Serum leptin and multi-detector computed tomography (MDCT)-measured bone attenuation among low BMI male patients with moderate-severity chronic obstructive pulmonary disease in exacerbation and stable states

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Introduction
Several studies have shown high prevalence of osteoporosis and weight loss in patients with chronic obstructive pulmonary disease (COPD). Leptin regulates bone metabolism, body weight, and pulmonary artery pressure. The association of bone density and serum leptin with BODE score in moderate COPD patients is still unclear.

Aim of the study
The aim of the study was to explore the association of serum leptin with average bone attenuation assessed by routine chest MDCT, and their correlation with clinical and echocardiographic parameters.

Patients and methods
The study included 54 male patients with low BMI and moderate COPD severity. Patients were divided into two groups: those with COPD exacerbations (24 patients; group I) and those with stable COPD (30 patients, group II). Twenty male volunteers of matched age and BMI were included as controls (group III). Calculation of BMI and BODE score was done. Spirometry and echocardiography were performed in all participants. Average bone attenuation of the thoracic spine was estimated by MDCT. Serum leptin was estimated.

Results
Group I and group II had significantly lower bone attenuation and higher BODE index, pulmonary artery systolic pressure (PASP), and right ventricle diameter (RVD) as compared with healthy controls ($P < 0.001$). Serum leptin level and leptin/BMI ratio were significantly increased in group I than in other groups ($P < 0.001$). Group II had significantly lower serum leptin than did controls. Serum leptin correlated positively with age, BMI, COPD severity, and bone attenuation and showed significant negative correlation with BODE score and serum calcium in group II. Meanwhile; it showed significant positive correlation with BMI and PASP in group I. In the stable COPD group, PASP, RVD, BMI, and bone attenuation were independent predictors of serum leptin, whereas BODE score, FEV_1, FEV_1/FVC, PASP, RVD, BMI, and serum leptin were independent predictors of bone attenuation.

Conclusion
COPD patients with moderate severity and low BMI had increased circulating leptin and low calcium level during exacerbation. Serum leptin level correlated with bone attenuation in stable but not in exacerbation states.

Keywords:
BODE score, bone attenuation, chronic obstructive pulmonary disease, exacerbations, serum leptin

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Introduction

Several studies have shown high prevalence of osteoporosis and weight loss among patients with severe chronic obstructive pulmonary disease (COPD) [1–3]. Recently, average attenuation of three thoracic vertebrae, numbers 4, 7, and 10, on routine chest computed tomography (CT) was seen to strongly correlate with the lowest bone mineral density (BMD) of the hip and lumbar spine on dual-energy x-ray absorptiometry (DXA) in patients with COPD [4,5]. Impaired lung function as measured by FEV_1 has been shown to be an independent predictor of osteoporosis among patients with more severe COPD [6]. However, data on moderate COPD (GOLD2) are few. Osteoporosis has been suggested to

be either a comorbidity, because of shared risk factors (e.g. older age, smoking, weight loss), or a systemic effect of COPD, including systemic inflammation and disturbance of the osteoprotegerin (OPG)/receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL) pathway [7,8].

Leptin is an adipokine that is related to the amount of body fat. Direct effect of circulating leptin promotes bone formation, at least partly, through the RANK/RANKL/OPG pathway [8]. However, through the central nervous system, leptin has the opposite effect on bone metabolism through its influence on satiety, decreasing body weight and increasing energy expenditure [9–11]. Clinical studies showed conflicting data on the association of serum leptin level with the prevalence and severity COPD [12]. In addition, circulating leptin concentrations rise during acute COPD exacerbations among average weight and obese patients under the control of systemic inflammatory response and return to baseline several days to weeks later in the stable state following the resolution of exacerbation [13–15]. Increased circulating leptin in acute exacerbation may contribute to anorexia and weight loss [16]. Systemic concentrations of leptin display a circadian rhythm, with a nadir at around 8 in the morning among healthy participants and noncachexic COPD patients [17]. Loss of circadian rhythm and normal physiologic release of leptin among underweight COPD patients may be a compensatory mechanism to maintain body fat content [18]. Data on the preservation of normal physiological inflammatory response and elevated leptin in acute exacerbation or its loss to maintain body weight in low BMI COPD is lacking. There are few data on the association of serum leptin, bone attenuation, and its correlation to clinical features among moderate COPD (GOLD2) male patients with low BMI in exacerbated and stable state.

The current study aimed to measure serum leptin level during stable and exacerbation status in GOLD2-COPD patients having low BMI and to explore the association of serum leptin with average bone attenuation as assessed by routine MDCT of the chest, and their correlations with studied clinical severity and echocardiographic scoring features.

Patients and methods

The current study is a prospective case–control study that was carried out in the Internal Medicine Department of Minia University Hospital during the period from May 2012 to January 2013. The study included 54 moderate COPD (GOLD2) male patients with low BMI (<21) aged above 40 years. Only men were chosen, in order to eliminate the effect of sex on routine chest MDCT-measured bone attenuation. They fulfilled the criteria of COPD (history of chronic productive cough, wheezy chest, gradual progressive dyspnea that responds partially to bronchodilators, and severe smoking index). COPD was diagnosed by postbronchodilator FEV_1/FVC less than 70 of predicted and reversibility of less than 12% and the severity of air flow limitation was assessed according to the GOLD criteria (2013) based on postbronchodilator FEV_1. Moderate COPD means GOLD2 = air flow limitation with 50%£FEV_1 <80% predicted [19]. The study has been approved by the local research ethics committee of Minia University, which conforms to the Declaration of Helsinki.

Patients were further subdivided to two main groups: patients with COPD exacerbations (24 patients, group I) and patients with stable COPD (30 patients, group II). Clinical stability was defined as no requirement for antibiotics, no flow limitation or oral steroids therapy, and no COPD exacerbations within the last 2 months [20]. An exacerbation is defined as the presence of at least 2 consecutive days of increase in any two 'major' symptoms (dyspnea, sputum purulence, or amount) or increase in one 'major' and one 'minor' symptom (wheeze, cough, symptoms of flu) [20]. Patients were further evaluated by calculation of BODE score severity index [21].

Exclusion criteria

Patients with other chronic illnesses that may affect BMD were excluded, such as diabetic patients and patients with chronic renal or liver disease, neoplastic disease, or cardiac failure. Patients with already known osteoporosis were also excluded.

Control group

Twenty healthy male volunteers of matched age and BMI were selected as controls with no past history of any chronic medical diseases that may affect BMD.

The selected patients and controls were briefed about the study and written informed consent was obtained.

Clinical study

All patients were examined within 24 h of inclusion and subjected to the following studies:

(1) Full history taking with special emphasis on smoking index, frequency of previous hospitalization (times/year), duration of illness (years), and dyspnea grade. Patients were asked about steroid intake, calcium and vitamin D intake (daily supplements and dietary intake), and history of spontaneous fractures.

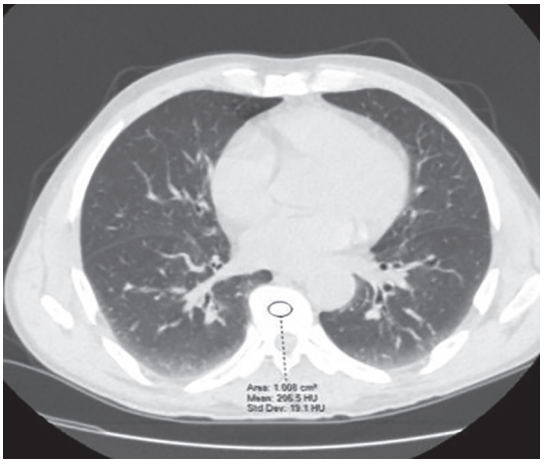
Clinical examination

(1) Body weight, height, and BMI (weight in kg/height in m^2) were calculated. Low BMI was defined as BMI of 21 kg/m^2 or less [20].
(2) Signs of corpulmonale [bilateral lower-limb edema, enlarged tender liver, increased jugular venous pressure, signs of right ventricular (RV) hypertrophy].
(3) Calculation of BODE score (the Computation of BMI, Degree of Airflow Obstruction and Dyspnea, and Exercise Capacity) [21] (Table 1).
(4) ECG, spirometry, and arterial blood gas analysis were carried out at the time of inclusion.
(5) Imaging studies:
    (a) *Plain chest radiograph*: The posteroanterior view was obtained for all patients on the first day of inclusion.
    (b) *Echocardiography*: All patients underwent echocardiography for measurement of RV dimensions and PSAP, using a GE Vivid 3 device (GE Medical Systems, Milwaukee, WI, USA). Using two-dimensional echocardiography, RV size can be measured from a four-chamber view obtained from the apical window at end-diastole. RV systolic pressure can be reliably determined from peak tricuspid jet velocity in the absence of a gradient across the pulmonic valve. PSAP is equal to RV systolic pressure. The normal cutoff value for invasively measured mean pulmonary artery (PA) pressure is 25 mmHg. Normal resting values are defined as a peak TR gradient of 2.8–2.9 m/s or a PSAP of 35–36 mmHg, assuming right atrium (RA) pressure of 3–5 mmHg [22].

MDCT chest

All patients were studied using a GE Bright-speed 16 multislice computed tomography machine (GE MedicalSystems, Milwaukee, WI, USA). A standard MDCT examination of the chest was performed with a slice thickness of 1.3 mm. No contrast was given. For every patient, we measured the bone density of the dorsal vertebrae in Hounsfield Units, as carried out in a previous study [5] (Figs 1, 2a and b).

Figure 1



Chest computed tomography (CT) image (pulmonary window) revealing increased fine reticulations in the periphery of both lung fields. Dorsal vertebral density in this patient is around 206.5 HU. Average density in this patient was 185.3 HU.

Laboratory studies

Venous blood samples were collected for estimation of the following:

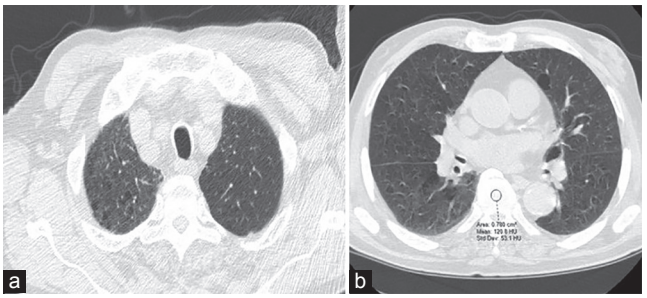
(1) Serum leptin:
    The DRG leptin enzyme-linked immunosorbent assay test was performed using Huma-reader plus

Table 1 Variables of BODE score

Variables	0	1	2	3
FEV1 % predicted†	≥65	50-64	36-49	≤35
Distance walked in 6 min (in meters)	≥350	250-349	150-249	≤149
The modified Medical Research Council (MMRC) Score‡	0-1	2	3	4
Body mass index§	>21	≤21		

The cut-off values for the assignment of points are shown for each variable. The total possible values range from 0 to 10. †FEV1% predicted denotes forced expiratory volume in one second as a percentage of the predicted value (the American Thoracic Society ‡Scores on the modified Medical Research Council (MMRC) [22] Dyspnea scale ranges from 0 to 4; 0 – "Not troubled with breathlessness except with strenuous exercise"; 1 – "Troubled by shortness of breath when hurrying on the level or walking up a slight-hill"; 2 – "Walks slower than people of the same age on the level because of breathlessness or has to stop for breath when walking at own pace on the level"; 3 – "Stops for breath after walking about 100 yards or after a few minutes on the level" and; 4 – "Too breathless to leave the house or breathless when dressing or undressing"; §The values for BMI were 0 or 1 because of the inflection point in the inverse relation between survival and BMI at a value of 21.

Figure 2



(a) Chest computed tomography (CT) image (pulmonary window) revealing increased fine reticulations of both lungs and patchy ground-glass opacification as well as emphysematous cysts. Dorsal vertebral density in this patient is around 120.8 HU. Average density in this patient was 145.8 HU. (b) Chest CT image (pulmonary window) revealing increased fine reticulations of both lungs and patchy ground-glass opacification as well as emphysematous cysts. Dorsal vertebral density in this patient is around 120.8 HU. Average density in this patient was 145.8 HU.

(USA). The normal value for men is 3.8 ± 1.79 ng/ml [23].
(2) Serum calcium, phosphorus, and alkaline phosphatase were also estimated.

Statistical study

Statistical analysis was carried out using SPSS version 20 (SPSS, Cary, North Carolina, USA). Data are presented as mean ± SD. *P* values of 0.05 or less were considered statistically significant. The independent-sample *t*-test was conducted for comparing parametric quantitative data between two groups. Pearson's correlation test was performed to evaluate for potential relationships. Multiple linear regression tests using leptin or bone attenuation as dependent factors were also conducted.

Results

Clinical characteristics of the studied groups are shown in Table 2. All patients were current smokers. Patients of group I and group II had significantly lower FEV_1% predicted, FEV_1/FVC, and bone attenuation and significantly higher BODE index, PASP, and RVD as compared with healthy controls ($P \leq 0.001$ for all). Group I had significantly higher serum leptin and serum leptin/BMI ratio compared with group II and group III ($P \leq 0.001$ for all). Serum calcium levels in both patient groups were significantly lower than in control participants. Also serum alkaline phosphatase and phosphorus levels were higher in the patient groups than in controls. Group I had significantly higher PASP than group II, but no significant difference was found between the two groups as regards age, smoking, FEV_1% predicted, FEV_1/FVC, RVD, BODE index, and bone attenuation. Group II had significantly lower serum leptin and serum leptin/BMI ratio than group III ($P \leq 0.001$).

Among COPD patients with exacerbation, serum leptin showed significant positive correlation with BMI, PASP, and serum calcium (P = 0.01, 0.005, and 0.016, respectively). This is shown in Table 3. Among COPD patients with stable status, serum leptin showed significant positive correlation with age, BMI, FEV_1, FEV_1/FVC, and bone attenuation ($P < 0.001$, $P < 0.01$, $P < 0.003$, $P < 0.02$, and $P < 0.001$, respectively) and significant negative correlation with BODE score ($P \leq 0.001$).

In group I, bone attenuation showed significant positive correlation with BMI, FEV_1% predicted, and FEV_1/FVC ratio ($P < 0.04$, $P < 0.001$, and $P < 0.001$, respectively) and significant negative correlation with age and BODE score ($P < 0.01$ and $P < 0.004$, respectively). This is shown in Table 4. In group II bone attenuation showed significant positive correlation with BMI, FEV_1% predicted, FEV_1/FVC ratio, and serum leptin ($P < 0.01$, $P < 0.003$, $P < 0.02$, and $P < 0.001$, respectively) and significant negative correlation with age and BODE score ($P < 0.001$ and $P < 0.001$, respectively).

Multiple regression analysis was performed for group II, with serum leptin as the independent factor and age, BMI, FEV_1, FEV_1/FVC, BODE score, PASP, RVD, and bone attenuation as dependent variables. This is shown in Table 5. PASP, RVD, BMI, and bone attenuation were dependent predictors to serum leptin ($P < 0.02$, $P < 0.04$, $P < 0.001$, and $P < 0.001$, respectively).

Table 2 Clinical, laboratory and echocardiographic characteristics of study population groups

Characteristic	Group I COPD with exacerbation (NO = 24)	Group II COPD with stable status (NO = 30)	Group III Healthy control (NO = 20)	*P* value		
				a	b	c
Age (years)	61.8 + 9.1	60.6 + 6.1	61.2 + 6.0	0.56	0.76	0.73
Smoking index	480 + 23.8	320 + 12.9	212 + 22.5	<0.01*	<0.001*	<0.001*
BMI (kg/m^2)	19.4 + 0.9	19.3 + 1.1	19.5 + 1.1	0.66	0.86	0.35
FEV1% predicted	56 .04 + 7.0	53. 23 + 4.0	87 + 0.01	0. 1	<0.001*	<0.001*
FEV1/FVC ratio	54.87 + 3.01	53.4 + 2.4.2	92.35 + 0.4	0.07	<0.001*	<0.001*
BODE score	5.7 + 1.3	5.6 + 1.02	0.75 + 0.2	0.7	<0.001*	<0.001*
RVD (cm)	4.08 + 0.49	4.07 + 0.15	1.86 + 0.25	0.9	<0.001*	<0.001*
PASP(mm /Hg)	63.7 + 7.3	59.6 ± 5.2	22.8 + 5.35	0.02*	<0.001*	<0.001*
Serum leptin (ng/ml)	15.67 + 4.9	1.4 + 0.9	3.31 + 0.78	<0.001*	<0.001*	<0.001*
Leptin /BMI ratio	0.83 + 0.3	0.07 + 0.02	0.17 + 0.14	<0.001*	<0.001*	<0.001*
Serum Ca^{+2} (mg/dl)	8.6 ± 0.72	8.8 ± 0.79	9.2 ± 0.34	0.677	<0.001*	<0.001*
Serum phosph (mg/dl)	5.83 ± 2.18	5.47 ± 1.44	3.95 ± 0.47	0.62	<0.001*	<0.001*
Serum alk. Phosph. (IU/L)	195.5 ± 55.7	184.7 ± 45.6	137.8 ± 13.16	<0.01*	<0.001*	<0.001*
Bone attenuation(HU)	208.25 + 43.5	193.8 + 47	249.7 + 41.5	0.39	<0.001*	<0.001*

Data are expressed as mean+SD and compared by independent t test; BMI, Body mass index; FEV1, forced expiratory volume in 1st second; FVC, forced vital capacity; RVD, right ventricular diameter; PASP, pulmonary artery systolic pressure; HU, Hounsefield Unit; a, *p* value when group I compared to group II; b, p value when group I compared to group III; c, p value when group II compared to group II

Multiple regression analysis among stable COPD patients with bone attenuation as the independent factor revealed BMI, FEV_1, FEV_1/FVC, BODE score, PASP, RVD, PASP, RVD, and leptin to be dependent predictors of bone attenuation ($P < 0.001$, $P < 0.01$, $P < 0.04$, $P < 0.003$, $P < 0.001$, $P < 0.007$, $P < 0.001$, respectively). This is shown in Table 6.

Discussion

The present study showed that low-BMI moderate-severity COPD patients with exacerbation exhibited increased serum leptin and leptin/BMI ratio compared with those with stable disease and healthy controls. This is in line with previous studies among average weight and overweight COPD patients who had elevated systemic leptin concentrations and adjusted leptin for percentage fat mass during acute exacerbations [13–15]. They also found that the levels returned to baseline several days to weeks later in the stable state following the resolution of the exacerbation. Increased leptin concentrations in acute exacerbation are due to infection and sepsis as a part of the systemic inflammatory response [24]. The inflammatory response in the lung in acute exacerbation may upregulate adipose tissue leptin expression by endogenous corticosterone elevation [25] and production of various proinflammatory cytokines, including tumor necrosis factor-α [26], which correlated with serum leptin in acute exacerbation of COPD [27]. Local pulmonary leptin production in exacerbation may be involved in elevated serum leptin levels. The concentration of leptin in bronchoalveolar lavage fluid is strongly correlated with serum levels, suggesting that leptin is also transported between blood and lungs by mechanisms that are not clearly

Table 3 Correlation between serum leptin and studied variables among COPD patients groups

Characteristic	Group I COPD with exacerbation (NO = 24)		Group II COPD with stable status (N0 = 30)	
	r	P value	r	P value
Age (years)	–0.36	0.08	0.47	0.001*
BMI (Kg/m2)	0.51	0.01*	0.71	0.01*
FEV1% predicted	–0.1	0.58	0.52	0.003*
FEV1/FVC ratio	–0.35	0.1	0.42	0.02*
Serum calcium	–0.310	0.016*	–0.290	0.05*
Serum phosphorus	0.148	0.258	0.160	0.245
Serum alkaline phosphatase	–0.485	0.107	–0.378	0.122
BODE score	0.01	0.58	–0.68	<0.001*
RVD (cm)	0.35	0.09	0.04	0.81
PASP (mmHg)	0.55	0.005*	–0.24	0.19
Bone attenuation (HU)	–0.35	0.08	0.74	<0.001*

BMI, Body mass index; FEV1, forced expiratory volume in 1st second; FVC, forced vital capacity; RVD, right ventricle diameter; PASP, pulmonary artery systolic pressure; HU, Hounsefield Unit

Table 4 Correlation between CT measured bone attenuation and studied parameters among COPD patients

Characteristic	Group I COPD with exacerbation (N0 = 24)		Group II COPD with stable status (N0 = 30)	
	r	*p*	*r*	*p*
Age (years)	–0.48	0.01*	–0.35	0.001*
BMI (Kg/m2)	0.42	0.04*	0.46	0.01*
FEV1% predicted	0.74	0.001*	0.82	0.001*
FEV1/FVC ratio	0.61	0.001*	0.72	0.004*
Serum calcium	0.260	0.15	0.257	0.18
Serum phosphorus	–0.107	0.71	–0.205	0.11
Serum alkaline phosph	–0.284	0.15	–0.260	0.10
BODE score	–0.53	0.007*	–0.91	<0.001*
RVD (cm)	–0.135	0.53	0.260	0.15
PASP (mm/Hg)	0.37	0.07	0.07	0.71
Serum leptin (ng/ml)	–0.36	0.09	0.77	<0.001*

BMI, Body mass index; FEV1, forced expiratory volume 1st second; FVC, forced vital capacity; RVD, right ventricular diameter; PASP, pulmonary artery systolic pressure

Table 5 Multiple regression analysis with the serum leptin as the independent factor among stable COPD

Model	Coefficients[a] Unstandardized Coefficients		Standardized Coefficients	*t*	*P*
	B	Std. Error	Beta		
(Constant))	–4.484	3.861		–1.161	.259
Age	0–.014	0.010	0.178	–1.474	.155
BODE score	0.113	0.143	.241	0.789	0.439
Serum Calcium	0.018	0.016	–3.298	3.317	0.016*
FEV1	–4.153	2.246	–0.354	–1.849	0.079
FEV1/FVC	0.019	.042	0.089	.445	0.661
PASP	0.038	0.016	0.419	2.397	0.026*
RVD	–0.926	0.431	–0.299	–2.147	0.044*
BMI	0.385	0.100	0.876	3.848	0.001*
Bone attenuation	0.007	0.002	0.720	4.162	0.000*

Table 6 Multiple regression analysis with the bone attenuation as the independent factor among stable COPD

Model	Coefficients[a] Unstandardized Coefficients		Standardized Coefficients	*t*	*P*
	B	Std. Error	Beta		
(Constant)	1069.902	288.696		3.706	0.001
Age	–0.183	.937	–.023	–.195	0.847
Smoking	24.07	22.10	0.534	2.340	0.05
BODE score	–36.860	10.836	–.791	–3.402	0.003
FEV1	540.465	192.445	.462	2.808	0.011
FEV1/FVC	–7.530	3.553	–.363	–2.119	0.046
PASP	–5.033	1.268	–.552	–3.969	0.001
RVD	111.828	37.097	.362	3.014	0.007
BMI	–40.705	8.321	–.928	–4.892	0.000
Leptin	62.663	15.056	.628	4.162	0.000
Serum calcium	61.342	14.17	0.688	4.220	0.000

understood [28–30]. Interestingly, leptin levels in serum and bronchoalveolar lavage are elevated following intratracheal challenge with *Klebsiella pneumoniae* [31]. Thus, we concluded that, despite loss of circadian rhythm and normal physiologic release of leptin as a compensatory mechanism to maintain body fat content among low-BMI COPD patients [18], the leptin inflammatory response is preserved with increased circulating level during exacerbation. This elevation may contribute to anorexia and further weight loss [16].

In the current study, stable COPD patients had significantly lower serum leptin levels compared with BMI-matched healthy controls. The data regarding the independent association between serum leptin concentrations and COPD prevalence or severity remain inconclusive [12]. Our results were in line with those of Yang *et al*. (2006) [32], Takabatake *et al*. (1999) [33], and Ugay *et al*. (2014) [34]. Also, leptin expression was decreased in participants with mild to severe COPD as compared with healthy nonsmoking participants [35]. In contrast, in another small Korean case–control study, no differences in serum leptin was found [36]. These studies involved only male COPD patients. Breyer *et al*. (2011) [37] found higher serum leptin concentrations among women but not men with stable COPD. Sex difference is attributed to the opposite effect of the sex hormone on serum leptin level. Leptin expression is inhibited by testosterone, whereas it is increased by ovarian sex steroids [37]. Low serum leptin among COPD patients in stable state may be explained by the chronic inflammation process, which, in contrast to acute inflammation, causes a reduction in leptin levels. Other contributing factors among them were anorexia and use of β_2 agonists [38].

The leptin receptor is expressed by human bronchial smooth muscle, submucosa, and alveolar epithelial cells [11]. The lung is therefore a likely target organ for leptin signaling. Leptin is a stimulant to ventilation and has an important role in lung maturation and development. Thus, leptin may have a pathogenic role in the occurrence of COPD and its severity [12]. Our findings indicated an association between serum leptin level and moderate COPD in the stable state and were in line with those of Brúsik *et al*. (2012) [39]. In support of our associations, both lower leptin/adiponectin ratio and single nucleotide polymorphisms of the leptin receptor gene were associated with lung function decline in patients with COPD in two independent Japanese and western cohort studies [40,41]. In contrast, others found no correlation between FEV_1 and leptin among average weight and obese COPD [42] patients (men and women). Yang *et al*. (2006) [32] reported a nonsignificant difference in serum leptin levels between moderate and severe COPD patients.

Leptin plays an important role in energy homeostasis, including control of body weight and energy expenditure [10]. We found that circulating leptin levels were positively correlated with BMI in both stable and exacerbation status. These were in agreement with a meta-analysis of Zhou *et al*. (2013) [27]. Thus, circulating leptin levels remain physiologically regulated in stable low-BMI COPD patients, as noted in other malnutritional states [43,44]. Presence of positive correlation among those with exacerbation is due to its synthesis mainly in white adipose tissue apparently in a proportion of stored fat [45].

A novel finding in our study is that serum leptin was positively correlated to PASP in the exacerbation group, and this group had significantly higher PASP compared with group II, despite the similarity between the two groups regarding the degree of airflow limitation and severity of COPD as presented by FEV_1 and BODE score, respectively. We also found PASP and RVD to be independent contributors to serum leptin in the stable group. Thus, associations of serum leptin level and PASP can be attributed to several mechanisms. Leptin activates the sympathetic nervous system with subsequent vasoconstriction [46–48]. It stimulates endothelin production by endothelial cells, which is a powerful vasoconstrictor of pulmonary circulation [49]. Leptin contributes to inflammatory changes in the pulmonary circulation and thereby increases pulmonary vascular resistance [48]. Also normal pulmonary arteries have little vasodilator response to leptin-induced endothelial nitric oxide production [50]. This little nitric oxide-mediated vasodilatory effect is further blunted because of pulmonary endothelial dysfunction in COPD patients. In a study by Tonelli *et al*. (2012) [51] patients having chronic pulmonary hypertension of other etiologies, such as idiopathic and thromboembolic, had increased leptin levels, which showed no associations with RV systolic pressure and other hemodynamic parameters.

The prevalence of osteoporosis, which is characterized by low BMD and microarchitectural changes [52], has been suggested to be higher in severe COPD patients than in control participants matched for age and sex [3]. The gold standard to diagnose osteoporosis is DXA [53]. Although DXA has a low radiation dose and is easy to use, previous studies have reported an underdiagnosis of osteoporosis [54]. To diagnose osteoporosis in an early stage, current research is focused on screening for osteoporosis using CT scans that were performed for other purposes. Abdominal and coronary CT studies have already been shown

to be useful for assessing BMD and osteoporosis screening [54–56]. Recently, average attenuation of the thoracic vertebrae on routine chest CT was strongly correlated with the lowest BMD of the hip and lumbar spine on DXA in patients with COPD [5]. In the present study, we assessed BMD by measuring average bone attenuation of thoracic spines by means of routine chest CT according to the method previously described by Ohara *et al*. (2008) [4] and Romme *et al*. (2012) [5]. Thus, it provides useful information on bone health in patients with COPD but it will not replace DXA because of the higher radiation dose and higher costs.

Our results showed that both COPD groups (stable and exacerbation) had lower bone attenuation of the thoracic vertebrae measured on low-dose chest CT compared with controls. Lower CT-measured bone attenuation was associated with lower FEV_1, FEV_1/FVC, and BMI and higher age and BODE score. Among stable COPD participants, FEV_1, FEV_1/FVC, leptin, and BMI were independently associated with CT-measured bone attenuation, and circulating leptin levels showed significant positive correlations with CT-measured bone attenuation. In line with previous data by DXA, and routine chest CT-measured bone attenuation [5] among severe and more severe COPD patients, we found that nonsevere COPD patients had lower bone attenuation compared with age-matched and BMI-matched controls. As some of the osteoporosis risk factors like advanced age, smoking, physical inactivity, and low body weight coincide in patients with COPD, it is not surprising that osteoporosis and COPD are strongly related. However, there is evidence that various COPD-specific factors such as systemic inflammation, vitamin D deficiency, use of corticosteroids, and disturbance of the OPG/RANK/RANKL pathway may be implicated [57]. Smoking aggravates disturbance of calcium metabolism in COPD, leading to hypocalcemia and hypercalciuria. Smoking can reduce the thickness of the cortical bone and gene expression of the bone matrix. In smokers with COPD, bone remodeling dysfunction is caused by suppression of osteogenesis and enhancement of resorption [57,58].

In our study, circulating leptin levels showed significant positive association with CT-measured bone attenuation in stable COPD patients. This finding might suggest that leptin acts as a mediator between fat mass and bone density in patients with COPD. Circulating leptin increases the proliferation and differentiation of osteoblasts and promotes bone nodule formation. It also regulates osteoclast development, at least partly, through the RANK/RANKL/OPG pathway, which results in net bone formation. However, leptin has the opposite effect on the central nervous system by influencing satiety and insulin secretion [9]. Thus, the local effects of leptin from adipose tissue are dominant over the central effects, resulting in a stronger skeleton. Our results are in line with the study by Pobeha *et al*. (2011) [58] and Ugay *et al*. (2014) [34]. The former group reported that COPD patients with osteoporosis had lower circulating leptin levels compared with COPD patients without osteoporosis, and adipose tissue leptin expression was correlated positively with bone density in COPD patients with or without osteoporosis. Ugay *et al*. (2014) [34] found significant positive correlation between serum leptin and BMD assessed by DXA. However, others reported conflicting results in different population groups.

We found among both groups of COPD participants that higher bone attenuation was associated with higher FEV_1% of predicted and FEV_1/FVC ratio. These were in line with findings of other authors [5,59,60]. Osteoporosis has been related to the severity of airflow obstruction, as skeletal and pulmonary systems share a common underlying mechanism such as impairment of Wingless tail/β-catenin signaling [60] or disturbance of the RANK/RANKL/OPG pathway due to systemic inflammation [61]. The prevalence of osteopenia and osteoporosis increased with more severe airflow obstruction according to the Third National Health and Nutrition Examination Survey, which included 9502 participants (NHANES III) [62]. They found significant association between vitamin D_2 levels and pulmonary functions after adjusting for variables such as age, sex, smoking history, height, ethnicity, history of COPD, and BMI [63]. More recently, a cross-sectional study with 95 COPD patients demonstrated that patients with osteoporosis had lower FEV_1 compared with patients with normal bone mass [64]. In contrast, some studies on patients with COPD did not find this relationship [65]. A novel finding of an inverse correlation between BODE score and BMD indicated an association of osteoporosis with severity, in addition to degree of airway obstruction, among nonsevere COPD patients [66,67]. Similarly, Malik *et al*. (2012) [68] found an association between many components of the BODE score and BMD among patients with pulmonary artery hypertension. They found that the osteopenic group had lower FEV_1 and a significantly decreased functional capacity.

We demonstrated significant association between pulmonary PASP, RVD, and bone attenuation among COPD patients. Ulrich *et al*. (2009) [69] reported that osteopenia was found in 60–80% of patients with pulmonary arterial hypertension of idiopathic and thromboembolic causes. Interestingly, they found a negative correlation of BMD with pulmonary vascular resistance, which was consistent with our results. In

contrast, Malik *et al*. (2012) [68] reported significant lower mean pulmonary artery pressure (P = 0.01) in osteopenic patients compared with nonosteopenic patients with pulmonary artery hypertension. This association may be explained by the metabolic effect of PASP on BMD [69]. Also serotonin may be a link between pulmonary hypertension and bone as it plays an important pathogenic role in various forms of pulmonary hypertension and recently has been reported to regulate bone mass [69–71]. Also, secondary hyperparathyroidism is highly prevalent in pulmonary hypertension and might contribute to bone and possibly pulmonary vascular disease [69].

Bone attenuation correlated positively with BMI in our study. This finding is supported by a study in which overweight and obese COPD patients had a decreased risk for osteoporosis, whereas cachectic COPD patients had an increased risk for osteoporosis compared with their normal weight peers [70–72]. Low BMI is a well-known risk factor for osteoporosis in COPD patients [73] and in the general population. Low body weight may lead to bone loss due to decreased mechanical loading or direct effects of loss of fat-free mass or fat mass on bone metabolism [74]. In a more recent study it was found that BMI, severe smoking index, and FEV_1 were independent risk factors for osteoporosis in COPD [75]. Many patients with end-stage COPD lose weight as the disease progresses because of decreased intake and increased energy requirements [67]. Furthermore, low bone mass was correlated with low fat-free mass in stage IV patients, and fat-free mass could thus be used as a determinant of bone loss in this population [67]. In contrast, Misof *et al*. (2015) [76] found unaffected mineralization processes in COPD and no significant negative effect of treatment with inhaled glucocorticoids on the bone mineralization pattern.

Regular evaluation of the biochemical markers of bone metabolism and BMD would be helpful for detecting any detrimental changes of bone in COPD patients under long-term inhaled corticosteroid therapy [75].

In the present study, bone attenuation decreased with age in COPD patients. This is in line with a previous study that showed that bone density of the thoracic vertebrae correlated negatively with age in male COPD patients [65]. In the general population, older age is a well-established risk factor of osteoporosis. In a healthy young skeleton, the rate of bone formation and matrix mineralization equals the rate of bone resorption and matrix degradation. However, during the aging process significant amounts of bone are lost because of enhanced resorption coupled with decreased formation, resulting in osteoporosis [74].

Limitations of the present study were the small number of subjects in each group, and lack of detailed history of drug intake of corticosteroid and β_2 agonists. Because of their high cost, serum levels of parathormone and 25-(OH) vitamin D were not included in the current study, but this drawback will be overcome in further studies based on the same group of patients.

Conclusion

Among COPD patients with moderate severity and low BMI, leptin inflammatory response is preserved with increased circulating levels during exacerbation. Serum leptin level correlated to bone attenuation in stable but not in exacerbation state. Both serum leptin level and bone attenuation correlated to BMI, degree of airway limitation, disease severity as assessed by BODE score index, and pulmonary artery pressure in stable COPD patients.

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Conflicts of interest

There are no conflicts of interest.

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