

Format for ANSWERING REVIEWERS

November 21, 2014



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13122-review.doc).

Title: Advanced glycation end-product expression is upregulated in the gastrointestinal tract of type 2 diabetic rats

Author: Peng-Min Chen, Hans Gregersen, Jing-Bo Zhao

Name of Journal: *World Journal of Diabetes*

ESPS Manuscript NO: 13122

To the editor

We have made numerous changes in the text to avoid redundant sentences from previous papers we published. We believe now that the percentage in all cases will be lower than 5%. We also made numerous changes to improve the spelling and grammar.

Please also notice that we changed the order of the authors and the corresponding author is now Professor Hans Gregersen.

You did not send us the formal reviews by the reviewers but we succeeded to find them on the journal homepage. Therefore, we have taken the reviews into account in the revised version we submit now.

Both reviewers expressed that the study was interesting.

Reviewer 02446566 wrote: This is an interesting study and I have some questions. 1) The life span of epithelial cells of the villi may be several days. How long does it take for the proteins in the epithelial cells are glycated or do the epithelial cells uptake AGE made in other part of the body? 2) To say the relationship with AGE and thickness of colon mucosa, comparison of the staining of colon muscle between GK and control is desirable in Fig 4. 3) Statistically, only the staining in duodenum is stronger in the GK group as shown in Fig 4 F. Therefore to say something about the increase of AGE and morphological or functional changes in diabetes needs more data (in the future).

1) We clearly demonstrate the expression of AGE in the intestinal villi, especially AGE in the villi of duodenum are up-regulated. However, it is well known that the protein glycation need long time whereas the villous epithelium are exchanged in relatively few days (3-5 days we believe). We do not have an explanation for this phenomenon but it caught our interest and we like to study it further in the future.

2) We agree with the reviewer's comment that it is desirable to compare the immuno-staining between GK and Control groups. However, no visible stained color was found in smooth muscle, consequently it is difficult to make such comparison. In future studies more types of AGE antibodies should be selected and the expression of AGE in the muscle layer and submucosa layer should be compared between normal and diabetic groups.

3) Correct, we only found significant increase in expression of AGE in the duodenal villi and crypt of diabetic GK group although the expression of AGE is also up-regulated (but not statistically significant, probably due type 2 error) in the ileal villi and crypts as well as the colon mucosa. Therefore, more detailed study with more samples are needed in the future to further demonstrate the relationship between AGE expression with histomorphological and functional changes related to diabetes.

Reviewer 01408945 wrote: This manuscript is well written. However, there are some criticisms. The reviewer's critiques are as follows. Major criticism: 1. The most important issue is that this study is not directly demonstrated the relationship between high density of AGE and the expression of RAGE in the intestine, and GI dysfunction. It is difficult in the animal models, as authors have mentioned in the text. Minor criticism: 1. There are no key words. 2. In the Statistically analysis section, authors mentioned that the results were expressed as meansSD unless indicated in the text. There is lack of " \pm ". 3. There are many English and English grammatical errors. Native English speakers should correct this manuscript. 4. In the references section authors should correct the texts, which were copied and pasted. 5. In the figure legends section, authors should explain abbreviation "GK" in the figure 1,2,3, and 4.

Reply to Major Criticism. As pointed out both by us and the reviewer, no causal relationship is demonstrated in this study. Unfortunately it was not possible to demonstrate the mechanisms but we hope in the future to deepen further into this subject.

Reply to minor criticism. All of the minor points have been corrected. Especially we paid attention to the spelling and grammar in the entire manuscript.

1, please find key words " Diabetes mellitus; Gastrointestinal complications; AGE; RAGE" at the end of Abstract section.

2, Now the "meansSD" is corrected to "means \pm SD". However, we sometime experience when uploading papers that special symbols disappear.

3, the spelling and grammar of English in the entire manuscript have been revised and corrected by corresponding author Professor Hans Gregersen.

4, The errors were corrected.

5, Abbreviation "GK" in the legends of figure 1,2,3, and 4 have been explained.

