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Systemic Inflammation and Ghrelin Levels in Chronic Obstructive Pulmonary Disease Patients with and without Pulmonary Hypertension

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Pulmonary hypertension (PH) is a common complication of chronic obstructive pulmonary disease (COPD). Ghrelin is an orexigenic hormone and endogenous vasodilatory peptide; however, no data exists on the potential role of ghrelin in PH secondary to COPD. It was aimed to determine serum ghrelin, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) levels in underweight and normal weight COPD patients with and without PH. Serum ghrelin, IL-6, and TNF-α levels were measured in underweight and normal weight 60 COPD patients with and without PH and in 15 healthy control subjects. IL-6, TNF-α and serum ghrelin levels were assessed by enzyme-linked immunosorbent assays (ELISA). Pulmonary artery pressure levels were measured by echocardiography. Serum IL-6 and TNF-α levels were significantly higher and ghrelin levels were significantly lower in COPD patients with or without PH compared to healthy control subjects. Serum ghrelin levels were statistically lower and IL-6 levels were higher in underweight COPD patients with PH than in underweight COPD patients without PH. No differences existed in serum ghrelin levels between the normal weight COPD patients with or without PH. Serum ghrelin levels were decreased in COPD patients with or without PH, particularly in underweight COPD patients with PH. It was concluded that ghrelin has a potential role in the development of PH, especially in underweight COPD patients.

***Key Words**: Ghrelin, pulmonary hypertension, chronic obstructive pulmonary disease, interleukin-6, tumor necrosis factor-alpha.*

Pulmoner Hipertansiyon Gelişen ve Gelişmeyen Kronik Obstrüktif Akciğer Hastalıklı Olgularda Sistemik İnflamasyon ve Grelin Düzeyleri

Pulmoner hipertansiyon (PH) Kronik Obstrüktif Akciğer Hastalığı'nın (KOAH) yaygın görülen bir komplikasyonudur. İştah açıcı ve endojen vazodilatör peptid hormon olan grelinin KOAH'a sekonder gelişen PH'de potansiyel rolü üzerine herhangi bir veri bulunmamaktadır. PH gelişen ve gelişmeyen kaşektik ve normal kilolu KOAH'lı olgularda serum grelin, tümör nekrozis faktör-alfa (TNF-α) ve interlökin-6 (IL-6) düzeylerinin belirlenmesi amaçlandı. PH gelişen ve gelişmeyen kaşektik ve normal kilolu 60 KOAH'lı ve 15 sağlıklı kontrol olguda serum grelin, IL-6 ve TNF-α düzeyleri ölçüldü. TNF-α, IL-6 ve grelin düzeyleri enzim immünoassay (ELISA) yöntemiyle, pulmoner arter basınçları ise ekokardiyografi ile ölçüldü. PH gelişen ve gelişmeyen KOAH'lı olgular sağlıklı kontrol olgular ile karşılaştırıldığında serum IL-6 ve TNF-α düzeyleri istatistiksel olarak anlamlı yüksek, serum grelin düzeyleri ise istatistiksel olarak anlamlı düşük bulundu. PH gelişen kaşektik KOAH olgularında PH gelişmeyen kaşektik KOAH olguları karşılaştırıldığında serum grelin düzeyleri istatistiksel olarak anlamlı düşük ve IL-6 düzeyleri istatistiksel olarak anlamlı yüksek olarak bulundu. Normal kilolu PH gelişen ve gelişmeyen KOAH'lı olgular arasında ise serum grelin düzeyleri açısından istatistiksel fark saptanmadı. PH gelişen kaşektik KOAH'lı olgularda daha belirgin olmak üzere, PH gelişen ve gelişmeyen tüm KOAH'lı olgularda serum grelin düzeyleri azalmıştır. Grelinin özellikle kaşektik KOAH olgularında olmak üzere, PH gelişmesine katkıda bulunan bir faktör olabileceği düşünülmüştür.

Anahtar Kelimeler: Grelin, pulmoner hipertansiyon, kronik obstrüktif akciğer hastalığı, interlökin-6, tümör nekrozis faktör alfa.

Introduction

Pulmonary hypertension (PH) develops in the majority of patients with chronic obstructive pulmonary disease (COPD), especially when airflow limitation is severe. In recent years, this traditional view has been questioned (1). Structural and functional changes in pulmonary arteries have been observed in normoxic patients with COPD (2). Inhaled noxious particles and gases in smokers lead to an inflammatory process in the wall of central and peripheral airways and pulmonary vascular lesions in patients with COPD (3, 4). Systemic inflammation contributes to the development of PH in patients with COPD; however, the role of systemic inflammation in the pathogenesis of PH in COPD is still controversial (5-7).

Ghrelin is an endogenous vasodilatory peptide also stimulating the release of growth hormone and has been demonstrated to attenuate the development of pulmonary arterial hypertension (PAH) in a monocrotaline-treated animal model of PAH (8). A previous study demonstrated that ghrelin improved endothelial dysfunction and increased endothelial NOS expression through a growth hormone (GH)-independent mechanism (9). Moreover, ghrelin improved cardiac function and cardiopulmonary-associated cachexia (10). Thus, it can be suggested that ghrelin may become a potential therapeutic agent for PH in the near future (11).

Pulmonary and systemic inflammation might contribute to weight loss in patients with COPD (12). Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are contributed to the development of weight loss in COPD patients. Several studies have demonstrated that plasma ghrelin levels are increased and positively correlated with TNF-α but negatively correlated with body mass index (BMI) in cachectic patients with chronic hearth failure (13) and cancer (14). A causal link between inflammatory status, weight maintenance and PH in COPD remains unclear.

We hypothesized that increased or decreased ghrelin levels may play a role on development of PH in patients with COPD. In addition changed ghrelin levels would be associated with evidence of an inflammatory response and weight loss in these patients. To test this we investigated the serum ghrelin levels and systemic inflammation by measuring serum levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in underweight and normal weight COPD patients with and without PH.

Materials and Methods

Subjects

Sixty patients with COPD from Firat University Hospital were enrolled when patients were clinically stable. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. Exclusion criteria were respiratory disorders other than COPD, pulmonary embolism, and left ventricular systolic or diastolic dysfunction. Long-term medication, including inhaled β_2 agonist, ipratropium bromide, xanthines and inhaled steroids was kept constant during this study protocol. COPD patients were grouped based on BMI and the presence of PH as Group Ia (underweight individuals [BMI<20 kg/m^2] with PH; n=15), Group Ib (underweight individuals [BMI<20 kg/m^2] without PH; n=15), Group Ic (normal weight individuals [BMI≥20 kg/m^2] with PH; n=15), and Group Id (normal weight individuals [BMI≥20 kg/m^2] without PH; n=15). The control group consisted of 15 healthy non-smoking subjects who had normal pulmonary function and did not have lung disease. All control subjects were randomly selected among the hospital staff. All participants gave informed consent to participate in the present study, and the study protocol was approved by the local ethics committee.

Pulmonary Function Testing

Pulmonary function parameters (forced expiratory volume in 1 second [FEV_1]; and forced vital capacity [FVC]) were measured using a spirometer (Ultima CPX 790705-205; Medgraphics Corporation, St. Paul, MN, USA). The values were interpreted according to the predicted values of the European Respiratory Society (15). Pulmonary function values were expressed as a percentage of the predicted values.

Arterial Blood Gases

Arterial blood gases were measured at rest with a blood gas analyzer (Rapid lab 348; Biobak, Chiron, Bayer Diagnostic, UK).

Fat-free mass

Fat-free mass (FFM) was measured by single-frequency (50 kHz) bioelectrical impedance analysis (Tanita TBF 300A Body Composition Analyzer, Tanita Corporation, Tokyo, Japan).

Measurement of serum IL-6, TNF-α and ghrelin levels

In all patients, venous blood samples from the antecubital vein were collected between 7:00 and 8:00 A.M. after overnight fasting and the patients seated and resting comfortably. The blood was centrifuged immediately at 4^0C and stored at -80^0C. Serum TNF-α and IL-6 levels were measured using appropriate commercial kits (Biosource, Biosource International Inc., Camarillo, CA, USA and Orgenium Laboratories, Helsinki, Finland, respectively) by enzyme-linked immunosorbent assay (ELISA) method (5). Plasma acylated and non-acylated ghrelin levels were measured with two commercially available ELISA kits (the Active Ghrelin ELISA kit and the Desacyl-Ghrelin ELISA kit, respectively), according to the manufacturer's protocol (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan). The Active Ghrelin ELISA Kit was used to measure *n*-octanoyl ghrelin. The Desacyl-ghrelin ELISA Kit was used for the measurement of desacyl-ghrelin. The minimal detection limits for acylated and desacyl ghrelin in this assay system were 2.5 and 12.5 fmol/ml, respectively. The intra-and inter-assay coefficients of variation were 6.5% and 9.8% for acylated ghrelin and 3.7% and 8.1% for desacyl ghrelin, respectively.

Echocardiography

Systolic pulmonary arterial pressure (P_{pas}) in patients with COPD were assessed by Doppler echocardiography (Acuson Sequa 512 device with a 3.5 MHz transducer; Acuson Corporation, Mountain View, CA, USA) by a single expert cardiologist who was blinded to the results of the biochemical analysis. While patients were in semi-supine position, continuous wave Doppler recordings of maximal velocity were obtained from apical, parasternal long-short axis, and subcostal transducer positions. Tricuspid regurgitant flow identified by color flow Doppler

techniques and the maximum jet velocity was measured by continuous-wave Doppler recording. Regurgitation flow obtained from the apical 4 cavity images by continuous-wave Doppler were inserted into the Bernoulli equation and tricuspid regurgitation was calculated automatically by device (16, 17).

∆PRV-RA ([the pressure gradient between the right atrium and right ventricle]=4[VTR]² [tricuspid regurgitant flow rate]) and right atrial pressure (appraisal of right atrial pressure was taken up to 10 mmHg) was added to this value to calculate the $P_{pas.}$ Systolic P_{pa} was calculated according to this equation and PH was defined as P_{pas} ≥30 mmHg (18-20).

Statistical Analysis

All statistical analyses were performed using the SPSS 12.0 program. The results were expressed as the mean±standard deviation (SD). Significance level was accepted as $p<0.05$. Kruskal-Wallis, Mann-Whitney U and chi-square (for sex distribution) tests were used to compare the data.

Results

The age and gender of the control subjects were similar to COPD patients. Sixty patients (52 males and 8 females) with COPD (mean age, 65.33±11.25 years; mean smoking history, 34.43±23.22 pack-years; and mean duration of disease, 5.11±5.57 years) were enrolled for the study. Mean age and gender ratios were similar between the patient population and the control subjects (10 males, 5 females, mean age, 67.47± 8.81 years, $p>0.05$).

Comparisons of the COPD patients with and without PH

There was no significant difference between COPD patients with or without PH in terms of age, BMI, and FFM ($p>0.05$). The FEV_1, FEV_1/FVC, and partial pressure of oxygen in arterial blood (PaO_2) values were significantly lower and the duration of disease and smoking history were significantly higher in COPD patients with PH compared to those without PH (Table 1).

Serum active ghrelin and TNF-α levels were significantly lower in COPD patients with PH than without PH patients. A trend toward higher IL-6 levels in COPD patients with PH compared to those without PH was noted; however, it did not reach statistically significance level (Table 1).

Comparisons of COPD patients with or without PH and control subjects

Serum IL-6 and TNF-α levels were significantly higher in COPD patients with or without PH group compared to control subjects. Serum active ghrelin levels were significantly lower in COPD patients with or without PH group than control subjects. In particular, the serum ghrelin levels were lower in patients with PH than those without PH and control subjects (Table 1).

Table 1. Demographic data and other parameters in COPD patients with or without pulmonary hypertension and control subjects.

	PH (+) COPD (n=30) (1)	PH (-) COPD (n=30) (2)	Control (n=15) (3)	P value (1 x 2)	P value (1x3)	P value (2x3)
Age (yr)	67.30 ± 10.40	63.37 ± 11.88	67.47 ± 8.81	NS	NS	NS
BMI (kg/m^2)	22.52 ± 6.64	23.73 ±± 6.19	26.64 ± 4.27	NS	0.016	NS
FFM (%)	12.73 ± 9.23	15.26 ± 10.62	21 ± 9.49	NS	0.008	0.043
PaO_2 (mmHg)	51.82 ± 8.85	61.55 ± 10.14	84.33 ± 2.25	< 0.001	< 0.001	< 0.001
$PaCO_2$ (mmHg)	42.04 ± 10.01	39.52 ± 6.09	38.66 ± 2.46	NS	NS	NS
pH	7.40 ± 0.04	7.40 ± 0.02	7.40 ± 0.01	NS	NS	NS
FEV_1 (%P)	34.70 ± 14.28	55.73 ± 20.82	90.20 ± 14.04	< 0.001	< 0.001	< 0.001
FEV_1/FVC (%P)	52.20 ± 9.48	59.63 ± 10.03	80.26 ± 7.62	0.005	< 0.001	< 0.001
TNF-α (pg/ml)	14.64 ± 5.80	19.97 ± 8.24	10.62 ± 1.31	0.009	0.002	< 0.001
IL-6 (pg/ml)	14.11 ± 9.69	12.21 ± 8.41	5.97 ± 4.16	NS	0.001	0.006
Active ghrelin (fmol/ml)	5.91 ± 2.59	8.01 ± 3.03	12.13 ± 4.51	0.007	< 0.001	0.002
Total ghrelin (fmol/ml)	125.83 ± 45.05	121.5 ± 30.72	129.67 ± 35.77	NS	NS	NS
Smoking history (pack/yr)	41.20 ± 26.73	27.17 ± 16.29	-	0.035	-	-
Duration of disease (yr)	6.60 ± 4.37	3.63 ± 6.28	-	< 0.001	-	-

BMI; body mass index, FFM; fat-free mass, PaO_2; arterial oxygen pressure, $PaCO_2$; arterial carbon dioxide pressure, FEV_1; forced expiratory volume in 1 second, FEV_1/FVC; the ratio of FEV_1/forced vital capacity (FVC), TNF-α; tumor necrosis factor-alpha IL-6; interleukin-6, yr; year, NS; non-significant

PH and cachectic status in patients with COPD

Underweight patients

There were no statistically significant differences between groups Ia and Ib in terms of age, duration of disease, smoking history, BMI, and FFM (p>0.05). Serum active ghrelin levels were significantly lower and IL-6 levels were significantly higher in group Ia than group Ib. Serum TNF-α levels were lower in group Ia compared with group Ib, but were not statistically significant (Table 2).

Normal weight patients

No differences existed between group Ic and group Id in terms of age, BMI, FFM, and smoking history (p>0.05) (Table 3). TNF-α and IL-6 levels were significantly lower in group Ic compared to group Id; however, there was no statistically significant differences in serum active ghrelin levels between the two groups.

Table 2. Demographic data and other parameters in underweight COPD patients with and without pulmonary hypertension.

	Group Ia (n=15)	Group Ib (n=15)	P value
Age (yr)	66.47 ± 12.08	64.47 ± 13.21	NS
BMI (kg/m^2)	17.21 ± 2.19	18.42 ± 1.35	NS
FFM (%)	5.88 ± 3.93	6.94± 2.54	NS
PaO_2 (mmHg)	50.22 ± 9.22	60.98 ± 10.90	0.012
$PaCO_2$ (mmHg)	39.97 ± 7.20	38.94 ± 4.02	NS
pH	7.41 ± 0.04	7.40 ± 0.03	NS
FEV_1 (%)	31.93 ± 10.24	51.86 ± 24.23	0.029
FEV_1/FVC (%)	49.86 ± 8.25	58.06 ± 9.80	0.016
TNF-α (pg/ml)	17.42 ± 6.59	19.82± 8.76	NS
IL-6 (pg/ml)	21.00 ± 8.48	9.92 ± 6.44	0.001
Active ghrelin (fmol/ml)	4.64 ± 1.79	7.22 ± 3.13	0.016
Total ghrelin (fmol/ml)	118.21 ± 32.31	138.49 ± 24.77	NS
Smoking history (pack/yr)	37.06 ± 16.02	28.06 ± 14.35	NS
Duration of disease (yr)	6.40 ± 4.46	5.60 ± 8.51	NS

BMI; body mass index, FFM; fat-free mass, PaO_2; arterial oxygen pressure, $PaCO_2$; arterial carbon dioxide pressure, FEV_1; forced expiratory volume in 1 second, FEV_1/FVC; the ratio of FEV_1/forced vital capacity (FVC), TNF-α; tumor necrosis factor-alpha IL-6; interleukin-6, yr; year, NS; non-significant

Table 3. Demographic data and other parameters in normal weight COPD patients with and without pulmonary hypertension.

	Group Ic (n=15)	Group Id (n=15)	P value
Age (yr)	68.13 ± 8.75	62.27 ± 10.74	NS
BMI (kg/m²)	27.82 ± 5.11	29.05 ± 4.14	NS
FFM (%)	19.58 ± 7.78	23.57 ± 8.89	NS
PaO_2 (mmHg)	53.42 ± 8.49	62.12 ± 9.66	0.009
$PaCO_2$ (mmHg)	42.11 ± 12.10	40.10 ± 7.75	NS
pH	7.39 ± 0.03	7.40 ± 0.02	NS
FEV_1 (%)	37.46 ± 17.35	59.60 ± 16.71	0.001
FEV_1/FVC (%)	54.53 ± 10.31	61.20 ± 10.36	NS
TNF-α (pg/ml)	11.85 ± 3.11	20.12 ± 8.00	0.002
IL-6 (pg/ml)	7.23 ± 4.60	14.50 ± 9.68	0.015
Active ghrelin (fmol/ml)	7.18 ± 2.70	8.79 ± 2.81	NS
Total ghrelin (fmol/ml)	133.45 ± 55,0	104.56 ± 26.91	NS
Smoking history (pack/yr)	45.33 ± 34.45	26.15 ± 18.83	NS
Duration of disease (yr)	6.80.± 4.42	1.66 ± 0.97	< 0.001

BMI; body mass index, FFM; fat-free mass, PaO_2; arterial oxygen pressure, $PaCO_2$; arterial carbon dioxide pressure, FEV_1; forced expiratory volume in 1 second, FEV_1/FVC; the ratio of FEV_1/forced vital capacity (FVC), TNF-α; tumor necrosis factor-alpha IL-6; interleukin-6, yr; year, NS; non-significant

There was no statistically significant difference in mean pulmonary artery pressures between groups Ia and Ic (59.93±16.14 and 56.46±12.33, respectively; p>0.05).

Discussion

Increased or decreased levels of ghrelin have been reported in patients with COPD; however, the relationship between ghrelin and cachexia is still controversial (21, 22). Recently, the relationship between ghrelin and PH has been the focus of attention. However, few data are available regarding the effect of ghrelin on the development of PH secondary to COPD. The present study demonstrated that serum active ghrelin levels decreased in COPD patients with PH. In particular, lower serum active ghrelin levels were determined in underweight COPD patients with PH.

Wiley and Davenport (23) have reported that ghrelin is a potent vasodilator in isolated human arteries. Nagaya et al. (24) have demonstrated that intravenous administration of ghrelin decreases systemic vascular resistance in rats, at least in part through GH-independent mechanisms. Several studies have demonstrated that ghrelin improves endothelial dysfunction and increases endothelial nitric oxide secretion through a GH-independent mechanism and ghrelin has a potent vasodilatory effect (9, 23-25). Thus, at least one ghrelin signaling pathway appears to be involved in the regulation of vascular tone (26). Previous experimental studies demonstrated that ghrelin was produced in the heart, lungs, and stomach of animals and the exogenous administration of ghrelin decreased the development of PH, right ventricular hypertrophy, and vascular remodeling in pulmonary arteries in animals (27, 28). Ghrelin has important direct cardiovascular effects that ghrelin administration reduced cardiac afterload and increased cardiac output in both normal subjects and patients with dilated cardiomyopathy (10, 29).

On the basis of the beneficial effects of ghrelin on the pulmonary vasculature and heart, we hypothesized that ghrelin might be beneficial in COPD patients with PH by improving cardiac and/or pulmonary vascular structure and function.

In the present study, it was demonstrated that serum active ghrelin levels significantly decreased in COPD patients with or without PH compared to control subjects. In particular, the decrease in serum active ghrelin levels was more prominent in COPD patients with PH. Thus, the serum active ghrelin level was decreased in patients with COPD and this decrease became more pronounced when COPD patients had PH. To our knowledge, there are no clinical studies evaluating the effects of ghrelin on the development of PH in COPD patients. For this reason, our results cannot be compared with other data. Surprisingly, the ability of systemic ghrelin in the regulation of its local production was apparently lost in PH. One experimental study demonstrated that endogenous production of ghrelin was almost abolished in normal rats treated with ghrelin. Furthermore, in PH the pulmonary expression of ghrelin was preserved and right ventricle myocardial expression was increased in monocrotalin-treated animals, and exogenous administration of ghrelin attenuated in PH and pulmonary vascular remodeling (27). Similarly, another experimental study demonstrated that exogenous ghrelin attenuated the progression of PH, which was induced by chronic hypoxia in rats (28). At the pulmonary level, ghrelin has a determinant role in fetal lung development (30), and the adult human lung is a major source of ghrelin mRNA gene expression (31). GH secretagogue receptors were detected in the adult lung parenchyma and pulmonary artery wall (32, 33). According to all these findings and our results, decreased ghrelin levels may contribute to the development of PH.

Ghrelin induces a positive energy balance and weight gain by decreasing fat utility and stimulating food intake (34, 35). When the serum active ghrelin levels were evaluated in both underweight and normal weight COPD patients with and without PH, it was demonstrated that the serum active ghrelin levels were lower in underweight COPD patients with PH than underweight COPD patients without PH. In contrast to these findings, there was no significant difference in serum active ghrelin levels between normal weight COPD patients with and without PH. These observations may implicate that ghrelin has a potential role in the development of PH, especially in underweight COPD patients. The potential role of inflammation in the pathogenesis of vessel disease was not well-studied until the past few years. Humbert et al (36), reported that elevated serum concentrations of IL-1 and IL-6, but not TNF- α, in patients with idiopathic pulmonary arterial hypertension (IPAH). Hoeper and Welte (7) explored that none of these cytokines was elevated in patients with IPAH. It has been recently shown that systemic inflammation in COPD appears to increase the risk for developing PH in COPD. Pro-inflammatory cytokines, such as IL-6, was correlated with the level of pulmonary artery pressure (6) and patients with significant pulmonary hypertension had higher levels of TNF-α (5). Inflammation, most likely involving IL-6, may contribute substantially to PH complicating COPD (37) Similarly, we also determined that serum IL-6 levels were significantly higher in underweight COPD patients with PH than those without PH; however, this relationship was not observed for TNF-α. In contrast to this finding, TNF-α and IL-6 levels were significantly lower in normal weight COPD patients with PH compared to normal weight COPD patients without PH. We believe that systemic inflammation may be at least partially responsible for pulmonary hypertension especially underweight COPD patients. Pro-inflammatory cytokines, such as TNF-α, was negatively correlated with plasma ghrelin in COPD (22). Decreased ghrelin levels in patients with COPD may contribute to alterations in metabolic status during inflammatory stress and may cause cachexia (22). Cachexia impacts both respiratory musculature and peripheral skeletal muscle function. Generalized muscle weakness is caused by deconditioning, malnutrition, electrolyte disturbances,

cardiac failure, and systemic inflammation (38). Malnutrition has a tremendous impact on respiratory functions. It affects respiratory muscle performance, lung structure, defense mechanisms, and control of ventilation and predisposes to respiratory failure (39). In addition, a decline in FFM in COPD is associated with worse lung function (40). It has been suggested that low body weight may be associated with decreased respiratory muscle function in COPD; however, the precise mechanism is not known (41). Nagaya et al. (42) demonstrated that repeated administration of ghrelin improves body composition, muscle wasting, and functional capacity. The present study results and previous data suggested that decreased ghrelin levels due to systemic inflammation and/or other reasons, such as right ventricular hypertrophy in cachectic COPD patients, contributed to the decreased respiratory function (22, 43). Ghrelin may act synergistically with severe airflow limitation effects and this may predispose to PH in patients with COPD especially underweight patients. However, in patients with severe PH frequently report loss of appetite and weight (44).

Ghrelin divided into two main groups according to octanoyl side chain is present. Octanoylated ghrelins have an endocrine activity (“active ghrelin”), but nonoctanoylated ghrelins do not have this activity (“inactive ghrelin”) (45). In the human stomach, the ratio of octanoylated to decanoylated ghrelin was found to be roughly 3:1. Because acylation of ghrelin is essential for its activity, the enzyme that catalyzes this modification step should be an important regulator of ghrelin biosynthesis. Plasma concentrations of active ghrelin constitute about 20% of total ghrelin levels and total ghrelin may reflect the level of the active form in plasma (22, 46). We measured in both total and active ghrelin levels. Our results showed that there was no significantly different between groups in total ghrelin levels despite active ghrelin levels changed. For this reason, measure of active ghrelin may be rely on active rather than total for the evaluation of ghrelin effects.

In conclusion, this was a pilot study which should generate additional ideas for future study. It was considered that ghrelin may be contributing factor to the development of PH other than the severity of COPD, hypoxemia, and systemic inflammation in underweight COPD patients but decreased ghrelin levels also might be actually reflect BMI that patients with more severe COPD have lost body weight. In addition, the loss of ghrelin as an endogenous pulmonary vasodilator leads to worsening PAP. Decreased ghrelin levels may be an important medical issue in cachectic COPD patients with PH. The role of ghrelin in weight loss and pathogenesis of PH in patients with COPD, and its potential beneficial effects on the treatment of COPD patients with PH should be investigated with further studies.

References

1. Wright JL, Levy RD, Churg A. Pulmonary hypertension in chronic obstructive pulmonary disease: current theories of pathogenesis and their implications for treatment. Thorax 2005; 60: 605-609.
2. Barbera JA, Riverola A, Roca J, *et al.* Pulmonary vascular abnormalities and ventilation-perfusion relationships in mild chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1994; 149: 423-429.
3. Santos S, Peinado VI, Ramírez J, *et al.* Characterization of pulmonary vascular remodelling in smokers and patients with mild COPD. Eur Respir J 2002; 19: 632–638.
4. Peinado VI, Barberá JA, Abate P, *et al.* Inflammatory reaction in pulmonary muscular arteries of patients with mild chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999; 159: 1605–1611.
5. Joppa P, Petrasova D, Stancak B, Tkacova R. Systemic inflammation in patients with COPD and pulmonary hypertension. Chest 2006; 130: 326–333.
6. Eddahibi S, Chaouat A, Tu L, *et al.* Interleukin-6 gene polymorphism confers susceptibility to pulmonary hypertension in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2006; 3: 475–476.
7. Hoeper MM, Welte T. Systemic inflammation, COPD, and pulmonary hypertension. Chest 2007; 131: 634–635.
8. Henriques-Coelho T, Roncon-Albuquerque Júnior R, Lourenço AP, et al. Ghrelin reverses molecular, structural and hemodynamic alterations of the right ventricle in pulmonary hypertension. Rev Port Cardiol 2006; 25: 55–63.
9. Shimizu Y, Nagaya N, Teranishi Y, *et al.* Ghrelin improves endothelial dysfunction through growth hormone-independent mechanisms in rats. Biochem Biophys Res Commun 2003; 310: 830-835.
10. Nagaya N, Kangawak. Ghrelin improves left ventricular dysfunction and cardiac cachexia in heart failure. Curr Opin Pharmacol 2003; 3: 146–151.
11. Puri A, McGoon MD, Kushwaha SS. Pulmonary arterial hypertension: current therapeutic strategies. Nat Clin Pract Cardiovasc Med 2007; 4: 319-329.
12. Eid AA, Ionescu AA, Nixon LS, *et al.* Inflammatory response and body composition in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001; 164: 1414-1418.
13. Nagaya N, Uematsu M, Kojima M, *et al.* Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. Circulation 2001; 104: 2034-2038.
14. Shimizu Y, Nagaya N, Isobe T, *et al.* Increased plasma ghrelin level in lung cancer cachexia. Clin Cancer Res 2003; 9: 774-778.
15. Standardized lung function testing. Official statement of the European Respiratory Society. Eur Respir J 1993; 6: 16.

16. Miyahara Y, Ikeda S, Yoshinaga T, *et al.* Echocardiographic evaluation of right cardiac function in patients with chronic pulmonary disease. Jpn Heart J 2001; 42: 483-493.

17. Moustapha A, Kaushik V, Diaz S, *et al* Echocardiographic evaluation of left ventricular diastolic function in patients with chronic pulmonary hypertension. Cardiology 2001; 95: 96-100.

18. Macnee W. Pathophysiology of cor pulmonale in chronic obstructive pulmonary disease Part one. Am J Respir Crit Care Med 1994; 150: 833–852.

19. Prichard JS. Pulmonary hypertension. In: Wetherall textbook of medicine. Wetherall DJ, Ledingham JGG, Warrell DA, (editors). Oxford: Oxford University Pres, 1996: 2505-2514.

20. Galie N, Torbicki A, Barst R, *et al.* Task Force: Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The task force on diagnosis and treatment of pulmonary arterial hypertension of the European Society of Cardiology. Eur Heart J 2004; 25: 2243-2278.

21. Itoh T, Nagaya N, Yoshikawa M, *et al.* Elevated plasma ghrelin level in underweight patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004; 170: 879-882.

22. Luo FM, Liu XJ, Li SQ, et al. Circulating ghrelin in patients with chronic obstructive pulmonary disease. Nutrition 2005; 21: 793-798.

23. Wiley KE, Davenport AP. Comparison of vasodilators in human internal mammary artery: ghrelin is a potent physiological antagonist of endothelin-1. Br J Pharmacol 2002; 136: 1146–1152.

24. Nagaya N, Uematsu M, Kojima M, *et al.* Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation 2001; 104: 1430–1435.

25. Okumura H, Nagaya N, Enomoto M, *et al.* Vasodilatory effects of ghrelin, an endogenous peptide from the stomach. J Cardiovasc Pharmacol 2002; 39: 779–783.

26. Zaloga GP. Ghrelin, diet, and pulmonary function. Chest 2005; 128; 1084-1086.

27. Henriques-Coelho T, Correia-Pinto J, Roncon-Albuquerque R Jr, *et al.* Endogenous production of ghrelin and beneficial effects of its exogenous administration in monocrotaline induced pulmonary hypertension. Am J Physiol Heart Circ Physiol 2004; 287: 2885–2890.

28. Schwenke DO, Tokudome T, Shirai M, *et al.* Exogenous ghrelin attenuates the progression of chronic hypoxia-induced pulmonary hypertension in conscious rats. Endocrinology 2008; 149: 237-244.

29. Nagaya N, Kojima M, Uematsu M, *et al.* Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. Am J Physiol Regul Integr Comp Physiol 2001; 280: 1483-1487.

30. Volante M, Fulcheri E, Allia E, *et al.* Ghrelin expression in fetal, infant and adult human lung. J Histochem Cytochem 2002; 50: 1013–1021.

31. Gnanapavan S, Kola B, Bustin SA, *et al.* The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002; 87: 2988–2991.

32. Papotti M, Ghe C, Cassoni P, *et al.* Growth hormone secretagogue binding sites in pheripheral human tissues. J Clin Endocrinol Metab 2000; 85: 3803–3807.

33. Katugampola S, Pallikaros Z, Davenport AP. [125I-His9]-ghrelin, a novel radioligand for localizing GHS orphan receptors in human and rat tissue; up regulation of receptors with atherosclerosis. Br J Pharmacol 2001; 134: 143–149.

34. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000; 407: 908-913.

35. Nakazato M, Murakami N, Date Y, *et al.* A role for ghrelin in the central regulation of feeding. Nature 2001; 409: 194-198.

36. Humbert M, Monti G, Brenot F, *et al.* Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. Am J Respir Crit Care Med 1995; 151:1628–1631.

37. Chaouat A, Savale L, Chouaid C, *et al.* Role for interleukin-6 in COPD-related pulmonary hypertension. Chest 2009; 136: 678-687.

38. Decramer M. Respiratory muscles in COPD: regulation of trophical status. Verh K Acad Geneeskd Belg 2001; 63: 577-602.

39. Ghignone M, Quintin L. Malnutrition and respiratory function. Int Anesthesiol Clin 1986; 24: 65-74.

40. Hopkinson NS, Tennant RC, Dayer MJ, *et al.* A prospective study of decline in fat free mass and skeletal muscle strength in chronic obstructive pulmonary disease. Respir Res 2007; 8: 25.

41. Nishimura Y, Tsutsumi M, Nakata H, *et al.* Relationship between respiratory muscle strength and lean body mass in men with COPD. Chest 1995; 107: 1232-1236.

42. Nagaya N, Itoh T, Murakami S, *et al.* Treatment of cachexia with ghrelin in patients with COPD. Chest 2005; 128: 1187-1193.

43. Tritos NA, Kissinger KV, Manning WJ, Danias PG. Association between ghrelin and cardiovascular indexes in healthy obese and lean men. Clin Endocrinol (Oxf) 2004; 60: 60-66.

44. Roux CW, Ghatei MA, Gibbs JSR, Bloom SR. The putative satiety hormone PPY is raised in cardiac cachexia associated with primary pulmonary hypertension. Heart 2005; 91: 241-242.

45. Hosoda H, Kojima M, Mizushima T, Shimizu S, Kangawa K. Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing. J Biol Chem 2003; 278: 64-70.

46. Espelund U, Hansen TK, Orskov H, Frystyk J. Assessment of ghrelin. APMIS Suppl 2003; 140-145.