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Serum myostatin levels and skeletal muscle wasting in chronic obstructive pulmonary disease

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KEYWORDS

Myostatin;
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Muscle wasting;
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Summary

Introduction: It is well confirmed that myostatin is a negative regulator of skeletal muscle mass and implicated in several diseases involved in muscle wasting and cachexia. Skeletal muscle wasting is an important systemic manifestation of chronic obstructive pulmonary disease (COPD), while the expression of circulating myostatin in COPD remains unclear. The aim of this study was to investigate the expression of circulating myostatin and its relationship with skeletal muscle wasting in COPD.

Methods: Seventy-one patients with stable COPD and sixty age-matched, healthy control subjects participated in the study. Total skeletal muscle mass (SMM) were calculated according to a validated formula by using age and anthropometric measurements. Serum levels of myostatin, tumor necrosis factor (TNF)- α and interleukin-6 were determined by ELISA.

Results: Serum myostatin levels were significantly elevated in COPD patients when compared to controls [(11.85 \pm 4.01) ng/ml vs. (7.46 \pm 2.21) ng/ml, $p < 0.01$], while total SMM was significantly decreased in COPD patients when compared to controls [(20.81 \pm 1.74) kg vs. (27.31 \pm 2.18) kg for male, and (11.70 \pm 0.56) kg vs. (19.89 \pm 1.47) kg for female] (both $p < 0.05$). Regression correlation analysis on all COPD patients showed that serum myostatin levels weren't significantly correlated with SMM, but correlated with TNF- α levels ($R^2 = 0.042$, $p = 0.048$). However, when stratified for gender, serum myostatin levels were correlated inversely both with SMM ($R^2 = 0.20$, $p = 0.000$) and with BMI ($R^2 = 0.084$, $p = 0.019$) in subgroup of male patients.

Conclusion: This study demonstrates that circulating myostatin levels are elevated in COPD and related to SMM in male patients, suggesting that myostatin contributes to skeletal muscle wasting in COPD.

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Introduction

Skeletal muscle wasting is one of the most important extra-pulmonary manifestations of chronic obstructive pulmonary disease (COPD),¹ which is recognized as contributing to an increase in morbidity and decrease in quality of life.^{2,3} Retrospective studies have demonstrated that skeletal muscle wasting is associated with an increase in mortality.⁴ Myostatin, a transforming growth factor-beta superfamily member, has been well confirmed as a negative regulator of skeletal muscle mass and mainly expressed in skeletal muscles,⁵ while there was also evidence of myostatin secretion and circulation in animals⁶ and human blood.⁷ In recent years, studies have shown that myostatin is implicated in several diseases involved in muscle wasting and cachexia.⁸ More recently, there were several reports showing that intramuscular myostatin expression accelerated in COPD patients, suggesting that intramuscular elevation of myostatin expression could induce muscle wasting in COPD patients.⁹ While expression of circulating myostatin in COPD and whether or not it contributes to whole-body skeletal muscle wasting in COPD remains unclear. The previous studies led us to hypothesize that an increase of myostatin expression in skeletal muscles of COPD patients may elevate circulating levels, resulting in muscle wasting of whole body. Therefore, the aims of this investigation were to evaluate the following questions in COPD patients: 1) Whether or not the circulating myostatin is increased; 2) Is the circulating level of myostatin correlated with the total-body skeletal muscle mass (SMM); and 3) Is the circulating level of myostatin correlated with TNF- α and IL-6, as the latter two were recognized as systemic cytokines associated with wasting in COPD.

Materials and methodology

Subjects and study design

This was an intersectional comparison study. The study was approved by the Research Committee of Human Investigation of Guangzhou Medical College and informed consent was obtained for each participant during the period from Mar 2007 to Oct 2008. Seventy-one patients with COPD were enrolled in the study. The diagnosis of COPD was according to the criteria of GOLD guidelines.¹⁰ Inclusion criteria for COPD included: 1) ratio of forced expiratory volume in 1st second to forced vital capacity (FEV₁/FVC) < 70% after bronchodilator; 2) FEV₁<80% of the predicted value; and 3) the reversibility testing with inhalation of β 2-agonist (200 mg salbutamol) showing to be <12% increase in FEV₁; 4) clinically stable for at least 3 months; 5) ex-smokers with abstaining from smoking for at least 3 years. The exclusion criteria were: 1) Patients with major co-morbidities or concomitant diseases that might alter nutritional status or muscle functions were excluded from the study. These co-morbidities included hepatic cirrhosis, uncontrolled diabetes, chronic renal failure, thyroid disease, neoplasm, and cancer, etc.; some co-morbidities such as osteoporosis, mild and moderate essential hypertension were not exclude from the study for these co-morbidities had no effects on nutritional status. 2) Patients receiving nutritional support therapy; 3) Patients

unwilling to participate or sign the informed consent. Sixty control subjects were recruited from the health check-up department of the hospital. The inclusion criteria were: 1) no history of major or chronic disorders; 2) with normal pulmonary function; 3) never smoked or had abstained from smoking for more than 10 years.

The medical treatment of the patients at the time of the study mainly included inhaled bronchodilator therapy in the form of long-acting agonists and/or anti-cholinergic agents. In addition, 85% of the patients were on inhaled corticosteroids (400–800 μ g budesonide equivalent dose/day). None of the patients was on regular systemic corticosteroids.

Pulmonary function evaluation

All patients and control subjects underwent spirometry and reversibility testing in COPD patients with inhalation of a short acting β 2-agonist of salbutamol (200 μ g).

Skeletal muscle mass

Total skeletal muscle mass (SMM) was evaluated according to a validated formula¹¹ by using age and anthropometric measurements; the latter included body height (in m) and skinfold corrected limb circumferences [upper arm, thigh, and calf girths (CAG, CTG, and CCG, respectively; in cm)]. Circumference measurements were made in the plane orthogonal to the long axis of the body segment being measured. Skinfold thickness of the triceps, thigh, and mid-calf were measured by using a skinfold caliper; circumferences of the mid-upper arm, mid-thigh, and mid-calf were measured with a flexible measuring tape, respectively, according the standardized anatomic locations.¹¹ The limb circumferences (C_{limb}) were corrected for subcutaneous adipose tissue thickness. The skinfold caliper measurement (S) was assumed to be twice the subcutaneous adipose tissue thickness. Corrected muscle circumferences (C_m) were thus calculated as $C_m = C_{limb} - \pi S$.¹² The formula was as follows:

$$SM(kg) = Ht \times (0.00744 \times CAG^2 + 0.00088 \times CTG^2 + 0.00441 \times CCG^2) + 2.4 \times sex + 0.048 \times age + race + 7.8$$

Where sex = 0 for female and 1 for male, race = -2.0 for Asian patients, according to the research by Lee et al.¹¹

Measurements of serum myostatin, TNF- α , and IL-6 levels

For each subject, peripheral venous blood was drawn between 7:00 and 8:00 am after fasting since 9:00 pm the previous night. After centrifugation at 1000 rpm for 5 min at 4 °C, serum samples were collected and subsequently stored at -80 °C until analyzed. Serum myostatin levels were determined by antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits (BioVendor Laboratory Medicine, Inc.), TNF- α by ELISA kits (Quantikine; R&D Systems, Minneapolis, MN), and IL-6 by ELISA kits (Bender Med Systems).

Level of daily life physical activity

Level of daily life physical activity (PA) was assessed by using a PA questionnaire adapted for the elderly in China. The original questionnaire on habitual PA consisted of 19 items, scored the past 3 year's household activities, sports activities, and other physically active leisure-time activities and gave an overall score.^{13,14} The subjects were asked to describe type of the PA, hours per week spent on it, and period of the year in which the PA was normally performed. There is an appendix for PA score criteria.

Statistical analyses

Statistical analysis was performed by using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) statistical package for windows and Graphpad Prism v. 5 (Graphpad software INC., San Diego, CA, USA). Data were expressed as mean \pm Sd. Student's *t* test was used for comparisons of unpaired parameters between patients and controls. Linear regression correlation analysis was used to determine the relationship of serum myostatin levels with other parameters. The level of statistical significance was set as $p < 0.05$.

Results

Characteristics of subjects

Clinical characteristics of all the patients with COPD and healthy controls are summarized in Table 1. Spirometric test showed the average FEV₁ was (37.76 \pm 14.93)% predicted, indicating that majority of the COPD patients were with severe airflow limitation. The severity distribution stratified by GOLD criteria were 13, 28 and 30 cases in GOLD stages II, III and IV respectively. There was no significant difference between patients and controls in terms of age, while a significant difference was observed in BMI between the two groups. Among all the COPD patients, 53 ones (74.65%) had malnutrition, as defined by BMI less than 21 kg/m².¹⁵

Skeletal muscle mass

Total body SMM was significantly decreased in patients as compared with controls, with regards to both its absolute value and its percentage of body weight, as shown in Table 2. Anthropometric measurements for limb circumferences and local skeletal muscles mass such as CAG, CTG and CCG were all significantly decreased in patients as compared with controls (Table 2).

Serum levels of myostatin, TNF- α and IL-6

The mean levels of serum myostatin were significantly elevated in COPD patients when compared to controls [(11.85 \pm 4.01) ng/ml vs. (7.46 \pm 2.21) ng/ml, $p < 0.001$], so were serum levels of TNF- α [(6.92 \pm 2.02) pg/ml vs. (4.80 \pm 3.45) pg/ml, $p < 0.001$], as shown in Fig. 1A and B, respectively. However, there was no significant difference in IL-6 levels between the two groups [(1.41 \pm 0.37) pg/ml vs. (1.34 \pm 0.70) pg/ml, $p = 0.19$]. Among patients with COPD, there was no significant difference in serum myostatin levels between the steroid-naïve patients and those on inhaled steroids [(10.17 \pm 3.24) ng/ml vs. (12.18 \pm 4.15) ng/ml, $p = 0.08$].

Correlations

Regression correlation analysis showed that serum myostatin levels were correlated neither with total-body SMM, nor with BMI in COPD group, but correlated with serum TNF- α levels ($R^2 = 0.042$, $p = 0.048$) in COPD group (Fig. 2). However, when stratified for gender, serum myostatin levels were inversely correlated both with total SMM ($R^2 = 0.20$, $p = 0.000$) and with BMI ($R^2 = 0.084$, $p = 0.019$) in COPD male patients, as shown in Fig. 3a and b, respectively; an inverse correlation was also observed between serum TNF- α levels and SMM in COPD male patients ($R^2 = 0.068$, $p = 0.032$).

In addition, total-body SMM was significantly correlated with BMI both in COPD group ($R^2 = 0.372$, $p = 0.000$), and in normal subjects ($R^2 = 0.185$, $p = 0.000$). When stratified

Table 1 General characteristics of COPD patients and controls.

	COPD ($n = 70$)	Control ($n = 60$)	Statistics	<i>P</i> value
Sex, M (%)	54 (76%)	21 (35%)	22.40 ^a	0.000
Age (years)	65.17 \pm 6.79	63.98 \pm 5.77	1.08	0.282
BMI (kg/m ²)	19.36 \pm 3.31	22.60 \pm 2.23	6.44	0.000
FEV ₁ %pred (%)	37.76 \pm 14.93	97.10 \pm 8.90	26.99	0.000
FEV ₁ /FVC (%)	43.97 \pm 12.44	88.52 \pm 8.41	23.56	0.000
PO ₂ (mmHg)	79.81 \pm 4.66	98.75 \pm 1.30	30.51	0.000
PCO ₂ (mmHg)	39.31 \pm 4.20	36.83 \pm 0.64	4.52	0.000
PH	7.40 \pm 0.30	7.39 \pm 0.10	0.807	0.421
Smoking (pack ^a years)	27.92 \pm 22.46	1.30 \pm 50.9	8.98	0.000
PA score (score)	5.25 \pm 1.28	7.97 \pm 1.21	12.45	0.000

BMI = body mass index; FEV₁ = forced expiratory volume at 1 s; FVC = forced vital capacity; %pred = percent of predicted value; PA = daily life physical activity; PO₂ = oxygen partial pressure; PCO₂ = carbon dioxide partial pressure.

The significant differences are vs. controls.

^a chi-square value; the others in the column all represent *t* values.

Table 2 Anthropometric measurements in COPD patients and controls.

	Sex	COPD patients	Controls	t value	P value
Age (years)	M	65.76 ± 7.38	65.14 ± 5.90	0.38	0.71
	F	63.29 ± 4.09	63.36 ± 5.67	0.048	0.95
BMI (kg/m ²)	M	20.00 ± 3.31	23.35 ± 1.63	4.40	0.000
	F	17.31 ± 2.38	22.20 ± 2.43	7.03	0.000
CAG (cm)	M	17.62 ± 1.88	24.27 ± 2.67	12.17	0.000
	F	14.90 ± 1.22	17.05 ± 1.79	5.24	0.000
CTG (cm)	M	30.46 ± 3.04	42.79 ± 2.26	19.14	0.000
	F	29.76 ± 2.04	36.13 ± 3.32	7.32	0.000
CCG (cm)	M	23.93 ± 1.50	28.53 ± 1.40	10.77	0.000
	F	21.60 ± 3.17	26.03 ± 2.08	7.06	0.000
SMM (kg)	M	20.81 ± 1.74	27.31 ± 2.18	12.24	0.000
	F	11.70 ± 0.56	19.89 ± 1.47	22.17	0.000
SMM/Wt% (%)	M	38.94 ± 4.21	41.81 ± 3.49	3.01	0.004
	F	28.81 ± 2.92	36.76 ± 3.77	8.57	0.000

CAG = skinfold corrected upper-arm girth; CCG = skinfold corrected calf girth; CTG = skinfold corrected thigh girth; SMM = total-body skeletal muscle mass; Wt = weight.

The significant differences are vs. controls.

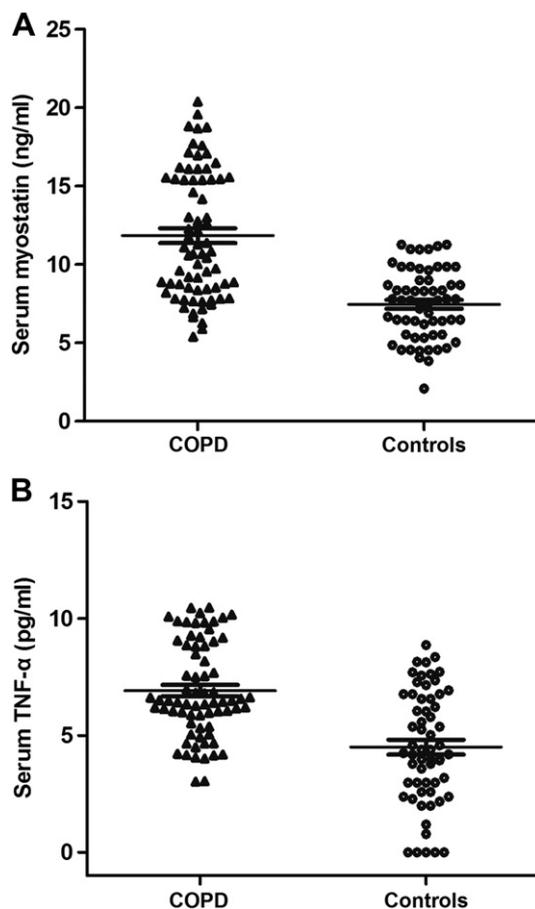


Figure 1 Serum levels of myostatin and TNF- α in COPD patients and controls. A: Serum myostatin levels were significantly elevated in patients relative to controls; B: Serum TNF- α levels were significantly elevated in patients when compared to controls.

for sex, the correlation coefficient (R^2 value) was even higher, with R^2 of 0.749 in female and 0.740 in male COPD patients, respectively, and 0.329 in female and 0.235 in male control subjects, respectively (all $p = 0.000$).

Discussion

In the present study, we analyzed serum myostatin levels in 71 patients with COPD and 60 control subjects. Our analysis yielded two main findings: (1) serum myostatin levels were significantly elevated in patients with COPD as compared with healthy controls. (2) The elevated myostatin levels were correlated inversely with total-body SMM and BMI in male COPD patients, while positively with TNF- α levels in all of the COPD patients. As far as we know, there were no reports about the relationship between serum myostatin and SMM in COPD patients in the previous studies.

In the present study, we evaluated total-body SMM according to a formula developed by Lee et al.¹¹ Our data showed that the total SMM was significantly correlated with

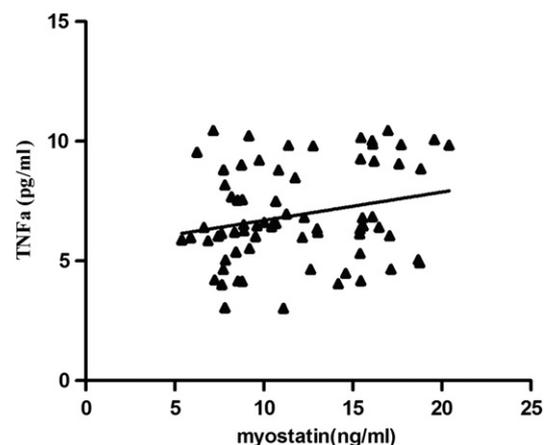


Figure 2 Correlation between serum myostatin and TNF- α levels in patients with COPD ($n = 71$). $R^2 = 0.042$, $p = 0.048$.

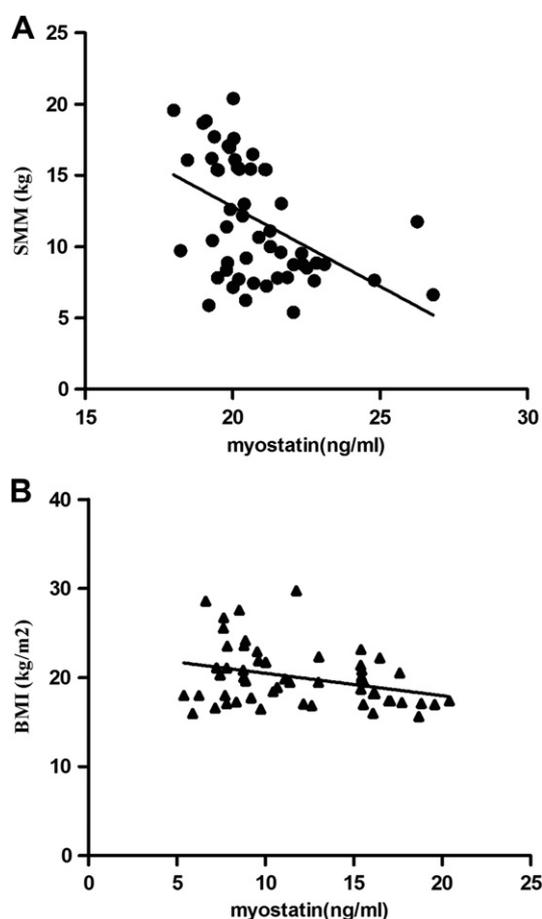


Figure 3 A: Inverse correlation between serum myostatin levels and total SMM in male patients with COPD ($n = 54$). $R^2 = 0.200$, $p = 0.000$. B: Inverse correlation between serum myostatin levels and BMI in male patients with COPD ($n = 54$). $R^2 = 0.084$, $p = 0.019$.

BMI in both COPD patients and controls, confirming the validity of the formula. Though we had tried all efforts to minimize the impact of co-morbidities, nutritional depletion were not avoidable for the patients; total-body SMM were significantly decreased in the patients as compared with controls, with regards to both its absolute value and its percentage of body weight so was BMI. The data was consistent with recent reports that skeletal muscle wasting and malnutrition were common and substantial in COPD patients.^{16,17} The results also showed that serum myostatin levels were significantly increased in COPD patients relative to controls. Myostatin is a new member of transforming growth factor-beta superfamily and first reported in 1997 by McPherron et al.¹⁸ Since its discovery, myostatin has quickly been attracted much attention as a key regulator of skeletal muscle mass in both animals¹⁹ and humans.²⁰ Recent studies have shown that myostatin is implicated in several diseases involved in muscle wasting and cachexia.^{21,22} Our study result that serum myostatin was significantly elevated in COPD patients was consistent with the report that myostatin mRNA levels and protein levels were elevated in quadriceps²³ and diaphragm²⁴ of COPD patients, respectively. Moreover, our data showed that serum myostatin levels were correlated inversely with total-body SMM in male COPD patients. The

similar study result showed that both the serum and intramuscular concentrations of myostatin were increased in HIV-infected men with weight loss compared with healthy men and correlated inversely with fat-free mass index in the patients.²⁵ These data support our hypothesis that serum levels of myostatin are consistent with those of gene expression in skeletal muscles and demonstrate that circulating myostatin may be associated with postnatal muscle wasting in body. In addition, our study showed that serum myostatin levels were even higher in the COPD patients with malnutrition than those with normal BMI, and correlated inversely with BMI in male COPD patients. Similarly, Schulte²⁶ reported that serum myostatin-immunoreactive protein levels were elevated in physically frail women and inversely correlated with lean mass. Yarasheski²⁷ reported that circulating levels of myostatin-immunoreactive protein were increased in elderly persons with weight loss. Together with these data, we suggest that circulating myostatin may also contribute to weight loss in COPD patients. Our hypothesis was supported by important messages from recent researches that myostatin cascade was involved not only in muscle atrophy but also in cachexia in mammalian,^{28,29} and that systemic over-expression of myostatin in adult mice induced profound muscle and fat loss similar to that seen in human cachexia syndromes.³⁰ Our findings, together with the previous reports of the association between elevated serum myostatin and lean muscle mass indicate a tight linkage of the circulating myostatin with skeletal muscle wasting and weight loss in COPD. However, the non-significant correlation of serum myostatin levels with SMM and BMI in female COPD patients was inconsistent with the above findings. The potential explanation could be the small sample size of female patients in our study.

With the evidence of elevation of intramuscular myostatin in COPD and our study result of elevation of circulating myostatin, we can conclude that myostatin is up-regulated in patients with COPD. There have been several reports concerning the risk factors related to up-regulation of intramuscular myostatin. The reported risk factors included cigarette smoking, muscle unloading and disuse, systemic corticosteroid treatment, and chronic infection.^{31–35} Very recently, Hayot et al.³⁶ reported an induction of myostatin expression in muscles of rats exposed to chronic hypoxia and demonstrated that myostatin up-regulation was associated with the skeletal muscle response to hypoxic stimuli. It is well known that the chronic progression of COPD is associated with recurrent infection during exacerbation periods. In severe COPD, chronic hypoxia, decreased physical activity and muscle disuse, as well as systemic corticosteroid treatment during acute exacerbation are commonly encountered. This means that COPD patients have the risk factors mentioned above to stimulate myostatin over-expression. In our study, ABG analysis showed a varying degree of hypoxia in COPD patients; PA scores proved a decreased physical activity in COPD patients. These findings in our study are consistent with the risk factors associated with myostatin over-expression in muscles. We thus speculate that the intramuscular elevation of myostatin may be the source of elevation of circulating myostatin, resulting in a whole body of muscle wasting in COPD patients. Our speculation was supported by the study result that cardiac-specific

over-expression of myostatin in heart could increase circulating levels of myostatin by 3- to 4-fold, resulting in a reduction in weight of whole-body muscles of rats with heart failure.³⁷ Moreover, the speculation was further supported by recent research evidences that resistance training could cause a significant decrease in serum myostatin levels and increase in muscle mass in both COPD patients and healthy men.^{38–40}

There have also been reports on the relationship of elevated TNF- α levels with myotrophy. Our data also showed that serum TNF- α levels were elevated and correlated with myostatin levels in COPD group. This finding was consistent with the study results by Lenk et al.⁴¹; they showed that TNF- α mRNA levels were increased and correlated positively with myostatin expression in skeletal muscles of chronic heart failure animals, moreover, they found that TNF- α could induce the expression of myostatin in differentiated C2C12 cells. TNF- α is a pleiotropic cytokine classically described as playing a central role in the pathophysiology of COPD.^{42,43} TNF- α is once named cachectin owing to its ability to induce tissue depletion and inflammatory weight loss.^{44–46} Currently, there were accumulating evidences suggesting that TNF- α plays an important role in skeletal muscle wasting in a number of systemic diseases including sepsis, COPD, cancer, and etc,^{47–49} as TNF- α is involved in the development of muscular abnormalities resulting in loss of skeletal muscle mass and function.^{50,51} Earlier study showed that circulating TNF- α levels were significantly higher in COPD patients with weight loss when compared to controls, and that the elevated TNF- α levels were correlated with BMI in the patients.⁵² Recent study found that the elevated TNF- α levels were correlated inversely with SMM (determined by creatinine-height index) in patients with COPD.⁵³ In accordance with the findings, our data showed an inverse correlation of serum TNF- α levels with total-body SMM in male COPD patients. Together with these literatures and our study results, we suggest that the elevated TNF- α might stimulate expression of myostatin in COPD, leading to a more significant skeletal muscle wasting in COPD. However, further studies need to investigate the exact relationship of TNF- α with myostatin and determine whether it is another inducer of myostatin in COPD. With regards to IL-6, our data failed to find out its elevation in COPD patients, which was in accordance with the one reported by Karadag et al.⁵⁴ However, Yende et al.⁵⁵ showed that IL-6 levels were significantly increased in COPD patients and correlated to body weight. The likely explanation for the inconsistency was that the patients in our study has been in stable stage for more than 3 months, thus the serum levels of IL-6 may be not significantly elevated. Further study should be taken to give the exact explanation, but we didn't focus on IL-6 in the study.

Limitation of the study

The major limitation of this study was the small sample size of the female patients with COPD. The conclusion should be confirmed by further studies in a larger population of female patients; although limited, is enough to gain some understanding of the myostatin in patients with COPD.

Conclusions

In summary, our study data demonstrates that circulating myostatin levels were significantly elevated in COPD patients, and that the elevated myostatin levels were correlated inversely with total-body SMM and BMI in COPD male patients. We conclude that the elevated circulating myostatin may contribute to muscle wasting and weight loss in patients with COPD.

Conflict of interest statement

None of the authors have a conflict of interest to declare in relation to this work.

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