

## RESPONSE TO REVIEWER'S COMMENTS – Manuscript 71667 – November 2021

Dear Professor Lian-Sheng Ma,

Thank you for considering publication of our manuscript (71667) entitled “Testosterone therapy reduces hepatic steatosis in men with type 2 diabetes and low serum testosterone concentrations” in the World Journal of Hepatology. Our point-by-point response is outlined below:

### Reviewer 1

*Comment: Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis, and is more common in subjects with type 2 diabetes mellitus (T2DM). Testosterone therapy has been explored in both animal and human studies with mixed results. Therefore, the aim of this study was to evaluate the effect of testosterone therapy on liver fat fraction and to determine other factors associated with changes in liver fat in a population of men with type 2 diabetes and low testosterone concentrations. Despite these limitations, the results showed that testosterone therapy was associated with a reduction in hepatic steatosis in men with diabetes and low serum testosterone. The paper is sound methodologically, however there are some issues that must be addressed as follows. 1) Due to the small sample size and the differences between participants in our study and those who did not have MRI scans, the results concluded from our study are difficult to generalize.*

Response: Thank you for your comment. We have acknowledged the small sample size and potential sampling bias in the discussion section, page 14, paragraph 3.

*Comment: 2) The baseline data did not match between the Testosterone group and the Placebo group, such as Lean mass and HDL.*

Response: We have acknowledged this difference in baseline data in page 9, paragraph 2.

*Comment: 3) The calculated free testosterone (cFT) should be more specifically illuminated in the Methods section.*

Response: The methods section has been updated with this information on page 7, paragraph 2.

*Comment: 4) The formats of the Tables are not standard, and they should be three-line tables with the table heads above the tables.*

Response: We have changed the tables and figures accordingly

Comment: 5) *The picture is not well annotated, so what do the red and green lines in Figure 1 and Supplementary Figure 1 represent? In addition, (A) and (B) in Figure 1 and Supplementary Figure 1 should be combined into one figure.*

Response: We have clarified the annotation of the figures to describe what the red and green lines and we have combined the (A) and (B) in the supplementary figures into one figure.

## **Reviewer 2**

Comment: *In this study, the authors highlighted that treatment of intramuscular testosterone undecanoate could significantly improve NAFLD in men with type 2 diabetes and low testosterone concentrations. In addition, the authors suggested that testosterone therapy increased lean muscle mass and reduced total fat mass. This study was a sub-analysis of a previous randomized controlled trial which had suggested that testosterone therapy could not improve glucose metabolism or visceral adiposity in obese men with moderately controlled T2D and only modest reductions in circulating testosterone levels typical for men with T2D. It was novel that the authors re-analyzed the patients with NAFLD, and using MRI scan for the assessment of hepatic steatosis. It seemed that testosterone therapy was involved with lipid metabolism, not only specifically affecting liver fat, but also body fat. However, visceral adiposity was unchanged. Could the authors explain more in detail about that?*

Response: Thank you for your comment. We have added a new reference that shows that the effect of testosterone therapy on targets of lipid and glucose metabolism appears to be tissue specific and does not affect visceral adiposity.

Comment: *Additionally, what might cause the differences in the participants in the index study who did not have MRI scans with lower baseline BMI, visceral adiposity, waist circumference and higher cholesterol levels in Table 2?*

Response: The lower BMI, visceral adiposity and waist circumference in our cohort is likely due to larger patients either not fitting in the MRI scanner or being apprehensive to undertake an MRI. We have outlined this in paragraph 1 on page 9. The higher cholesterol levels in our study cohort are likely a false positive association.