pm$ 15.37	37.56 $\pm$ 15.82
median (range)	30 (5-60)	37 (15-90)
Occupational hazard		
non present		47 (81.0%)
present		11 (19.0%)
FEV <sub>1</sub> % pr.		
mean $\pm$ SD,		53.99 $\pm$ 16.25
median (range)		54.95 (25.24-98.00)
FVC %		
mean $\pm$ SD,		72.43 $\pm$ 16.50
median (range)		71.50 (39.56-114.90)
FEV <sub>1</sub> /FVC %		
mean $\pm$ SD,		56.40 $\pm$ 10.49
median (range)		58.50 (31.60-70.00)
GOLD stage (2016)		
I		2 (3.4%)
II		36 (62.1%)
III		17 (29.3%)
IV		3 (5.2%)
GOLD ABCD groups (2017)		
A		10 (17.2%)
B		40 (69.0%)
C		0 (0%)
D		8 (13.8%)
CAT test scores		
mean $\pm$ SD,		14.09 $\pm$ 3.99
median (range)		14 (7-25)
Symptomatic disease (CAT test)		
non-symptomatic (CAT <10)		9 (15.5%)

symptomatic (CAT $\geq$ 10)	49 (84.5%)
Exacerbations (during the last 12 mo)	
none or one	34 (58.6%)
two or more	24 (41.4%)
Numbers of hospitalizations	
none	48 (82.8%)
one	10 (17.2%)

According to GOLD 2017, the airflow limitation severity in our COPD patients was as follows: most patients were with COPD GOLD II - 36 (62.1%), followed by patients with GOLD III - 17 (29.3%) and GOLD IV - 3 (5.2%). Only 2 (3.4%) patients were with COPD GOLD I. Based on the ABCD assessment GOLD 2017, in our patient group we had predominance of those from group B (40, 69%). There were 10 patients from group A (17.2%), and 8 (13.8%) from group D. None of our patients belonged to group C.

### Laboratory methods

Two millilitres of peripheral blood from the patients and controls were collected without anticoagulant, kept at 4°C for 30 min and centrifuged to obtain serum. The serum samples were stored at -80°C until the assays. The serum leptin concentration was measured by enzyme-linked immunosorbent assay (ELISA kit, R&D Systems, Inc., Minneapolis, MN, USA). The results were expressed as optical density (OD) at 450 nm and concentration was calculated in pg/ml according to the OD of the standards and standard curve.

### Statistical analysis

Statistical analysis was performed using SPSS, 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables were analyzed for normality by Kolmogorov-Smirnov and Shapiro-Wilk tests. The Student's t test and analysis of variance (ANOVA) test LSD post hoc test were applied for comparing the continuous variables with normal distribution in two or more independent groups, respectively, whereas the Mann-Whitney U test and Kruskal-Wallis test were applied for continuous variables with non-normal distribution. The frequencies of distribution of categorical variables in contingency tables were analyzed using  $\chi^2$  test and Fisher's exact test. Correlations were performed by Pearson or Spearman's test depending on the normality of the continuous variables. The Receiver Operating Characteristics (ROC) curve was created for determining the proper cut-off values for dichotomizing the groups and the logistic regression analysis was also performed. Factors with  $p < 0.05$  were considered statistically significant.

## RESULTS

The serum levels of leptin varied both in the group of controls and in COPD patients. Despite this, the levels in COPD patients were higher than in controls ( $221.52 \pm 24.28$  (SE) vs.  $165.04 \pm 26.01$  pg/ml,  $p = 0.197$ ), but the difference could not reach statistical significance. When the patients' and control groups were divided according to genders, we found statistically significant difference in leptin levels between female COPD patients and controls ( $414.60 \pm 60.63$  vs.  $219.40 \pm 44.15$  pg/ml,  $p = 0.038$ ), while there was no significant difference between men with and without COPD ( $171.15 \pm 20.85$  vs.  $137.86 \pm 30.68$  pg/ml,  $p = 0.425$ ) (Fig. 1).

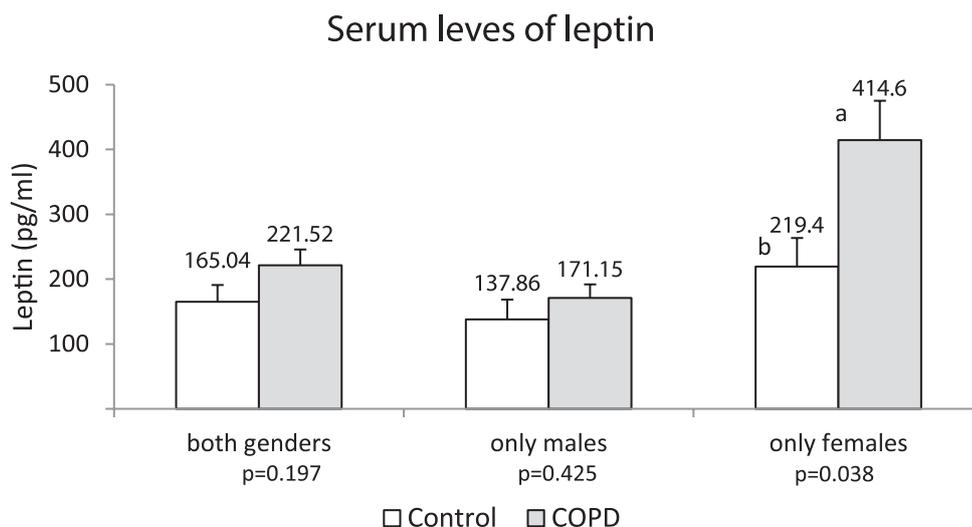
The levels of leptin were greatly dependent on the BMI in COPD patients ( $p < 0.0001$ , ANOVA test): they were the highest in obese subjects ( $341.35 \pm 48.04$  pg/ml), and differed significantly from those with overweight ( $209.21 \pm 27.86$  pg/ml,  $p = 0.007$ , LSD post hoc test) and especially from patients with normal BMI ( $71.43 \pm 14.28$  pg/ml,  $p < 0.0001$ , LSD post hoc test) (Fig. 2).

Analogous results were observed in controls ( $p = 0.024$ , ANOVA test): obese controls had significantly higher leptin serum levels ( $285.82 \pm 37.12$  pg/ml) than overweight controls ( $119.85 \pm 29.95$  pg/ml,  $p = 0.011$ , LSD post hoc test) and controls with normal BMI ( $134.75 \pm 44.28$  pg/ml,  $p = 0.018$ , LSD post hoc test) (Fig. 2).

In the COPD group several associations were observed. COPD female patients had significantly higher leptin levels than the male ones ( $p < 0.0001$ , Student t-test), particularly in overweight and obese COPD patients (Fig. 3). Because there was only one female COPD patient with normal BMI, analysis was not eligible for this subgroup.

The serum leptin levels in COPD non-smokers ( $n = 5$ ) were considerably higher ( $440.00 \pm 47.88$  pg/ml) than those in ex-smokers ( $n = 36$ ,  $205.64 \pm 27.00$  pg/ml,  $p = 0.007$ ) and especially than those in current smokers ( $n = 17$ ,  $190.88 \pm 51.45$  pg/ml,  $p = 0.007$ ) (Fig. 4). Similar results were found in the control group without statistical significance ( $209.01$  vs.  $168.20$  vs.  $139.91$  pg/ml,  $p = 0.544$ ).

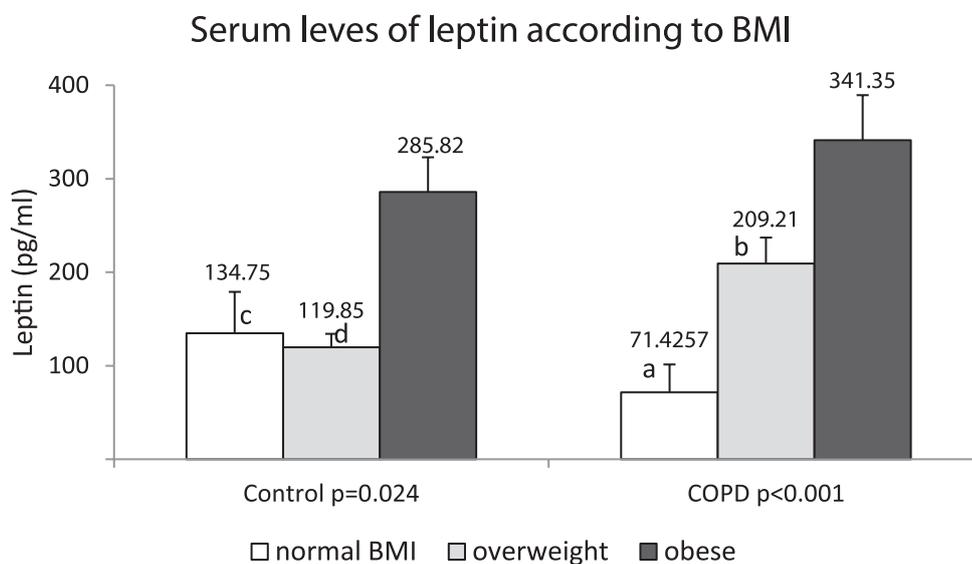
In the group of overweight or obese patients ( $n = 44$ ), those with mild disease (GOLD II,  $n = 26$ ) had more elevated serum leptin levels ( $317.33 \pm 40.26$  pg/ml) than those in the patients with severe or very severe COPD (GOLD III



**Figure 1.** Comparing leptin serum levels between patients with COPD and control individuals. The data are presented as mean ± SE (standard error) and are compared using the Student t test.

**a** - females vs. males in COPD,  $p < 0.0001$ ;

**b** - females vs. males in controls,  $p = 0.144$



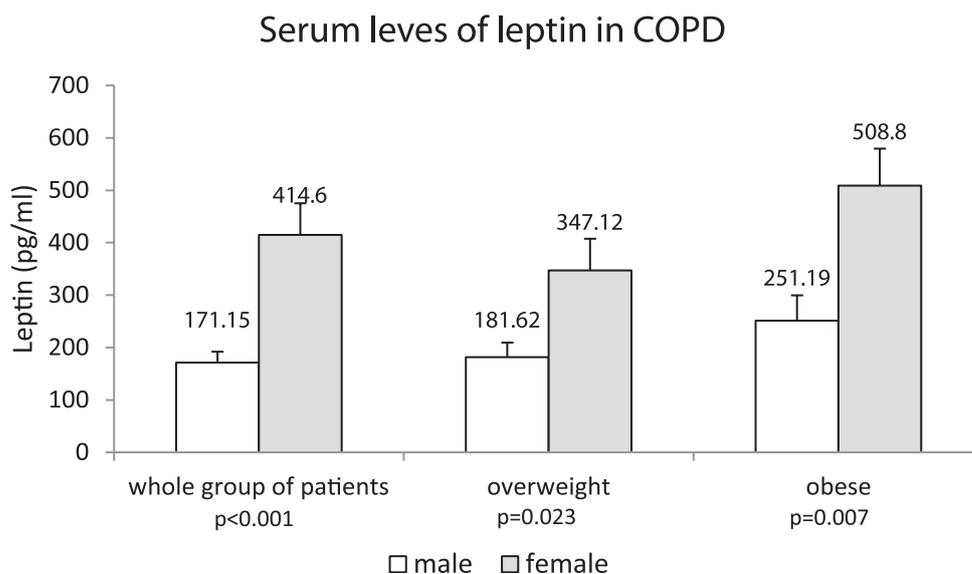
**Figure 2.** The serum levels of leptin according to the BMI in patients with COPD and in control individuals. The data are presented as mean ± SE (standard error) (AN test). The differences between groups were analyzed by LSD post hoc test.

**a** -  $p < 0.0001$ , obese vs. normal BMI COPD patients

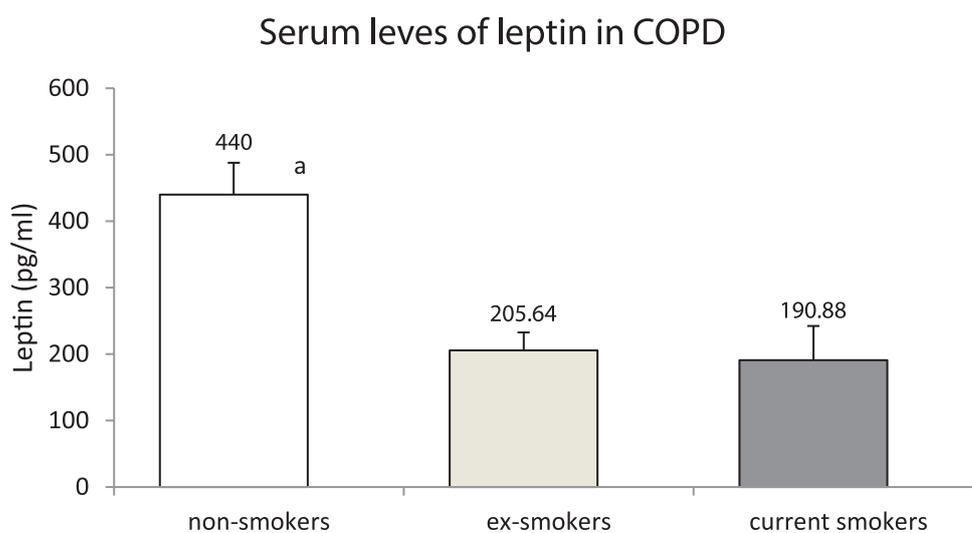
**b** -  $p = 0.007$ , obese vs. overweight COPD patients

**c** -  $p = 0.011$ , obese vs. normal BMI controls

**d** -  $p = 0.018$ , obese vs. overweight controls



**Figure 3.** Comparing leptin serum levels between genders in the whole group of patients with COPD and in the subgroups of those with overweight or obese. The data are presented as mean  $\pm$  SE (standard error) and are compared using the Student *t* test.



**Figure 4.** Comparing leptin serum levels between patients with different smoking habits. The data are presented as mean  $\pm$  SE (standard error) and are compared using ANOVA ( $p = 0.018$ ) and LSD post hoc test; **a** -  $p = 0.007$ , non-smokers vs. ex-smokers and non-smokers vs. current smokers.

and IV,  $n=18$ ,  $199.86 \pm 30.07$  pg/ml,  $p=0.038$ ). Similar observation was found for the patients with normal BMI, but without significance ( $p=0.246$ ).

When analyzing only overweight and obese patients (BMI above 25), the Receiver Operating Characteristics (ROC) curve produced area under the curve (AUC) of 0.662 with 95% CI of 0.501-0.824 ( $p=0.070$ ) (Fig. 5). The value below 300.00 ng/ml determined more severe COPD (GOLD III/IV) with sensitivity of 78.8% and with specificity of 53.8% ( $p=0.036$ ): 78.8% of the patients with GOLD III/IV and 46.2% of those with GOLD I/II had leptin value lower than 300.00 ng/ml. The regression analysis showed that the leptin levels below 300.00 ng/ml determined 4.08-fold higher risk for more severe COPD (OR=4.083, 95% CI 1.056 - 15.791) in patients with BMI above 25.

The same group of patients (with BMI higher than 25) had also moderate positive correlations between leptin serum levels and spirometric indices, such as FEV<sub>1</sub>% ( $r = 0.304$ ,  $p=0.045$ , Pearson correlation) and FEV<sub>1</sub>/FVC ratio ( $r = 0.348$ ,  $p=0.021$ , Pearson correlation). The leptin levels in overweight/obese patients correlated negatively with a marginal significance with the ABCD GOLD groups (presented in the scale format, Spearman's Rho= -0.300,  $p=0.043$ ) and with the CAT points (Spearman's Rho=-0.258,  $p=0.091$ ). There were also tendencies for lower leptin in those who exacerbated more frequently ( $219.02 \pm 35.25$  vs.  $315.16 \pm 41.53$  ng/ml,  $p=0.088$ ) and in those being at least once hospitalized during the previous year ( $166.59 \pm 48.47$  vs.  $292.09 \pm 31.64$  ng/ml,  $p=0.085$ ) in comparison to the non-common exacerbators or to these without hospitalization, respectively.

## DISCUSSION

In the past years, a growing number of studies have examined the potential role of leptin in the respiratory system, including COPD.<sup>12</sup> Leptin, a 16KDa protein of 167 amino acids, is the product of the *ob* gene localized in humans on chromosome 7. Leptin belongs structurally to the cytokine family, which includes interleukin-6 (IL-6), G-CSF, and oncostatin M. The protein leptin is synthesized and secreted mainly by white adipose tissue proportionally to fat stores, and because of this is considered an adipokine.<sup>12</sup>

Leptin is recognized as a regulatory mediator with a large functional pleiotropy, but its main role is believed to be the regulation of body fat mass by inhibiting the appetite and influencing the metabolism via balancing intake and expenditure of energy.<sup>14</sup> Leptin has been reported to participate in a variety of other physiological functions in both the central nervous system and the periphery, such as endocrine function, immune response, wound healing, reproduction, cardiovascular pathophysiology, and respiratory tissue development, remodeling, and function.<sup>14</sup> Besides the adipose tissue, leptin is produced in lower amounts by other tissues, such as the placenta, gastric fundic mucosa, and pancreas. Studies on animal models, have

demonstrated that *ob* gene is expressed in fetal lung tissue in baboons, and fetal rat lung fibroblasts.<sup>12</sup> Vernooij JH and Bruno have demonstrated the production of leptin in human peripheral lung tissue, namely bronchial epithelial cells, alveolar type II pneumocytes, and lung macrophages. Interestingly, enhanced pulmonary leptin expression was observed in patients with severe COPD and asymptomatic smokers, whereas the reduced leptin was associated with severe asthma.<sup>15</sup>

In our study we found that in both patients and control groups the obesity was related to the highest serum levels of leptin and decreased with decrease of BMI. These results confirm the well-documented observations that obesity is characterized by increased circulatory leptin concentrations and central leptin resistance due to reduced brain leptin transport and/or down-regulation of its receptors in the central nervous system.<sup>6</sup> Researchers have reported associations between leptin, body mass (BM) and body composition parameters - fat mass (FM) and fat mass index (FMI), lean tissue mass (LTM), lean tissue mass index (LTMI) and bone mineral density (BMD) in 67 male COPD patients. A positive association between leptin and FMI and BMD was found only in non-obese COPD patients, while a positive correlation was observed between leptin and the total hip T score only in obese COPD patients. All these result have suggested protective role of leptin on the skeleton of obese COPD patients.<sup>6</sup>

Takabatake et al. examined the circadian rhythm of circulating leptin in COPD and documented its absence in cachexic COPD patients, while it was preserved in normal weight COPD subjects.<sup>16</sup>

In a study with healthy adults from both genders, Paul et al. have assessed the possible association between serum leptin levels and BMI, and have found a progressive increase in serum leptin concentration along with an increase in BMI. Moreover, they have reported significant difference in leptin concentrations between the genders with remarkable increase in females than males in all subgroups - in normal, overweight and obese subjects.<sup>17</sup>

Later on, in 2013, similar results were reported for Rawalpindi population: the serum leptin levels correlated with BMI and obese and non-obese women had significantly higher levels of leptin than men with corresponding body mass indexes.<sup>18</sup> The results of our current study confirmed also the same observations about the gender difference in serum leptin levels with significant enhancement of leptin levels in females, independently on the body mass composition.

It is well established that smoking is a major risk factor in the development of COPD. Evidence on the association of leptin and smoking is limited and discordant. Recently, some reports have linked leptin to tobacco craving and withdrawal-related symptoms.<sup>19-21</sup> Very few studies have examined leptin levels prospectively in accordance to the smoking habits in healthy individuals of both genders and in patients with COPD. In such a prospective study Lemieux A et al. have found that leptin concentration en-

hances during a period of 48 hours in females after quitting smoking, but remains stable in blood of non-smokers, in relapsers and in male abstainers. Interestingly, leptin was found to correlate negatively with withdrawal symptoms and increased leptin was associated with decreased risk of relapse.<sup>19</sup> Analogous results were reported by Kryfti M et al., who described an increase of serum leptin levels within a period of 3 months after smoking cessation and decreasing in the following 3 months (until month 6 after abstinence from smoking).<sup>20</sup>

Reports have proven that smokers usually have a lower body weight compared to non-smokers.<sup>21</sup> Moreover, in most cases, smoking cessation is associated with weight gain. In a randomized controlled trial, it was found that serum leptin significantly increased after a year of smoking cessation compared to the baseline levels independently of the extent of the weight gain. The authors suggested that chronic tobacco use and physical activity might modulate the leptin dynamics.<sup>21</sup> However, the mechanism involved in the effect of smoking cessation on weight variations remain not fully clarified and more larger studies is warranted to be performed.

Experimental evidence for the effect of cigarette smoking on body weight and serum leptin level was reported by Esquivé et al.<sup>22</sup> In a rat model on concomitant sucrose diet and cigarette smoke exposure the authors have found that these conditions have led to a decrease in the body weight, visceral fat, adipokines as leptin and resistin and pro-inflammatory cytokines (IL-1 $\beta$  and IFN- $\gamma$ ), while all these markers have been increased in rats on a sucrose diet only.<sup>22</sup>

In our study, we found that COPD patients who had never smoked had the highest leptin levels, followed by the ex-smokers and then by current smokers. Similar results we found for the control group, too. In our patients' group only 5 individuals were non-smokers and all of them were with BMI above the normal score (29.9). Because of the limited number of the subgroups, we were not able to evaluate the effect of smoking on leptin level in COPD patients divided according to the BMI.

In the group of the COPD patients, we found that the overweight and obese patients with mild disease had elevated serum leptin levels than these with severe and very severe disease. Moreover, in the same subgroup of overweight and obese patients, the leptin concentration correlated positively with FEV<sub>1</sub>% and FEV<sub>1</sub>/FVC ratio. These results suggest that the decrease of leptin might be associated with aggravation of the disease.

To some extend, our results are consistent with those reported by Mohan et al., describing an enhancement of plasma leptin during clinical recovery of 82 patients admitted to the hospital with a diagnosis of acute exacerbation of COPD: the levels of leptin are significantly higher at the discharge of the patients compared to the baseline levels at the admission.<sup>10</sup> Earlier, in smaller groups of patients (27 with acute exacerbation of COPD and 24 with stable COPD), opposite results were obtained: the serum

levels in the exacerbation patients are significantly higher than in patients with stable COPD and healthy controls.<sup>13</sup> In other studies, however, no associations of blood leptin levels with the severity of COPD were found: such results were obtained in a large population-based study comprising 5175 controls and 1240 COPD patients of both genders from USA.<sup>2</sup> Only a few studies have explored the correlations of blood leptin levels with some lung function indexes of COPD patients. The study of Oh et al. has reported a significant inverse correlation of plasma leptin with initial FEV<sub>1</sub> (L)<sup>7</sup>, while others have not found any correlations<sup>5</sup>.

## CONCLUSION

Based on the results of our cross-sectional study, we can confirm that the serum leptin levels depend on the BMI and are influenced by gender and smoking habits. However, due to the absence of significance in the levels between controls and patients, we propose that this adipokine cannot be considered a reliable serum biomarker for distinguishing COPD, but the decrease of serum leptin might be associated with aggravation of the disease, especially in overweight and obese patients.

Based on the main limitations of our study, particularly on the limited number of COPD patients and controls, we consider that larger cross-sectional and especially prospective study with higher number of traced individuals must be designed and performed.

## ACKNOWLEDGMENTS

This study was supported by project No 3/2018 of the Medical Faculty, Trakia University, by Trakia University Infrastructural Project 3/2017 and by the National Scientific Program of Young scientists and postdoctoral specialists of Trakia University, Stara Zagora, Bulgaria.

## REFERENCES

1. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2017 Report, 2017.
2. Sueblinvong V, Liangpunsakul S. Relationship between serum leptin and chronic obstructive pulmonary disease in US adults: results from the third National Health and Nutrition Examination Survey. *J Investig Med* 2014; 62(7): 934-7.
3. Petkova D, Stratev V. [Inflammation in COPD – Alea iacta est. Therapeutic possibilities.] *In Spiro* 2013; 2(21):31-4. [In Bulgarian].
4. Francisco V, Pino J, Campos-Cabaleiro V, et al. Obesity, fat mass and immune system: role for leptin. *Front Physiol* 2018; 9: 640.
5. Kumor-Kisielewska A, Kierszniewska-Stepien D, Pietras T, et al. Assessment of leptin and resisting levels in patients with chronic obstructive pulmonary disease. *Pol Arch Med Wewn* 2013; 123(5): 215-20.
6. Korsic M, Kusec V. Serum leptin and skeletal differences between

- obese and non-obese patients with chronic obstructive pulmonary disease. *Obes Facts* 2014; 7(6): 399-407.
7. Oh YM, Jeong BH, Woo SY, et al. Association of plasma adipokines with chronic obstructive pulmonary disease severity and progression. *Ann Am Thorac Soc* 2015; 12(7): 1005-12.
  8. Rehman Khan A, Awan FR. Leptin resistance: a possible interface between obesity and pulmonary-related disorders. *Int J Endocrinol Metab* 2016; 14(1): e32586.
  9. Tong X, Ma Y, Zhou Q, et al. Serum and tissue leptin in lung cancer: A meta-analysis. *Oncotarget* 2017; 8(12): 19699-711.
  10. Mohan A, Arora S, Uniyal A, et al. Evaluation of plasma leptin, tumor necrosis factor-alpha, and prealbumin as prognostic biomarkers during clinical recovery from acute exacerbations of chronic obstructive pulmonary disease. *Lung India* 2017; 34(1): 3-8.
  11. Coats V, Despres JP, Almeras N, et al. Ectopic adiposity and cardio-metabolic health in COPD. *Int J Chron Obstruct Pulmon Dis* 2018; 13: 3331-40.
  12. Malli F, Papaioannou AI, Gourgoulianis KI, et al. The role of leptin in the respiratory system: an overview. *Respir Res* 2010; 11: 152.
  13. Liang R, Zhang W, Song YM. Levels of leptin and IL-6 in lungs and blood are associated with the severity of chronic obstructive pulmonary disease in patients and rat models. *Mol Med Rep* 2013; 7(5): 1470-6.
  14. Vernooij JH, Ubags ND, Brusselle GG, et al. Leptin as regulator of pulmonary immune responses: involvement in respiratory diseases. *Pulm Pharmacol Ther* 2013; 26(4): 464-72.
  15. Vernooij JH, Drummen NE, van Suylen RJ, et al. Enhanced pulmonary leptin expression in patients with severe COPD and asymptomatic smokers. *Thorax* 2009; 64(1): 26-32.
  16. Takabatake N, Nakamura H, Minamihaba O, et al. A novel pathophysiologic phenomenon in cachexic patients with chronic obstructive pulmonary disease: the relationship between the circadian rhythm of circulating leptin and the very low-frequency component of heart rate variability. *Am J Respir Crit Care Med* 2001; 163(6): 1314-9.
  17. Paul RF, Hassan M, Nazar HS, et al. Effect of body mass index on serum leptin levels. *J Ayub Med Coll Abbottabad* 2011; 23(3): 40-3.
  18. Kazmi A, Sattar A, Hashim R, et al. Serum leptin values in the healthy obese and non-obese subjects of Rawalpindi. *J Pak Med Assoc* 2013; 63(2): 245-8.
  19. Lemieux A, Nakajima M, Hatsukami DK, et al. Changes in circulating leptin levels during the initial stage of cessation are associated with smoking relapse. *Psychopharmacology* 2015; 232(18): 3355-61.
  20. Kryfti M, Dimakou K, Toumbis M, et al. Effects of smoking cessation on serum leptin and adiponectin levels. *Tob Induc Dis* 2015; 13: 30.
  21. Gonseth S, Locatelli I, Bize R, et al. Leptin and smoking cessation: secondary analyses of a randomized controlled trial assessing physical activity as an aid for smoking cessation. *BMC Public Health* 2014; 14: 911.
  22. Esquivel AL, Perez-Ramos J, Cisneros J, et al. The effect of obesity and tobacco smoke exposure on inflammatory mediators and matrix metalloproteinases in rat model. *Toxicol Mech Methods* 2014; 24(9): 633-43.

# Возможная роль сывороточного лептина в качестве биомаркера при ХОБЛ

Яница А. Желязкова<sup>1</sup>, Таня Т. Тачева<sup>1</sup>, Димо М. Димов<sup>2</sup>, Деница Г. Влайкова<sup>1</sup>, Анелия В. Биволарска<sup>3</sup>, Татьяна И. Влайкова<sup>1,3</sup>

<sup>1</sup>Кафедра химии и биохимии, Факультет медицины, Фракийский университет, Стара Загора, Болгария

<sup>2</sup>Кафедра здравоохранения, Факультет медицины, Фракийский университет, Стара Загора, Болгария

<sup>3</sup>Кафедра биохимии, Факультет фармации, Медицинский университет - Пловдив, Пловдив, Болгария

**Адрес для корреспонденции:** Татьяна И. Влайкова, Кафедра химии и биохимии, Факультет медицины, Фракийский университет, ул. „Армейска“ № 11, 6000, Стара Загора, Болгария E-mail: tvlaykova@abv.bg; Тел: +359 888002438

**Дата получения:** 04 апреля 2019 ♦ **Дата приемки:** 13 июня 2019 ♦ **Дата публикации:** 31 декабря 2019

**Образец цитирования:** Zhelyazkova YA, Tacheva TT, Dimov DM, Vlaykova DG, Bivolarska AV, Vlaykova TI. Possible role of serum leptin as biomarker in COPD. Folia Med (Plovdiv) 2019;61(4):512-21. doi: 10.3897/folmed.61.e47745.

## Абстракт

Лептин является одним из адипокинов, который, как было показано, оказывает значительное влияние на респираторные заболевания, включая хроническую обструктивную болезнь лёгких (ХОБЛ).

Целью данного исследования было оценить вероятную роль сывороточного лептина в качестве биомаркера при ХОБЛ.

Уровни лептина в сыворотке измеряли у 58 пациентов со стабильной ХОБЛ и у 21 из контрольной группы методом ELISA.

Уровни лептина были выше, хотя и незначительными, у пациентов с ХОБЛ, чем в контрольной группе ( $221,52 \pm 24,28$  (SE) против  $165,04 \pm 26,01$  pg/ml,  $p = 0,197$ ). Уровни лептина были сильно зависимыми от ИМТ как у пациентов с ХОБЛ ( $p < 0,001$ ), так и у контрольных ( $p = 0,024$ ): они были самыми высокими у людей с ожирением и снижались при снижении ИМТ.

В группе пациентов с ХОБЛ уровень лептина у женщин был значительно выше, чем у мужчин ( $p < 0,0001$ ), независимо от ИМТ. Некурящие имели значительно более высокие уровни лептина, чем бывшие курильщики ( $p = 0,007$ ) и активные курильщики ( $p = 0,007$ ). У пациентов с ИМТ выше 25 наблюдалось несколько ассоциаций: у пациентов с лёгкой формой ХОБЛ уровень лептина в сыворотке был выше, чем у пациентов с тяжёлой или очень тяжёлой формой ( $p = 0,038$ ); Уровни лептина положительно коррелировали с FEV1% ( $r = 0,304$ ,  $p = 0,045$ ) и соотношением FEV1 / FVC ( $r = 0,348$ ,  $p = 0,021$ ) и отрицательно с группировками ABCD по классификации GOLD ( $Rho = -0,300$ ,  $p = 0,043$ ) и с баллами оценочного теста ХОБЛ (COPD Assessment Test, CAT) и ( $Rho = -0,258$ ,  $p = 0,091$ ); Уровень лептина ниже 300 ng/ml определял в 4,08 раза больший риск развития более тяжёлой формы ХОБЛ.

Результаты нашего исследования подтверждают, что уровень лептина в сыворотке значительно зависит от ИМТ и зависит от пола и курения. Однако этот адипокин не может быть использован в качестве сывороточного биомаркера для определения пациентов с ХОБЛ, но его снижение может быть связано с ухудшением заболевания.

## Ключевые слова

ХОБЛ, лептин, биомаркер, ELISA