Elevated Plasma Adiponectin Levels in Patients with Chronic Obstructive Pulmonary Disease

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Running title: Plasma adiponectin levels in COPD

Word count: 2615

Number of tables: 4

Number of figures: 2

Number of references: 38

Keywords: Adiponectin, Chronic obstructive pulmonary disease, C-reactive protein, Interleukin-6, Interleukin-8, Lung function

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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

stable and acute exacerbation of COPD.[13,14] However, in one study, adiponectin was found to suppress TNF-α and MMP-12 production in alveolar macrophages and absence of adiponectin led to an emphysema-like lesion in adiponectin knock-out mice.[15]

In this study, we hypothesized that circulating levels of adiponectin and other conventional inflammatory biomarkers might be associated with lung function in ever-smokers with or without COPD. Thus, we studied patients with stable COPD who were ever-smokers, and healthy ever-smokers and non-smokers as controls to investigate circulating levels of adiponectin, IL-6, IL-8 and CRP, and the correlations between these biomarkers and lung functions.

METHODS

Study Subjects

Three groups of men, a total of 184, were randomly chosen from our database of the COPD study conducted by the COPD Study Group of the Hong Kong Thoracic Society between 2005 and 2006:[16] (1) healthy life long non-smokers; (2) healthy ever-smokers: either current smokers or ex-smokers (defined as those who had not smoked within the last 12 months) with $FEV_1/FVC \geq 70$ and $FEV_1 \geq 80$ (% predicted), and no chronic respiratory symptoms; and (3) stable COPD patients, who are ever-smokers and defined as $FEV_1/FVC < 70$ and/or $FEV_1 < 80$ (% predicted) according to the diagnostic criteria of Global Initiative for Chronic Obstructive Lung Disease (GOLD).[17] Stable COPD patients were defined as those who did not have exacerbation within the last 12 weeks prior to recruitment. The healthy subjects, irrespective of smoking habits, were recruited from those attending churches and community centers for the elderly across Hong Kong. COPD patients were recruited from outpatient respiratory clinics. Lung function tests were performed in all control subjects and patients using standardized methods according to the American Thoracic Society guidelines.[18] The predicted values were based on reference values obtained from our local population.[19] Information about smoking habits, respiratory symptoms and other diseases such as cardiovascular diseases

were obtained from a detailed questionnaire. Subjects were excluded if they had a history of asthma or other lung illnesses. Four of the COPD patients had coronary artery disease. There was no patient from our cohort had stage 1 disease [$FEV_1/FVC < 70$, $FEV_1 \geq 80$ (% predicted)] and were subdivided into three groups according to disease severity based on GOLD criteria (stage 2: $50\% \leq FEV_1 < 80\%$ predicted; stage 3: $30\% \leq FEV_1 < 50\%$ predicted; stage 4: $FEV_1 < 30\%$ predicted).[16] Patients were also subdivided into two groups based on body mass index (BMI): BMI < 18.5 kg/m^2 and BMI ≥ 18.5 kg/m^2 according to WHO criteria.[20] Every participant signed the informed consent form and this study was approved by the Ethics Committee of The University of Hong Kong.

Blood Sampling and Analysis

Venous blood samples were taken from all subjects, centrifuged immediately at 1600x g for 10 min at 4°C and stored at -70°C. Plasma adiponectin (R&D Systems Inc., MN, USA), IL-6, IL-8 ((BD Biosciences Pharmingen, San Diego, CA, USA) and CRP (Diagnostic Systems Laboratories Inc., Texas, USA) were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits respectively.

Statistical Analysis

Data were expressed as mean ± SD or median (interquartile range; IQR) for normally or non-normally distributed variables, respectively, unless specified. The normality was tested by the method of Kolmogorov-Smirnov. Demographic data were compared between any two groups by either Student *t* test or χ^2 statistics. Plasma levels of adiponectin, IL-6, IL-8 and CRP were compared by Mann-Whitney U test. All data including those whose readings were below the detection limit were included in these comparisons. In COPD patients and healthy ever-smokers, the relationships between adiponectin, IL-6, IL-8 or CRP and the lung function measures or other demographic variables were first investigated by the Spearman rank-order correlation using data from all subjects. If similar results were obtained by the Pearson product-moment correlation, the Pearson partial correlation between log-transformed adiponectin, IL-6, IL-8 or CRP and the lung function measures with adjustment for cofounders was then estimated using data from those with positive values. Multiple linear regression analyses were performed to study the relationships between COPD severity and plasma adiponectin, IL-6, IL-8 or CRP (in log scale), adjusting for age, BMI, smoking status and pack-years smoked within COPD patients only. Stages 2, 3 and 4 COPD patients were coded with values 1, 2 and 3, respectively, and entered the regression model as a continuous independent factor.

All *p*-values were not adjusted for multiple testing due to the exploratory nature of this study. SPSS for Windows version 16.0 statistical package (SPSS, 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

was found to have positive correlations with age (r = 0.424 and 0.303 respectively, $p \leq 0.001$) and pack-year smoked (r = 0.254 and 0.338 respectively, $p < 0.01$) and inverse correlation with BMI (r = -0.623 and -0.396, $p < 0.001$). After controlling for age, BMI, smoking status and pack-year smoked, plasma IL-6 remained positively correlated with plasma CRP (r = 0.356, $p < 0.001$). Ex-smokers regardless of lung function status also had higher plasma adiponectin levels than current smokers (median: 3.26, IQR: 1.96-5.65 μg/ml versus median: 2.24, IQR: 1.03-4.43 μg/ml, $p = 0.016$, Mann-Whitney U test).

Pulmonary function parameters, FEV_1 (% predicted), FVC (% predicted) and FEV_1/FVC ratio, correlated negatively with plasma adiponectin, IL-6 and CRP but positively with plasma IL-8 (data not shown). After controlling for age, BMI, smoking status and pack-year smoked, these lung function parameters remained inversely correlated with plasma adiponectin, IL-6 and CRP levels, and positively correlated with plasma IL-8 levels (Table 3).

There were no significant differences in age, BMI and pack years smoked among COPD patients according to disease severity (data not shown). We found an increase in plasma adiponectin and CRP levels with disease severity (Figure 1A and 1D). Stage 4 COPD patients had the highest median of plasma adiponectin levels compared to stage 2 and stage 3 COPD patients. No difference was found in plasma

IL-6 among different stages (Figure 1B). Plasma IL-8 levels show a non-significant decrease with disease severity (Figure 1C).

The COPD patients with BMI < 18.5 kg/m^2 had significantly elevated plasma adiponectin levels in comparison to those with BMI ≥ 18.5 kg/m^2 (Figure 2A). They also showed higher median of plasma IL-6 and IL-8, and lower CRP levels than COPD patients with BMI ≥ 18.5 kg/m^2 but not reaching statistical significance (Figure 2B-D).

After adjusting for age, BMI, smoking status and pack-year smoked, multiple linear regression analyses showed that plasma adiponectin levels still increased with the COPD staging (Table 4) but plasma IL-6, IL-8 or CRP levels were not related to COPD staging.

DISCUSSION

In this study, we found that COPD patients who were ever-smokers had significantly higher plasma levels of adiponectin, IL-6 and CRP than healthy ever-smokers and non-smokers. Plasma levels of adiponectin, IL-6 and CRP were negatively correlated with FEV_1 (% predicted) in COPD patients and healthy ever-smokers.

Our findings that COPD patients had a significantly higher plasma adiponectin levels and that the more severe COPD patients had even higher levels suggest that adiponectin might be inappropriately secreted in this disease. The exact role of adiponectin could not be elucidated in this study since it is a cross-sectional study. In contrast to our findings, Tomoda *et al*[13] and Kirdar *et al*[14] observed no relationship between lung function and plasma adiponectin. This may probably be due to the limited sample size in their studies. Although the function of adiponectin remains controversial, adiponectin was recently found positively correlated with IL-6 in dialysis patients[21], in line with our findings. Additionally, we found that plasma adiponectin levels were inversely correlated with BMI in the group of healthy ever-smokers and those with COPD as reported in previous studies.[13] COPD patients with BMI < 18.5 kg/m^2 had a significant elevation of circulating adiponectin compared to those with BMI ≥ 18.5 kg/m^2 as previously reported.[13] This

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]

CONCLUSION

We found an inverse relationship between FEV_1 (% predicted) and plasma adiponectin, IL-6 or CRP levels in ever smokers. In COPD patients, we found elevated plasma adiponectin, IL-6 and CRP levels and the more severe the disease, the higher the adiponectin levels. These findings suggest that these biomarkers might be associated with COPD. As this study provides evidence of association rather than of causation, prospective studies are required to assess biological significance of these associations.

Acknowledgements

This work was supported partly by the Hong Kong Lung Foundation. The authors wish to thank all nurses and laboratory staffs who took part in this study; all of the subjects for their participation. KHC designed, coordinated and carried most of the work, ELISA and the statistical analysis, drafted the manuscript. SCY performed ELISA. TJY gave advice on performing the statistical analysis. AHKC helped the recruitment of the study subjects. MSMI and MMWC-Y helped to improve the final manuscript. JCWM conceived of the study, aided technical trouble shooting, helped to perform the statistical analysis, and drafted and edited the manuscript. All authors read and approved the final manuscript.

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Table 1. Characteristics of study subjects

	Healthy non-smokers	Healthy ever-smokers	COPD
	n = 51	n = 62	n = 71
Age	45 ± 13	56 ± 15[#]	70 ± 9[†,‡]
Smoking status (current/ex-smoker)	NA	31/31	19/52[ξ]
Pack-years smoked	NA	23 ± 15	48 ± 34[‡]
BMI	24.6 ± 3.5	23.7 ± 3.2	20.7 ± 3.7[†,‡]
FEV_1 % predicted	105 ± 14	102 ± 15	37 ± 13[†,‡]
FVC % predicted	104± 14	102 ± 13	72 ± 21[†,‡]
FEV_1/ FVC	81 ± 5	78 ± 6[#]	39 ± 9[†,‡]

Data are expressed as mean ± SD.

[#] $p < 0.01$ 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.

[ξ] $p = 0.01$ by Chi-square test with continuity correction.

Table 2. Plasma levels of inflammatory mediators

	Healthy non-smokers n = 51	Healthy ever-smokers N = 62	COPD 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 2.98 (2.75-3.50)	n = 20 3.09 (2.77-3.84)	n = 56 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)†,‡
≥ 3.1ϕ only	n = 16 7.50 (4.31-12.58)	n = 55 14.64 (8.35-30.42)	n = 55 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

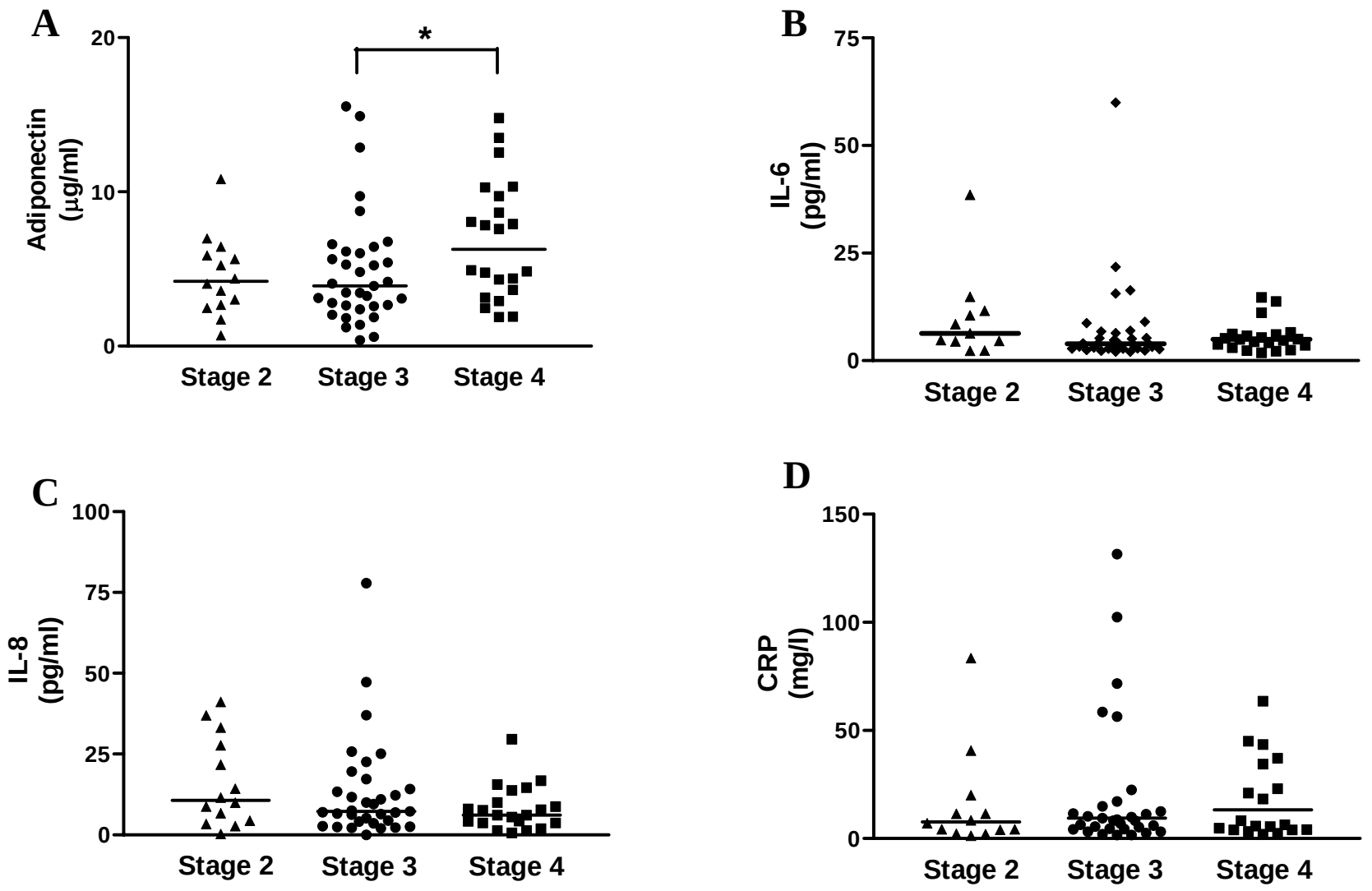
A
Adiponectin (μg/ml)
20
10
0
*
Stage 2
Stage 3
Stage 4
B
IL-6 (pg/ml)
75
50
25
0
Stage 2
Stage 3
Stage 4
C
IL-8 (pg/ml)
100
75
50
25
0
Stage 2
Stage 3
Stage 4
D
CRP (mg/l)
150
100
50
0
Stage 2
Stage 3
Stage 4

Figure 2

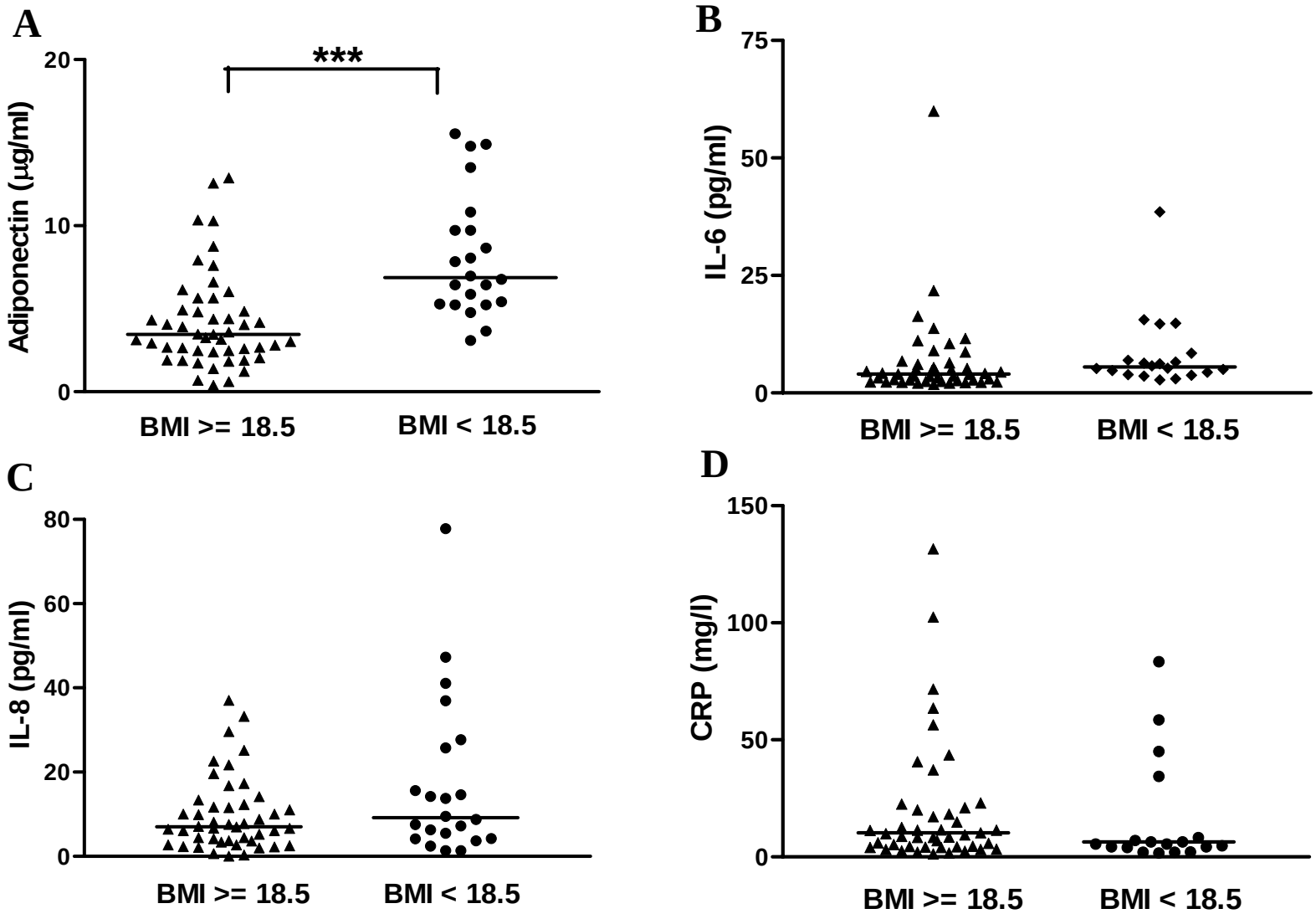
A
***
Adiponectin (µg/ml)
20
10
0
BMI >= 18.5
BMI < 18.5
B
IL-6 (pg/ml)
75
50
25
0
BMI >= 18.5
BMI < 18.5
C
IL-8 (pg/ml)
80
60
40
20
0
BMI >= 18.5
BMI < 18.5
D
CRP (mg/l)
150
100
50
0
BMI >= 18.5
BMI < 18.5

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 ≥ 18.5 kg/m^2 (n = 49) and BMI < 18.5 kg/m^2 (n = 22). *** $p < 0.001$ between BMI ≥ 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 ≥ 18.5 kg/m^2 (n = 45) and BMI < 18.5 kg/m^2 (n = 20). Four negative values from BMI ≥ 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 ≥ 18.5 kg/m^2 (n = 45) and BMI < 18.5 kg/m^2 (n = 22). Four negative values from BMI ≥ 18.5 kg/m^2 were not included in the plot. (D) Plasma CRP in COPD patients with BMI ≥ 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 ≥ 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.