ORIGINAL ARTICLE

Assessment of leptin and resistin levels in patients with chronic obstructive pulmonary disease

Anna Kumor-Kisielewska, Dorota Kierszniewska-Stępień, Tadeusz Pietras, Jadwiga Kroczyńska-Bednarek, Zofia Kurmanowska, Adam Antczak, Paweł Górski

Department of Allergology and Pulmonary Diseases, Medical University of Lodz, Łódź, Poland

KEY WORDS

chronic obstructive pulmonary disease, leptin, resistin

ABSTRACT

INTRODUCTION Chronic obstructive pulmonary disease (COPD) is the most common chronic lung disease in the world. The increasing severity of inflammatory processes in the respiratory tract leads to exacerbation of COPD. This process may be associated with changes in the synthesis of adipokines, the peptides that participate in immune processes.

OBJECTIVES The aim of this study was to identify more sensitive and specific laboratory markers useful in diagnosing inflammatory processes in patients with COPD.

PATIENTS AND METHODS The study involved 33 patients with COPD without exacerbation. During the previous year, 1 episode of exacerbation was reported in 15 patients and no exacerbations were reported in the remaining 18 patients. Serum concentrations of adipokines were measured using an enzyme-linked immunosorbent assay (ELISA).

RESULTS In patients with COPD, we observed a 2-fold increase in leptin levels compared with healthy controls (18.8 ±10.2 ng/ml vs. 9.06 ±4.33 ng/ml; $P = 0.042$). Mean resistin levels in these patients were also 2-fold higher than those in controls (8.24 ±4.18 ng/ml vs. 3.58 ±1.51 ng/ml, respectively; $P = 0.027$). Significant positive correlations between C-reactive protein (CRP) and leptin as well as CRP and resistin levels were observed in patients with COPD ($r = 0.75$ and $r = 0.83$, respectively; $P < 0.05$). Moreover, a statistically significant negative correlation between the forced expiratory volume in 1 second (FEV_1) and resistin was noted in this group ($r = 0.62$; $P < 0.05$). There was no correlation between FEV_1 and leptin levels either in patients with COPD or in healthy controls.

CONCLUSIONS A significant increase in leptin and resistin levels in patients with COPD may suggest that these adipokines are involved in the inflammatory process underlying the disease.

Correspondence to:
Anna Kumor-Kisielewska, MD, PhD, Klinika Pneumonologii i Alergologii, Uniwersytet Medyczny w Łodzi, ul. Kopcińskiego 22, 90-153 Łódź, Poland, phone: +48-42-677-69-26, fax: +48-42-678-76-07, e-mail: anna_kumor@wp.pl
Received: February 2, 2013.
Revision accepted: April 15, 2013.
Published online: April 24, 2013.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2013; 123 (5): 215-220

INTRODUCTION Chronic obstructive pulmonary disease (COPD) is one of the most frequent chronic lung diseases worldwide. In Poland, at least 10% of the subjects above 40 years of age are diagnosed with COPD.[1]

The disease is characterized by progressive restriction of air flow through the respiratory tract, which is associated with the pathology of the small airways and damage to the pulmonary interstitium (emphysema), varying in severity, which leads to impairment of systemic gas exchange. The process of interstitial damage typically follows an inflammatory reaction that is associated with the pathological response of the respiratory tract to harmful dusts and substances that patients are exposed to during their life.[2,3] The changes affect primarily the terminal portions of the airways and the pulmonary blood vessels. They present as a characteristic inflammatory condition with increased counts of neutrophils in the airway lumen, macrophages in the lumen and airway walls as well as in the pulmonary parenchyma, and $CD8^+$ T lymphocytes in the airway lumen and pulmonary parenchyma (the count of $CD4^+$ T lymphocytes is also increased, but to a lower extent than that of $CD8^+$;

an increase in the CD8+/CD4+ ratio is observed). During exacerbations, increased counts of eosinophils are also observed in the sputum and airway walls, and in the case of a simultaneous infection – also of B lymphocytes.[4,5]

The above inflammatory cells cause direct damage to the pulmonary tissue. Additionally, they produce mediators enhancing the inflammatory response, increasing the migration of inflammatory cells from the bloodstream and causing structural remodeling of the surrounding tissues. The mediators involved in the pathogenesis of COPD include proinflammatory cytokines such as interleukin (IL) 6, tumor necrosis factor-α (TNF-α), and IL-17.[6]

The increasing severity of inflammatory processes in the respiratory tract is associated with exacerbation of COPD. This, in turn, is manifested by impairment of the patient's ventilation, which triggers the pathway leading to hypercatabolism, a condition closely associated with the mechanism of cachexia, commonly observed in patients with COPD exacerbations. The pathologic changes involve excessive weight loss with the consequent reduction of the adipose tissue, which is one of the most important endocrine systems in the human body. It is responsible for the synthesis of adipokines, the peptides that play an important role in systemic metabolism. They demonstrate a regulatory effect mainly on lipid metabolism and immune processes.[7,8]

One of adipokines, discovered recently but relatively well-known, is leptin, characterized by multidirectional effects both within the central nervous system and in the periphery. Leptin synthesis determines the appropriate expenditure of energy, regulates many basic life processes such as pubescence and reproductive functions. It is also an important factor involved in angiopoiesis, modulation of the production of cytokines by T-helper (Th) lymphocytes, and regulation of the cell cycle.[9,10] The serum levels of this polypeptide correlate with the systemic adipose tissue mass. The involvement of leptin in the regulation of the hypothalamus–pituitary–adrenal axis and its similarity to the cytokine family may suggest the role of that hormone in acute-phase processes.[11] Recent studies have demonstrated possible immune effects of leptin, exerted by the stimulation of immunocompetent cells. It is particularly significant that the hormone is synthesized by the bone marrow stromal cells and that it stimulates the growth of hematopoietic cells. Leptin can also activate the synthesis of proinflammatory cytokines, such as IL-2, interferon-γ, by Th1 and inhibit the synthesis of cytokines such as IL-4 by Th2. Administration of leptin has been demonstrated to reduce an immunosuppressive effect of this hormone in fasting subjects.[12]

Resistin is a newly identified class of cysteine-ich proteins collectively termed resistin-like molecules. Significant expression of resistin in humans is mainly found in mononuclear leukocytes, macrophages, spleen, and bone marrow cells. Increasing evidence indicates that resistin plays an important regulatory role in insulin resistance and in a variety of pathological processes including cardiovascular disease, autoimmune disease, malignancy, asthma, or inflammatory bowel disease. Some proinflammatory markers, such as TNF-α or IL-6 can regulate resistin gene expression.[13] Other evidence linking resistin to inflammation is that plasma resistin levels were found to be associated with numerous inflammatory agents in a number of pathophysiological conditions. According to one study, individuals with clinical signs of severe inflammation showed significantly higher concentrations of resistin compared with healthy individuals.[13,14]

The aim of the current study was to identify more sensitive and specific laboratory markers useful in diagnosing inflammatory processes, especially in COPD patients with uncertain prognosis. Therefore, we decided to assess changes in the concentrations of adipocytokines, leptin and resistin, with the hope that they may become new markers useful in the monitoring of COPD.

PATIENTS AND METHODS

Patients The study was conducted in 33 patients with COPD, aged from 50 to 87 years (mean, 67 ±11 years), treated in the Department of Allergology and Pulmonary Diseases at the Medical University of Lodz, Łódź, Poland, and later at an outpatient clinic. COPD was confirmed based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) document of 2010. During the previous year, 1 episode of exacerbation was reported in 15 patients and no exacerbations were reported in the remaining 18 patients. Exacerbations in the course of this study were excluded in all patients on the basis of laboratory tests (blood cell count with smear, C-reactive protein [CRP]) and clinical presentation (absence of dyspnea, normal body temperature, no green sputum expectoration). Lung tumors were excluded by radiological modalities (X-ray, computed tomography). All patients were ex-smokers. In the study group, smoking history was between 25 and 40 years and the mean number of the packs of cigarettes was 25 pack per year.

The control group consisted of 17 age-matched healthy volunteers.

The study protocol was approved by the local ethics committee (approval no. RNN/4/12/KE).

Methods The analysis of data obtained from the study groups was conducted using the STATISTICA v. 10 software (StatSoft, Inc., United States). The characteristics were assessed using the *t* test (parametric variables) or the Mann-Whitney test (nonparametric distribution). Correlations between variables were evaluated by the Pearson correlation test. The results were considered to be statistically significant at a P value of less than 0.05.

Anthropometry In all patients, body weight and height were measured to calculate the body mass

TABLE 1 Anthropometric parameters in the study groups

Parameter	COPD patients n = 33	Controls n = 17
women, n (%)	13 (39)	8 (47)
men, n (%)	20 (61)	9 (53)
age, y	67 ±11	65 ±14
age, women, y	65 ±10	62 ±13
age, men, y	69 ±9	68 ±15
weight, kg	75.2 ±22.5	81.2 ±14.6
BMI, kg/m^2	26.8 ±6.90	24.18 ±4.58
WHR	0.92 ±0.09	0.94 ±0.07

Abbreviations: BMI – body mass index, COPD – chronic obstructive pulmonary disease, WHR – waist-to-hip ratio

index (BMI), and waist and hip circumference to calculate the waist-to-hip ratio (WHR).

Gasometry Gasometry was performed in the arterial blood. Patients were prepared according to the required standards, and the test was performed using a Corning 348 blood gas analyzer (Ciba Corning, United States).

Biochemical investigations Serum CRP levels were measured using a turbidimetric method (OLYMPUS AU 400 biochemical analyzer, Olympus, United States). Leptin and resistin levels were measured by an enzyme-linked immunosorbent assay (Quantikine Human Leptin and Quantikine Human Resistin kits, R&D, United States).

Spirometry Spirometry was performed in clinically stable patients without the signs of infection using LUNGTEST 1000 spirometer. If long-acting β-agonists were administered, they were withdrawn 12 hours before the test, while if short-acting β_2-agonists were administered they were withdrawn 6 hours before the test. Spirometry was performed after a minimum 15-minute rest.

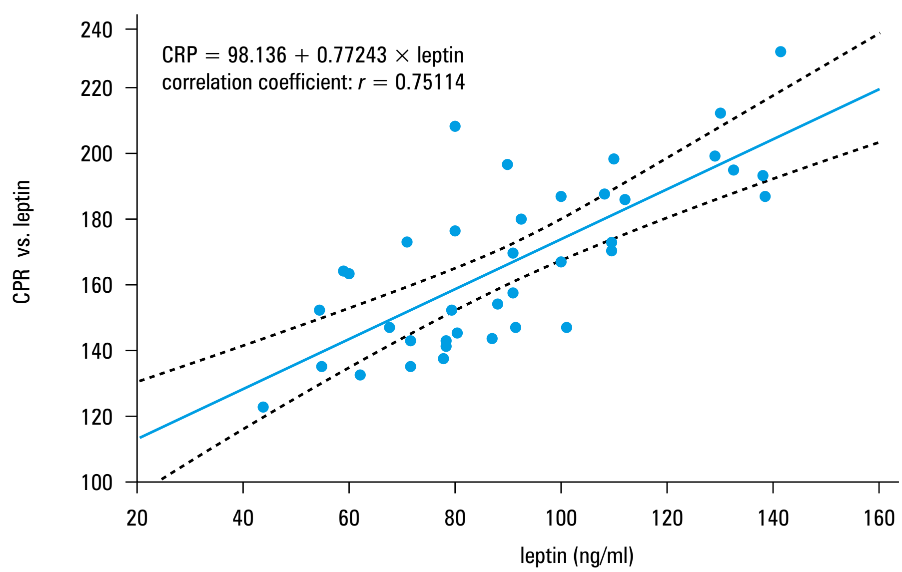
RESULTS Patients with COPD had a significant 2-fold increase in leptin levels compared with healthy controls (18.8 ±10.2 ng/ml vs. 9.06 ±4.33 ng/ml, respectively; $P = 0.042$). Of note, no correlations were observed between leptin levels and the BMI in patients with COPD ($r = 0.32$; $P < 0.05$). A similar trend was observed for resistin: its mean levels in patients with COPD were twice higher than those in the control group (8.24 ±4.18 ng/ml vs. 3.58 ±1.51 ng/ml, respectively). No correlation between resistin levels and the BMI was noted ($r = 0.28$; $P < 0.05$). No significant differences in the BMI and WHR between the 2 groups were observed (**TABLE 1**).

Considering the above findings, we decided to investigate the correlation of leptin and resistin with CRP, which is a commonly accepted inflammatory marker. We observed an increase in CRP levels in patients with CRP but it was not statistically significant when compared with the control group (6.47 ±5.03 ng/ml vs. 3.40 ±1.99 ng/ml, respectively; $P = 0.064$). Significant positive correlations between CRP and leptin as well as CRP and resistin levels were observed in patients with COPD ($r = 0.75$ and $r = 0.83$, respectively, $P < 0.05$; **FIGURES 1** and **2**). No such correlation was noted in the control group.

We also evaluated correlations between leptin and resistin levels and respiratory function parameters. No correlations were observed between adipokines and oxygen pressure in any of the groups. However, a significant negative correlation between the forced expiratory volume in 1 second (FEV_1) and resistin levels was noted in the group of patients with COPD ($r = 0.62$; $P < 0.05$) (**FIGURE 3**). The values of FEV_1 in the study groups are presented in **TABLE 2**. FEV_1 values did not correlate with leptin levels either in patients with COPD or in controls.

DISCUSSION The aim of our study was to assess whether adipokines, leptin and resistin, can be useful as markers of the inflammatory process in

FIGURE 1 Correlation between C-reactive protein (CRP) and leptin in patients with chronic obstructive pulmonary disease



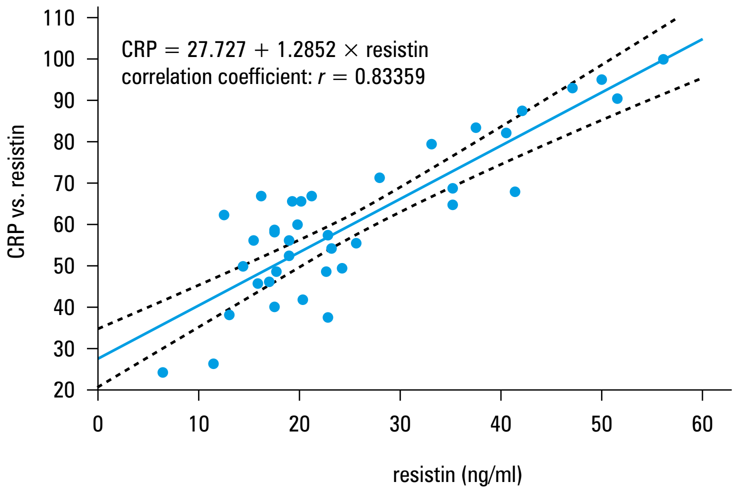


FIGURE 2 Correlation between resistin and C-reactive protein (CRP) in patients with chronic obstructive pulmonary disease

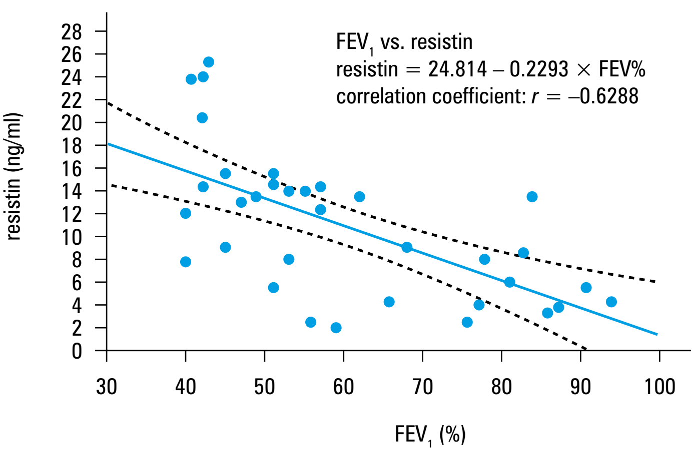


FIGURE 3 Correlation between forced expiratory volume in 1 second (FEV_1) and resistin in patients with chronic obstructive pulmonary disease

TABLE 2 Spirometric parameters in the study groups

Parameter	COPD patients	Controls
$\%FEV_1$	48.60 ±11.3	77.63 ±8.15
FEV_1	1.29 ±0.09	2.71 ±0.66

Abbreviations: FEV_1 – forced expiratory volume in 1 second, others – see TABLE 1

COPD. Our results confirmed this hypothesis because the levels of both adipokines were significantly higher and demonstrated a positive correlation with CRP, which is a commonly recognized acute-phase protein. Numerous studies have shown that inflammatory markers are independently associated with circulating leptin and resistin levels in patients with a chronic inflammatory process.[15,16] CRP, a marker of inflammation, was reported to be positively correlated with resistin levels in several pathophysiological conditions.[17] This finding may support the involvement of adipokines in the inflammatory process, which has already been confirmed by a number of studies.[15-17] Moreover, our study confirmed a strong positive correlation between CRP and leptin and CRP and resistin levels.

However, it should be emphasized that our study investigated a full-blown inflammatory process as one of only few papers addressing the changes in leptin and resistin levels in COPD.[18,19] Poulain et al.[20] also observed elevated leptin concentrations in patients with COPD; however, in contrast to our results, these changes demonstrated a positive correlation with the BMI. Different results were reported by Yang et al.,[21] who observed significantly lower leptin levels in patients with COPD than in control subjects, especially in the subgroup of patients with cachexia. Of note, our patients showed no signs of cachexia and had similar BMI values to those in controls. This is important because the changes of leptin and resistin levels are dependent on the adipose tissue. As a result, the process of cachexia may be associated with the decreased levels of adipokines.

Similarly to leptin, we obtained different results for resistin compared with the data presented in the literature. In particular, decreased resistin levels were associated with decreased BMI.[20,21] In contrast, Breyer et al.[22] observed no changes in leptin and resistin levels in patients with COPD and the obtained values were comparable to those in the control group.[22]

The role of leptin and resistin as inflammatory markers can be confirmed by a positive correlation with CRP, which was also shown in the study of COPD patients by van de Borst et al.[23] In addition, a positive correlation between resistin levels and FEV_1, which coincides with a positive correlation between resistin levels and CRP, may indicate the association between resistin levels and the severity of COPD.

In conclusion, a significant increase in leptin and resistin levels observed in patients with COPD may suggest that these adipokines are involved in the inflammatory process underlying the disease.

Acknowledgments These studies were supported by grant KBN no N N402 188135.

REFERENCES

1 Nizankowska-Mogilnicka E, Mejza F, Buist A, et al. Prevalence of COPD and tobacco smoking in Malopolska region - results from the BOLD Study in Poland. Pol Arch Med Wewn. 2007; 117: 402-410.

2 Jahnz-Różyk K, Targowski T, From S, et al. [Costs of chronic obstructive pulmonary disease in patients treated in ambulatory care in Poland]. Pneumonol Alergol Pol. 2011; 79: 337-342. Polish.

3 Boros PW, Lubiński W. Health state and the quality of life in patients with chronic obstructive pulmonary disease in Poland: a study using the EuroQoL-5D questionnaire. Pol Arch Med Wewn. 2012; 122: 73-81.

4 Fischer BM, Pavlisko E, Voynow JA. Pathogenic triad in COPD: oxidative stress, protease-antiprotease inbalance, and inflammation. Int J Chron Obstruct Pulmon Dis. 2011; 6: 413-421.

5 Tilemann L, Gindner L, Meyer F, et al. Differences in local and systemic inflammatory markers in patients with obstructive airways disease. Prim Care Respir J. 2011; 20: 407-414.

6 Langereis JD, Schweizer RC, Lammers JW, et al. A unique protein profile of peripheral neutrophils from COPD patients does not reflect cytokine-induced protein profiles of neutrophils in vitro. BMC Pulm Med. 2011; 11: 44.

7 Lainscak M, Von Haehling S, Doehner W, et al. Body mass index and prognosis in patients hospitalized with acute exacerbation of chronic obstructive pulmonary disease. J Cachexia Sarcopenia Muscle. 2011; 2: 81-86.

8 Sood A. Obesity, adipokine, and lung disease. J Appl Physiol. 2010; 108: 744-753.

9 Friedman JM. Obesity in the new millennium. Nature. 2000; 404: 632-634.

10 Unger RH. Leptin physiology: a second look. Regul Pept. 2000; 92: 87-95.

11 Martin SS, Qasim A, Reilly MP. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. J Am Coll Cardiol. 2008; 52: 1201-1210.

12 Kolasa-Trela R, Miszalski-Jamka T, Grudzień E, et al. Adiponectin, leptin, and resistin in patients with aortic stenosis without concomitant atherosclerotic vascular disease. Pol Arch Med Wewn. 2011; 121: 352-360.

13 Larochelle J, Freiler J, Dice J, et al. Plasma resistin levels in asthmatic as a marker of disease state. J Asthma. 2007; 44: 509-513.

14 Fang C, Meng Q, Wu H, et al. Resistin-like molecule-β is a human airway remodeling mediator. Eur Respir J. 2012; 39: 458-466.

15 Vondracek SF, Voelkel NF, McDermott MT, Valdez C. The relationship between adipokines, body composition, and bone density in men with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2009; 4: 267-277.

16 Ehtesham NZ, Nasiruddin M, Alvi A, et al. Treatment and point determinants for pulmonary tuberculosis: human resistin as a surrogate biomarker. Tuberculosis (Edinb). 2011; 91: 293-299.

17 Lacoma A, Prat C, Andreo F, et al. Value of procalcitonin, C-reactive protein, and neopterin in exacerbations of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2011; 6: 157-169.

18 Kythreotis P, Kokkini A, Aygeropoulou S, et al. Plasma leptin and insulin-like growth factor I levels during acute exacerbations of chronic obstructive pulmonary disease. BMC Pulm Med. 2009; 5: 9-11.

19 Al Mutairi SS, Mojiminiyi OA, Shihab-Eldeen A, et al. Putative roles of circulating resistin in patients with asthma, COPD, and cigarette smokers. Dis Markers. 2011; 31: 1-7.

20 Poulain M., Doucet M., Drapeau V, et al. Metabolic and inflammatory profile in obese patients with chronic obstructive pulmonary disease. Chron Respir Dis. 2008; 5: 35-41.

21 Yang YM, Sun TY, Liu XM. The role of serum leptin and tumor necrosis factor-alpha in malnutrition of male chronic obstructive pulmonary disease patients. Chin Med J (Engl). 2006; 119: 628-633.

22 Breyer MK, Rutten EP, Vernooy JH, et al. Gender differences in the adipose secretome system in chronic obstructive pulmonary disease (COPD): a pivotal role of leptin. Respir Med. 2011; 105: 1046-1053.

23 van den Borst B, Gosker HR, Wesseling G, et al. Low-grade adipose tissue inflammation in patients with mild-to-moderate chronic obstructive pulmonary disease. Am J Clin Nutr. 2011; 94: 1504-1512.

ARTYKUŁ ORYGINALNY

Ocena stężeń leptyny i rezystyny u pacjentów chorych na przewlekłą obturacyjną chorobę płuc

Anna Kumor-Kisielewska, Dorota Kierszniewska-Stępień, Tadeusz Pietras, Jadwiga Kroczyńska-Bednarek, Zofia Kurmanowska, Adam Antczak, Paweł Górski

Klinika Pneumonologii i Alergologii, Uniwersytet Medyczny w Łodzi, Łódź

SŁOWA KLUCZOWE

leptyna, przewlekła obturacyjna choroba płuc, rezystyn

STRESZCZENIE

WPROWADZENIE Przewlekła obturacyjna choroba płuc (POChP) jest najczęstszą przewlekłą chorobą płuc na świecie. Nasilenie procesów zapalnych w obrębie dróg oddechowych prowadzi do zaostrzenia POChp. Proces ten może być związany ze zmianami syntezy adipohormonów, które odgrywają ważną rolę w procesach odpornościowych.

CELE Celem prowadzonych badań jest znalezienie bardziej czułych i specyficznych swoistych markerów laboratoryjnych pozwalających ocenić nasilenie procesów zapalnych u pacjentów chorych na POChP.

PACJENCI I METODY Badania przeprowadzono w grupie 33 chorych na POChP, bez cech zaostrzenia choroby. 15 chorych miało 1 incydent zaostrzenia choroby w ciągu ostatniego roku, natomiast u pozostałych 18 pacjentów nie zaobserwowano zaostrzeń w tym czasie. Stężenie adipohormonów w surowicy oceniane było z wykorzystaniem metody kanapkowej ELISA.

WYNIKI U chorych na POChP zaobserwowano dwukrotny wzrost stężenia leptyny w porównaniu z grupą kontrolną (18,8 ± 10,2 *vs* 9,06 ±4,33 ng/ml; p = 0,042). Średnie stężenie rezystyny u tych chorych było dwukrotnie wyższe niż stężenie w grupie kontrolnej (8,24 ± 4,18 ng/ml *vs* 3,58 ± 1,51ng/ml, odpowiednio; p = 0,027). Stwierdzono statystycznie znamienną dodatnią korelację pomiędzy stężeniami białka C-reaktywnego (*C-reactive protein* – CRP) i leptyny jak również CRP i rezystyny w grupie chorych na POChP (r = 0,75 oraz r = 0,83, p <0,05; odpowiednio). Ponadto stwierdzono ujemną korelację w tej grupie między wartością nasilonej 1-sekundowej objętości wydechowej (*forced expiratory volume in 1 second* – FEV_1) i rezystyną (r = 0,62; p <0,05). Nie stwierdzono korelacji między wartościami FEV_1 i stężeniami leptyny zarówno w grupie chorych jak i w grupie kontrolnej.

WNIOSKI Znaczny wzrost stężenia leptyny i rezystyny obserwowany u pacjentów z POChP może sugerować rolę tych adipohormonów w procesie zapalnym leżącym u podłoża tej choroby.

Adres do korespondencji:
dr n. med. Anna Kumor-Kisielewska, Klinika Pneumonologii i Alergologii, Uniwersytet Medyczny w Łodzi, ul. Kopcińskiego 22, 90-153 Łódź, tel.: 42-678-76-07, fax: 42-677-69-26, e-mail: anna_kumor@wp.pl
Praca wpłynęła: 02.02.2013.
Przyjęta do druku: 15.04.2013.
Publikacja *online*: 24.04.2013.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2013; 123 (5): 215-220