Author’s response to reviews

Title: CHANGES IN PLASMA ALDOSTERONE LEVEL AFTER WEIGHT LOSS BY BARIATRIC SURGERY IN MORBIDLY OBESE PATIENTS

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Author’s response to reviews:

Dear Editor,

Please reconsider our study ‘Changes in testosterone levels and sex hormone-binding globulin levels in extremely obese men after bariatric surgery’ for future publication. We would like to thank the reviewers for their comments. Please find a detailed response to each questions/comments below.

Comments of the first reviewer

1. Subjects and methods: please clarify how heart failure was ruled out. Most of overweight/obese patients may have diastolic heart failure rather than systolic heart failure. How did you make the diagnosis?
   • A sentence “Symptomatic heart failure was excluded by careful history and physical examination” was added to the subjective and methods section accordingly.

2) How was the waist circumference measure? By whom? Was it done twice and average? Please clarify.
   • A sentence “Waist circumference was measured by a single investigator according to WHO steps protocol” was added to the subjective and methods section accordingly.
3) How was the blood pressure measure? Manual or automated? How many times? Did the participant sit and relax prior to being measured? Was the protocol followed JNC or SPRINT trial? Please clarify.

- A sentence “Blood pressure was measured during a clinic visit using a Omron machine (automated device) with appropriate cuff size, blood pressure was recorded three times and the mean of second and third records were analysed. The participants rested for three minutes between each readings according WHO steps protocol” was added to the subjective and methods section accordingly.

4) Please specify what kind of assay being used to measure electrolyte, glucose and lipid profile?

- Details of types of assay were added to the methods section as suggested.

5) Please comment on using bioelectrical impedance analysis to measure body fat? Do you have data on other body composition?

- A sentence “Body composition was measured using bio electrical impedance analysis by Inbody 720. Body composition analyzed when the subject is fasting” was added to the subjective and methods section accordingly.

6) Four patients had hypertension prior to the surgery. Did the patients receive treatment? What medications?

- “Four patients had hypertension prior to surgery. Their previous medications were as follow; losartan, manidipine and amlodipine. Then the medications were changed to doxazosin and/or verapamil prior to the measurement of plasma aldosterone concentration(PAC)/direct renin activity(DRC)” was added to the results section.

7) The authors reported that all patients had obstructive sleep apnea. Did the patients receive or adhere to the treatment? Because OSA can activate RAS, could it be possible that reduction in PAC was due to improvement in OSA or the treatment of OSA?

- All participants were required to wear CPAP with demonstrated good compliance on the machine downloading at least 2 months prior to the surgery. Unfortunately, we do not have information on CPAP use after the surgery, so we could not give a comment if this would have an effect on PAC.

8) Do you have data on dietary intake? It is important to quantify sodium intake prior and after the surgery.

- Unfortunately, we did not collect the data on sodium intake.

9) Please report renal function and GFR. How many patients were in stage 1 and 2?
• Data were added to table 2 as suggested.

10) Could you please comment why your study's result differs from Ruano et al where there was a correlation between PAC and BMI?

• Our subjects were all Asians with a very high BMI at baseline (57kg/m2). In our study, percent fat was correlated with PAC rather than BMI. BMI may not be a good indicator of obesity in our Asian subjects.

11) Page 4, line 21-23: How can the author conclude that the decrease in PAC suggesting the local RAS rather than systemic RAS activation?

• We saw a major reduction in PAC but no significant change in DRC. If major drive from systemic RAS, we would have seen a change in DRC.

12) Page 4, line 35-37: how can the author conclude that there was no essential hypertension in the study?

• All our subjects were able to discontinue all anti-hypertensive medications after bariatric surgery.

13) Page 4, line 41-42: Could you further hypothesize the differences of weight loss surgery would affect PAC?

• “The difference in the magnitude of changes in the gut hormones such as ghrelin and glucagon-like peptide-1 (GLP-1) in RYGB and SG may have an effect on blood pressure regulation as well” was added to the discussion section.

14) Page 4, line 44-45: the sentence started with "Nonetheless, the reduction of leptin…" is out of place here. Suggested to move up to the discussion.

• Changes have been made as suggested.

15) Page 2, line 33: recommended "A prospective study was conducted…"

• Changes have been made as suggested.

16) Page 2, line 34: "…adults…”

• Changes have been made as suggested.

17) Page 2, line 33: please add Bangkok, Thailand where the King Chulalongkorn Memorial Hospital is located.

• Changes have been made as suggested.
18) Page 2, line 41: should be "All subjects were given written informed consent…” Please also specify where the body of local ethic committee was. For example, it was approved by King Chulalongkorn Memorial Hospital, Chulalongkorn University’s ethical committee.

- Changes have been made as suggested.

19) Table 1: recommended "Baseline characteristics of the study participants"

- Changes have been made as suggested.

20) Table 1: please specify ethnicity

- “All participants were Asians” This was added to the result section.

21) Table 2: recommended "Baseline and changes in clinical and laboratory parameters at 3 and 6 months after bariatric surgery"

- Changes have been made as suggested.

22) Table 2: recommend reporting clinical parameters before laboratory parameters. Please also give abbreviation for BW

- Changes have been made as suggested.

23) Why wasn't the percent fat capture at 6 months. Please clarify in method.

- The measurement of body composition at 6 months was not included in our study protocol.

Comments of the second reviewer

1) As mentioned in your limitations section, you have included RYGB and SG as surgical procedures. Despite the small numbers it would be beneficial to add a subgroup analysis that separately analyses the effects of RYGB and SG on Aldosterone levels

- Unfortunately, we could not do a subgroup analysis due to a small numbers of subjects when breaking down to each group.

2) Do you have any data regarding renal function disorders. If so, this should be mentioned in the methods and results section, as renal function (disorders) are an important factor in the occurrence of hypertension

- Data were added to Table 2 as suggested.
3) For the discussion; you mention the sympathetic activity hypotheses, however it should be mentioned that there is a significant influence of incretins on the resolution of comorbidities (see studies from M. Alpert and T. Kindel). You also should discuss the evidence from the GATEWAY trial (by Schiavon and colleagues)

- “The reduction in the inflammatory cytokine and the improvement in insulin sensitivity could play a role in the reduction in blood pressure after RYGB as demonstrated in the GATEWAY study.” was added to the discussion section as the reviewer suggested.

All the changes in the manuscript were highlighted in yellow.

Sincerely,

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