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# Elevated Plasma Adiponectin Levels in Patients with Chronic Obstructive Pulmonary Disease

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**ABSTRACT**

**Background:** Adiponectin is an anti-inflammatory adipokine and may play a role in COPD pathogenesis. This study was to investigate the relationship between adiponectin, interleukin (IL)-6, IL-8 and C-reactive protein (CRP) and COPD by evaluating these biomarkers in ever-smokers with or without the disease.

**Method:** Plasma levels of adiponectin, IL-6, IL-8 and CRP were measured using commercial available kits respectively in COPD patients (n=71), healthy ever-smokers (n = 62) and non-smokers (n = 51).

**Results:** There were significant increases in plasma adiponectin, IL-6 and CRP in COPD patients [median (IQR): 4.39 μg/ml (2.68-6.98 μg/ml), 4.19 pg/ml (<2.40-6.40 pg/ml), 8.75 mg/l (4.26-40.63 mg/l) respectively] compared to healthy ever-smokers [1.90 μg/ml (0.86-2.86 μg/ml), <2.40 pg/ml (<2.40-2.77 pg/ml), 3.71 mg/l (1.97-10.37 mg/l) respectively; $p < 0.001$] and non-smokers [1.76 μg/ml (1.34-2.52 μg/ml), <2.40 pg/ml (<2.40-2.78 pg/ml), 3.12 mg/l (2.11-5.71 mg/l) respectively; $p < 0.001$]. COPD patients had lower plasma IL-8 levels than healthy ever-smokers. Among ever-smokers with or without COPD, plasma adiponectin, IL-6 and CRP levels were inversely correlated with $FEV_1$ (% predicted) after adjustment for age, BMI, smoking status and pack-years.

**Conclusion:** Our findings suggest that in COPD patients, adiponectin might be

associated with COPD pathogenesis.

# INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major global disease and has been estimated to be the third leading cause of mortality worldwide by 2020.[1] It is a disease characterized by slowly progressive airflow limitation and includes both emphysema and chronic bronchitis.[2] Cigarette smoking is the most important risk factor, contributing to more than 90% of COPD cases.[3] There is increasing evidence of systemic inflammation in patients with COPD. However, the main cause for the presence of systemic inflammation in COPD patients is still unclear but systemic hypoxia due to the progression of COPD has been suggested to be a possibility. Several disease biomarkers have been found to be helpful in assessing systemic and local inflammation including interleukin (IL)-6, IL-8 and C-reactive protein (CRP).[4-8]

Adiponectin is a secretary 30kD protein synthesized by adipocytes in healthy subjects. Three isoforms (trimer, hexamer and high molecular weight complex) were found in the circulation with different biochemical properties.[9] Its role in inflammation is controversial since its plasma concentration decreases in diseases such as metabolic syndrome and type II diabetes[10] but increases in some inflammatory diseases like rheumatoid arthritis and systemic lupus erythematosus.[11,12] Elevation of plasma adiponectin level was found in patients with

20    stable and acute exacerbation of COPD.[13,14] However, in one study, adiponectin was

21    found to suppress TNF-$\alpha$ and MMP-12 production in alveolar macrophages and

22    absence of adiponectin led to an emphysema-like lesion in adiponectin knock-out

23    mice.[15]

24         In this study, we hypothesized that circulating levels of adiponectin and other

25    conventional inflammatory biomarkers might be associated with lung function in

26    ever-smokers with or without COPD. Thus, we studied patients with stable COPD

27    who were ever-smokers, and healthy ever-smokers and non-smokers as controls to

28    investigate circulating levels of adiponectin, IL-6, IL-8 and CRP, and the

29    correlations between these biomarkers and lung functions.

30 **METHODS**

31 *Study Subjects*

32        Three groups of men, a total of 184, were randomly chosen from our

33 database of the COPD study conducted by the COPD Study Group of the Hong

34 Kong Thoracic Society between 2005 and 2006:[16] (1) healthy life long non-smokers;

35 (2) healthy ever-smokers: either current smokers or ex-smokers (defined as those

36 who had not smoked within the last 12 months) with $FEV_1/FVC \geq 70$ and $FEV_1 \geq 80$

37 (% predicted), and no chronic respiratory symptoms; and (3) stable COPD patients,

38 who are ever-smokers and defined as $FEV_1/FVC < 70$ and/or $FEV_1 < 80$ (%

39 predicted) according to the diagnostic criteria of Global Initiative for Chronic

40 Obstructive Lung Disease (GOLD).[17] Stable COPD patients were defined as those

41 who did not have exacerbation within the last 12 weeks prior to recruitment. The

42 healthy subjects, irrespective of smoking habits, were recruited from those attending

43 churches and community centers for the elderly across Hong Kong. COPD patients

44 were recruited from outpatient respiratory clinics. Lung function tests were

45 performed in all control subjects and patients using standardized methods according

46 to the American Thoracic Society guidelines.[18] The predicted values were based on

47 reference values obtained from our local population.[19] Information about smoking

48 habits, respiratory symptoms and other diseases such as cardiovascular diseases

49  were obtained from a detailed questionnaire. Subjects were excluded if they had a

50  history of asthma or other lung illnesses. Four of the COPD patients had coronary

51  artery disease. There was no patient from our cohort had stage 1 disease [$FEV_1/FVC$

52  $< 70$, $FEV_1 \geq 80$ (% predicted)] and were subdivided into three groups according to

53  disease severity based on GOLD criteria (stage 2: $50\% \leq FEV_1 < 80\%$ predicted;

54  stage 3: $30\% \leq FEV_1 < 50\%$ predicted; stage 4: $FEV_1 < 30\%$ predicted).[16] Patients

55  were also subdivided into two groups based on body mass index (BMI):   BMI $<$

56  18.5 kg/m$^2$ and BMI $\geq$ 18.5 kg/m$^2$ according to WHO criteria.[20] Every participant

57  signed the informed consent form and this study was approved by the Ethics

58  Committee of The University of Hong Kong.

59

60  *Blood Sampling and Analysis*

61      Venous blood samples were taken from all subjects, centrifuged immediately

62  at 1600x g for 10 min at 4°C and stored at -70°C. Plasma adiponectin (R&D

63  Systems Inc., MN, USA), IL-6, IL-8 ((BD Biosciences Pharmingen, San Diego, CA,

64  USA) and CRP (Diagnostic Systems Laboratories Inc., Texas, USA) were measured

65  by commercially available enzyme-linked immunosorbent assay (ELISA) kits

66  respectively.

67

*Statistical Analysis*

69       Data were expressed as mean ± SD or median (interquartile range; IQR) for

70    normally or non-normally distributed variables, respectively, unless specified. The

71    normality was tested by the method of Kolmogorov-Smirnov. Demographic data

72    were compared between any two groups by either Student $t$ test or $\chi^2$ statistics.

73    Plasma levels of adiponectin, IL-6, IL-8 and CRP were compared by Mann-Whitney

74    U test. All data including those whose readings were below the detection limit were

75    included in these comparisons. In COPD patients and healthy ever-smokers, the

76    relationships between adiponectin, IL-6, IL-8 or CRP and the lung function

77    measures or other demographic variables were first investigated by the Spearman

78    rank-order correlation using data from all subjects. If similar results were obtained

79    by the Pearson product-moment correlation, the Pearson partial correlation between

80    log-transformed adiponectin, IL-6, IL-8 or CRP and the lung function measures with

81    adjustment for cofounders was then estimated using data from those with positive

82    values. Multiple linear regression analyses were performed to study the relationships

83    between COPD severity and plasma adiponectin, IL-6, IL-8 or CRP (in log scale),

84    adjusting for age, BMI, smoking status and pack-years smoked within COPD

85    patients only. Stages 2, 3 and 4 COPD patients were coded with values 1, 2 and 3,

86    respectively, and entered the regression model as a continuous independent factor.

87      All *p*-values were not adjusted for multiple testing due to the exploratory

88  nature of this study. SPSS for Windows version 16.0 statistical package (SPSS,

89  Chicago, IL) was used for statistical analyses.

## RESULTS

Demographic characteristics of the study subjects are summarized in Table 1. All the recruited COPD patients were current or ex-smokers. COPD patients were significantly older and had a significantly lower BMI than healthy non-smokers or ever-smokers. COPD patients also had higher pack-year smoked than healthy ever-smokers. There were 5 missing values for pack-years smoked due to incomplete information in the questionnaire. As expected, there were significant reductions of $FEV_1$ (% predicted), FVC (% predicted) and $FEV_1$/FVC ratio in COPD patients compared with healthy ever-smokers, irrespective of smoking status. Plasma adiponectin, IL-6 and CRP levels were significantly elevated in COPD patients compared with healthy ever-smokers or non-smokers. Plasma IL-8 levels were significantly increased in COPD patients and healthy ever-smokers compared with healthy non-smokers. Healthy ever-smokers also had higher levels of plasma IL-8 compared with healthy non-smokers while COPD patients had lower plasma IL-8 levels than healthy ever-smokers (Table 2).

In ever-smokers with or without COPD, Spearman's correlation analysis did not show pair-wise correlations among plasma adiponectin, IL-8 and CRP ($r < 0.18$ and $p > 0.05$). However, plasma IL-6 showed pair-wise correlations with adiponectin and CRP ($r = 0.374$ and $0.284$ respectively, $p < 0.01$). Plasma adiponectin and IL-6

11

109 was found to have positive correlations with age (r = 0.424 and 0.303 respectively, *p*

110 ≤ 0.001) and pack-year smoked (r = 0.254 and 0.338 respectively, *p* < 0.01) and

111 inverse correlation with BMI (r = -0.623 and -0.396, *p* < 0.001). After controlling for

112 age, BMI, smoking status and pack-year smoked, plasma IL-6 remained positively

113 correlated with plasma CRP (r = 0.356, *p* < 0.001). Ex-smokers regardless of lung

114 function status also had higher plasma adiponectin levels than current smokers

115 (median: 3.26, IQR: 1.96-5.65 µg/ml versus median: 2.24, IQR: 1.03-4.43 µg/ml, *p* =

116 0.016, Mann-Whitney U test).

117 Pulmonary function parameters, $FEV_1$ (% predicted), FVC (% predicted) and

118 $FEV_1$/FVC ratio, correlated negatively with plasma adiponectin, IL-6 and CRP but

119 positively with plasma IL-8 (data not shown). After controlling for age, BMI,

120 smoking status and pack-year smoked, these lung function parameters remained

121 inversely correlated with plasma adiponectin, IL-6 and CRP levels, and positively

122 correlated with plasma IL-8 levels (Table 3).

123 There were no significant differences in age, BMI and pack years smoked

124 among COPD patients according to disease severity (data not shown). We found an

125 increase in plasma adiponectin and CRP levels with disease severity (Figure 1A and

126 1D). Stage 4 COPD patients had the highest median of plasma adiponectin levels

127 compared to stage 2 and stage 3 COPD patients. No difference was found in plasma

128    IL-6 among different stages (Figure 1B). Plasma IL-8 levels show a non-significant

129    decrease with disease severity (Figure 1C).

130         The COPD patients with BMI < 18.5 kg/m$^2$ had significantly elevated

131    plasma adiponectin levels in comparison to those with BMI $\geq$ 18.5 kg/m$^2$ (Figure

132    2A). They also showed higher median of plasma IL-6 and IL-8, and lower CRP

133    levels than COPD patients with BMI $\geq$ 18.5 kg/m$^2$ but not reaching statistical

134    significance (Figure 2B-D).

135         After adjusting for age, BMI, smoking status and pack-year smoked,

136    multiple linear regression analyses showed that plasma adiponectin levels still

137    increased with the COPD staging (Table 4) but plasma IL-6, IL-8 or CRP levels

138    were not related to COPD staging.

**DISCUSSION**

139

140    In this study, we found that COPD patients who were ever-smokers had

141    significantly higher plasma levels of adiponectin, IL-6 and CRP than healthy

142    ever-smokers and non-smokers. Plasma levels of adiponectin, IL-6 and CRP were

143    negatively correlated with $FEV_1$ (% predicted) in COPD patients and healthy

144    ever-smokers.

145    Our findings that COPD patients had a significantly higher plasma

146    adiponectin levels and that the more severe COPD patients had even higher levels

147    suggest that adiponectin might be inappropriately secreted in this disease. The exact

148    role of adiponectin could not be elucidated in this study since it is a cross-sectional

149    study. In contrast to our findings, Tomoda *et al*[13] and Kirdar *et al*[14] observed no

150    relationship between lung function and plasma adiponectin. This may probably be

151    due to the limited sample size in their studies. Although the function of adiponectin

152    remains controversial, adiponectin was recently found positively correlated with

153    IL-6 in dialysis patients[21], in line with our findings. Additionally, we found that

154    plasma adiponectin levels were inversely correlated with BMI in the group of

155    healthy ever-smokers and those with COPD as reported in previous studies.[13] COPD

156    patients with BMI < 18.5 kg/m$^2$ had a significant elevation of circulating

157    adiponectin compared to those with BMI ≥ 18.5 kg/m$^2$ as previously reported.[13] This

14

158    could be the consequence of severely decreased body fat as reported in patients with

159    anorexia nervosa and cachexia.[22,23]

160        We demonstrated elevated plasma IL-8 levels in ever-smokers with or

161    without COPD compared with those of healthy non-smokers; but COPD patients

162    had lower plasma IL-8 levels than healthy ever-smokers. Our results are in contrast

163    to that of previous researchers who found marginally higher plasma IL-8 levels in

164    COPD patients compared with those of healthy smokers[24], and elevated IL-8 levels

165    in induced sputum of COPD patients.[25,26] This discrepancy might be explained by

166    the fact that local and systemic inflammations are differentially regulated.[27] In

167    studies done by Yoshikawa *et al*[28], chemotactic activity and migration of neutrophils

168    from blood of severe COPD patients were lower than that of less severe patients or

169    healthy smokers. Fietta and colleagues[29] also found that the number of functional

170    neutrophils and monocytes was reduced in chronic bronchitis. Our finding of

171    reduced plasma IL-8 levels in more severe COPD patients suggests that a reduction

172    of chemoattractant might be present in severe COPD cases. Another possible

173    explanation is that the release of IL-8 is suppressed by an endogenous inhibitor

174    which could be adiponectin as adiponectin has been found to inhibit IL-8

175    production.[30] Moreover, IL-8 might also be suppressed by CRP as reported by an *in*

176    *vitro* study.[31]

177        Ever-smokers with COPD had the highest plasma IL-6 and CRP levels

178    compared with those of healthy non-smokers and ever-smokers, in line with

179    previous publications.[7,32,33] $FEV_1$ (% predicted) was found to be inversely correlated

180    to plasma IL-6 and CRP levels as in previous report,[8,32] suggesting that systemic

181    inflammation might play a role in the development of airway obstruction. Plasma

182    IL-6 also showed a positive correlation with CRP, which is consistent with previous

183    findings,[32] since IL-6 is a positive regulator of CRP by triggering acute-phase

184    response in liver.[34]

185        The strength of our study is that we have investigated the potential role of

186    adiponectin alongside that of other inflammatory biomarkers (IL-6, IL-8 and CRP)

187    in relation to the lung function in the same subjects with a larger sample compared

188    with previous studies.[13,14] In addition, we have used all-male study population to

189    avoid the sex differences in the plasma levels of different forms of adiponectin[9] and

190    the disease state of emphysema due to a heterogeneous population.[35] However, the

191    sample size is still relatively small for subgroup analysis after stratification by

192    disease severity but this is the first study to demonstrate the relationship between

193    plasma adiponectin and COPD severity. Our study also has several limitations.

194    Firstly, this is a cross-sectional study that limits the interpretation of a causal link

195    between the markers adiponectin, IL-6, IL-8 and CRP, and lung function changes.

196    As reported by Summer and coworkers,[15] mice deficient in adiponectin was more

197    susceptible to develop emphysema, which implied that adiponectin might be

198    involved in tissue repair rather than disease development. However, there are no

199    association studies for adiponectin gene polymorphisms and COPD in determining

200    which polymorphism causes the functional effect. Prospective studies in smokers

201    with or without COPD are required to fully address the role of adiponectin in the

202    development and progression of COPD. Secondly, we carried out the measurements

203    in plasma, which reflects only systemic changes and may not adequately reflect the

204    local concentrations in the lungs. Further studies involving biological samples such

205    as BAL, induced sputum and exhaled breath condensate, might shed more light

206    locally. Thirdly, we measured total adiponectin levels in plasma instead of its

207    different isoforms in the present samples. High molecular weight (HMW) isoform

208    was found to have greater clinical significance than the other two isoforms in

209    obesity-related diseases.[36] A similar pattern of total adiponectin and HMW isoform

210    was recently observed with the HMW isoform being the most abundant

211    adiponectin.[37] In this study, we could not rule out whether a specific isoform such as

212    HMW isoform involves in COPD progression or not, however, the measurement of

213    total adiponectin or a specific isoform in plasma has been demonstrated to produce

214    similar results.[38]

215 **CONCLUSION**

216        We found an inverse relationship between $FEV_1$ (% predicted) and plasma

217  adiponectin, IL-6 or CRP levels in ever smokers. In COPD patients, we found

218  elevated plasma adiponectin, IL-6 and CRP levels and the more severe the disease,

219  the higher the adiponectin levels. These findings suggest that these biomarkers

220  might be associated with COPD. As this study provides evidence of association

221  rather than of causation, prospective studies are required to assess biological

222  significance of these associations.

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**References**

1. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997; 349:1498-1504.

2. Pauwels RA, Buist AS, Calverley PM, Genkins CR, Hurd SS; GOLD Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO global initiative for chronic obstructive pulmonary disease (GOLD) workshop summary. Am J Respir Crit Care Med 2001; 163:1256-1276.

3. Sethi JM, Rochester CL. Smoking and chronic obstructive pulmonary disease. Clin Chest Med 2000; 21:67-86.

4. Kolsum U, Roy K, Starkey C, et al. The repeatability of interleukin-6, tumor necrosis factor-alpha, and C-reactive protein in COPD patients over one year. Int J Chron Obstruct Pulmon Dis 2009; 4:149-56.

5. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med 1996; 153:530-534.

6. Yamamoto C, Yoneda T, Yoshikawa M, et al. Airway inflammation in COPD assessed by sputum levels of interleukin-8. Chest 1997; 112:505-510.

7.    Man SF, Connett JE, Anthonisen NR, Wise RA, Tashkin DP, Sin DD. C-reactive

protein and mortality in mild to moderate chronic obstructive pulmonary disease.

Thorax 2006; 61:849-853.

8.    Karadag F, Kirdar S, Karul AB, Ceylan E. The value of C-reactive protein as a

marker of systemic inflammation in stable chronic obstructive pulmonary disease.

Eur J Int Med 2008; 19:104-108.

9.    Schraw T, Wang ZV, Halberg N, Hawkins M, Scherer PE. Plasma adiponectin

complexes have distinct biochemical characteristics. Endocrinology 2008;

149:2270-2282.

10.   Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and

adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome.

J Clin Invest 2006; 116:1784-1792.

11.   Otero M, Lago R, Gomez R, et al. Changes in plasma levels of fat-derived

hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid

arthritis. Ann Rheum Dis 2006; 65:1198-1201.

12.   Rovin BH, Song H, Hebert LA, et al. Plasma, urine, and renal expression of

adiponectin in human systemic lupus erythematosus. Kidney Int 2005;

68:1825-1833.

13.   Tomoda K, Yoshikawa M, Itoh T, et al. Elevated circulating plasma adiponectin

in underweight patients with COPD. Chest 2007; 132:135-140.

14. Kirdar S, Serter M, Ceylan E, Sener AG, Kavak T, Karadag F. Adiponectin as a biomarker of systemic inflammatory response in smoker patients with stable and exacerbation phases of chronic obstructive pulmonary disease. Scand J Clin Lab Invest 2009; 69:219-224.

15. Summer R, Little FF, Ouchi N, et al. Alveolar macrophage activation and an emphysema-like phenotype in adiponectin-deficient mice. Am J Physiol Lung Cell Mol Physiol 2008; 294:L1035-L1042.

16. Chan-Yeung M, Ho AS, Cheung AH, et al. Determinants of chronic obstructive pulmonary disease in Chinese patients in Hong Kong. Int J Tuberc Lung Dis 2007; 11:502-507.

17. Rabe KF, Hurd S, Anzueto A, et al. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2007; 176:532-555.

18. Standardization of spirometry. 1994 Update. American Thoracic Society. Am J Respir Crit Care Med 1995; 152:1107-1136.

19. Ip MS, Ko FW, Lau AC, et al. Updated spirometric reference values for adult Chinese in Hong Kong and implications on clinical utilization. Chest 2006;

129:384-392.

20. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004; 363:157-163.

21. Ayerden Ebinç F, Ebinç H, Derici U, et al. The relationship between adiponectin levels and proinflammatory cytokines and left ventricular mass in dialysis patients. J Nephrol 2009; 22: 216-223.

22. Housova J, Anderlova K, Krizova J, et al. Serum adiponectin and resistin concentrations in patients with restrictive and binge/ purge form of anorexia nervosa and bulimia nervosa. J Clin Endocrinol Metab 2005; 90:1366-1370.

23. Plaisance EP, Grandjean PW, Judd RL, Jones KW, Taylor JK. The influence of sex, body composition, and nonesterified fatty acids on serum adipokine concentrations. Metabolism 2009; 58: 1557-1563.

24. Vernooy JH, Kucukaycan M, Jacobs JA, et al. Local and systemic inflammation in patients with chronic obstructive pulmonary disease: soluble tumor necrosis factor receptors are increased in sputum. Am J Respir Crit Care Med 2002; 166:1218-1224.

25. Yamamoto C, Yoneda T, Yoshikawa M, et al. Airway inflammation in COPD assessed by sputum levels of interleukin-8. Chest 1997; 112:505-510.

26. Rutgers SR, Timens W, Kaufmann HF, van der Mark TW, Koëter GH, Postma D S. Comparison of induced sputum with bronchial wash, bronchoalveolar lavage and bronchial biopsies in COPD. Eur Respir J 2000; 15:109-115.

27. Higashimoto Y, Iwata T, Okada M, Satoh H, Fukuda K, Tohda Y. Serum biomarkers as predictors of lung function decline in chronic obstructive pulmonary disease. Respir Med 2009; 103:1231-1238.

28. Yoshikawa T, Dent G, Ward J, et al. Impaired neutrophil chemotaxis in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2007;175 :473-9.

29. Fietta A, Bersani C, De Rose V, et al. Evaluation of systemic host defense mechanisms in chronic bronchitis. Respiration 1988; 53: 37-43.

30. Kobashi C, Urakaze M, Kishida M, et al. Adiponectin inhibits endothelial synthesis of interleukin-8. Circ Res 2005; 97:1245-1252.

31. Nan JL, Li JJ, He JG. C-reactive protein decreases interleukin-8 production in human endothelial progenitor cells by inhibition of p38 MAPK pathway. Chin Med J 2009; 122: 1922-1928.

32. Yende S, Waterer GW, Tolley EA, et al. Inflammatory markers are associated with ventilatory limitation and muscle dysfunction in obstructive lung disease in well functioning elderly subjects. Thorax 2006; 61:10-16.

33. 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.

34. Kishimoto T. The biology of interleukin-6. Blood 1989; 74: 1-10.

35. Camp PG, Coxson HO, Levy RD, et al. Sex differences in emphysema and airway disease in smokers. Chest 2009; 136:1480-1488.

36. Wang Y, Lam KS, Yau MH, Xu A. Post-translational modifications of adiponectin: mechanisms and functional inmplications. Biochem J 2008; 409:623-633.

37. Leung K-C, Xu A, Craig ME, Martin A, Lam KS, O'Sullivan AJ. Adiponectin isoform distribution in women – relationship to female sex steroids and insulin sensitivity. Metab Clin Exp 2009; 58:239-245.

38. Devaraj S, Swarbrick MM, Singh U, Adams-Huet B, Havel PJ, Jialal I. CRP and adiponectin and its oligomers in the metabolic syndrome: evaluation of new laboratory-based biomarkers. Am J Clin Pathol 2008; 129:815-822.

**Table 1. Characteristics of study subjects**

| | Healthy non-smokers | Healthy ever-smokers | COPD |
|---|---|---|---|
| | n = 51 | n = 62 | n = 71 |
| Age | $45 \pm 13$ | $56 \pm 15^{\#}$ | $70 \pm 9^{\dagger,\ddagger}$ |
| Smoking status (current/ex-smoker) | NA | 31/31 | $19/52^{\xi}$ |
| Pack-years smoked | NA | $23 \pm 15$ | $48 \pm 34^{\ddagger}$ |
| BMI | $24.6 \pm 3.5$ | $23.7 \pm 3.2$ | $20.7 \pm 3.7^{\dagger,\ddagger}$ |
| $FEV_1$ % predicted | $105 \pm 14$ | $102 \pm 15$ | $37 \pm 13^{\dagger,\ddagger}$ |
| FVC % predicted | $104 \pm 14$ | $102 \pm 13$ | $72 \pm 21^{\dagger,\ddagger}$ |
| $FEV_1$/ FVC | $81 \pm 5$ | $78 \pm 6^{\#}$ | $39 \pm 9^{\dagger,\ddagger}$ |

Data are expressed as mean $\pm$ SD.

$^{\#}p < 0.01$ between healthy non-smokers and ever-smokers, $^{\dagger}p < 0.001$ between healthy non-smokers and COPD patients and $^{\ddagger}p < 0.05$ between healthy ever-smokers and COPD patients by either t-test or Mann-Whitney U test.

$^{\xi}p = 0.01$ by Chi-square test with continuity correction.

**Table 2. Plasma levels of inflammatory mediators**

| | Healthy non-smokers | Healthy ever-smokers | COPD |
| --- | --- | --- | --- |
| | n = 51 | N = 62 | N = 71 |
| Adiponectin (μg/ml) | 1.76 (1.34-2.52) | 1.90 (0.86-2.86) | 4.39 (2.68-6.98)[†,‡] |
| IL-6 (pg/ml) (all) | <2.40 (<2.40-2.78) | <2.40 (<2.40-2.77) | 4.19 (2.67-6.40)[†,‡] |
| ≥ 2.4[*] only | n = 18<br>2.98 (2.75-3.50) | n = 20<br>3.09 (2.77-3.84) | n = 56<br>4.98 (3.61-8.11) |
| IL-8 (pg/ml) (all) | < 3.10 (<3.10-4.14) | 13.84 (6.86-29.73)[#] | 7.26 (3.63-14.25)[†,‡] |
| ≥ 3.1[φ] only | n = 16<br>7.50 (4.31-12.58) | n = 55<br>14.64 (8.35-30.42) | n = 55<br>9.92 (6.30-17.33) |
| CRP (mg/l) | 3.12 (2.11-5.71) | 3.71 (1.97-10.37) | 8.75 (4.26-40.63)[†,‡] |

Data are expressed as median (IQR).

[*] The detection limit for IL-6 was 2.4 pg/ml. There were 33 healthy non-smokers, 42 healthy ever-smokers and 15 COPD patients whose IL-6 was < 2.4 pg/ml.

[φ] The detection limit for IL-8 was 3.1 pg/ml. There were 35 healthy non-smokers, 7 healthy ever-smokers and 16 COPD patients whose IL-8 was < 3.1 pg/ml.

[#] $p < 0.01$ between healthy non-smokers and ever-smokers,[†] $p < 0.001$ between healthy non-smokers and COPD patients and [‡] $p < 0.05$ between healthy ever-smokers and COPD patients by either t-test or Mann-Whitney U test.

**Table 3. Relationship between plasma adiponectin, IL-6, IL-8 or CRP and lung function parameters in ever-smokers with or without COPD (Pearson partial correlation)[a]**

|  | Adiponectin | IL-6[b] | IL-8[c] | CRP |
|---|---|---|---|---|
| $FEV_1$ (% predicted) | -0.370[***] | -0.381[***] | 0.208[*] | -0.303[**] |
| FVC (% predicted) | -0.262[**] | -0.189 | 0.196[*] | -0.187 |
| $FEV_1$/FVC ratio | -0.302[**] | -0.368[***] | 0.193[*] | -0.284[**] |

[*]$p < 0.05$, [**]$p < 0.01$ and [***]$p < 0.001$. [a]adjusted for age, BMI, smoking status and pack-years smoked.

[b]All patients with data on smoking status were included, except 21 with negative IL-6 values.

[c]All patients with data on smoking status were included, except 7 with negative IL-8 values.

**Table 4. Associations between plasma levels of adiponectin, IL-6, IL-8 or CRP and disease severity in patients with COPD adjusted for age, BMI, smoking status and pack-years smoked by multiple linear regression analysis**

| | Adiponectin | | | IL-6[a] | | | IL-8[b] | | | CRP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\beta$[#] | SE* | $p$ | $\beta$[#] | SE* | $p$ | $\beta$[#] | SE* | $p$ | $\beta$[#] | SE* | $p$ |
| Age | 0.004 | 0.009 | 0.623 | -0.002 | 0.011 | 0.874 | 0.009 | 0.017 | 0.593 | -0.011 | 0.024 | 0.639 |
| BMI | -0.114 | 0.02 | **<0.001** | -0.047 | 0.024 | 0.058 | -0.021 | 0.036 | 0.556 | 0.008 | 0.053 | 0.884 |
| Smoking status | 0.203 | 0.175 | 0.251 | -0.121 | 0.212 | 0.571 | -0.013 | 0.320 | 0.968 | -0.119 | 0.464 | 0.798 |
| Pack-year smoked | -0.002 | 0.002 | 0.436 | 0.003 | 0.003 | 0.218 | 0.002 | 0.004 | 0.669 | 0.002 | 0.006 | 0.687 |
| Disease severity | 0.220 | 0.103 | **0.037** | -0.183 | 0.131 | 0.166 | -0.237 | 0.189 | 0.215 | 0.210 | 0.273 | 0.444 |

[#]Unstandardized coefficients; *Standard error

[a,b] All COPD patients with data on smoking status were included, except 6 with negative IL-6 values and 4 with negative IL-8 values.
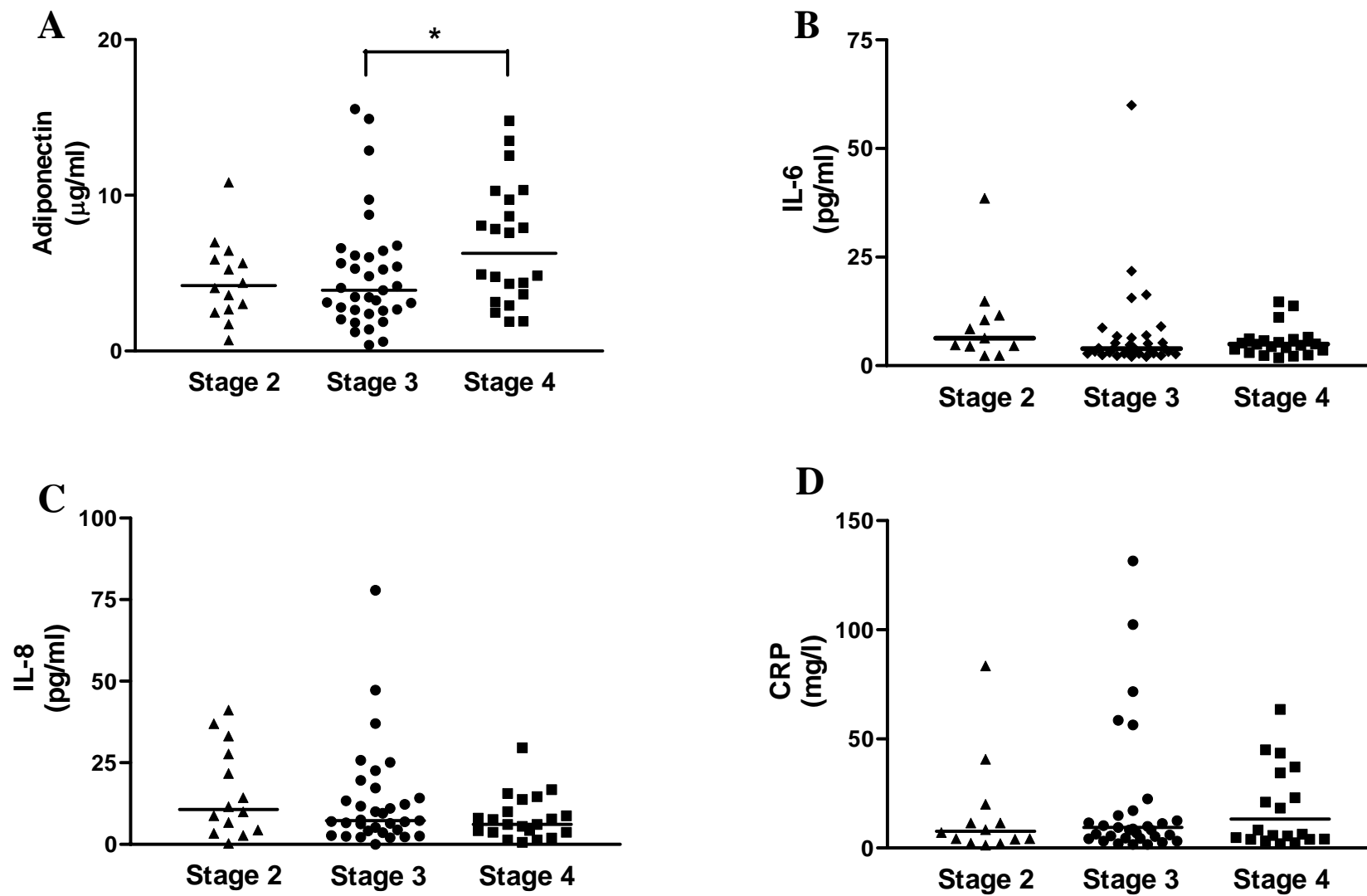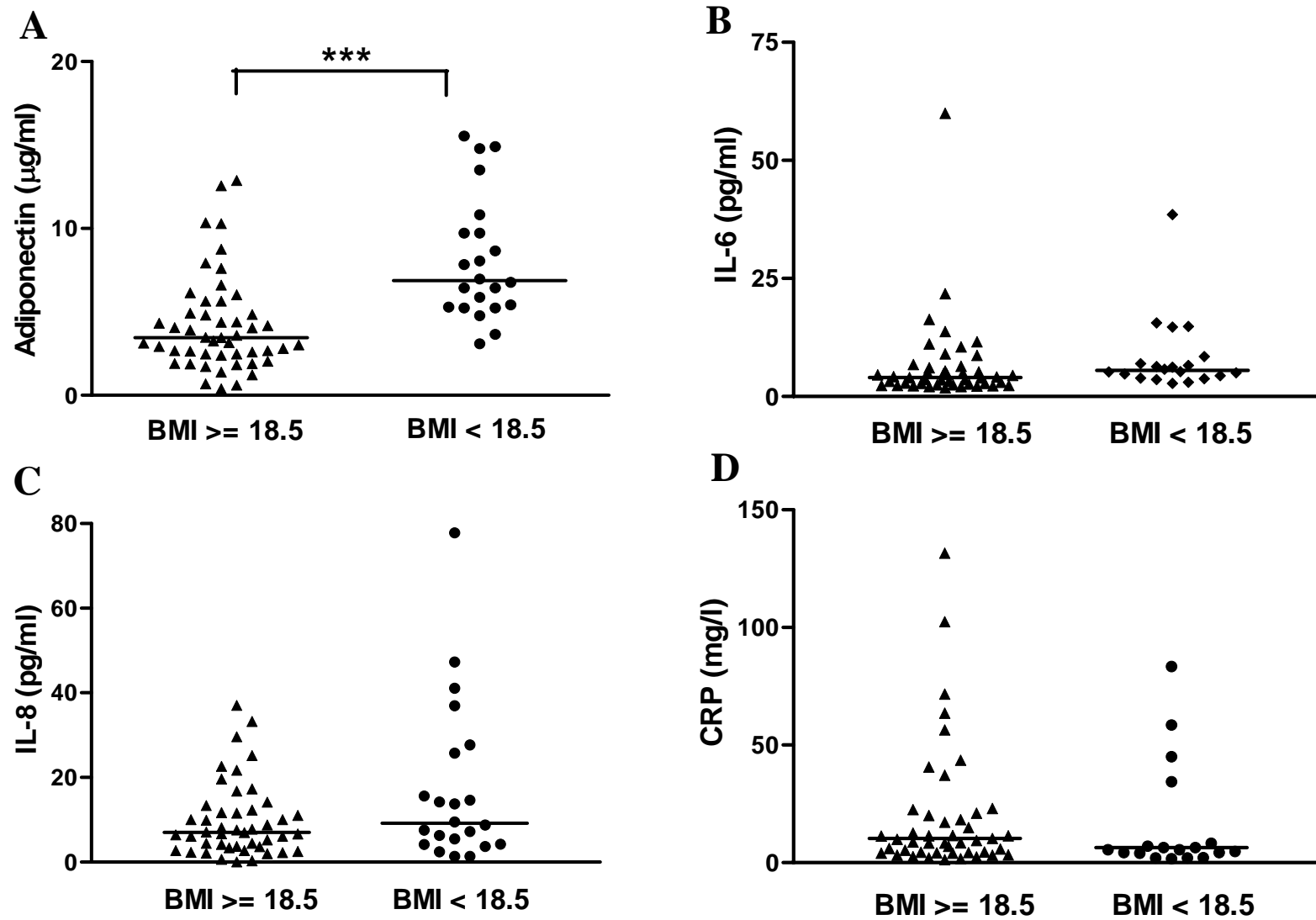
Figure 1

Figure 2

**Figure legends**

**Figure 1.** Plasma adiponectin, IL-8, IL-6 and CRP levels according to disease severity.

(A) Plasma adiponectin in stage 2, 3, and 4 COPD patients with n = 14, 35, and 22

respectively. [*] $p < 0.05$   by Mann-Whitney U test. (B) Plasma IL-6 in stage 2, 3, and

4 COPD patients with n = 11, 33, and 21 respectively. Three negative values from

stage 2, two negative values from stag 3 and one negative values from stage 4 were

not included in the plot. (C) Plasma IL-8 in stage 2, 3 and 4 COPD patients with n =

14, 32, and 21 respectively. Three negative values from stage 3 and one negative

value from stage 4 were not included in the plot. (D) Plasma CRP in stage 2, 3 and 4

COPD patients with n = 14, 35 and 22 respectively). The y-axis of plasma CRP was

restricted to 150 mg/l; one subject from stage 2 (251 mg/l), four subjects from stage 3

(196, 373, 411 and 551 mg/l) and three subjects from stage 4 (165, 199 and 414 mg/l)

were not shown in the plot. The horizontal line represents median values.


**Figure 2.** Plasma levels of adiponectin, IL-8, IL-6 and CRP according to BMI. (A)

Plasma adiponectin in COPD patients with BMI $\geq$ 18.5 kg/m$^2$ (n = 49) and BMI <

18.5 kg/m$^2$ (n = 22). [***] $p < 0.001$ between BMI $\geq$ 18.5 kg/m$^2$ and BMI < 18.5 kg/m$^2$

by Mann-Whitney U test. (B) Plasma IL-6 in COPD patients with BMI $\geq$ 18.5 kg/m$^2$

(n = 45) and BMI < 18.5 kg/m$^2$ (n = 20). Four negative values from BMI $\geq$ 18.5 kg/m$^2$

and two negative values from BMI < 18.5 kg/m$^2$ were not included in the plot. (C)

Plasma IL-8 in COPD patients with BMI $\geq$ 18.5 kg/m$^2$ (n = 45) and BMI < 18.5 kg/m$^2$

(n = 22). Four negative values from BMI $\geq$ 18.5 kg/m$^2$ were not included in the plot.

(D) Plasma CRP in COPD patients with BMI $\geq$ 18.5 kg/m$^2$ (n = 49) and BMI < 18.5

kg/m$^2$ (n = 22). The y-axis of plasma CRP was restricted to 150 mg/l; four subjects

from BMI $\geq$ 18.5 kg/m$^2$ (196, 199, 441 and 551 mg/l) and four subjects from BMI <

18.5 kg/m$^2$ (165, 251, 373 and 411 mg/l) were not shown in the plot. The horizontal

line represents median values.