Reviewer ID: 02455955

1. In the abstract part, the authors presented evidence that humanin increases insulin sensitivity, improves beta cell survival, and delays the onset of diabetes. Is this an vivo study or vitro study? The reason for this review alone does not seem to be convincing. The author should concisely summarize the convincing evidence in the abstract section for review and discussing the current research on humanin and diabetes.

It is a conclusion coming out of both in vivo and in vitro studies. We tried to summarize all the evidence regarding humanin and diabetes.

2. The author focuses on the discovery process of humanin in the background part. I think the detailed discovery process of humanin is not necessary for this review. The background part should focus on the physiological role of humanin, the current epidemiology and the pathogenesis of diabetes, and the role of humanin in diabetes already found in previous studies. Please focus on the research topic in this part.

Thank you for the comment. We added the parts: "Subsequently…response factors.", "Diabetes...for its treatment" in order to add data on the physiological role of humanin, the current epidemiology and the pathogenesis of diabetes, and the role of humanin in diabetes.

3. The description of humanin structure is not combined to the research outcome. What are the characteristics of the structure of humanin to support its role in diabetes? What are the effects of different isoforms on its mechanism of action?

For both of these questions we added the paragraph: "Especially concerning diabetes…insulin sensitivity" and the paragraph entitled "Role of humanin in the pathogenesis of type 1 diabetes".

4. Mechanisms of action: In the second paragraph of this part, the author first explained that humanin is regulated by IGF-1 and GH playing a role in various diseases (including diabetes). Then the author discusses that humanin is related to mitochondrial dysfunction, affecting ROS, playing a role in many diseases (excluding diabetes). Are these two mechanisms both related to diabetes? If not, what are the reasons stating the second mechanism? The writing logic is very confusing and cannot be understood well.

Diabetes is one of the major clinical manifestations/metabolic disorders of the metabolic syndrome, which were mentioned among the age-related diseases. Consequently, these two mechanisms of action are both related to diabetes.

5. Role of humanin in type 2 diabetes: 5. The title of the article indicates that the outcome of the study is diabetes, but it seems that the author only describes type 2 diabetes. In addition to type 2 diabetes, diabetes also includes subtypes such as type 1 diabetes and gestational diabetes.

Thank you for your comment. We corrected the title to: "Role of humanin in the pathogenesis of type 2 diabetes" and we added the paragraph titled "Role of humanin in the pathogenesis of type 1 diabetes".

6. Please describe in detail the current epidemiology of diabetes.

We added that in the introduction, as we also described above ("The number of people with diabetes… for its treatment").

7. 7. The purpose of this paragraph is to explain the role of humanin in the pathogenesis of type 2 diabetes. The title of the paragraph should be revise to avoid misunderstand.

We have changed that into "Role of humanin in the pathogenesis of type 2 diabetes".

8. Clinical trials 8.Since the author looks ahead to the prospects of humanin in the treatment of diabetes in the conclusion part, the existing treatment methods for diabetes should also be in detail be described.

We added the text: “Besides the initial and principal… in diabetes prevention and treatment.”.

9. The author also describes increased humanin levels in patients with mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), and chronic progressive external ophthalmoplegia (CPEO), is this related to the outcome of this review?

We added the text: “These conditions are related to… MELAS and CPEO.”.

10. The existing studies listed by the author do not seem to be clinical trials, but rather observational studies. There are no examples of humanin being used in the treatment of diabetes., which is contrary to the author’s prospects for the use of humanin in the treatment of diabetes.

Yes, as we added and mentioned: “Altered HN concentrations in diabetes could serve as a potential biomarker. Nevertheless, no clinical trials investigating the effects of HN or its analogues (e.g. HNGF6a) administration have thus far been published, albeit it would be an innovative and promising breakthrough in diabetes prevention and treatment.”

Reviewer ID 02623025

The article of Chrysoula Boutari et al. entitled "Humanin and Diabetes Mellitus: A review of in vitro and in vivo studie".is an interesting manuscript describing the role and suggesting potential new functions of the Humanin, a Mitochondrial derived peptides (MDPs), on diabetes mellitus. The work is well done but some points need to be revised:

1. It would be appropriate to realize a specific figure of the mechanisms of action of Humanin in diabetes mellitus.

Thank you for the recommendation. We prepared the Figure 1. Mechanisms Of Action Of Humanin In Diabetes Mellitus.

2. It should be explain or hypothesize an explanation for the difference in blood values by comparing type 1 diabetes and type 2 diabetes.

As we highlighted at the end of the paragraph about the "Role of humanin in the pathogenesis of type 1 diabetes", "Yet, no studies juxtaposing the HN levels in T1DM and T2DM have been published thus far." However, we described the mechanisms which impair humanin's levels ("The beta cells destruction,…in NOD mice in vivo" and "Particularly, hyperglycemia causes extended free…agents that are inhibited by HN." and "As for the changes in HN levels with ageing,…mitochondria may upregulate HN levels."

3. Are there any clinical trials on Humanin or analogues such as HNGF6a?

As we added "no clinical trials investigating the effects of HN or its analogues (e.g. HNGF6a) administration have thus far been published, albeit it would be an innovative and promising breakthrough in diabetes prevention and treatment."

4. It is possible that age can influence Humanin hematic values in the diabetic population, can you discuss on it.

Thank you for the comment. We wrote the paragraph: "As for the changes in HN levels with ageing, Voigt et al. [67] showed that HN decreased with age among individuals attending a diabetes complications screening clinic suggesting a protective function of HN and this observation was consistent with a previous study among human and mice [23]. On the contrary, circulating levels of HN increase in age-associated diseases such as T2DM. With disease progression and further oxidative stress, mitochondria may upregulate HN levels."

5. It should be nice to deepen the correlations between Humanin and adiponectin

We tried to deepen with the part: "Adiponectin levels were positively correlated with HN. Adiponectin concentrations decrease in pre-diabetes and DM [81]. It has also been demonstrated that adiponectin knockout mice have reduced mitochondrial content combined with insulin resistance [82]. In addition adiponectin may impair mitochondrial biogenesis [83]. Thus the affected mitochondrial function may be a result of the low adiponectin levels."

Reviewer ID 02459759

This is a review of Humanin's in vivo and in vitro studies in diabetes. The article summarizes its effects and mechanisms, existing in vivo and in vitro studies, and clinical trials. It has a certain degree of innovation, provides new ideas for the treatment of diabetes in the future, and has certain clinical significance. The only drawback is the lack of some insights. The discussion part can be richer in content, even better.

Thank you for your comments. We added several parts and more aspects related to the topic.

Reviewers ID 05426937 and 03906428 did not make any comments/questions/recommendations