Author's response to reviews

Title: Effect of octanoic acid-rich formula on plasma ghrelin levels in cachectic patients with chronic respiratory disease

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Effect of octanoic acid-rich formula on plasma ghrelin levels in cachectic patients with chronic respiratory disease

Responses to Reviewer 1:

1) **Participants:** We agree with the reviewer’s comment that multiple causes may influence ghrelin system function. We have therefore added information on the underlying diseases of the patients, duration of illness, and smoking habits to provide readers with a better understand of the characteristics of each patient (page 5, lines 5-8).

2) **Study protocol:** We have added the BMI of each patient group and replaced body weight with BMI in the Table. We also agree with the comment from the reviewer that plasma leptin levels may be helpful in understanding the low fat mass and acyl-ghrelin level in cachectic patients. However, we were unable to measure this parameter, as we had not obtained informed consent for such measurements and no samples remain for measurement of leptin levels.

3) **Blood sampling and assays:** To compare ghrelin levels in patients with chronic respiratory diseases and normal subjects, we have included the range and mean ± SD of acyl- and desacyl-ghrelin levels in age and sex matched controls in the Results section (page 8, lines 1-7).

4) **Discussion:** To avoid confusion, we have used acyl- and desacyl-ghrelin levels instead of total ghrelin levels in the Discussion section. In addition, we have added comments regarding the implications of 24-h ghrelin profile in the Figure Legends, to clarify this issue for readers.

5) **Table:** Based on the comments from the reviewer, we have changed the title of the Table and have added details to the figure legend.

6) The manuscript has been carefully rewritten based on the reviewer’s comments, and
has subsequently been checked by a native speaker to avoid grammatical errors.
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Responses to Reviewer 2

1) We have added patient profiles, including underlying diseases, duration of illness and smoking habits to provide readers with a better understanding of the characteristics of each patient (page 5, lines 5-8). We have also added changes to catecholamines levels to the Results section and the Table (page 8, line 25). We were unable to measure levels of pro-inflammatory cytokines, as no informed consent was obtained for such measurements.

2) As the participants in the first study were the only 4 in-patients in the study, we had deleted statistical assessments in the Figure from the original manuscript. Based on the comments from the reviewer, we have added statistical analysis to the Figure.
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**Responses to Reviewer 3**

1) We provided a definition for “cachectic patients” in the Methods section of the original manuscript (page 5, lines 16-19).

2) We have added patient profiles including underlying diseases, duration of illness and smoking habits to the text, to clarify the characteristics of patients (page 5, lines 5-8).

3) We had checked appetite scores of patients using a VAS scale, but did not include this information in the original manuscript. We agree with the reviewer’s comment that appetite is an important marker related to increases in acyl-ghrelin levels. We have thus added appetite scores to the Table. In the first trial, 4 inpatients were examined under each condition (with or without the formula). Calorie intake was limited to 1,800 kcal/day without the formula. With the formula, 400 kcal/day was added. To avoid confusion, we have amended the Methods section in the revised text (page 6, lines 6-7).

4) We have renamed “Figure 1” as simply “Figure”.

5) We have added titles to the X and Y axes.

6) We have provided statistical analysis in the Figure.

7) No clear relationships were seen between acyl-ghrelin levels and growth rate in other parameters after administration of the formula. This has been described in the Results section (page 8, lines 22-23).

8) We have corrected the spelling of the term “orexigenic” in the revised text.
Responses to Reviewer 4

1) We agree with reviewer’s comment that the comparison between energy match formulas with or without octanoic acids is necessary. Previous reports have shown that acyl-ghrelin levels decreased due to weight gain. However, in our study, body weight and acyl-ghrelin levels increased simultaneously after 2-week administration of octanoic acids. We would like to emphasize this finding.

2) Both chronic respiratory disease and malignancy are common in clinical settings and are well known to lead to cachexia in the later stages. As emphasized in the Introduction section, many reports have described ghrelin levels in patients with malignancy, but none in cachectic patients with chronic respiratory disease (page 4, lines 20-23). This is the reason we selected patients with chronic respiratory disease as subjects.

3) Currently, no methods are available for measuring octanoic acid levels. We thus could not investigate relationships between plasma octanoic acid and acyl-ghrelin in patients.