Author's response to reviews

Title: Body Mass Index in Chronic Heart Failure: Association with biomarkers of neurohormonal activation, inflammation and endothelial dysfunction

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Cover letter regarding the revised manuscript:

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Body Mass Index in Chronic Heart Failure: Association with biomarkers of neurohormonal activation, inflammation and endothelial dysfunction

Comments by referee #1

Reviewer's report
Title:
Body Mass Index in Chronic Heart Failure: Association with biomarkers of neurohormonal activation, inflammation and endothelial dysfunction

This is a retrospective, observational, monocentric clinical study of well-characterized patients entered in a management program of systolic HF. The methods and results are properly presented and the conclusions supported by data and well-balanced.

The limited number of patients enrolled in the present study should be acknowledged as a limitation in an ad-hoc section (see major comment #1).

I have several major comments.
Major Compulsory Revisions:
1. The authors should briefly discuss about the sample size of their study and provide their statistical power (minimal strength of detectable associations between BMI and biomarkers).

Thank you for raising this relevant comment. We do agree that the limited number of patients is a limitation. We have added a section in the Discussion, where this issue is addressed.
Discussion section, page 10:

There are some limitations to this study. First, there are a limited number of patients enrolled in the current study, which might diminish the statistical power of detecting associations between BMI and biomarkers. Second, this study was monocentric and only BMI and no other anthropometric data were available in this CHF cohort.

Furthermore, the present analyses reflect post hoc analyses on data collected for other purposes (C Kistorp et al. Circulation 2005) for which reason power calculations were not performed and confidence intervals should be noted. We recognize that post hoc analyses increase the risk for at Type I error, and this point is now mentioned in the limitation paragraph at page 10 in the revised draft:

Finally, it should be noted that the present analyses are post hoc analyses on data collected for other purposes [6]. This may increase the risk for a Type I error. Whether we have overlooked a
small effect of BMI on α-defensins due to a low sample size (a Type II error) can neither be excluded and our findings should be confirmed in larger cohorts.

2. The authors may want to quote and comment the findings of a previous study that evaluated the association between MR-proANP and BMI in a large population of patients with chronic HF (Masson et al., Eur J Clin Invest 2011;41:1330).

Thank you for bringing this article to our attention. We have added a comment on the findings of the abovementioned study.

Discussion section, page 8:

We found a gradual decline in plasma MR-proANP levels corresponding to an increase in BMI, which is in accordance with findings of a previous study by Masson et al [27].

3. In the Methods section, briefly describe the reagents and analytical procedures used to assay the circulating biomarkers. What is the specificity of the alpha-defensins assay?

Thank you for this very relevant comment. We have added information on assays used to determine the circulating biomarker and the specificity of the alpha-defensins assay.

In the methods section, page 5, Laboratory measurements:

Laboratory measurements

All patients met at the outpatient clinic for blood sampling following an overnight fast (8 hrs). Venous blood was drawn and stored as EDTA-plasma at -80 °C in aliquots until analysis. A urine sample was collected. To determine the plasma level of α-defensins we used a validated, in-house, solid-phase RIA. Using microtiter plates (from Nunc, Roskilde, Denmark) incubated overnight at 5 °C with anti-mouse IgG (Sigma-Aldrich, Copenhagen, Denmark). After washing, all wells were added 50 µL of standard (purified α-defensins-1, Sigma-Aldrich) or diluted plasma (1 in 25), 50 µL of 125I-labeled α-defensins (~10,000 cpm) and 100 µL (200 µg/L) of a specific monoclonal antibody, which recognizes α-defensins-1, -2, and -3 (clone DEF 3, BMA, Augst, Switzerland). The intra- and inter-assay coefficients of variation (CVs) were 6% and 9%, respectively. Repetitive freezing and thawing (nine cycles) on serum and plasma levels of α-defensins did not affect the α-defensins levels [20]. All blood samples were measured within the same assay-run. HsCRP was measured with a latex-particle-enhanced immunoassay (Roche Diagnostics, Germany). Plasma NT-proBNP was measured by using a highly sensitive and specific immunoassay based on double-antibody sandwich technique (Roche Diagnostics, Mannheim, Germany). The intra-assay and inter-assay coefficients of variation were 1.3% and 4.8%, respectively [21]. To determine plasma level of total adiponectin we used a validated, in-house, time resolved immunofluorometric assay (TR-IFMA) based on commercially available antibodies and recombinant human adiponectin obtained from R&D Systems, Abingdon, UK. The detection limit is <1.5 µg/l. The within-assay coefficient of variation (CV) of standards and unknown samples averaged <5%; the in-between assay CV was 8-
12% depending on concentrations of adiponectin [22]. Plasma concentrations of midregional proANP (MR-proANP), MR-proADM, and copeptin, were measured on the Kryptor Compact platform (B-R-A-H-M-S, Henningsdorf, Germany). Regarding MR-proANP; the interassay CV was <6.5% at all concentration ranges, with respect to MR-proADM; 5-20 %, and for copeptin <15%, according to manufacturer [23-25].

Discretionary Revisions:

1. What is the rationale for investigating the association between alpha-defensins and BMI in CHF? Are there previous observations in different settings?

This is a relevant question raised by the referee. Knowing that pro-inflammatory cytokines such as interleukin (IL) -6 stimulate the ubiquitin-proteasome system and seem to have a direct negative effect on fat free mass (muscles), and therefore make IL-6 a potential player in the muscular wasting process in patients with cardiac cachexia, we wished to explore if components of the innate immune system also could be related to BMI and potentially be associated to weight loss seen in some patients with CHF (cardiac cachexia).

2. Since the paper focuses on the hormonal interplay between adipose tissue, adiponectin and natriuretic peptides, it would be interesting to show the relations of circulating biomarkers with anthropometric (waist circumference) or biological (visceral fat mass, body fat content) estimated of adipose tissue content.

We do agree that it would have been interesting to determine the associations between circulating biomarkers and anthropometric data such as total fat mass, android- and gynoid fat mass.

Unfortunately, we did not have anthropometric data in this study cohort, which is a limitation in the current study.

Minor Essential Revisions:

1. Data shown in Figure 1 are duplicates of those presented at the bottom of Table 1. Please choose either one.

To avoid duplication of data, we have revised Table 1.

2. Use consistent concentration unit for NT-proBNP though the paper (expressed either as ng/L or pmol/mL)

We have streamlined the unit used for NT-proBNP concentrations.

3. Reference #18 is incomplete.

Reference # 18 has now been completed.

4. Number the pages
5. Revise carefully English style and spelling (see for instance the conclusive sentence or “pathophysiological”).

We have scrutinized English style and spelling in the current paper.

6. Y-label of Figure 1, panel C, looks incorrect.

The Y-label of Figure 1, panel C is correct. The descriptive medians for alpha-defensins (ng/ml) are: 547 (466-617), 493 (434-577), 483 (407-603) according to BMI categories. P-value = 0.35.

7. Since BMI is the stratification variable in Table 1, do not show the p value for this variable.

The BMI variable has been removed in Table 1.

8. Table 2: check the abbreviation for NYHA class; provide SE for standardized beta-coefficient.

SE for standardized beta-coefficient has been added to Table 2 and spelling has been corrected.

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Reviewer’s report

Title:
Body Mass Index in Chronic Heart Failure: Association with biomarkers of neurohormonal activation, inflammation and endothelial dysfunction
Version: 1
Date: 7 August 2013
Reviewer: GULTEN TACOY
Reviewer’s report:

1) The reason of CHF is not clear. Atherosclerotic coronary artery disease or idiopathic dilated cardiomyopathy may have different effects on biomarkers. The authors must detailed evaluated this issue in discussion.

Thank you for this comment, briefly we have added information on the share of patients with HF based on ischemic heart disease in the Result section, baseline characteristics, page 7:

Clinical characteristics according to BMI are presented in Table 1. The patients were older in the lower BMI categories, they had the lowest prevalence of DM, and had the highest levels of circulating adiponectin, NT-proBNP, and MR-proANP. A low BMI (<21 kg/m²) was associated
with markedly elevated hsCRP concentrations. Fifty-seven % of the patients had ischemic heart disease (IHD).

Further we have discussed this issue raised by referee#2, in the Discussion section, page 2) Only there is one important issue which the authors demonstrated that "Plasma levels of #-defensins were not affected by BMI in the present study thus, indicating that the immune system is not directly linked to the progressive weight loss observed in CHF with cachexia." It is true for this study but not enough. With only one biomarker this assumption is not completely true.

We do agree that plasma alpha-defensins association to BMI is the most interesting result in the current study and we do know that these data are preliminary. However, this is the first study on alpha-defensins and BMI which makes this study interesting at least for the physicians and scientists that have interest in metabolic disturbances in CHF with weight loss. Another not yet well investigated issue is the role of MR-proANP and BMI in CHF patients, where this study is confirmative of the study by Masson et al.