BODY COMPOSITION AND PLASMA LEVELS OF INFLAMMATORY

BIOMARKERS IN COPD

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ABSTRACT

Previous studies suggest a relationship between systemic inflammation and body composition in COPD. We examined the relationships between body composition (fat free mass index (FFMI, kg/m2), and fat mass index (FMI, kg/m2)) and three plasma inflammatory markers C-Reactive Protein (CRP), soluble tumor necrosis factor receptor one (sTNF-R1), and osteoprotegerin (OPG) in 409 stable COPD patients (aged 40-75, GOLD categories II-IV, 249 male) from the Bergen COPD Cohort Study in Western Norway. FFMI and FMI were measured by bioelectrical impedance. Plasma CRP (ug/mL), soluble TNF-R1 (pg/mL) and OPG (ng/mL) were determined by enzymeimmunoassays. Correlations and Kruskal Wallis tests were used for bivariate analyses. Linear regression models were fitted for each of the three markers, CRP, sTNF-R1, and OPG, with FFMI and FMI as explanatory variables including as potential confounders sex, age, smoking habits, GOLD category, hypoxemia, Charlson comorbidity index, and inhaled steroid use. CRP and sTNF-R1 levels correlated positively with both FFMI and FMI. The adjusted regression coefficients for an increase in logCRP per unit increase in FFMI was 1.23 (1.14, 1.33), and for sTNF-R1 24.9 (11.8, 38.1). Higher FMI was associated with a lower OPG, with adjusted regression coefficient -0.14 (-0.23, -0.04), whereas FFMI was unrelated to OPG.

In conclusion, COPD patients with low FFMI, had lower, not higher plasma levels of CRP and sTNF-R1, whereas higher fat mass was associated with higher CRP and sTNF-R1 and lower OPG.

Abbreviations:

BMI - Body mass index

COPD - Chronic obstructive pulmonary disease

CRP - C reactive protein

EIA - Enzyme immunoassay

FEV1 - Forced expiratory volume in 1 second

FFMI - Free fat mass index

FMI - Fat mass index

FVC - Forced vital capacity

GOLD - Global initiative for chronic obstructive lung disease

IL - Interleukin

OPG - Osteoprotegerin

RANK - Receptor activator of nuclear factor-κB

RANKL - Receptor activator of nuclear factor-κB ligand

sTNF-R1 - Soluble tumor necrosis factor receptor 1

TNFα - Tumor necrosis factor alpha

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the airways and lung parenchyma, estimated to affect 9-10% of adults over the age of 40 years [1]. COPD causes both significant mortality [2] and morbidity [3].

Approximately 25% of patients with COPD experience a pathological loss of lean body mass [4]. COPD patients with abnormally low lean body mass are at an increased risk of death compared to those with normal lean body mass [5]. The term cachexia is often used to describe the state of pathologically low lean body mass. However, some authors define cachexia as a state in which protein is lost specifically due to pro-inflammatory processes like those driven by tumor necrosis factor (TNF)α and IL-6 [6]. Although an increase in systemic inflammation arguably is the prevailing theory for loss of lean body mass in some COPD patients, this theory remains unproven [4].

Support for the role of systemic inflammation comes from cross-sectional studies in which higher blood levels of TNFα [7-10], IL-6 [9, 10] and C-reactive protein (CRP) [9] were seen in underweight COPD patients compared with normal weight patients. However, in the earlier studies [7-9], body mass index (BMI) was used to stratify patients. This is imperfect, since patients can have significant muscle wasting without a significant decrease in total body weight, if there is a corresponding increase in fat mass [11].

Four later studies have included measurements of fat free mass index (FFMI) by bioelectrical impedance [12-15]. Among these studies, there was no statistically significant relationship between levels of TNFα and body composition in the three studies which measured TNFα [12-

14]. In the fourth study, CRP was negatively associated with FFMI in bivariate analyses, but not after adjustment for confounders age, number of lymphocytes, handgrip strength, walking distance on 6 minute walk test, and percent low-attenuating area on HR CT scans [15]. The total number of COPD patients in these studies was generally small and varied between 20 [13] and 99 [12].

Because of confounding variables, there is still considerable uncertainty regarding the question of whether COPD patients with low FFMI have different blood levels of important inflammatory markers or not. To shed further light on this question, it is necessary to have a sample size that allows for multivariate analysis. The Bergen COPD Cohort Study (BCCS) has enrolled more than 400 COPD patients and is powered for a multivariate analysis. It has therefore provided an opportunity to assess the relationship between pro-inflammatory markers and body composition defined by both FFMI and fat mass index (FMI). We have recently published the levels of six inflammatory markers in COPD patients and subjects without COPD [16]. Of these, CRP, soluble TNF receptor 1 (sTNF-R1), and osteoprotegerin (OPG) were potentially related to body composition in COPD based on previous studies for CRP and sTNF-R1 [7-11, 13-15, 17], and for OPG based on its role in bone metabolism [18], and in metabolic disorders like diabetes [19]. Thus, the current paper reports on the relationship between these three markers and body composition in the more than 400 COPD patients at entry into the Bergen COPD Cohort Study.

METHODS

Study population

The first phase of the Bergen COPD Cohort study was undertaken in 2006 through 2007, and the selection of the study population and data collection has recently been published in greater detail

[16]. Altogether 433 COPD patients from western Norway, aged 40-75, were included in the first phase of the BCCS. Roughly 75% of the COPD patients were recruited through the outpatient clinic of the Department of Thoracic Medicine, Haukeland University Hospital. The remaining 25% were recruited from surrounding hospitals in western Norway, or from referrals from three private lung physicians outside the hospital.

All patients had a clinical diagnosis of COPD, a smoking history of more than 10 pack years, a post-bronchodilator FEV_1/FVC ratio < 0.7, and an FEV_1 of less than 80% predicted based on Norwegian pre-bronchodilation reference values [20]. Patients with known cancer the last five years, or with a known inflammatory disorder like rheumatoid arthritis, systemic lupus erythematosus or inflammatory bowel disease were not included. Common chronic comorbid disorders with known inflammatory components, like coronary heart disease, hypertension and diabetes, were not cause for exclusion. No COPD patients were included if judged by the study physician to have had an exacerbation the last four weeks prior to enrollment.

All patients received written information prior to participation, and all patients signed informed consent. The regional ethical committee approved the study.

Data collection

A physician examined all patients and recorded a full medical history including smoking history and comorbidities. The physician performed arterial blood gas sampling, and immediately analyzed the sample on a Radiometer ABL 520 blood gas analyzer. Lung function measurements were performed with a Viasys Masterscope before and 15 minutes after an inhalation of 0.4 mg salbutamol. Bioelectrical impedance was used to measure fat free mass after overnight fasting,

using a Bodystat 1500 (Bodystat Ltd, Isle of Man, UK). The fat free mass index (FFMI) was defined as the fat free mass (kg) divided by the square of height (m^2), and similarly for the fat mass index (FMI).

Plasma samples were centrifuged within 30 minutes at 2150g (15 minutes at 4°C). All samples were stored at –80°C and thawed <3 times. The inflammatory markers CRP, sTNF-R1, and OPG were analyzed by enzyme immunoassays (EIAs), as previously described [16].

Statistical analyses

Stata 10.1 was used for computation [21]. Based on the medical history, Charlsons Comorbidity Index was calculated [22, 23]. Since women and men differ in body composition with regard to fat mass, msucle mass and bone density, quartiles of FFMI and FMI were calculated for women and men separately, and then pooled into one variable for both sexes for FFMI and FMI quartiles respectively. Bivariate associations between the levels of each of the three markers and FFMI and FMI in quartiles were tested with the Kruskal Wallis non-parametric test. For the multivariate analyses, linear multivariable regression was performed with the three markers as the outcome variables respectively. Due to the non-normal distribution of the residuals, logCRP was used as the outcome in the regression analysis examining the effects of FFMI and FMI on CRP. Examined independent variables were sex, age, smoking (ex versus current), FEV1 in percent predicted, hypoxemia, Charlson comorbidity index, and use of inhaled steroids. Multicollinarity prevented the the simultaneous inclusion of FFMI and FMI directly in the multivariable regression models. Instead, the method of residualization was used to correct for multicollinarity. The residuals from a regression of FMI and FMI squared on FFMI was included in the models predicting the effect of FFMI on the levels of the markers, and vice versa for FMI. Thus, for each

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model we examined the effect of either FFMI or FMI, adjusted for the residuals of the other body composition index as well as all mentioned potential confounders. Age, FEV1 in percent predicted, FFMI and FMI were all treated as continuous variables after testing the linearity assumption with the method of fractional polynomials [24].

Bioelectrical impedance was not measured in five patients: Two had metal implants (pacemaker), and three patients refused measurement. The EIA analyses were performed before inclusion of seven patients, and blood sampling was unsuccessful in another six patients. For six patients we ran out of plasma before completing the analyses for either sTNF-R1 or OPG. Thus, the final multivariable models are based on 409 patients.

RESULTS

The baseline characteristics of the 409 COPD patients are given in table 1. Male patients were slightly older, had slightly lower percent predicted FEV_1, had more comorbidity, but were less likely to use inhaled steroids. Even though BMI was not significantly different between the sexes, FFMI was higher in men while FMI was higher in women. 31.0% of women had FFMI below 14 kg/m2, and 27.5% of men had FFMI below 17 kg/m2 (table 1). These FFMI cutoff values indicate the 5th percentile for FFMI in normal adult Caucasian male and female populations [25], and form a useful definition of cachexia [4].

When considered separately, a close relationship was found between FFMI and FMI in both men and women, shown in figure 1: With increasing FFMI, there was an increase in FMI. However, the relationship differed somewhat between the sexes, where men had a narrower range of FMI for a wider range of FFMI (figure 1).

Association between markers of infammation and body composition – bivariate analyses

Mean plasma levels of CRP, sTNF-R1, and OPG, by quartiles of FFMI, FMI, and the FFMI/FMI ratio respectively are shown in figures 2 through 4. CRP and sTNF-R1 increased significantly with increasing FFMI and also with increasing FMI (figures 2-3), whereas OPG decreased significantly with increasing FFMI, but did not vary significantly with FMI (figure 4). The analyses were also performed separately for women and men, but there was no significant sex difference in the relationship between the quartiles of FFMI and FMI and the three markers (data not shown).

To further attempt to disentangle the effects of FFMI and FMI, we looked at the association between FFMI and each of the three markers in men with a FMI below 10 kg/m2 only. This was done in an attempt to separate the influences of FMI and FFMI on the biomarkers. The rationale was that, in this subgroup of subjects, there was a wide variation in FFMI, but only small variation in FMI (figure 1). Thus, this group might provide the opportunity to better assess the effect of FFMI on the three markers. In this group, the correlation coefficient between FFMI and CRP was only 0.05 (p=0.5), that between FFMI and sTNF-R1 was only 0.02 (p=0.8), and that between FFMI and OPG was -0.10 (p=0.14). Thus, in this sub-sample there was no significant association between FFMI and the three markers. However, despite the small variation in FMI in this sub-sample, there was still a significant positive association between FMI and CRP (correlation coefficient 0.16, p=0.02) and between FMI and sTNF-R1 (correlation coefficient 0.22, p<0.01), but no relationship between FMI and OPG (correlation coefficient 0.07, p=0.33).

Association between markers of infammation and body composition – multivariate analyses

The effect (regression coefficients) of FFMI and FMI on the levels of the three markers CRP, sTNF-R1, and OPG in the COPD patients, after adjustment for sex, age, smoking, FEV_1, hypoxemia, Charlson comorbidity index, and use of inhaled steroids are shown in table 2. In both models, adjustment was made for the other body composition index by way of residualization, to reduce the effect of multicollinearity between FFMI and FMI.

For both CRP and sTNF-R1, the multivariate analyses confirmed the findings from the bivariate analyses. Higher FFMI and higher FMI were both associated with higher plasma levels of CRP and sTNF-R1 after adjustment for potential confounders. For OPG, the results were reversed compared with the bivariate analyses after adjustment for confounders. Higher FMI was significantly associated with lower levels of OPG. For FFMI, the association with OPG did not reach statistical significance.

DISCUSSION

In this cross-sectional study of more than 400 COPD patients, including more than 100 with cachexia, circulating plasma levels of the two inflammatory markers CRP and sTNF-R1 were not elevated in patients having lower FFMI, as noted both in the bivariate analyses, and in the multivariate analyses with adjustment for sex, age, smoking, FEV_1, hypoxemia, comorbidities, use of inhaled steroids, and FMI. Rather, we found the exact opposite. Our analyses imply that fat mass in addition to fat free mass was related to the plasma levels of CRP and sTNF-R1. In contrast to the pattern for CRP and sTNF-R1, low FMI was associated with high OPG levels, whereas no association was found between FFMI and OPG.

COPD is a heterogeneous disease, with substantial individual variation in symptomatology, progression of lung function decline, exacerbation frequency, and development of complications

like cachexia. Roughly 25% of COPD patients develop cachexia [4], a serious complication associated with increased mortality within the COPD patient population. The reasons why some COPD patients develop cachexia and others do not remain elusive. One prominent theory suggested by several authors [26-28], is that pathological weight loss is at least partly explained by an increase in systemic inflammation reflected in enhanced plasma or serum levels of inflammatory markers.

Several cross-sectional studies have examined systemic levels of inflammatory markers in COPD patients with and without cachexia. Most of the studies have focused on TNFα [7-10, 12-14], and/or the TNFα receptors [8, 9, 14, 17, 29-31], whereas two examined CRP [15, 32]. The earliest published studies found elevated levels of TNFα [7-10] in cachectic patients, whereas in later studies [12, 13], no such difference was found. In addition, over time the reported levels of TNFα have decreased, from 6-70 pg/mL [7, 8] in the earlier studies, to 0.5-1.2 pg/mL in the later studies [12, 13]. This decrease likely reflects improvement in assay specificity over time, and suggests that group differences in the earliest studies should be confirmed by more specific assays.

Measurement of the two soluble TNFα receptors, sTNF-R1 and sTNF-R2, is easier than that for TNFα, in large part because of much higher concentrations in serum and plasma. Also, whereas TNFα typically is pulse-secreted, the receptors provide a more stable and reliable measurement of the activity of the TNFα pathway [33]. In the two previous studies that examined cachexia in COPD patients by systemic levels of the TNFα receptors, one study found that the receptors were elevated in cachectic COPD patients compared with non-cachectic COPD patients [9] and the

other study did not [12]. However, the numbers of cachectic patients in these two studies were only 29 and 35, respectively.

Loss of muscle mass may not be the only factor when cachectic patients experience a loss of lean body mass, loss of bone mass may be important too. COPD patients with cachexia have a large increase in risk for developing osteoporosis [34]. OPG is a soluble member of the TNF receptor superfamily that inhibits osteoclastogenesis by binding the receptor activator of nuclear factor-κB ligand (RANKL), acting as a decoy receptor to competitively inhibit RANKL interaction with its receptor RANK. However, while RANKL circulates in very low levels, OPG is a more stable overall measure of RANKL/RANK activity than RANKL, potentially reflecting inflammatory as well as bone and matrix remodelling activity induced by RANKL. We have previously shown that plasma levels of OPG were lower in COPD patients than subjects without COPD [16]. We are not aware of previous studies examining the relationship between systemic OPG and body composition. In the current study high OPG was associated with low FMI, whereas no association was found between plasma levels of OPG and FFMI after adjusement for confounders. This finding could reflect a higher turnover of bone metabolism in COPD patients with low FMI, that OPG reflected inflammatory pathways not reflected by CRP or sTNF-R1, or an hitherto unrecognized metabolic effect of the RANKL/OPG/RANK axis. Unfortunately we did not have measurements of bone density in this study, and we could therefore not compare levels of OPG to degrees of measured osteoporosis. This could be an interesting topic for future studies.

The largest challenge in interpreting the results from the current study is the close relationship between FFMI and FMI. BMI is an imperfect tool in assessing body composition, as it does not separate lean body mass from fat mass, and was therefore not used. Two subjects can vary widely

in fat mass, and still have the same BMI. To try to separate the effects of FFMI and FMI, we looked at the relationship between FFMI and the three markers in the subgroup of men with a FMI below 10 kg/m2. In this group, where the variation of FFMI was great but the variation of FMI small, FFMI was not related to the levels of the three markers. In addition, the multivariable analyses indicated that both FFMI and FMI had independent effects on the levels of CRP and possibly sTNF-R1. Thus, these analyses support the conclusion that CRP and sTNF-R1 is not elevated in cachectic COPD patients.

Tthe current study highlights the likely contribution of metabolically active fat tissue in systemic inflammation in COPD. Adipose tissue may promote low-grade systemic inflammation and inflammation may promote dysfunction of adipocytes and adipose tissue. Previous studies have shown that overweight subjects have elevated CRP, both in healthy population studies [35] and in studies on other diseases like diabetes [36]. Further, TNFα has been shown to be produced by adipocytes, and its expression increase with adipocyte mass [37, 38]. TNFα increase insulin resistance and may be upregulated as a consequence of a high fat diet. Elevated levels of sTNF-R1 could be seen as a marker of this mechanism. There are not many studies looking specifically at obesity in COPD. However, in a small Canadian study on 28 COPD patients, overweight patients had higher systemic levels of CRP, TNFα and IL-6 [39]. Thus, we suggest that inflammation in COPD may also be related to obesity within the COPD population, potentially contributing to the increased occurence of obesity related comorbidities such as coronary heart disease. This will need to be confirmed in longitudinal studies.

Some methodological shortcomings must be acknowledged. First, the study was cross-sectional. Therefore, there is the possibility that COPD patients prone to develop cachexia had higher systemic levels of inflammation prior to their loss of lean body mass, but as cachexia developed, levels of the markers fell. This can only be resolved in future longitudinal studies. Second, being an observational study, we cannot say for certain whether the observed relationships are physiological or pathological. Third, by design we only have COPD patients with GOLD stage II or worse, and the results may not be applicable to patients with mild disease (GOLD stage I). Fourth, we did not measure the levels of soluble TNF-R2 in the plasma of our patients. In previous studies on levels of TNF receptors in COPD [8, 9, 12, 14, 29-31], no real difference have been found between the two receptors, with regard to levels in COPD patients versus controls, and with regard to cachexia or no cachexia. However whether that would be true also in the current cohort we cannot know. Ideally, future studies should include measurements of both TNF receptors and TNFα as well as TNFα bioactivity.

To conclude, the current study does not support the theory that cachexia in COPD patients is mediated through an increase in TNFα related systemic inflammation. Rather, the study results allow the hypothesis that high fat mass may also be a predictor of high plasma levels of CRP and sTNF-R1 in COPD patients. The relationship between FMI and OPG in COPD patients is novel, and could be an indication that fat metabolism is a factor in the development of osteoporosis in COPD patients. However, these findings will need to be confirmed in longitudinal studies.

REFERENCES

1.   Halbert RJ, Natoli JL, Gano A *et al*. Global burden of COPD: systematic review and meta-analysis. *Eur Respir J* 2006; 28: 523-532.

2.   Calverley PM, Walker P. Chronic obstructive pulmonary disease. *Lancet* 2003; 362: 1053-1061.

3.   Ferrer M, Alonso J, Morera J *et al*. Chronic obstructive pulmonary disease stage and health-related quality of life. The Quality of Life of Chronic Obstructive Pulmonary Disease Study Group. *Ann Intern Med* 1997; 127: 1072-1079.

4.   Wagner PD. Possible mechanisms underlying the development of cachexia in COPD. *Eur Respir J* 2008; 31: 492-501.

5.   Schols AM, Broekhuizen R, Weling-Scheepers CA *et al*. Body composition and mortality in chronic obstructive pulmonary disease. *Am J Clin Nutr* 2005; 82: 53-59.

6.   Debigare R, Cote CH, Maltais F. Peripheral muscle wasting in chronic obstructive pulmonary disease. Clinical relevance and mechanisms. *Am J Respir Crit Care Med* 2001; 164: 1712-1717.

7.   Di Francia M, Barbier D, Mege JL *et al*. Tumor necrosis factor-alpha levels and weight loss in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1994; 150: 1453-1455.

8.   Takabatake N, Nakamura H, Abe S *et al*. Circulating leptin in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 159: 1215-1219.

9.   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.

10. 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.

11. Schols AM, Soeters PB, Dingemans AM *et al*. Prevalence and characteristics of nutritional depletion in patients with stable COPD eligible for pulmonary rehabilitation. *Am Rev Respir Dis* 1993; 147: 1151-1156.

12. Broekhuizen R, Grimble RF, Howell WM *et al*. Pulmonary cachexia, systemic inflammatory profile, and the interleukin 1beta -511 single nucleotide polymorphism. *Am J Clin Nutr* 2005; 82: 1059-1064.

13. Van Helvoort HA, Heijdra YF, Thijs HM *et al*. Exercise-induced systemic effects in muscle-wasted patients with COPD. *Med Sci Sports Exerc* 2006; 38: 1543-1552.

14. Shin KC, Chung JH, Lee KH. Effects of TNF-alpha and leptin on weight loss in patients with stable chronic obstructive pulmonary disease. *Korean J Intern Med* 2007; 22: 249-255.

15. Kurosaki H, Ishii T, Motohashi N *et al*. Extent of emphysema on HRCT affects loss of fat-free mass and fat mass in COPD. *Intern Med* 2009; 48: 41-48.

16. Eagan TM, Ueland T, Wagner PD *et al*. Systemic inflammatory markers in chronic obstructive pulmonary disease - results from the Bergen COPD Cohort Study. *Eur Respir J* 2010; 35: 540-548.

17. Broekhuizen R, Vernooy JH, Schols AM *et al*. Leptin as local inflammatory marker in COPD. *Respir Med* 2005; 99: 70-74.

18. Horowitz MC, Xi Y, Wilson K *et al*. Control of osteoclastogenesis and bone resorption by members of the TNF family of receptors and ligands. *Cytokine Growth Factor Rev* 2001; 12: 9-18.

19. Nabipour I, Kalantarhormozi M, Larijani B *et al*. Osteoprotegerin in relation to type 2 diabetes mellitus and the metabolic syndrome in postmenopausal women. *Metabolism* 2009;

20. Johannessen A, Lehmann S, Omenaas ER *et al*. Post-bronchodilator spirometry reference values in adults and implications for disease management. *Am J Respir Crit Care Med* 2006; 173: 1316-1325.

21. StataCorp. Stata Statistical Software. 2007;

22. Charlson ME, Pompei P, Ales KL *et al*. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373-383.

23. Charlson M, Szatrowski TP, Peterson J *et al*. Validation of a combined comorbidity index. *J Clin Epidemiol* 1994; 47: 1245-1251.

24. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol* 1999; 28: 964-974.

25. Schutz Y, Kyle UU, Pichard C. Fat-free mass index and fat mass index percentiles in Caucasians aged 18-98 y. *Int J Obes Relat Metab Disord* 2002; 26: 953-960.

26. Balasubramanian VP, Varkey B. Chronic obstructive pulmonary disease: effects beyond the lungs. *Curr Opin Pulm Med* 2006; 12: 106-112.

27. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Respir J* 2009; 33: 1165-1185.

28. Wouters EF, Creutzberg EC, Schols AM. Systemic effects in COPD. *Chest* 2002; 121: 127S-130S.

29. Bolton CE, Ionescu AA, Shiels KM *et al*. Associated loss of fat-free mass and bone mineral density in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004; 170: 1286-1293.

30. Cohen RI, Marzouk K, Berkoski P *et al*. Body composition and resting energy expenditure in clinically stable, non-weight-losing patients with severe emphysema. *Chest* 2003; 124: 1365-1372.

31. Schols AM, Buurman WA, Staal van den Brekel AJ *et al*. Evidence for a relation between metabolic derangements and increased levels of inflammatory mediators in a subgroup of patients with chronic obstructive pulmonary disease. *Thorax* 1996; 51: 819-824.

32. Pinto-Plata VM, Mullerova H, Toso JF *et al*. C-reactive protein in patients with COPD, control smokers and non-smokers. *Thorax* 2006; 61: 23-28.

33. Dempsey PW, Doyle SE, He JQ *et al*. The signaling adaptors and pathways activated by TNF superfamily. *Cytokine Growth Factor Rev* 2003; 14: 193-209.

34. Graat-Verboom L, Spruit MA, van den Borne BE *et al*. Correlates of osteoporosis in chronic obstructive pulmonary disease: An underestimated systemic component. *Respir Med* 2009;

35. Nguyen XM, Lane J, Smith BR *et al*. Changes in inflammatory biomarkers across weight classes in a representative US population: a link between obesity and inflammation. *J Gastrointest Surg* 2009; 13: 1205-1212.

36. Hofso D, Ueland T, Hager H *et al*. Inflammatory mediators in morbidly obese subjects: associations with glucose abnormalities and changes after oral glucose. *Eur J Endocrinol* 2009; 161: 451-458.

37. Kern PA, Saghizadeh M, Ong JM *et al*. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 1995; 95: 2111-2119.

38. Skolnik EY, Marcusohn J. Inhibition of insulin receptor signaling by TNF: potential role in obesity and non-insulin-dependent diabetes mellitus. *Cytokine Growth Factor Rev* 1996; 7: 161-173.

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

Table 1. Baseline characteristics of the 409 COPD patients

	Women (n= 160)	Men (n = 249)	p*
Age			
mean, SD	62.3 (6.6)	64.2 (7.0)	<0.01
Body composition			
mean BMI (kg/m2), SD	24.9 (6.2)	25.7 (4.9)	0.16
mean FMI (kg/m2), SD	10.1 (4.3)	7.3 (2.6)	<0.01
mean FFMI (kg/m2), SD	14.8 (2.4)	18.4 (3.3)	<0.01
Percentage of patients having pathologically low FFMI†	31.0	27.5	0.45
Smoking habits, %			0.57
ex	55.0	57.8	
current	45.0	42.2	
FEV1 in percent predicted			
mean, SD	50.4 (13.9)	47.8 (14.4)	0.08
Hypoxemia (PaO2 < 8.0 kPa)¥			0.31
No	84.8	15.2	
Yes	88.4	11.6	
Daily use of inhaled steroids, %	75.0	64.7	0.03
Charlson Comorbidity Index, %			0.01
1 (COPD only)	63.1	52.2	
2	25.0	23.7	
3	8.8	14.5	
4-6	3.1	9.6	
Inflammatory markers			
mean CRP (ug/ml), SD	8.9 (12.5)	7.7 (10.8)	0.78
mean sTNF-R1 (pg/ml), SD	699 (219)	758 (250)	0.01
mean OPG (ng/ml), SD	6.0 (2.1)	5.7 (2.3)	0.16

* t-test for age, body composition, FEV1; Chi square for percentage of patients with low FFMI, smoking habits, use of inhaled steroids, hypoxemia and Charlson's comorbidity index; and Wilcoxon Rank sum test for CRP, sTNF-R1, and OPG

† below 14 kg/m2 in women and below 17 kg/m2 in men, based on reference values from a normal population (Scutz et al reference number 24)

¥ 38 subjects refused measurement, or attempt was unsuccesful

Table 2. The regression coefficients (95% CI) from six multivariable regression analyses* assessing the association of FFMI and FMI to the level of three plasma markers

	logCRP	p	sTNF-R1	p	OPG	p
FFMI (kg/m2)	**1.23**	<0.01	**24.9**	<0.01	-0.06	0.32
	(1.14, 1.33)		(11.8, 38.1)		(-0.19, 0.06)	
FMI (kg/m2)	**1.12**	<0.01	**15.4**	<0.01	**-0.14**	<0.01
	(1.06, 1.19)		(5.6, 25.3)		(-0.23, -0.04)	

* All models included sex, age, smoking, FEV1, Charlson comorbidity Index, Hypoxemia, use of inhaled steroids and the residuals from the other body composition index than the one in question (see text for further details)

Legend to figures:

Figure 1. The distribution of fat mass index by fat free mass index in women and men in the baseline phase of the Bergen COPD Cohort Study
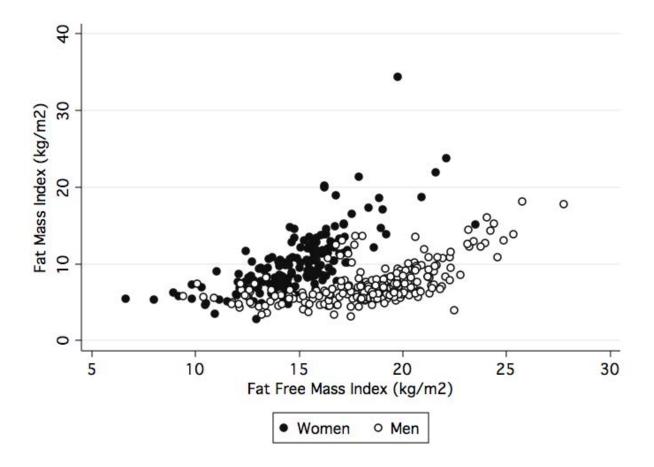
Figure 2. The mean (SE) plasma level of CRP, by quartiles of fat free mass index (FFMI) and fat mass index (FMI) among COPD patients

p<0.001

mean (SE) CRP (μg/mL)

Q1 (lowest)　Q2　Q3　Q4 (highest)

Quartiles of fat free mass index (kg/m2)

p<0.001

mean (SE) CRP (μg/mL)

Q1 (lowest)　Q2　Q3　Q4 (highest)
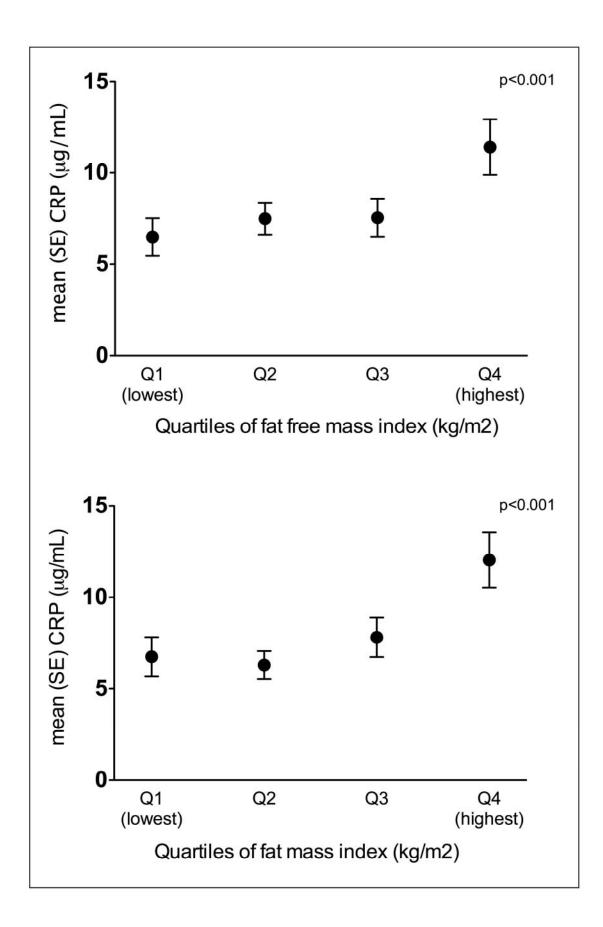
Quartiles of fat mass index (kg/m2)

Figure 3. The mean (SE) plasma level of sTNF-R1, by quartiles of fat free mass index (FFMI) and fat mass index (FMI) among COPD patients
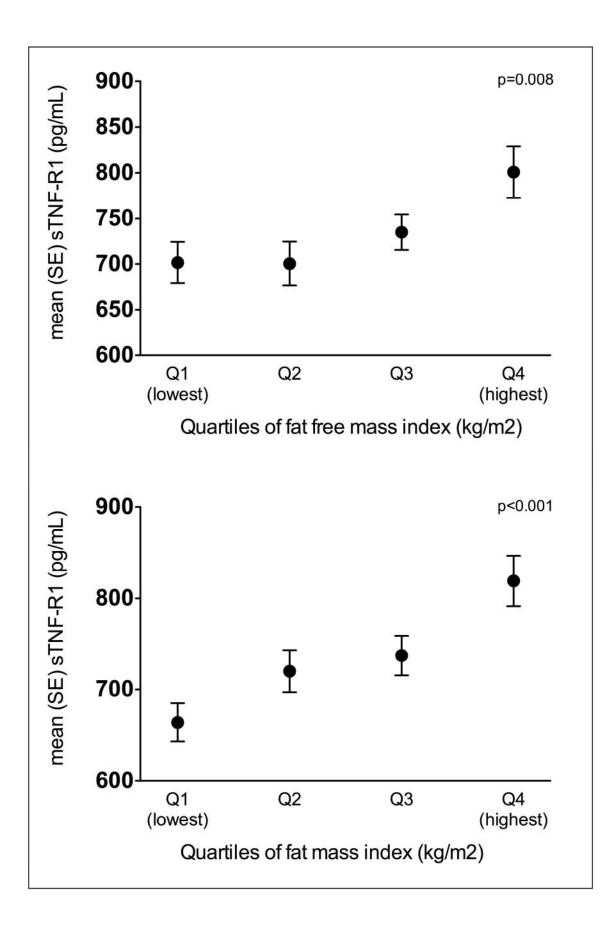
Figure 4. The mean (SE) plasma level of Osteoprotegerin (OPG), by quartiles of fat free mass index (FFMI) and fat mass index (FMI) among COPD patients