

Dear Editors and Reviewers:

Thank you for your letter and comments concerning our manuscript. Those comments are valuable and helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made corrections which we hope meet with approval. Revised portions have been highlighted in the paper. The main corrections in the paper and the responses to the reviewer's comments are as follows:

### **Reply to the reviewer's comments**

**Comments 1.** The authors indicated that "RBP4 could involve in the improvement of diabetic atherosclerosis by regulating JAK2/STAT3 signaling pathway" in the conclusion section in the Abstract. I think that "improvement" is not correct and that "development" and "initiation or progression" are correct. The authors should revise the word adequately.

**Reply:** Thanks for the reviewer's advice, we have revised "improvement" to "initiation or progression" in the abstract section.

**Comments 2.** When recombinant RBP4 was given intraperitoneally, it is not clear how the RBP4 levels in serum or adipose tissue were increased. In addition, were the RBP4 levels in aortic tissues elevated? The authors should indicate the above concerns clearly.

**Reply:** The ectopic expression of RBP4 can be achieved after being

intraperitoneally injected with recombinant RBP4. The specific performance is that the expressions of serum RBP4 and adipose tissue RBP4 were higher in group DA than those in group NC and group DM.

The reason why we chose adipose tissue RBP4 and serum RBP4 as the main indexes is that RBP4 is released from hepatocytes and adipocytes. However, this comment is helpful and valuable. It is indeed better adding the expression of RBP4 in aortic tissues to reveal the elevation of RBP4 in diabetic atherosclerosis rats, we will take this suggestion in our further investigation.

**Comments 3.** The authors showed protein expression of cyclin D1 and B-cell lymphoma-2 (Bcl2) by immunohistochemistry. However, I think that the ratio of the positive staining area to one visual field area is not adequate and that the ratio of the positive staining area to vascular smooth muscle in the media is adequate. The authors should evaluate the expression levels adequately. Moreover, it is not clear where are positive areas in Figure 6. The authors should show the more adequate figures to indicate the expression of cyclin D1 and Bcl2.

**Reply:** Thanks for the reviewer's advice, we have recalculated the ratio of the positive staining area in table 3, which can be seen at Page 15. Positive protein expressions in Figure 6 are dyed blue and purple granules distributed throughout the nucleus, and the corresponding description is added in the revised manuscript. In order to indicate the expression of

cyclin D1 and Bcl-2 more clearly, we have uploaded original pictures in the attachment.

**Comments 4.** The order of the bar graphs is different depending on the each figure. Namely, the first is NC, second is DM and the last is DA in Figure 2. On the other hand, the first is DA, second is NC and the last is DM in Figure 3. The authors should standardize the order in the each figure. The order such as Figure 2 is recommended for me.

**Reply:** Thanks for the reviewer's suggestion. We have standardized the order in each figure as the one in figure 2 in the revised manuscript.

**Comments 5.** The description of relative expression levels are different in the each figure. The authors should standardize the description in the each figure. Fold activation relative to the left bar graph (NC) is recommended for me.

**Reply:** Thanks for your suggestion. The reviewer's comment is indeed a better choice for the figures of this manuscript. The fact is that since the sampling order in our practical experiments is fixed as "DA-NC-DM", the corresponding results, i.e., the Western Blot bands, are difficult to be changed. However, all protein expression figures are standardized and all histograms are also consistent.

**Comments 6.** Expression levels of mRNAs of JAK2 and STAT3 were increased in Figure 4. However, expression levels of protein of JAK2 and STAT3 were not increased in Figure 5. The authors should indicate how

we interpret the discrepancy of expression levels between mRNA and protein.

**Reply:** In fact, mRNA expression detected by PCR is not always exactly consistent with the level of protein evaluated by western blot, and the levels of protein and phosphorylated protein can both be detected by western blot. However, our research indicates that the expression trend of JAK2 mRNA and STAT3 mRNA in each group is consistent with that of activated JAK2 (phosphorylated-JAK2) and activated STAT3 (phosphorylated-STAT3).

**Comments 7.** The authors showed the correlation between RBP4 and the other indicators in Table 4. However, I do not know which RBP4 was serum or adipose tissue. The authors should indicate which one was used in Table 4.

**Reply:** Results of the correlation analysis revealed that the serum and adipose RBP4 were both positively correlated with TG, TC, LDL-c, FINS, HbA1C, P-JAK2, P-STAT3, Bcl-2, CyclinD1, AI and HOMA-IR and negatively correlated with HDL-c (Table 4). The related description has been updated in the revised manuscript.

**Comments 8.** It is written that the number was 33 in the title of Table 4. However, it is written that the number was 50 in Table 4. The authors should indicate which number is true.

**Reply:** We are so sorry to make this mistake. The number in the title of

Table 4 is correct, and we have revised 50 to 33 in Table 4.

**Comments 9.** It is written that the number was 55 in the title of Table 5. However, it is written that the number was 50 in type 2 DM groups in the methods. The authors should indicate which number is true.

*Reply:* The correct number is 50. We have modified 55 to 50 in the title.

**Comments 10.** Discussion section is redundant. The authors should revise it more concisely.

*Reply:* The discussion section has been revised according to the reviewer's suggestion.

**Comments 11.** I know that JAK2, STAT3, Bcl2 and Cyclin D2 are the predictors of diabetic atherosclerosis. However, the causal relationships among them were not examined in this study. Therefore, the authors should examine the causal relationships among them by using specific inhibitors, etc.

*Reply:* STAT3 is a cytoplasmic transcription factor that becomes activated by phosphorylation. Once phosphorylated by JAK, STAT3 dimerizes and translocates to the nucleus, where it activates the transcription of target genes such as Bcl-2 and cyclin D1. We have added the description about the relationships among them in the manuscript.

In the present study, the trend of JAK2, STAT3, Bcl2 and Cyclin D1 were consistent in each group. RBP4 can trigger the phosphorylation of JAK2 and STAT3, which leads to the upregulation of the expression of

the STAT target genes such as Bcl-2 and cyclin D1, and promotes VSMC proliferation ultimately. However, the reviewer's comment is a valuable suggestion, we would like to use some specific inhibitors in the further researchs

**Comments 12.** There are some grammatical and typographical errors in this manuscript. Therefore, the manuscript should be reviewed by the native speaker of English.

**Reply:** Thanks for the reviewer's suggestion, the manuscript has been revised with the help of a professional English language editing company.

**Round-2:**

Dear Professor:

Thank you for your letter and comments concerning our manuscript. We have studied the comments carefully and have made corrections which we hope meet with approval. The responses to the reviewer's comments are as follows: Reply to the reviewer' comments The authors tried to respond my concerns adequately. The manuscript has been getting better due to their efforts. However, some important points have not been revised until now.

**Comments 1.** The authors reported that positive protein expressions in Figure 6 are dyed in blue and purple granules distributed throughout the nucleus. However, I do not know where the positive staining areas are.

The authors should indicate the areas more clearly by using some arrows etc.

**Reply:** Thanks for the reviewer's advice, we have indicated the positive staining areas by adding some arrows. Besides, we feel ashamed that we had made a mistake in previous description of the color dyed in Fig.6. In fact, the positive protein expressions of Cyclin D1 were brown-yellow granules distributed throughout the nucleus, and the positive protein of Bcl-2 were also stained brown-yellow in the cytoplasm.

**Comments 2.** It is known that the expression levels of mRNA and protein are not necessarily paralleled by posttranslational modification etc. Therefore, I know that protein levels of JAK2 and STAT3 in DM and DA models were not increased compared with those in NC model, even though mRNA levels of JAK2 and STAT3 in DM and DA models were significantly increased compared with those in NC model. However, phosphorylated protein levels indicate how much is activated in total amount of the target molecules. Therefore, the description in Response to Reviewers is not correct: "The expression trend of JAK2 mRNA and STAT3 mRNA in each group is consistent with that of activated JAK2 (phosphorylated-JAK2) and activated STAT3 (phosphorylated-STAT3)." The authors should pay more attention to the interpretation of results.

**Reply:** Thanks for the reviewer's advice. In our study, the total protein levels of JAK2 and STAT3 in DM and DA models were not increased

compared with those in NC model. However, compared with NC model, mRNA and phosphorylated protein levels of JAK2 and STAT3 in DM and DA models were both significantly increased, and simultaneously, the p-JAK2/JAK2 ratio and the p-STAT3/STAT3 ratio were also increased. Therefore, we speculate that elevated RBP4 can improve the transcription level of JAK2 and STAT3 and increase the degrees of phosphorylation of JAK2 and STAT3, but has no effect on the total protein level.

**Comments 3.** The authors showed the correlation between RBP4 and the other indicators in Table 4. I know that RBP4 levels of both serum and adipose tissue show the similar trend. However, I do not know which RBP4 was analyzed in Table 4, serum or adipose tissue. The authors should indicate which one was used in Table 4.

*Reply:* Indeed, the description was not so clear. Serum RBP4 was analyzed in Table 4. Thanks for the reviewer's advice.

**Comments 4.** The manuscript has been getting better by a professional English language editing. However, there are some misspelling in this manuscript. Therefore, the manuscript should be revised more carefully.

*Reply:* We have already re-checked the manuscript repeatedly and finished revising, thank you for your kindly suggestion.

### **Reply to the editorial office's comments**

**Comments 1.** The language classification is Grade C. Please visit the

following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>;

**Reply:** Thank you, according to the suggestion, we have finished the professional English language editing through the recommend website.

**Comments 2.** The “Author Contributions” section is missing. Please provide the author contributions

**Reply:** Thank you, we have added the “Author Contributions” section.

**Comments 3.** The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s)

**Reply:** Thank you, we have upload the approved grant application form.

**Comments 4.** The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor

**Reply:** Thank you, we have arranged the original pictures using PowerPoint.

**Comments 5.** PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout

**Reply:** Thank you, we have added the PMID and DOI numbers and listed

all authors in the reference section.

**Comments 6.** The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text

**Reply:** Thank you, we have added the “Article Highlights” section at the end of the main text.

**Comments 7.** Please upload the primary version (PDF) of the Institutional Animal Care and Use Committee’s official approval in Chinese version.

**Reply:** Thank you, we have uploaded the PDF of the Institutional Animal Care and Use Committee’s official approval in Chinese version.

### **Reply to company editor-in-chief’s comments**

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Diabetes, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office’s comments and the Criteria for Manuscript Revision by Authors. Before its final acceptance, the author(s) must provide the Chinese version of the ethical approval document. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following

website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>.

***Reply:*** Thanks. We have already supplemented the Chinese version of the ethical approval document, and finished the English language editing via the recommend website.