Reviewer's report

Title: Physiological Effects of Ghrelin in Cachectic COPD: substudy of a multicenter, randomized, double-blind, placebo-controlled trial of ghrelin treatment

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Reviewer: Friedhelm Sayk

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In the present study the authors describe that a 3-week treatment with Ghrelin bd significantly increased maximal oxygen-uptake in COPD-patients with improved exertional capacity and improvements in ventilatory-cardiac parameters. The present results were retrieved from 10 patients (single center) and 10 controls out of a multicenter study that comprised 33 patients only http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0035708. Patients were investigated prior to and after treatment at various pre-defined conditions (rest, work rate at iso-time, iso-oxygen, peak incremental exercise). The principle finding is well in line with previous results from the same research team. Previously they found improvement of the 6-min walking test in COPD-patients after 7 weeks of Ghrelin treatment.

Overall, the topic is highly relevant for a major health problem in modern society. The manuscript is well-written. From a descriptive point of view these results are of considerable interest. However, there are several concerns that need clarification:

1) The previous multicenter study was designed to comprise 60 patients but was finished after 33 patients. Post-hoc power calculation justified this change of protocol. In the present subgroup, again, sample size was corrected due to new intercurrent (?) power calculations.

Given this limited sample size, some parameters revealed significant differences compared to placebo while others did not. Is this a problem of underpower for some parameters?

2) Given the potential future pharmacotherapeutical impact of this study, the method of randomization should be clarified.

3) The authors focused on a multitude of related parameters at diverse time points and exercise conditions – some showed significant differences (pre- vs. post-treatement or ghrelin vs. placebo) while others did not without any obvious biophysiological explanation (NE-levels in iso-time vs. iso-oxygen vs. peak incremental exercise vs. rest). This bears some risk of 'significance' due to the stochastic probability of p<0.05 at multiple testing. Is there any chance to calculate covariances?
4) The authors primarily report the differences between treatment groups, or between time points of measurement. Absolute values are only found for baseline characteristics in Tab. 1. Data in Tab. 2-5 are limited to the net changes (e.g. NE-level). Therefore, the reader has difficulties to estimate the absolute Ghrelin effect on ventilatory-cardiac parameters and to conclude the message by oneself, despite the good structure of the manuscript.

5) The statistical analysis was performed by an external company. Please provide a disclosure statement concerning conflicts of interest for Mr. K. Tsuguchi (Nihon Ultmarc Inc. Tokyo, Japan).

6) The chosen methods to conclude that ghrelin might modulate the neurohumoral balance are weak. Plasma NE is a rather crude marker of sympathetic activity. Therefore, value of the correlation calculated against VO2 is of limited descriptive value. However, studies using highly specific microneurographic techniques have recently strengthened the hypothesis that ghrelin exerts sympathoinhibitory effects.

Taken together, the results are of rather descriptive value pointing towards beneficial effects of ghrelin in cachectic COPD patients. They are helpful to generate hypotheses. Definitely, this warrants proper confirmation in a large scale prospective randomized controlled trial with a sound statistical intention-to-treat analysis.

Minor concerns:
Discussion, first paragraph: “iii) the mean changes from pre-treatment in #VE/#Vco2, #VE/#Vo2, and VD/VT were significantly decreased compared with the placebo group at peak exercise” does not make sense. According to Tab. 3, the mean changes from pre-treatment were bigger, e.g. #VE/#Vo2 – ghrelin -3.7 (3.7)###placebo 0.5 (4.2), treatment effect -4.2 (CI -7.9 to -0.5; 0.030).

The title might be more precise, to provide a better estimate what topic the article is about, e.g. Effects of Ghrelin treatment on exercise capacity in Cachectic COPD

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests