Plasma leptin and adiponectin in COPD exacerbations: Associations with inflammatory biomarkers

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KEYWORDS
leptin;
adiponectin;
adipose tissue;
COPD;
exacerbation;
Systemic inflammation

Summary
Background: Various systemic inflammatory markers have been evaluated for their value in acute exacerbations of chronic obstructive pulmonary disease (COPD). Leptin and adiponectin have been linked to acute exacerbations and stable COPD.

Objectives: To assess plasma leptin, adiponectin and their ratio in acute exacerbations of COPD and to study possible associations with inflammatory biomarkers.

Methods: Plasma leptin, adiponectin and their ratio (L/A) and serum biomarkers of systemic inflammation C-reactive protein (CRP), Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) were assessed at three time points (admission, resolution and stable phase 8 weeks after resolution) in a selected cohort of 63 COPD patients hospitalized for acute exacerbations. Subjects with comorbidities related to adipose tissue hormones were meticulously excluded.

Measurements and main results: All systemic inflammatory biomarkers, leptin and L/A ratio were elevated during admission compared to resolution and stable phase (mean L/A ratio 2.6 vs. 1.57 vs. 1.22, respectively; p < 0.0001), whereas adiponectin was elevated at resolution compared to admission. Log leptin, adiponectin and L/A ratio were significantly associated with variables of systemic inflammation, after proper adjustments, both on admission and in stable condition. In stepwise multiple linear regression models, IL-6 and TNF-α present the most significant associations with leptin, adiponectin and their ratio.

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Introduction

Chronic obstructive pulmonary disease (COPD) is considered to be a disease which profoundly affects worldwide mortality and morbidity. Exacerbations of COPD (ECOPD) are associated with worsening of lung function, decreased health-related quality of life, increased systemic inflammation and significant impact on survival.

It is well appreciated that there is an up-regulation of airway and systemic inflammation in COPD. Various biomarkers are reported to be higher during COPD compared to baseline measurements. Systemic inflammatory parameters like interleukin-6 (IL-6) and C-reactive protein (CRP) correlate with selected airway inflammatory parameters and seem to be higher in the presence of respiratory tract infections. Despite the above evidence, many aspects of the underlying mechanism of increased systemic inflammation in COPD remain speculative.

Adipose tissue is a highly active organ and there is evidence that it secretes a large variety of proteins, including cytokines, chemokines, and hormone-like factors such as leptin, adiponectin and resistin. Leptin is a circulating hormone produced by adipose tissue acting both centrally and peripherally to regulate several metabolic and inflammation-related functions. Adiponectin is the adipokine that is mainly involved in the regulation of insulin sensitivity. Adiponectin has also anti-inflammatory properties, by reducing inflammatory cytokines and inducing anti-inflammatory ones. Increased levels of leptin were reported in stable COPD as well as in ECOPD. However, limited data is available on the role of adiponectin in COPD, with the exception of an increase in its levels in underweight COPD patients and a marginal difference between stable phase and exacerbation.

We hypothesized that adipose tissue is an important contributor to the systemic inflammation of COPD particularly to that observed in ECOPD. Given the opposing effect of leptin and adiponectin, we hypothesized that their ratio may be of greater interest in this direction instead of the single adipokines. The aim of the present study was to evaluate the levels of leptin, adiponectin and their (leptin/adiponectin [L/A]) ratio at the onset and the resolution of an ECOPD, as well as at a stable phase 8 weeks later; measurements were performed in a selected cohort of COPD patients without comorbidities in order to eliminate possible bias from diseases where adipose tissue hormones are also implicated. Additionally, associations between leptin, adiponectin and L/A ratio with biomarkers expressing the systemic inflammatory process, such as serum IL-6, CRP and tumor necrosis factor alpha (TNF-α), were additionally studied.

Methods

Study subjects

COPD patients admitted to two University Hospitals for ECOPD were evaluated for the present study. All patients were diagnosed for COPD according to Global initiative for Obstructive Lung Diseases (GOLD) guidelines, and ECOPD were graded as level II–III according to ERS/ATS consensus criteria. All patients fulfilling Anthonisen’s criteria for type 1 ECOPD. The management of all patients was in accordance with the ERS/ATS guidelines, including bronchodilators, systemic corticosteroids (30–40 mg prednisolone) for 10 days and antibiotics. Patients with significant comorbidities, including tuberculosis or other lung disease except from COPD, apparent heart failure, coronary artery disease, renal or liver impairment or failure, diabetes mellitus, history of cancer in any site, metabolic syndrome, collagen and vascular disorders were excluded. Patients receiving oral corticosteroids and those with respiratory tract infection or ECOPD in the past 8 weeks prior to admission were also excluded. Study was approved by scientific committees of both hospitals and subjects provided informed consent.

Study design

Patients were evaluated at three time points: on admission, on resolution and on stable state, 8 weeks after resolution. On admission, detailed medical history, clinical examination, identification of the cause of exacerbation, evaluation for comorbidities, as well as treatment regimens, including long term oxygen therapy (LTOT), were obtained and blood samples were drawn prior to the initiation of treatment. On resolution and on stable phase samples were drawn in the morning between 8 and 10 am. Simple spirometry (Vicat-test, Model VEP2; Mijnhardt; Rotterdam, Holland) pre- and post-bronchodilatation to determine forced expiratory volume in one second (FEV1)% pred. and FEV1/forced vital capacity (FVC) ratio was performed on stable phase. Arterial blood gases (Ecosys II, Eschweiler compact BGA, Kiel, Germany) were obtained in the three study phases. FiO2 was additionally calculated. Hypoxia was determined by arterial oxygen tension (PaO2)/FiO2 ratio since some of the patients were already receiving oxygen on admission.

Definitions of clinical status at three time points

Resolution of AECOPD was defined as completion of treatment with corticosteroids and antibiotics, return of symptoms to baseline and no requirement of increased doses of bronchodilatation. Stable state was considered as no requirements for increases in treatment and no significant changes in symptoms apart from expected daily variation 8 weeks after the resolution.

Measurement of serum and plasma biomarkers

Blood samples were immediately centrifuged at 4 °C and stored at –80 °C. Plasma leptin and adiponectin, and serum TNF-α and IL-6 were measured by an enzyme-linked immunosorbent assay (R&D systems, Abington, UK).
of detection were 7.8 pg/ml, 0.246 ng/ml, 0.12 pg/ml and 0.039 pg/ml, respectively. For leptin and adiponectin, samples were further diluted 1:100, according to manufacturer’s guidelines, so that the minimum detectable doses were 0.78 ng/ml and 0.025 μg/ml respectively. CRP (mg/dl) was measured using highly sensitive nephelometry (Da de Herrig 035041, Marburg, Germany) with normal values <0.3 mg/dl.

Statistical analysis

Normally distributed data are presented as mean ± standard deviation (SD), whereas skewed data are presented as median (interquartile ranges). Normality of distribution was checked with Kolmogorov-Smirnov test. Comparisons of biomarkers among the three times evaluated were performed with Friedman’s test for repeated measures, with appropriate post-hoc multiple comparison tests (Dunn’s), since data were skewed. For the evaluation of associations between leptin, adiponectin and L/A ratio with inflammatory biomarkers, their values were log-transformed to obtain normal distribution. Linear regression analyses were performed using log leptin, log adiponectin and log L/A ratio as dependent variables and CRP, IL-6 and TNF-α as independent variables, after adjustment for age, gender, body mass index (BMI), smoking habit (in pack years), treatment regimens prior to admission (including LTOT) and prescribed treatment after discharge (used only for regression analysis in stable phase), PaO2/FiO2 ratio and arterial carbon dioxide tension (PaCO2). In order to identify the most significant predictors of leptin, adiponectin and L/A ratio, stepwise multiple linear regression analyses were performed. Statistical analysis was performed using SPSS 15.0 and Graph Pad Prism 5.

Results

The flow chart of the patients included in the study is presented in Fig. 1. Sixty-three patients were included in the final analysis. The demographic characteristics of the 63 study participants are presented in Table 1.

Levels of leptin, adiponectin and inflammatory cytokines

Table 2 and Fig. 2 present the time-course of leptin, adiponectin and their ratio. The levels of leptin on admission were higher compared to resolution and to the stable state 8 weeks later (p < 0.0001, Fig. 2A). In contrast, the levels of adiponectin presented a significant increase on resolution compared to the admission and the stable phase at 8 weeks (p < 0.0001, Fig. 2B). The L/A ratio presented significant reduction on resolution compared to the admission and the stable phase at 8 weeks (p < 0.0001, Fig. 2C). Leptin, adiponectin and L/A ratio levels did not differ between resolution of ECOPD and stable phase despite a tendency of lower leptin levels in the stable phase (p > 0.05).

All three inflammatory cytokines studied (CRP, IL-6, and TNF-α) presented a significant reduction of their levels on resolution compared to the admission, with no further alteration of their levels between at resolution and at 8 weeks later (Table 2, p > 0.05 between resolution and stable phase).

Table 1 Demographic characteristics of the patients included in the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>63</td>
</tr>
<tr>
<td>Age</td>
<td>67.4 ± 9.1</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>9/54</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 ± 5.3</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>42.8 ± 13.4</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>64.6 ± 8.3</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>54.8 ± 6.8</td>
</tr>
<tr>
<td>Smoking habit (pack years)</td>
<td>92.5 ± 49.8</td>
</tr>
<tr>
<td>Current/ex-smokers</td>
<td>38/25</td>
</tr>
<tr>
<td>Treatment prior to admission</td>
<td>ICS 44/63</td>
</tr>
<tr>
<td></td>
<td>LABA 36/63</td>
</tr>
<tr>
<td></td>
<td>LAMA 37/63</td>
</tr>
<tr>
<td></td>
<td>LTOT 29/63</td>
</tr>
<tr>
<td></td>
<td>SABA 34/63</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation (SD). Spirometric data represent post-bronchodilator values on stable condition. BMI: Body mass index, FEV₁: forced expiratory volume in one second, FVC: forced vital capacity. ICS = Inhaled steroids, LABA = Long acting beta-two agonists, LAMA = Long acting muscarinic antagonists, LTOT = Long term oxygen treatment, SABA = Short acting beta-two agonists.

Associations of leptin, adiponectin and L/A ratio on admission (Table 3)

After proper adjustments, log leptin presented significant positive associations with CRP (p = 0.025), IL-6 (p = 0.001) and TNF-α (p = 0.006). In a stepwise multiple linear regression model, the most significant predictor of log leptin on admission was IL-6 (R = 0.416, p = 0.001).

After proper adjustments, log adiponectin presented significant negative associations with CRP (p = 0.006), IL-6 (p = 0.008) and TNF-α (p = 0.036). In a stepwise multiple linear regression model, the most significant predictor of log adiponectin on admission was IL-6 (R = −0.433, p = 0.002). Finally, after proper adjustments log L/A ratio presented significant positive associations with CRP (p = 0.025), IL-6 (p = 0.002) and TNF-α (p = 0.016). In a stepwise multiple linear regression model, the most significant predictors of log L/A ratio on admission were TNF-α and IL-6 (R = 0.412, p = 0.001).
Associations of leptin, adiponectin and L/A ratio on stable state (Table 4)

After proper adjustments including prescribed treatment after discharge, log leptin presented significant positive associations with IL-6 (p = 0.013) and TNF-α (p = 0.022). In a stepwise multiple linear regression model, the most significant predictors of log leptin on stable state were BMI and IL-6 (R = 0.456, p = 0.001).

After proper adjustments, log adiponectin presented significant negative associations with CRP (p = 0.034) and IL-6 (p = 0.034). In a stepwise multiple linear regression model, the most significant predictor of adiponectin on stable state was IL-6 (R = −0.286, p = 0.023).

Finally after proper adjustments, log L/A ratio presented significant positive associations with CRP (p = 0.036), IL-6 (p = 0.009) and TNF-α (p = 0.017). In a stepwise multiple linear regression model, the most significant predictors of log L/A ratio on stable state were TNF-α and BMI (R = 0.424, p = 0.003).

Discussion

In this prospective study we have identified that leptin and adiponectin may represent blood biomarkers during ECOPD. According to our data, leptin is increased on admission for ECOPD and is reduced on resolution, whereas adiponectin is reduced on admission and increased on resolution, with their ratio (L/A) presenting a decrease between admission and resolution. Additionally we have shown that leptin and adiponectin levels are associated with the levels of biomarkers of systemic inflammation, and those associations are independent from confounding factors. A possible important observation of our data is that the increased leptin/adiponectin ratio is associated with an increase of systemic inflammation during ECOPD.

The underlying cellular or molecular mechanisms of ECOPD are still not well understood and, according to current evidence, the reason is a further amplification of the inflammatory process by infectious (bacterial or viral) stimuli. It is still under consideration whether this increase represents an overspill from the lungs or is amplified by the interaction of mechanisms not always known for their regulatory role in inflammation. In animal models, leptin, a cytokine-like hormone with pleiotropic actions, modulates increased production of cytokines, like
TNF-α and IL-6, in response to inflammatory stimuli related to bacterial infection. Data from another study, however, suggest that the production of inflammatory cytokines modulates the expression of leptin and indirectly stimulates circulating leptin levels. Adiponectin is a modulator of multiple obesity-related diseases by attenuating excessive inflammatory responses in a variety of tissues. It is found in the circulation at the highest concentration of all adipokines. Adiponectin reduces the production and activity of TNF-α, inhibits IL-6 production, and induces the production of many anti-inflammatory cytokines. Plasma CRP levels negatively correlate with plasma adiponectin levels, while hypoadiponectinemia is associated with increased IL-6 and TNF-α levels. In a similar manner to leptin, experimental studies demonstrated that pro-inflammatory cytokines like TNF-α and IL-6 negatively modulate the production of adiponectin. Based on the aforementioned data, adiponectin may have an anti-inflammatory role in several disease entities. Taking into consideration the data from current literature, combined to our results, we can think of adipose tissue as an important contributor to the systemic inflammation of ECOPD. Indeed, the inflammatory/anti-inflammatory effects of adipokines highlight the importance of body composition in the pathogenesis of COPD. The inverse time-course of adipokines and the complex interrelationship among them and the systemic inflammatory process observed in this study might indicate a possible predisposing factor for the development of ECOPD or alternatively a systemic defense mechanism particularly in infectious origin ECOPD.

However, several issues regarding the involvement of leptin and adiponectin in the whole inflammatory process need to be addressed. An important issue which is not clear is whether the systemic inflammation directly affects or regulates adipose tissue imbalance or the imbalance of adipokines regulates the inflammatory process. Another important issue is whether systemic inflammation is the only significant parameter in the interaction between ECOPD and adipokines, or other factors like metabolic alterations play additional significant role. As recently suggested, both leptin and adiponectin are up-regulated during periods of catabolism in order to control energy expenditure, a situation that is present during ECOPD of infectious origin.

According to our data, IL-6 and TNF-α present the most significant associations with leptin and adiponectin levels in stepwise multiple linear regression models. The issue which needs clarification is the fact that IL-6 presents the most significant associations with the single components of the ratio while TNF-α with ratio irrespective of the study phase. It has been estimated that approximately 20% of the total circulating concentration of IL-6 originates from adipose tissue. In contrast to IL-6, the expression of TNF-α from human adipose tissue remains controversial, since some experimental studies as well as an in vivo study failed to detect significant differences. Based on the above data supporting that IL-6 is the cytokine that is mostly related to the adipose tissue, combined to the data from the stepwise multiple regression analysis in our study, we believe that IL-6 represents an important mediator in the interactions between the adipose tissue and the inflammatory process during ECOPD. The significant association between L/A ratio, TNF-α and BMI might represent the contribution of the energy component when assessing the ratio. The strong association between leptin and L/A ratio with BMI at the stable phase confirms previous data showing a close relation between adiposity and leptin. Future

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Associations between leptin, adiponectin and their ratio and the levels of inflammatory cytokines on admission.</th>
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</thead>
<tbody>
<tr>
<td>log Leptin</td>
<td>log Adiponectin</td>
</tr>
<tr>
<td>B</td>
<td>95% CI for B</td>
</tr>
<tr>
<td>CRP</td>
<td>0.030</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.051</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.137</td>
</tr>
</tbody>
</table>

Associations are presented after adjustment for age, gender, BMI, smoking habit, treatment regimens before admission, PaO2/FiO2 ratio and PaCO2. B represents the unstandardized coefficient. Bold figures represent statistically significant linear relations. CI: confidence intervals, CRP: C-reactive protein, IL-6: interleukin-6, TNF-α: Tumor necrosis factor alpha.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Associations between leptin, adiponectin and their ratio and the levels of inflammatory cytokines on stable state, at the resolution of the exacerbation (8 weeks).</th>
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</thead>
<tbody>
<tr>
<td>log Leptin</td>
<td>log Adiponectin</td>
</tr>
<tr>
<td>B</td>
<td>95% CI for B</td>
</tr>
<tr>
<td>CRP</td>
<td>0.094</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.046</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.191</td>
</tr>
</tbody>
</table>

Associations are presented after adjustment for age, gender, BMI, smoking habit, treatment regimens after discharge, PaO2/FiO2 ratio and PaCO2. B represents the unstandardized coefficient. Bold figures represent statistically significant linear relations. CI: confidence intervals, CRP: C-reactive protein, IL-6: interleukin-6, TNF-α: Tumor necrosis factor alpha.
studies need to address the role of adipose tissue hormones in ECOPD of different origin and in the presence of comorbidities. Another possible limitation of the present study, and of other studies evaluating adipokines, is the several parameters that may affect their levels in serum. We have addressed this limitation by drawing blood samples at a specific time in the morning and by proper adjustments of leptin and adiponectin associations with inflammatory parameters for all confounding factors in the regression analysis, including BMI, smoking, gender, hypoxia, treatment regimens prior to admission and after discharge, hypercapnia and age. Another possible confounding factor is the use of systemic corticosteroids; however, all our patients were treated in a similar manner receiving similar doses of corticosteroids in similar time intervals. In addition, previously published data suggest that steroids increase leptin levels and inhibit adiponectin release from adipocytes, which was not the case with our study showing reduction of leptin and increase of adiponectin with treatment of the ECOPD.

In conclusion, in the present study we have shown that both leptin and adiponectin are associated with the systemic inflammatory process of exacerbations of COPD and their most significant associations seem to be those with IL-6 and TNF-α. Our data provide evidence for a significant association between the two adipokines and the inflammatory process, suggesting a possible additional role of the adipose tissue, besides its energy storage capacity, during ECOPD.

Conflict of interest

None of the authors have any conflicts to disclose.

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