Dear Editor,

We would like to express gratitude for the important and valuable comments regarding the content of our manuscript (MS:1877990296587582 titled “The effect of endogenously released glucose, insulin, glucagon-like peptide 1, ghrelin on cardiac output, heart rate, stroke volume, and blood pressure”). We present a point-to-point response to reviewer comments and description of the changes made. We hope that our revisions have improved the manuscript, and that it will now be accepted for publication in its present form. We are, however, prepared to make additional changes if deemed necessary.

Point-to-point response to reviewer comments and description of the changes made.

Reviewer 1

The article by Hlebowicz et al addresses a simple but important question that is related to the postprandial changes in blood flow, cardiac output and blood pressure. I found the article interesting for the implication in metabolic studies.

Major Compulsory Revisions

- The major comment is that the conclusion of the abstract is understating the message of this paper. Many metabolic studies have been recently carried out in postprandial state, also because GLP-1 and GIP hormone are released after a
meal. Several studies have investigated the effect of GLP-1 agonist on heart function and this should be highlighted in the discussion of this paper.

Response; We are grateful for the kind comment by the reviewer. The effect of GLP-1 agonist on heart function and is now highlighted in the discussion. This has added to the revised manuscript “Treatment with GLP-1 receptor agonist in patients with type 2 diabetes has been associated with a lower risk of CVD events and hospitalizations that treatment with other glucose-lowering therapies. Also, treatment with GLP-1 receptor agonist in subjects with type 2 diabetes has been associated with lower systolic blood pressure without effects on HR.”

- Circulatory models, ie based on blood flow and cardiac output, have been recently proposed to study postprandial metabolism. The assumption to use these models is that CO is constant throughout the experiment. This article clearly shows that this is not the case and thus those models should not be used to evaluate postprandial state.

Response; We do not agree with you that CO is constant. Our study shows that there are variations in CO. A limitation in our study is that we did not have a control-group that was not served a meal.

- in the abstract “In men, the postprandial changes in glucose levels were positively correlated to systolic blood pressure, the GLP-1 levels to SV, the insulin levels to CO, and the ghrelin levels to HR. Was the correlation insulin-CO and ghrelin levels significant only in men or in the entire cohort? It is not clear.

Response; Significant correlations were found only in men. this has been changed in the revised manuscript. The sentence now read “No statistically significant correlations were seen in women.”

The correlation should be performed in the entire cohort as multiple regression by correcting per gender and BMI. These results should be better discussed and try to interpret the result of correlation

Response; We do not agree with you that it is necessary to use multiple regression by correcting per gender and BMI. We have consulted a statistician from the University of Lund that think it would be more appropriate to use the Pearson correlation performed for men and women separately. We have performed multiple regression in the entire cohort by correcting per gender and BMI. The BMI does not affect the results. However, the gender affects the results. We present the significant multiple regression results for the AUCs.

AUC CO 30min Regression 0.000
AUC ghrelin 30 min 0.411
gender 0.000
BMI 0.303
AUC CO 30min Regression 0.000
AUC GLP-1 30 min 0.998
- Glucose and insulin concentrations after the meal were not very high. Given that insulin stimulates blood flow and (see for example Autonomic and hemodynamic responses to insulin in lean and obese humans., Muscelli E, JCEM 1998) and affects autonomic control of cardiac function, you should discuss this issue, since different meal could have higher insulin responses.

Response; This has added to the revised manuscript “There seems to be an effect of meal size on postprandial increase in CO. Also, physiological hyperinsulinemia affects autonomic control and reduces diastolic blood pressure, increase HR and CO”.

- Page 10. “To our best knowledge, this is the first study to examine at the effect of endogenously released glucose, insulin, glucagon-like peptide 1, ghrelin levels on CO, SV, HR, systolic and diastolic blood pressure in both men and women” please eliminate glucose from this sentence since after a mixed meal glucose production is suppressed so the great part of glucose measured in the systemic blood is exogenous, so the authors do not look at “endogenously released glucose”.

Response; This has been changed in the revised manuscript. The sentence now read “To our best knowledge, this is the first study to examine at the effect of, endogenously released insulin, glucagon-like peptide 1, ghrelin levels on CO, SV, HR, systolic and diastolic blood pressure in both men and women”.

- Table 1 and results. What was the rational to measure the variable of table 1 at 0, 30 and 110 instead of 120min?

Response; It was not possible to perform the echocardiography, measure the blood pressure and take blood samples at the same time. Therefore are the blood samples taken at 120 min.

-Figure 1 Were the AUC of GLP-1 different in men and women?

Response; The AUC of GLP-1 were not different in men and women.
Was blood for GLP-1 assessment collected with DPP-IV inhibitor? This is crucial a commercial kit?

Response: Dipeptidyl peptidase-IV inhibitor was added to the low pressure blood test bottles before collection of blood for GLP-1. This has been added to the revised manuscript.

Reviewer 2

In the present study authors describe the haemodynamic effects of post-prandial endogenous release of glucose, insulin, GLP-1 and ghrelin and conclude that they significantly affect heart rate, diastolic blood pressure, stroke volume and output. They conclude that patients should not be evaluated after eating since this condition may affect cardiovascular parameters. Such an influence is more marked in men than in women. Although not highly original, these results may be of clinical interests. There are a few issues that authors should address:

1. In the results section of the abstract there are no numbers. Please add.

Response; This has been changed in the revised manuscript. The result section of the abstract now read “In men, significant correlations were found between GLP-1 level at 30 min and SV at 30 min (P = 0.015, r = 0.946), and between ghrelin levels and HR (P = 0.013, r = 0.951) at 110 min. Significant correlations were also found between the change in glucose level at 30 min and the change in systolic blood pressure (P = 0.021, r = -0.681), and the change in SV (P = 0.008, r = -0.748) relative to the fasting in men. The insulin 0-30 min AUC was significantly correlated to the CO 0-30 min AUC (P = 0.002, r = 0.814) in men. Significant correlations were also found between the 0-120 min ghrelin and HR AUCs (P = 0.007, r = 0.966) in men. No statistically significant correlations were seen in women.”

Please give more details on the patient population under investigation.

Response; This has been changed in the revised manuscript. The result section of the abstract now read “Eleven healthy men and twelve healthy women ((mean ± SEM) aged: 26 ± 0.2 y; body mass index: 21.8 ± 0.1 kg/m2)) were included in this study.”

2. Data presentation is difficult to follow and most of the results would be better depicted in graph format, such as the correlation between CV parameters and metabolic ones.

Response; We have added Figure 4. The mean (± SEM) cardiac output (CO), heart rate (HR), and stroke volume (SV) and Figure 5. The mean (± SEM) systolic and diastolic blood pressure. However, we do not agree with you that it is necessary to present the correlations in graph format. There are already 5 Figures in this manuscript.

3. The main flaw of the present study may be related to the intra and inter-observer variability of the echo measurements. Test and re-test data should be given. In other words, the differences observed may be related to difference in measurement over time. Please address.
Response; inter-observer variability is not an issue in the current study as all echocardiography examinations were performed by one single physician. We performed a separate intra-observer variability study for stroke volume (SV) and cardiac output (CO) measurements (which was not a problem because multiple acquisitions were made during each exam and stored digitally). This intra-observer variability study concluded low variability <5% for both CO and SV. We have added following sentence in the method section “A separate intra-observer variability study concluded low variability for CO and SV (<5%).”

4. The tables are difficult to follow and values should be reported in the results section of the manuscript. Please include all values together and then separate on gender basis.

Response; The values are now reported in the results section of the manuscript or figures. However, we do not agree with you that it is necessary to include all values together and the separate on gender basis. There are already too many results needed to be reported.

5. The post-prandial glucose, insulin, GLP-1 and ghrelin response paragraph should me moved as first part of results.

Response; This has been changed in the revised manuscript.

6. Use the AUC in graph format for the most significant data

Response; The AUC values for the significant data are now reported in the results section of the manuscript. However, we do not agree with you that it is necessary to use the AUC in graph format. There are already five figures in this manuscript.

7. Please acknowledge in the discussion section of the manuscript the limited number of healthy subjects studied.

Response; This has been added in the revised manuscript. The sentence now read “The present study had some limitations: the small sample size of healthy young subjects and the fact that it was not possible to perform the echocardiography or gastric ultrasound examinations at the same time.”

8. Please expand the pathophysiologic mechanisms at the basis of the gender difference observed.

Response; This has been added in the revised manuscript “There are also known gender differences in cardiovascular diseases and this may be related to sex hormones, estrogen and testosterone [52]. “

9. The meal effect is well established in the study of endothelial function and fasting state is recommended. Should this recommendation extended to any study having as an endpoint cardiovascular parameters?

Response; The metabolic effects of GLP-1 receptor agonist has been evaluated on endothelial dysfunction (Koska et al. Diabetes Care 2010:33(5):1028-1030). The postprandial endothelial function was improved significantly.
11. Due to the nature of the journal it would be important to have sample images.

Response: We have added two images and corresponding legends. Figure 1. LVOT. Representative image of measurement of the left ventricular outflow tract diameter. Figure 2. VTI. Representative measurement of the left ventricular outflow tract velocity-time integral.

Please do not hesitate to contact me if you have any questions.

Sincerely,

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