PEER-REVIEW REPORT

Name of journal: World Journal of Diabetes

Manuscript NO: 61244

Title: Elevated retinol binding protein 4 levels are associated with atherosclerosis in diabetic rats via the JAK2/STAT3 signalling pathway

Reviewer's code: 00503260

Position: Editorial Board

Academic degree: MD, PhD

Professional title: Assistant Professor

Reviewer's Country/Territory: Japan

Author's Country/Territory: China

Manuscript submission date: 2020-11-30

Reviewer chosen by: Xi-Fang Chen (Quit in 2021)

Reviewer accepted review: 2021-01-05 13:53

Reviewer performed review: 2021-01-09 13:55

Review time: 4 Days

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [Y] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The authors demonstrated that elevated retinol binding protein 4 (RBP4) levels were associated with diabetic atherosclerosis by Janus kinase 2 (JAK2) / signal transducer and activator of transcription 3 (STAT3).   This manuscript is important, because the precise mechanisms for the occurrence and development of diabetic atherosclerosis have not been clarified until now.   However, some concerns have been raised in the manuscript. (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. (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. (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. (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. (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. (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. (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. (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. (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. (10) Discussion section is redundant. The authors should revise it more concisely. (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. (12) There are some grammatical and typographical errors in this manuscript. Therefore, the manuscript should be reviewed by the native speaker of English.

RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: World Journal of Diabetes

Manuscript NO: 61244

Title: Elevated retinol binding protein 4 levels are associated with atherosclerosis in diabetic rats via the JAK2/STAT3 signalling pathway

Reviewer's code: 00503260

Position: Editorial Board

Academic degree: MD, PhD

Professional title: Assistant Professor

Reviewer's Country/Territory: Japan

Author's Country/Territory: China

Manuscript submission date: 2020-11-30

Reviewer chosen by: Chen-Chen Gao

Reviewer accepted review: 2021-02-04 10:34

Reviewer performed review: 2021-02-06 08:03

Review time: 1 Day and 21 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

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. (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. (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. (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. (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.