

Point-by-point responses to each of the issues raised in the peer-review reports.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: This manuscript summarizes the positive effect of Klotho on diabetic retinopathy. However, as a narrative review, this manuscript lacks a rigorous format and the authors are recommended to make revisions. Additionally, it is suggested that the authors delve deeper into the related targets and mechanisms of Klotho's effect on improving DR.

Reply: We thank you for the comment. We carefully and extensively revised the manuscript. In addition, we better described the mechanisms through which Klotho exerts protective effects in DR. We hope that the revision improves the manuscript and meets your requests.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: Thanks for the invitation for reviewing the paper. Major concerns: 1. The logical structure of the article is loose. There are 5 parts of this article: KLOTHO, KLOTHO AND DIABETES, DIABETIC RETINOPATHY, KLOTHO AND RETINAL HOMEOSTASIS, KLOTHO AND DIABETIC RETINOPATHY. As we know, the topic of the article is "klotho" and "diabetes retinopathy". Some part is a little bit digress from the main subject. Some parts can be merged into one part. For instance, in part "klotho", author mentioned 3 proteins of klotho, which one is closely related to diabetes or diabetes retinopathy should point out. In part "diabetic retinopathy", it contains some unnecessary repetition.

Reply: We thank you for the comment. We carefully and extensively revised the manuscript, delving deeper some topics and removing the unnecessary parts. In addition, we better clarified which isoform of Klotho exerts beneficial effects in diabetes.

If simply talk about "klotho" or diabetic retinopathy or diabetes and can't sufficiently prove the relationship of "klotho" and "diabetes retinopathy", the novel point of this review can't be shown and I will reject this article.

Reply: In this review we focused on the role of Klotho in DR, highlighting the importance of Klotho in maintaining retinal homeostasis and its positive effects on several mechanisms involved in DR onset and progression. We better described the mechanisms through which Klotho may exerts protective effects in DR. We hope that the revision improves the manuscript and meets your requests.

2. Insufficient literature citation. Only 20 out of the 85 references were published within the past 3 years, more fresh references were needed. The proportion of the literature in the last 5 years is better to be more than 50%, and try to avoid citing review articles as references. Search for latest research of diabetic retinopathy, and figure out possible target with klotho.

Reply: We added recent references. Now, the references published in the last 3 years are more than 30, and the proportion of the cited literature referred to the last 5 years is almost the 50%.

Page 10, line 28 : About autophagy, more recent studies in diabetic patients can be further elaborated.

Reply: We delved deeper the section related to autophagy (pages 11-12).

3. Add pictures and tables for display "Klotho, : therapeutic target; diabetic retinopathy"

Reply: We thank you for the suggestion, we added a table that summarizes the main effects due to depletion or addition of Klotho on retinal cells; moreover, we added a figure that shows the protective effects that Klotho exerts in DR.

Minor concerns 1. Page 4, line 25: The logic here is not clear, the increased level of Klotho during the worsening of diabetic CKD versus the increased level of Klotho inhibit the progression of kidney diseases , this two conditions can be further elaborated.

Reply: Thank you for the comment, we better explained the relationship between levels of Klotho during worsening of diabetic nephropathy (pages 4-5).

2. Page 4, line 27: For the early biomarker of diabetic nephropathy (DN) , the author fail to explain exactly how Klotho can be detected and measured, and to what level can be called increase or decrease.

Reply: In the experimental models Klotho has been evaluated in serum and cell media using ELISA (for instance see references 19, 24, 54, 56, 57 and 60). In general, the levels of Klotho are expressed in comparison to those of a control (cells or population). To our knowledge, there isn't a specific cutoff at which Klotho is considered elevated or decreased.

3. Page 5, line 20: Proliferative diabetic retinopathy (PDR) , the author should not start the name of a disease with abbreviated term, which makes understanding of the sentence difficult.

Reply: Thank you for the comment, we corrected it.

4. Page 8, line 18: The logic here is not clear. The lower level of the index in diabetic patients with retinopathy does not mean that the index must be protective and relevant when the level is elevated. Final comment Klotho (KI) is considered an antiaging gene.

Reply: Thank you for the comment, we better explained why the functional “KL-VS” variant of the Klotho gene may be protective against retinopathy incidence, page 10: “...On the other hand, the incidence of the functional “KL-VS” variant of the Klotho gene, which is associated with higher longevity in humans, is lower in people with diabetic retinopathy and is associated with reduced serum levels of inflammatory markers and pro-angiogenic factors, suggesting that this genotype may be protective against retinopathy incidence [61].....”

This paper reviews “klotho” from a new perspective that relationship between klotho and retinopathy. The conclusion of the paper is not convinced. This paper need major revision.

Reply: we thank you for your constructive comments, we hope that the revision meets your requests.

Reviewer #3:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Comments: The authors reviewed the relation between Klotho and DR. They focused on the role of Klotho in DR, highlighting the importance of Klotho in maintaining retinal homeostasis and its positive effects on several mechanisms involved in DR onset and progression. Therefore, they proposed Klotho could be a novel biomarker and a good candidate for the treatment of DR. This manuscript is interesting and creative. I have several suggestions.

1. Please provide a figure concerning the signal pathway related with Klotho.

Reply: We thank you for the suggestion, we added a table that summarizes the main effects due to depletion and addition of Klotho on retinal cells; moreover, we added a figure that shows the protective effects that Klotho exerts in DR.

2. Please add the full name of POS when it first appears.

Reply: We thank you for the comment, we added the full name of POS when it first appears.

3. Please delete irrelevant part of Klotho and DR.

Reply: We thank you for the suggestion, we removed the unnecessary parts in section relative to DR.

Authors need to re-focus the and better structure the content. Especially, Table and figures could be an effective way to follow the topic directly.

Reply: We thank you for the suggestions. We carefully revised the manuscript. We hope that the revision improves the manuscript and meets your requests.

Reviewer #4:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (High priority)

Specific Comments to Authors: The authors have performed a complete systematic review and yielded a well written well connected easy to read manuscript about a subject that has not been well known among clinicians and this authoritative paper would entice researchers to pursue further research in this promising avenue. I need to enhance the paper by adding one more sentence with 3 references: To add more evidence for Klotho as biomarker for diabetic retinopathy, the authors need to add that Klotho is also considered as a new biomarker for early diabetic nephropathy and as an emergent exerkin. This systematic review was the first to evaluate the relationship between sKlotho levels and DN. The sKlotho level was significantly lower in the early stages of DN, indicating that sKlotho might be a new biomarker of DN in the future. Xin C, Sun X, Li Z, Gao T. Relationship of Soluble Klotho and Early Stage of Diabetic Nephropathy: A Systematic Review and Meta-Analysis. *Front Endocrinol (Lausanne)*. 2022 May 27;13:902765. doi: 10.3389/fendo.2022.902765. Piwkowska A, Zdrojewski Ł, Heleniak Z, Dębska-Ślizień A. Novel Markers in Diabetic Kidney Disease-Current State and Perspectives. *Diagnostics (Basel)*. 2022 May 11;12(5):1205. doi: 10.3390/diagnostics12051205. Available evidence suggests that exercise increases Klotho levels, regardless of the condition or intervention, shedding some light on this anti-aging protein as an emergent and promising exerkin. Corrêa HL, Raab ATO, Araújo TM, Deus LA, Reis AL, Honorato FS, Rodrigues-Silva PL, Neves RVP, Brunetta HS, Mori MADS, Franco OL, Rosa TDS. A systematic review and meta-analysis demonstrating Klotho as an emerging exerkin. *Sci Rep*. 2022 Oct 20;12(1):17587. doi: 10.1038/s41598-022-22123-1.

Reply: We thank you for your positive comments. During the revision of the manuscript we cited the references that you suggested at page 3 ref [10]: “.....*The shed soluble form of Klotho seems to be dominant on both the secreted and the membrane forms in humans [8]. It has been proposed that the soluble forms of Klotho function as a hormone [9]. Moreover, since circulating levels of sKl increase following exercise training, it has been also hypothesized that klotho may be related to the antiaging effects of physical activity [10].*”; and at page 5 ref [25] and [26]: “....*A recent meta-analysis of data on sKl amount in patients with Diabetic nephropathy (DN) confirms that levels of sKl are further lowered in the early stage of DN [25], suggesting that Klotho might be considered as an early biomarker of diabetic nephropathy [23, 26].....*”.

We hope that the revision improves the value of the manuscript.

6 EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) *Science editor:*

The manuscript has been peer-reviewed, and it's ready for the first decision.

(2) *Company editor-in-chief:*

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 final acceptance, the author(s) must add a table/figure to the manuscript. There are no restrictions on the figures (color, B/W) and tables. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the RCA. RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: <https://www.referencecitationanalysis.com/>.

Reply: Dear Editor,

We carefully revised and performed all changes suggested by reviewers. In particular, we delved deeper some topics including the latest research results and removing the unnecessary parts. Moreover, we added a table that summarizes the main effects due to depletion and addition of Klotho on retinal cells and a figure that shows the protective effects that Klotho exerts in DR. All the revisions to the manuscript has been marked up using the "Track Changes". We hope that this revised version of our manuscript is now potentially reconsidered as suitable for publication in World Journal of Diabetes. We are indebted with reviewers for their careful reading and pertinent comments, which improved the manuscript.