Author's response to reviews

Title: The effects of treatment with liraglutide on atherothrombotic risk in obese young women with polycystic ovary syndrome and controls: a case control study.

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Author's response to reviews: see over
Dear Dr De Souza and reviewers,

Thank you for reviewing our submission and for the comments of the referees that have improved the manuscript.

We have amended the manuscript in line with your recommendations. All the changes are highlighted in red in the manuscript.

Yours sincerely,

Dr. Hassan Kahal

(on behalf of the authors)

**Editorial Requirements:**

1. Ethics committee: Please update your ethics statement to include the name of the ethics committee that approved your study. Thank you. This has been updated.

2. TRN (Trial Registration Number): Please add the TRN as the last line on the abstract section. Thank you. TRN has now been added to abstract section.

3. Title Page: Please include on the title page, at minimum, the names, institutions, countries and email addresses of all authors, and the full postal address of the submitting author. Thank you. This has been updated.
This manuscript reports the results of an important pilot study investigating the effects of GLP-1 agonist liraglutide on weight loss in PCOS and matched controls. The study reports interesting results; primarily that although changes in weight loss are small and similar between the two groups, they were statistically significant and coupled with reduction in atherosclerosis markers. The author’s hypothesis is that women with PCOS and weight/age matched controls respond to liraglutide in a similar fashion.

Major Reviews

1. Please include a reference for the statement “Weight loss has been found to reduce many CV risk markers in PCOS including inflammation, and insulin resistance (IR).”; lines 53-55. A suitable reference would be Thomson et al, Hum Repro 2012 27(7):2169-76. Thank you for, we have added the reference suggested.

2. Please update the reference used on line 63 of the paper, there are several large trials of liraglutide published recently that would be a good addition to this older reference (ie. Wadden et al, Int J Ob 2013 Nov; 37(11):1443-51). Thank you, we have updated the reference.

3. Please include some explanation for the dose selected in this trial. Several doses are seen in the literature of similar trials. Thank you for comment. As liraglutide is currently only licensed for the treatment of type 2 diabetes, we have chosen the highest dose recommended for the treatment of that condition (1.8mg od); a dose with known safety profile and adverse reactions.

We have included this in the discussion section, lines: 223 – 226. It reads:

(The weight loss of 3 – 4% achieved in our study is in accord with other published data [1], and although a higher dose of liraglutide might have resulted in more pronounced weight loss [2], we have chosen the highest dose currently licensed for the treatment of people with type 2 diabetes (1.8mg od) [3]; a dose with well-known safety profile and adverse reactions.)


4. Power calculations: the power calculations provided are only for a single trait (P-selectin expression). Although this is important, the results from a number of other traits are reported. Perhaps a power table showing multiple effect sizes would give the reader a better opportunity to interpret the results of this study. The authors should also comment on the difference between the number of subjects needed per the power calculation and the number of subjects who completed the study, and how this difference affects the interpretation of the results of the study.

Thank you for comments.

Please find a graph highlighting the effect of sample size on study power:

As the dropout rate in the study was 31% (n=25) we are aware that this may have compromised the study power.

We have now highlighted this in the manuscript:

1. In the paragraph discussing how platelet P-selectin expression was only reduced in the control group (lines 253 -256), it reads:
   (However, it is worth noting that between group comparisons were not significant and it is so possible that the lack of significant change in platelet P-selectin expression in the PCOS group after treatment is related to higher than anticipated dropout during the study).

2. In the study limitations paragraph (lines 302 – 307), it reads:
   (The main limitation to our study was the higher than anticipated drop out during the study which may have compromised the study power. We are aware that interpretation of non-
significant findings is challenging. Another limitation is the absence of a placebo-treated group, with equal amount of weight loss achieved, to clarify if the changes observed were related to liraglutide per se or to the associated weight loss. However, the results of this study would now allow a larger study to be powered appropriately to include a placebo.

5. In defining differences between the two groups at baseline, the addition of significant P values to Table 1 (as a symbol) would be extremely helpful. Although the P values (of presumably all significant traits) are given in line 162-164, they would be more informative in the context of the table. There is also no mention of any difference in baseline glucose between the two groups. Thank you for comments. We have highlighted all the significant differences between the two groups at baseline in table 1 with an asterisk. The fasting plasma glucose levels were not significantly different between the two groups at baseline.

6. In comparing the response of the two groups the addition of P values in the same fashion requested above is needed. A statistical test to compare the changes in the traits between cases and controls and the respective P value should be included in both the text of the manuscript (line 172-175) and line 202 and Table 1. At very least this should be given for weight loss (delta weight). Thank you. Changes between the two groups were not statistically significant. We have:
   - defined how between groups comparisons were measured in the statistical analysis section (lines 159 - 162).
   - It reads: (Between groups’ comparisons after intervention were as follows: for each group (PCOS and controls) a difference between baseline and 6 months was calculated. The between group differences were compared using the independent t-test (or Mann-Whitney U test for non-normally distributed data).)
   - We have represented the data in two tables, instead of the previous tables and figures, highlighting the between groups comparisons. Please see table 1 and 2.
   - highlighted that between groups changes were not significant for each parameter measured in the abstract, results and discussion sections.

7. Figure 1: please define the abbreviation SEM. Please note figure 1 has been replaced with table 2.

8. Figure 2: the legend panel should either be added to each panel or included once, not included in three of the five panels. Please move the legend explaining the asterisk used into the text of the figure footnote. Please note figure 2 has been replaced with table 2.

Minor Essential Reviews

1. Please define the abbreviation SD (line 145). Done.

2. It seemed quite interesting that there was no increase in HOMA-%B. While this is an imperfect measure of beta cell function, it might be helpful to include a comment in the discussion on the lack of improvement in HOMA-%B in this study (including if other studies detect an increase in this trait). Thank you for comments. We have updated the discussion (lines 284 - 294). It reads:

   (Although treatment with liraglutide, in our study, has resulted in significant reduction in fasting plasma glucose, insulin, and insulin resistance (HOMA-IR), there was no improvement in beta cell
function (HOMA-β). Preclinical studies suggest that liraglutide treatment increases beta cells mass [1]. Treatment with liraglutide, in a 20 week trial, increased HOMA-β by 5 – 24% in obese men and women [2]. The study included more participants (90 per group) than ours, which may explain the different results. Interestingly, there was no association between the dose of liraglutide and the change in β-cell function after treatment (median increase by 27.8% for liraglutide 1.8mg od and 8.4% for 2.4mg od), nor did the changes in HOMA-β correlate with HOMA-IR (which did not change during the study) [2]. It is worth noting that HOMA-β, although simple and commonly used, has many limitations including the use of a fasting parameter of β-cell function, which reflects basal rather than glucose-stimulated insulin secretion, and is also affected by alterations in insulin clearance [3-5].


3. There isn’t a clear and concise statement in the opening of the discussion that reintroduces the reader to the initial hypothesis of the paper (stated in lines 73-74) and comments how the primary findings of the paper fit with it. Adding this to the beginning of the discussion would significantly improve the first paragraph of the discussion. Thank you for comment. The opening paragraph (lines 217 – 222) for the discussion section now reads:

(Our data suggest that young obese women with PCOS and weight matched controls respond equally to treatment with liraglutide. Six months treatment with liraglutide (1.8mg od) resulted in a small, though significant, 3 – 4% weight loss that was associated with significant reductions in IR, inflammation, oxidative stress and improvement in several CV risk markers in young obese women with and without PCOS. Our findings are important as the majority of previous reports suggest the need for moderate weight loss (≥10%) to achieve reduction in atherothrombotic risk [1, 2].)


4. The discussion would benefit from a few in depth sentences describing the mechanism of action of GLP-1 in insulin secretion, glucose homeostasis and body weight. Thank you. We have updated the discussion (lines 226 - 235). It reads:

The effects of liraglutide on weight loss are thought to be mainly mediated through delayed gastric emptying and reduced appetite, rather than a change in energy expenditure [1]. While its effects on glucose metabolism are secondary to an increase in insulin secretion in a glucose-dependent manner, suppression of glucagon secretion, enhanced hepatic insulin action, and reduced β-cell apoptosis [2-4]. Similar to native GLP-1, liraglutide is widely believed to exert its actions through the GLP-1 receptor (GLP-1R) and the activation of cyclic adenosine monophosphate (cAMP) dependent pathway [5]. This is supported by the wide expression of GLP-1R including in the pancreas, stomach, and brain [4]. However, the underlying mechanisms for GLP-1 mediated weight loss remain poorly understood and may involve direct, GLP-1R mediated, and indirect, e.g. neuronal, pathways [4].


5. In comparing the results of this study to previous studies (line 241) the authors should add that the cited study also included diet and/or exercise interventions, and these could be important in cIMT. Thank you. We have updated the sentence (lines 271 - 272). It reads:

(The change in BMI in our study was smaller, 1.0 - 1.4 kg/m², the follow up duration was shorter, and no diet and/or exercise interventions were included which may account for the discrepancy.)

6. In the footnote of Table 1 please add that the P values listed are from comparing baseline to 6m within each group. (Also see Major revisions for requests to add P values comparing the groups at baseline and 6m). Only significant P values need to be added to this table with symbols. Thank you, we have clarified the P values.
Reviewer 2:

Dear Editor,

In this study, authors assessed the effects of liraglutide treatment on obesity, and CV risk markers in obese women with PCOS and matched controls. The aim, idea and the materials-methods are all O.K.

But there is problem with statistical analysis. The first one is the power of the study. Power of the study is weak. Because according to power analysis, min. 18 cases were needed for both case and control groups to get 80% power. But only 13 PCOS and 12 controls were completed study which is not enough to get 80% power. Another statistical problem is that authors did not compared time dependent parameters between case and control groups, instead they compared time-dependent parameters in each group in itself. In order to see if there is a significant difference between case and control groups, they must be compared in terms of time-dependent parameters (tests that were done 6 month before and 6 months later)

Thank you for comments.

In answer to comment 1 about study power, please find a graph highlighting the effect of sample size on study power:

As the dropout rate in the study was 31% 25 participant completed, we are aware that this may have compromised the study power.

We have now highlighted this in the manuscript:
1. In the paragraph discussing how platelet P-selectin expression was only reduced in the control group (lines 253 -256), it reads:
(However, it is worth noting that between group comparisons were not significant and it is so possible that the lack of significant change in platelet P-selectin expression in the PCOS group after treatment is related to higher than anticipated dropout during the study).

2. In the study limitations paragraph (lines 302 – 307), it reads:
(The main limitation to our study was the higher than anticipated drop out during the study which may have compromised the study power. We are aware that interpretation of non-significant findings is challenging. Another limitation is the absence of a placebo-treated group, with equal amount of weight loss achieved, to clarify if the changes observed were related to liraglutide *per se* or to the associated weight loss. However, the results of this study would now allow a larger study to be powered appropriately to include a placebo.)

In answer to comment 2 about between groups comparisons:

The changes between the two groups were not statistically significant. We have:

- defined how between groups comparisons were measured in the statistical analysis section (lines 159 - 162).
  It reads: (Between groups’ comparisons after intervention were as follows: for each group (PCOS and controls) a difference between baseline and 6 months was calculated. The between group differences were compared using the independent t-test (or Mann-Whitney U test for non-normally distributed data).)
- We have represented the data in two tables, instead of the previous tables and figures, highlighting the between groups comparisons. Please see table 1 and 2.
- highlighted that between groups changes were not significant for each parameter measured in the abstract, results and discussion sections.