Author's response to reviews

Title: Plasma leptin and insulin-like growth factor I levels during acute exacerbations of Chronic Obstructive Pulmonary Disease

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Version: 3 Date: 2 December 2008

Author's response to reviews: see over
Dear editor,

I am very pleased to resubmit our manuscript entitled “Plasma leptin and insulin-like growth factor I levels during acute exacerbations of Chronic Obstructive Pulmonary Disease”. The issues raised by the reviewers and our responses are detailed below. The comments from each reviewer are dealt with in turn.

I am looking forward to your response.

Yours sincerely,

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Reviewer’s report (1)
Title: Plasma leptin and insulin-like growth factor I levels during acute exacerbations of Chronic Obstructive Pulmonary Disease

Version: 2 Date: 23 September 2008

Reviewer: Richard Debigare

Reviewer’s report:
Overview of the paper
In a group of patients with COPD, leptin and IGF-1 levels were measured at day 1 and day 15 during an exacerbation. In addition, inflammatory cytokines were also measured. Overall, authors found that IGF-1 levels were decreased in patients with COPD compared to healthy subjects. This decrease was still present at day 15. In a similar fashion, leptin levels were increased at day 1 and day 15 in patients with COPD. A relationship between TNF and leptin levels is found at day 1 in COPD. This relationship is lost at day 15. Authors conclude that inappropriately increased circulating leptin levels along with decreased IGF-1 levels occur during acute exacerbations of COPD. Compared to chronic bronchitis, patients with emphysema have lower circulating IGF-1 levels at the onset of the exacerbation but also two weeks later.

1. Is the question posed by authors well defined?
The authors present leptin, TNF and IGF-1 biology in their introduction. The association between these factors and the pulmonary disease is not well connected. As a consequence, the research question is difficult to sort out. The introduction has to be rewritten in order to better link the different concepts and to better define the research question in accordance to the problematic previously exposed in the introduction.
Answer: The introduction has been rewritten.

2. Are the methods appropriate and well described?
The methods used to measure inflammatory cytokines, leptin and IGF-1 are appropriate. However, description in the selection of the patient and treatment is incomplete. Is it 52 consecutive patients? Is there any dropout during the study? Is oxygen therapy used during the hospitalization? What is the smoking status of the control subjects? How the control group was recruited? Why they did not measure IGF binding proteins?

Leptin is known to affect energy expenditure. There is no energy expenditure measurement in the study.

**Answer:** No, they are not 52 consecutive patients. As mentioned in the Methods section, all COPD admitted due to an exacerbation, were evaluated for comorbidities and were accordingly included or excluded from the study. There were also 2 dropouts, one patient who developed nosocomial pneumonia and another one who died because of myocardial infarction on Day 8. Data regarding oxygen therapy have been added to the Methods. Control subjects were not smokers had no medical illnesses, had normal physical examinations and showed no symptoms or signs of infection at the time of the study. They were recruited from the outpatient clinic. We did not measure IGF-I binding proteins, since we were not aware of possible changes in IGF-I levels. We refer to IGF-I binding proteins in the discussion and it seems reasonable, based on our results, that IGF-I binding proteins should be measured. Our study was not designed to examine energy expenditure and the relationship of it with leptin.

**3. Are the data sound?**
The data presented by the authors are duplicated in table 2 and 3. Table 3 should be deleted. The p column in the table is not necessary. I would suggest the superscript annotation instead. This would also clarify the table 2 where two p columns are presented but not explained. How these values are comparable to what have already been published in patients with COPD. The authors chose to use the median (25-75 percentiles) to present the data. Using this nomenclature, the reader does not know if there were values under the level of detection. This information should be added in results section.

**Answer:** Table 3 has been deleted. The superscript annotation has been used instead of p columns. There were no values under the level of detection in either leptin, IGF-I or cytokines. This information has been added to the Methods section.

**4. Does the manuscript adhere to the relevant standards for reporting and data deposition?**
No comment

**5. Are the discussion and conclusions well balanced and adequately supported by the data?**
This is a major point in this manuscript. Most of the data presented in this paper are not novel and already known. The discussion is not well focused on the research question. Since the research is not clear, this is probably a reflection of it. It is difficult to follow the rational in their finding and on the interpretation of their data. For instance, on page 12, line 22 we read: “Our data support a role for IGF-1 in acute exacerbations of COPD”. This sentence
is not explained and not supported by any argumentation. What kind of role? I am not sure that this study was designed to test this hypothesis.

Answer: The discussion has been modified according to our main findings. Possible implications of these findings are also discussed. Leptin levels have been measured in COPD exacerbations. However, IGF-I circulating levels have not and there are no studies evaluating a possible relationship between IGF-I and cytokines or IGF-I and leptin.

6. Are the limitations of the work clearly stated?
Authors mentioned fact about IGF-1 level and concurrent biological state that could explain its variation in their cohort.

Answer: This limitation has been addressed in the discussion section.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Reference 20 is incomplete. The relationship between inflammation-leptin and inflammation-IGF-1 is scientifically weak. Literature is abundant and should be carefully reviewed to strengthen the introduction and clearly define the research question.

Answer: The reference has been completed.

8. Do the title and abstract accurately convey what has been found?
No comment

9. Is the writing acceptable?
The structure of the introduction has to be carefully revised. Same comment for the discussion.

Answer: The introduction and discussion have been modified.

DISCRETIONARY REVISIONS
Please add page numbering in the manuscript.

Answer: Page numbers have been added.

Introduction:
Some points in the introduction are unreferenced. For instance, page 3, 4th line starting with:” In a subgroup of COPD....”

Answer: The reference has been added (Vermeeren et al, ERJ 1997).

Methods:
Why no measure of FFM? The tool to measure FM also measure FFM. This data could be added to the manuscript.

Answer: FFM can be calculated by subtracting FM from body weight. Both data (body weight and FM) are included in the tables. However, if the reviewer thinks that FFM is essential we can add these data as well.

MINOR ESSENTIAL REVISIONS
In addition to the previous comments,

Introduction:
page 3 line 29-30. Increased IGF mRNA levels occur during an acute
exacerbation, but you cannot assert causativeness.  
**Answer:** This is one of the limited references regarding IGF-I and COPD exacerbation. We agree that one cannot assert causativeness. We aimed to evaluate circulating IGF-I levels during COPD exacerbations and whether they were related to systemic inflammation.

Page 3 line 32. reference 13 (also in discussion). What is the relevance of hypoxia to COPD in regards to your argument? Yes COPD patients show increased hypoxia and hypoxia in small animals can be used as a model for hypoxia in COPD but without explaining that you cannot introduce this point. **Answer:** Patients with COPD in general and a large portion of our COPD study group had hypoxia. Small animals with hypoxia can be used as a model for hypoxia in COPD. A comment to this effect has been added in the discussion.

Poor use of terminology in the introduction. Cachexia, weight loss and anorexia are different by their very definition. Pick one and stick with it, if this article is about leptin and regulation of fat mass, use weight loss. If using cachexia, muscle mass should be considered. Also, in a paper about weight loss, leptin and COPD should the obesity paradox not be mentioned/considered?  
**Answer:** The reviewer is right. The term “weight loss” is the most appropriate in our study. Regarding obesity, we evaluated possible correlations of the measured parameters with BMI and %FM.

Methods:  
pg6 line 6 ambient air?  
**Answer:** Arterial blood gases were obtained with the patients breathing room air (FiO\textsubscript{2}: 0.21) as mentioned in the methods section (page 6, line 9). The word “ambient” has been added.

pg6 line 18 (and in general) where observers blinded?  
**Answer:** Yes, the two observers who assessed the presence and extent of emphysema in CT scans were blinded.

Statistical analysis. Why were nonparametric tests used with such a large data pool? Would have thought that the data pool was large enough for the central limit theorem to apply, allowing the assumption of normality.  
**Answer:** The data pool is sufficiently large to use parametric tests. However, since there are no reference values for normals or patients for IGF-I, we believe that our findings are strengthened by using nonparametric tests in our statistical analysis.

Results:  
Present key results as figures not tables. Show correlations as figures.  
**Answer:** We have added a figure regarding IGF-I levels in the three groups (chronic bronchitis, emphysema, healthy controls), and a figure for the TNF-\(\alpha\) – leptin correlation.
pg9 line 17. ‘relationship between’, not ‘relation’.
Answer: “Relation …” has been replaced by “Relationship …”

pg9 line 20. Was the significant correlation positive or negative? Figures would help.
Answer: The correlation was positive. The word “positive” has been added. A figure also has been added for the correlation between leptin and TNF-α.

Discussion:
pg11 line 30. ‘limited’, not ‘few’.
Answer: “Few…” has been replaced by “Limited …”

pg12 line 27, conclusions that lower IGF levels may lead to “decreased muscle mass loss” do authors mean either “decreased muscle mass” or “muscle mass loss”
Answer: Yes, we meant to write “… decreased muscle mass…” The word “loss” has been deleted.

MAJOR COMPULSORY REVISIONS
Introduction:
Overall, the introduction doesn’t clearly lead to the research question, doesn’t convince the reader for the case that the authors are trying to present. The introduction is not well constructed in this regard, the writing has to be revised.
Answer: The introduction has been modified in order to address clearly the research question.

Results
While the data from the exacerbation group at D15 is physiological meaningful and interesting it does not provide a reliable internal baseline measure as these values may have been affected by the prior exacerbation (ie is the differences seen between COPD vs control at D15 due to recovery from exacerbation or just normal for COPD?). A control group of stable COPD patients would provide a better comparison than healthy normal disease free adults.
Answer: It is true that the values measured on Day 15 may reflect the prior exacerbation and recovery from it or may be just normal for COPD. From this point, a group of stable COPD patients would be of help. However, on the one hand systemic inflammation is ameliorated within 15 days, so that there are no differences between COPD and normal subjects (with the exception of IL-8, that is normalised after one more week – data not shown-) and on the other hand we are interested in the changes found in circulating leptin and IGF-I levels which are attributed to the exacerbation (as shown in Day 1).

Discussion
pg12 line 27, the reference to cachexia and muscle mass loss seen in COPD is not conclusive in this cohort of patients. No cachexia was seen based on estimates of fat free mass (FFM) of 49.6 vs 51.3 kg (COPD vs control; estimate from authors data, based of body mass – FM, table 1). The relevance of referring to cachexia and muscle mass loss in COPD in the
closing sentences of the discussion in a manuscript that does not deal with muscle mass may be incorrect. Muscle mass was not measure in this cohort and was not part of the research question. This incongruence in the concepts (leptin level vs inflammation; leptin level vs muscle mass) has to be reviewed in order to clarify the message of the paper.

**Answer:** The reviewer is right. In our manuscript, cachexia or muscle mass loss was not measured. However, one should mention based upon differences found between chronic bronchitis and emphysema and changes of leptin and IGF-I levels during an exacerbation, that these changes may be implicated in the metabolic derangements and weight loss seen in COPD patients. The closing sentences have been modified, so that they would not refer to muscle mass.

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:** I declare that I have no competing interests
Reviewer's report (2)
Title: Plasma leptin and insulin-like growth factor I levels during acute exacerbations of Chronic Obstructive Pulmonary Disease

Version: 2 Date: 4 November 2008
Reviewer: Martijn A. Spruit

Reviewer’s report:
With interest I have read your paper. I have the following major and minor comments:
1. Please add page numbers.
   Answer: Page numbers have been added.

2. Please provide name of the ethics committee and number of the approval.
   Answer: The Ethics Committee of Sotiria Chest Hospital (acts as a scientific committee as well in our hospital) gave approval for this study (3/10/2005).

3. Please explain how co-morbidities were assessed?
   Answer: We included in our study COPD patients with no major comorbidities, especially those that could possibly affect leptin and IGF-I levels or those associated with some degree of systemic inflammation, possibly cofounding cytokine levels. Accordingly, patients with diabetes mellitus, congestive heart failure, lung cancer, collagen vascular diseases and disturbances in thyroid function (known to influence leptin levels) were excluded from our study group.

4. Please explain how written permission was obtained in the participants.
   Answer: The participants were informed in detail about the study as soon as they were admitted to the department (soon after their examination at the A & E department) and signed the informed consent.

5. Did you directly stop the prednisoline after 7 days?
   Answer: Yes, prednisolone was stopped directly at Day 7. As explained in the discussion (p. 11, line 23), samples on Day 1 were obtained before corticosteroid administration and on Day 15 7 days after the last intravenous administration of prednisolone. A comment has been added in the methods section, that prednisolone was directly stopped after Day 7.

6. How many patients did receive oxygen during their stay in the hospital?
   Answer: 38/52 patients received oxygen therapy during their hospital stay (21/29 with chronic bronchitis and 17/23 with emphysema). A comment has been added to the methods section.

7. Did the patients already receive oral steroids before arrival at the emergency room?
   Answer: No, they did not. All patients received the first steroid dose intravenously inside the hospital and after obtaining the blood sample for Day 1.
8. Please specify for each test when they were performed (day of admission, etcetera...)
Answer: Blood samples for the measurement of TNF-α, IL-1β, IL-6, IL-8, leptin and IGF-I were obtained in the morning of Day 1 between 8.30-9.30AM with the patients being in the fasting state for at least 10 hours. Blood samples were also obtained in the morning of Day 15 between 9.30-10.30AM, again with the patients being in the fasting state for 10 hours overnight.

9. Did you also assess (high-sensitivity) CRP?
Answer: High sensitivity CRP was not assessed in our study.

10. I am interested in the possible correlation between the change in biomarkers of systemic inflammation and change in IGF-I between D1 and D15.
Answer: As mentioned in the correlation analyses in the Results section (page 9, line 33) IGF-I was not related to any of the biomarkers of systemic inflammation neither on Day 1 nor on Day 15.

11. Second paragraph discussion: please specify that IL-8 is still increased compared to healthy subjects. Please also explain why
Answer: Although IL-8 levels were decreased on Day 15 in COPD patients, they still differed significantly from healthy subjects. A comment has been added to the discussion section (page 10, line 11).

12. Please explain why the authors did not assess the patients 3 months after discharge. Or even a group of clinically stable patients.
Answer: The aim of our study was to examine whether COPD exacerbations were accompanied by changes in leptin and IGF-I circulating levels and whether these levels were related to biomarkers of systemic inflammation during the exacerbation. We may have assessed our patients at a later time point as well, as suggested by the reviewer. However, as shown in our results by Day 15 systemic inflammation has been notoriously ameliorated, even IL-8 that was still higher compared to healthy subjects was significantly decreased when compared to Day 1. That’s why we did not use a subgroup of clinically stable COPD patients, since we believe that in terms of systemic inflammation, they did not differ from our patients on Day 15.

13. Engelen et al also compared emphysema with chronic bronchitis.
Answer: Engelen et al compared emphysema with chronic bronchitis and found that emphysematous patients had lower body mass index (compatible with our group) and lower lean mass due to increased lean mass depletion compared to chronic bronchitis (Clin Nutr 1999).

14. Table 3 can be deleted from the manuscript (too much overlap with table 2).
Answer: Table 3 has been deleted, as suggested by the reviewer.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.