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Circulating Plasma Ghrelin and Obestatin Levels Are Low in Chronic Obstructive Pulmonary Disease Patients with Pulmonary Artery Hypertension

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Abstract: *Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor, was firstly identified from rat stomach and cloned in rats and humans. Recently, ghrelin has been shown to participate in cardiovascular and sympathic regulations and play a useful role in some cardiovascular diseases including heart failure, myocardial infarction and pulmonary hypertension. We investigated the fasting plasma ghrelin and obestatin levels and their relationships between them and some other hemodyanmic parameters in chronic obstructive pulmonary disease (COPD) patients with PAH. We found that fasting plasma ghrelin and obestatin levels were significantly lower in PAH group compared with those of the control group (253.8 ± 2.2 pg/ml vs 266.1 ± 2.9 pg/ml; 21.3 ± 0.2 pg/ml vs 22.4 ± 0.3 pg/ml; respectively). In a multiple regression model analysis, only insulin was an independent predictor of fasting ghrelin in COPD patients with PAH (standardized coefficient = 0.555, P = 0.021). We thought that there was imbalance expression of ghrelin and obestatin in COPD patients with PAH and ghrelin and obestatin might play a role in the pathophysiologic progress of COPD patients with PAH.*

Keywords: *ghrelin; obestatin; chronic obstructive pulmonary disease; pulmonary artery hypertension*

1. INTRODUCTION

Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor, was firstly identified from rat stomach and cloned in rats and humans [1]. Subsequently, many studies have suggested that ghrelin has a multiplicity of functions such as stimulate appetite and initiate food intake, modulate energy metabolism, control gastric motility [2]. Recently, ghrelin has been shown to participate in cardiovascular and sympathic regulations and play a useful role in some cardiovascular diseases including heart failure and myocardial infarction [3]. Schwenke DO *et al.* found that exogenous ghrelin was able to attenuate the magnitude of pulmonary arterial hypertension (PAH), pulmonary vascular remodeling and right ventricular hypertrophy in conscious rats [4]. We also found that circulating plasma ghrelin level was negatively correlated with pulmonary arterial pressure in atrial septal defect (ASD) patients [5]. These results suggest that ghrelin might play a role in the pathophysiologic progress of PAH.

In 2005, Zhang *et al.* firstly reported that they found a ghrelin-associated peptide, encoded by the same gene as ghrelin, and named it obestatin [6]. Obestatin, although derived from the same peptide precursor as ghrelin, displayed some reverse effects and antagonized the actions of ghrelin when both peptides were coadministered [6]. Recently, Anderwald-Stadler *et al.* found that plasma obestatin was negatively correlated with systolic blood pressure in human and we also found that plasma ghrelin and obestatin levels were correlated with blood pressure in rats and humans [7, 8, 9]. In 2013, we found that fasting plasma obestatin level was higher in ASD patients with PAH compared with ASD patients without PAH [5]. Based on the above results, we thought that obestatin also might play a role in the cardiovascular regulation in rats and humans and that the disturbances of both ghrelin and obestatin in

the circulation of patients could develop some diseases.

To the best of our knowledge, plasma ghrelin and obestatin levels and the correlationships between them and some other hemodynamic parameters in COPD patients with PAH have not been explored. The aim of this study was to investigate the fasting plasma ghrelin and obestatin levels and their relationships between them and some other hemodynamic parameters in COPD patients with PAH.

2. METHODS

2.1. Subjects

Forty patients, ranging in age from 55 to 84 years old (mean age: 69.6±1.6 years), were recruited from those who were diagnosed COPD between Octobert 2013 and February 2014. All patients had no evidence of liver or kidney diseases based on the laboratory examinations. These COPD patients were taken to the ultrasound department for echocardiography according to the guideline of ESC [10]. The estimation of systolic pulmonary arterial pressure (PASP) is based on the peak velocity of the jet of tricuspid regurgitation [10]. Based on the results of echocardiography, the patients were divided into two groups: twenty-two patients were enrolled in the PAH group (PASP>50mmHg), the rest seventeen patients were enrolled in the control group (PASP<36mmHg).

2.2. Anthropometry

Height and weight were measured according to standard techniques in each subject [11]. Body mass index (BMI) was calculated.

2.3. Echocardiography

All the patients were examined at rest in the supine position or left lateral decubitus position. A complete M-mode and 2D echocardiography study was performed by a senior sonographer using a standardized protocol on the same machine (Vivid 7, Vingmed-General Electric, Horten, Norway) according to the American Society of Echocardiography guidelines [12]. The left ventricular contractility (fractional shortening, FS and ejection fraction, EF) was measured by M-mode echocardiography performed from the parasternal long-axis view.

2.4. Protocol and Methods

Blood samples were taken from the antecubital vein in the morning between 7:00 AM and 8:00 AM after an overnight fast, because plasma ghrelin and obestatin levels have been shown to be altered by food intake [13, 6]. The blood was immediately transferred into a chilled glass tube containing EDTA-2Na (1 mg/ml) and aprotinin (Phoenix Pharmaceuticals, Belmont, CA; 100μl containing 0.6 trypsin inhibitor units per milliliter of blood), then immediately centrifuged at 1600×g for 15 min at 4°C. Plasma samples were frozen and stored at −80°C until assayed. The fasting blood level of glucose was measured in the clinical laboratory of our hospital. The plasma glucose levels were measured by an automated glucose oxidase method (Automatic Analyzer 7600-020; Hitachi, Tokyo, Japan).

2.5. Hormone Assay

Plasma ghrelin, obestatin, and insulin levels were measured by enzyme linked immunosorbent assay (ELISA) method according to the manufacturer's instructions. The reagent kits were manufactured by R& D Systems (Minneapolis, USA). Insulin resistance was calculated by the homeostasis model of assessment for insulin resistance (HOMA-IR) approach, calculated as fasting insulin (micro units per mil-liter) × fasting blood glucose (mill moles per liter)/22.5 [14].

2.6. Statistical Analysis

Data are expressed as the mean ± SE. Comparisons between the parameters of patients with PAH and those of patients with normal PAP were performed with unpaired Student's *t* test. The relationships between ghrelin, obestatin, PASP, PADP, mPAP, insulin, and various clinical parameters were examined by bivariate correlations (Pearson's correlation coefficient). Multiple regression analysis was further used to assess the relationships between mPAP, ghrelin, obestatin, iusulin, and clinical variables. A value of $P < 0.05$ was considered significant. All of the analyses were performed using SPSS for windows (version 10.0; SPSS Inc., Chicago, IL).

3. RESULTS

3.1. Subjects (Table 1)

Table 1 depicts the characteristics of the study population. There were no significant differences in age, BMI, and glucose between the PAH group and the control group. The PAH group had higher EF and insulin level compared with that of the control group. However, the insulin resistance as calculated by HOMA-IR approach was not found to show significant difference between the PAH group and the control group.

Table1. *Characterization of the subjects included in the study.*

Parameters	PAH group	Control group	*P* value
Number	20	20	
Age(y)	69.1±2.2	70.3±2.4	0.72
BMI(Kg/m^2)	20.1±0.7	20.4±1.1	0.80
EF (%)	59.8±1.4	64.5±1.5	0.03
Fasting glucose(mmol/liter)	5.5±0.23	5.1±0.18	0.19
Fasting insulin(μIu/ml)	10.3±0.14	11.0±0.14	0.002
HOMA-IR	2.49±0.11	2.47±0.10	0.88

Data are means ± SE and P <0.05 was regarded as statistically significant.

3.2. Differences in Ghrelin, Obestatin, and Ghrelin to Obestatin Ratio at Fasting (Fig. 1)

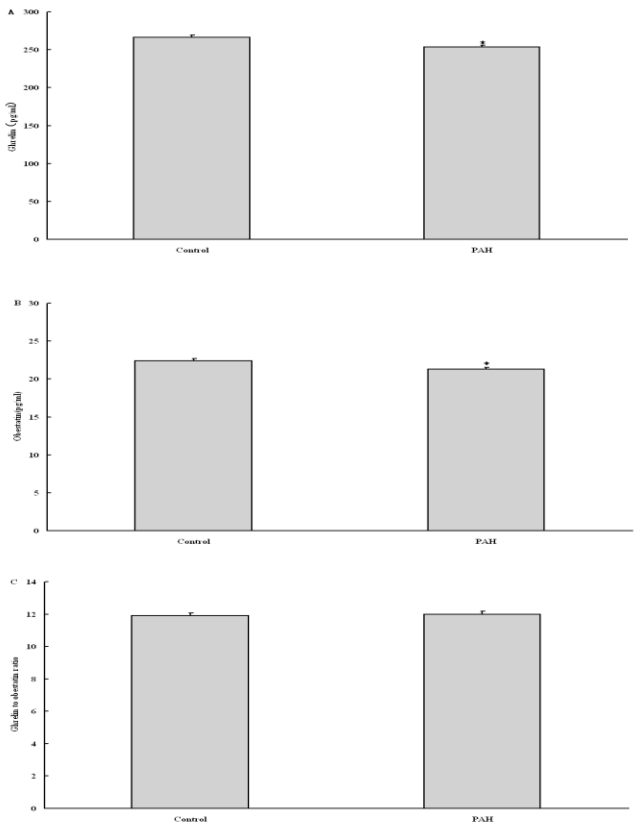

Figure1. *Fasting plasma ghrelin levels (A), obestatin levels (B), ghrelin to obestatin ratio (C) in PAH group and control group.* $^*P<0.05$ *compared with control group.*

The fasting plasma concentration of ghrelin was 253.8 ± 2.2 pg/ml and 266.1 ± 2.9 pg/ml in PAH group and control group, respectively, and there was a significant difference between them (P<0.05). The fasting plasma concentration of obestatin was 21.3 ± 0.2 pg/ml and 22.4 ± 0.3 pg/ml in PAH group and control group, respectively, and the difference between them was also significant (P<0.05). However, the ratio of ghrelin to obestatin was found no significant difference in PAH group compared with control group (12.0 ± 0.2 vs 11.9 ± 0.2, P>0.05).

3.3. Correlations of Ghrelin, Obestatin, and Ghrelin to Obestatin Ratio with Various Parameters (Table 2)

Fasting glucose, obestatin, EF, BMI, and age were not found to be correlated with ghrelin in the PAH group. In the control group, fasting ghrelin was found to be positively correlated with insulin. Based on the simple correlation coefficients, the relationships between ghrelin, obestatin, and related variables in the control group were further assessed in multiple regression models. In a multiple regression model including fasting obestatin, insulin, HOMA-IR, EF, and BMI, only insulin was an independent predictor of fasting ghrelin (standardized coefficient = 0.555, $P = 0.021$), whereas other parameters did not show significant correlations with ghrelin.

Fasting insulin, obestatin, EF, and ghrelin to obestatin ratio were found to be positively correlated with ghrelin in the study population. In a multiple regression model including age, fasting glucose, insulin, obestatin, HOMA-IR, EF, ghrelin to obestatin ratio, and BMI, ghrelin to obestatin ratio,obestatin, and BMI were found to be independent predictors of ghrelin (standardized coefficient =1.278, P <0.001; standardized coefficient =1.231, P <0.001; standardized coefficient = 0.023, P = 0.048; respectively). In another multiple regression model including age, fasting glucose, insulin, ghrelin, HOMA-IR, EF, ghrelin to obestatin ratio, and BMI, ghrelin to obestatin ratio, ghrelin, and BMI were found to be independent predictors of obestatin (standardized coefficient =-1.037, P <0.001; standardized coefficient = 0.809, P <0.001; standardized coefficient = -0.018, P = 0.049; respectively).

Table2. *Correlations of ghrelin, obestatin, and ghrelin to obestatin ratio with various parameters in the study population.*

Parameters	Fasting Ghrelin		Fasting Obestatin		Ghrelin to obestatin ratio	
	r	p	r	p	r	p
Age	0.158	0.335	0.152	0.355	-0.007	0.968
BMI	0.136	0.408	-0.158	0.337	0.241	0.140
Fasting glucose	-0.070	0.673	-0.305	0.059	0.224	0.171
Fasting insulin	0.484	0.002	0.570	<0.001	-0.169	0.303
HOMA-IR	0.111	0.501	-0.106	0.520	0.174	0.290
EF	0.328	0.042	0.181	0.271	0.083	0.616
Fasting ghrelin	1		0.351	0.029	0.439	0.005
Fasting obestatin	0.351	0.029	1		-0.686	<0.001
Ghrelin to obestatin	0.439	0.005	-0.686	<0.001	1	

4. DISCUSSION

The aim of this study was to investigate the plasma ghrelin and obestatin levels and their relationships between them and some clinical parameters in COPD patients with PAH. To the best of our knowledge, we are the first to explore the relationships between ghrelin, obestatin, and clinical parameters in COPD patients with or without PAH.

In this study, we found that fasting plasma ghrelin and obestatin levels were significantly lower in PAH group compared with those of the control group. This suggests that the imbalance expression of both ghrelin and obestatin was also existed in COPD with PAH patients and might play a role in the pathophysilogical process in COPD patients with PAH. In this study, we also found that the ratio of ghrelin to obestatin was lower in PAH group compared with that of the control group, however, the difference between them was not found to be significant. In our previous study, we found that fasting plasma ghrelin level was significantly lower in patients with hypertension compared with that of the patients with normal blood pressure [9]. In 2013, we also found that fasting plasma ghrelin level and the ratio of ghrelin to obestatin were significantly lower in PAH group compared with those of the control group in atrial septal defect (ASD) patients [5]. However, fasting plasma ghrelin and obestatin levels were significantly higher in spontaneously hypertensive rats compared with those of the Wistar-Kyoto rats [8]. Based on the small amount of available data at present, the differences among the above results may be due to the different cohorts enrolled.

In this study, we also explored the relationships between ghrelin and obestatin and some clinical parameters. We found that insulin, obestatin, and EF were positively correlated with ghrelin in the

study population. Moreover, ghrelin to obestatin ratio and BMI were also found to be independent predictors of ghrelin and obestatin in the study population in a multiple regression model analysis. The above results could be explained by the fact of both ghrelin and obestatin deriving from the same gene to some degree.

In this study, we found that fasting insulin level was significantly higher in PAH group compared with that of the control group. This is in accordance with the previous study of Zamanian et al in 2008, which revealed that insulin resistance was more prevalent in patients with PAH [15]. However, the insulin resistance as calculated by HOMA-IR approach was not found to show significant difference between the PAH group and the control group.

There were several limitations in this study. First, we measured the total plasma ghrelin level in humans. As we know, there are two kinds of ghrelin, des-acyl ghrelin and acyl ghrelin, and des-acyl ghrelin constitutes most circulating ghrelin and mediates no effects. If we measured the concentration of the two kinds of ghrelin in fasting plasma, we might find more information about ghrelin and clinical hemodynamic parameters. Second, the results of this study should be tested in a larger sample of patients where subgroup comparisons could be allowed (i.e. female vs male, different levels of PAP). Third , the pulmonary arterial pressure level of the COPD patients in this study were estimated by echocardiography method and the patients were simply divided into two groups based on the results of echocardiography examination. If right heart catheterization examination was used to estimate the pulmonary arterial pressure level accurately in this study, we might find more information between ghrelin, obestatin and some other clinical hemodynamic parameters. Finally, the results of this study should be tested in a larger sample of patients where subgroup comparisons could be allowed (i.e. female vs male, different levels of PAP).

To our knowledge, this is the first study to explore the expression of ghrelin and obestatin and the relationships between them and some other clinical parameters. The results of this study suggested that there was imbalance expression of ghrelin and obestatin in COPD patients with PAH. Future studies to confirm or refute our initial results are eagerly anticipated.

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