Author's response to reviews

Title: The relationship between bile acid concentration, glucagon-like-peptide 1, fibroblast growth factor 15 and bile acid receptors in rats during progression of glucose intolerance

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Author’s response to reviews:

Dear Editor,

We have studied the valuable comments from you and reviewers carefully, and tried our best to revise the manuscript. The point to point responds to the reviewer’s comments are listed as following:

Responds to the reviewers’ comments:

Reviewer 1 :

Comment 1: English is not the first language of the authors, and thus many corrections are necessary. I will list some below.

Response: We have had the manuscript polished with a professional assistance in writing.

Comment 2: The results of these studies are correlations, rather than cause and effect. Thus, this is a descriptive manuscript.

Response: This paper is exactly a descriptive manuscript, we will try to explore the cause and effect in future work.

Comment 3: It is known that some bile acids are agonists and some are antagonists of FXR. Thus, it would be better to quantify individual bile acids than total bile acids.

Response: Thank you for your valuable advice. According to references we have reviewed, most bile acids such as CDCA, LCA, DCA and CA are agonists of FXR, while TβMCA with a low
concentration is an antagonists of FXR in rodents. So we only quantified total bile acids instead. We will quantify individual bile acids to analyze the correlation with FGF15 in future work.

Comment 4: First 3 lines: bile acids are involved in activation of the G protein----A few researchers have explored changes TGR5.....

Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 5: Abstract line 20: intolerance in rats.....lipid, TBAs, alanine.

Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 6: Introduction, line 6: In 1999, bile acids were discovered.

Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 7: Line 11. The FXR pathway... by bile acids synthesized in hepatocytes or ....pathway that suppresses hepatic.....epithelial cells, mainly.....

Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 8: Line 50: in a glucose.

Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 9: Page 3, line 1 bile acids on lipid and glucose homeostasis, we hypothesized that an alteration of bile .....glucose intolerance. Little is known in regard to changes TR5....TGR5 and Fxr in intestine.

Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 10: Throughout, whenever a company is given, also indicate city and state.

Response: According to the reviewer’s comment, we have added the city and state of the company.

Comment 11: Line 39: Thirty-three four-week old male.

Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 12: Throughout: high-fat, high-sugar

Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 13: Page 4, line 1: groups to assess insulin.....blood glucose was measured at ....diabetes. Blood glucose.
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 14: Line 17 considered to have developed type
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 15: Line 42: at the 5-hour time point were
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 16: line 50: used for quantifying
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 17: Line 12: Remove THE at the beginning of the two sentences.
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 18: Line 17: instructions......with a DPP4
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 19: Line 42: Remove THE...
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 20: Line 56: System (remove s)
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 21: Line 28: What is RIPA?
Response: RIPA means Radio Immunoprecipitation Assay. It is composed of 50mM Tris, 150mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, sodium orthovanadate, sodium fluoride, EDTA, and leupeptin.

Comment 22: Page 7, line 11: At the 4th week
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 23: Line 36. levels
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 24: No discussion of figure 1 is provided.
Response: Figure 1 is the result of OGTT test which showed the successful modeling of IGT in the IGT and DM groups after 4 weeks of high-fat, high-sugar diet. And this is the base of successful modeling of T2DM before intraperitoneally injection of STZ. We thought this was just a precondition of following experiment, so we didn’t discuss figure 1.

Comment 25: Page 8 line 1 and 2: Change smaller to less in both lines
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 26: Page 9, line 31: demonstrated changes.....levels were increased
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 27: Page 9,last line: to the FGR in
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 28: Page 11, line 6: studies discovered a relationship
Response: According to the reviewer’s comment, we have corrected the sentence.

Comment 29: In contrast most scientists, the authors have indicated the importance of the large intestine in these correlations. They might want to indicate the first manuscript that suggested the importance of the large intestine of these parameters in germ-free mice---see Selwyn in Drug Metab and Dispos 43: 1544, 2015.

Response: We did find the expression of FXR and TGR5 changed more significantly in colon than ileum, with causes unknown. Maybe BA transporters’ changes in large intestine in GF mice seen in “Importance of Large Intestine in Regulating Bile Acids and Glucagon-Like Peptide-1 in Germ-Free Mice” have some relationship with it.

Comment 30: Fig 3B: Is the statistics on the right two bars not significant?
Response: We didn’t compared statistics in ileum and colon of FXR or TGR5 expression. Maybe the differences of FXR or TGR5 expression between ileum and colon in the same group are significant, while we didn’t find relationship of the differences with bile acid, GLP-1 or FGF15.

Reviewer 2

Comment 1: Authors mention that a group of animals were allowed normal chow, provide appropriate description (for example - fat/carbohydrates ratio and energy provided in KJ/g)
Response: The normal chow provided energy 16.1KJ/g.

Comment 2: Need to provide energy in KJ for HFD.
Response : The HFD provided energy 18.7KJ/g.

Comment 3: What ethical approvals were obtained prior to commencement of animal experiments?

Response : All animal experimental protocols were approved by the Animal Ethics Committee of Animal Center of East Hospital, Tongji University, and all the animal experiments were carried out in strict accordance with the Guidelines for Care and Use of Laboratory Animals of the Animal Ethics Committee of Animal Center of East Hospital, Tongji University.

Comment 4: What was the rational for carrying out OGTT a day prior to STZ injections? As the animals should have been stress free 3-4 days prior to STZ administrations.

Response: Thank you very much to point out the defect in the experiment. All rats in three groups had received the same stress from OGTT, and the rats in NC and IGT group were intraperitoneally injected with the same dose of sodium citrate. So the differences caused by stress may be avoided.

Comment 5: Need citation, when using 30mg/kg BW STZ.

Response: We have listed the reference when using 30mg/kg STZ.

Comment 6: What was the rational for carrying out body weight measurements every 2 weeks?

Response: In fact, we carried out body weight and glucose measurements every week. Because we didn’t give any drug except SZT in the experiment, so we thought the changes of body weight every week had no significant impact on the results.

Comment 7: There are numerous incidences in the manuscript where unscientific English is used (for example line 48 - authors say that "rats were killed at the end of study", Line 45 - authors say that "something else"). Authors need to make considerable effort in re-writing parts of manuscript.

Response: Thank you for your advice. We have had the manuscript polished with a professional assistance in writing.

Comment 8: In method section, a mere mention that blood for measurements were collected from heart is not suffice. Please provide what method/equipment/consumables were used for this.

Response: Blood was collected with Na-heparin injectors into Na-heparin tubes from the heart left ventricles.

Comment 9: Please provide citation for 2 h meal test.

Response: We have provided citation for 2h meal test.
Comment 10: Table 1 legend - Provide more information such as the day on which measurements were taken.

Response: These measurements were taken on the day rats were sacrificed.

Comment 11: When coming to figures, there are a number of issues related to uniformity, such as fonts used in Figure 2-4 are different and not consistent. There are no mention of Figure number at some incidences, X-axis in Fig 2A does not contain units, at some places there are gridlines while in others there are none, all line graphs should start at conjoint axis point 0 - lot of variation among different graphs. Uniformity and consistency is stressed.

Response: We are sorry for the uniformity of the figures. We have uniformed the figures according to the requests.

Thank you for advices on the manuscript, they are very important for us in the research. I hope the responses will meet your requests, and the manuscript will be accepted by you!

Yours sincerely

Bo Feng