Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript Number: 26392

Manuscript Title: Effects of diet on intestinal microbiotas and nonalcoholic fatty liver disease development

Dear Editor,

Thank you for your letter and for the reviewers' comments concerning our manuscript. Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. Based on these comments and suggestions, we have made careful modifications on the original manuscript. Revised portion are marked in red in the paper. Below you will find our responses to the reviewers' comments.

We hope that these revisions are satisfactory and that the revised version will be acceptable for publication.

Thank you very much for your work concerning our paper.

Sincerely yours,

Ya-Ni Yin

Answer to Reviewers :

Authors are thankful to the reviewers. Their comments will definitely help to improve the quality of the manuscript.

Below are the answers to each specific point.

List of Revisions Made to ESPS Manuscript No. 26392

Reviewer's code: 00503561

Comment 1: Provide the possible difference of SD rats from different supplier; ideally the author check or monitor several colonies randomly besides this study sets. If there are some information in the literature on rodent microbiota, include them in the reference and discussion.

Response: Thank you very much for your suggestion. Host genotype is an important factor that can affect intestinal microbiota composition. For example, the intestinal microbiota of our SD rats is different from C57BL/6J mice (compared to reference 41). However, these differences may due to many other factors, such as diet, experimental environment and sequencing technique. Most studies compared the diversity of intestinal microbiota in the same species of rodent, according to different host-genotype. The differences among speices may be varied and lack of a consistent condition. This shortage leads a future research direction, and more profound study is clearly warranted.

Comment 2: Address the possible problem when they extrapolate their data to human beings.

Response: Thanks for your very thoughtful suggestion. We have added following sentences to address the possible problem when extrapolate our data to human beings in discussion section.

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“It should be pointed out that the results of the present study were obtained from rats and host genotype also can affect intestinal microbiota composition, so it may not be appropriate to apply our results directly to humans.”

(Page16 line 1 to line 4)

Reviewer’s code: 03464856

Comment 1: Although restrictive high-fat and high-sugar diet also induced NAFLD, the changes were very small as compared with FFAT. Therefore, the authors emphasized the independency from calories, but it seems to be not so important. The importance of the findings should be described more.

Response: Thank you for your comments. The traditional opinion is that excess caloric intake is the main cause of obesity and associated with NAFLD. Diet composition may plays an important role in development of NAFLD. The unbalanced diet instead of caloric intake may be a reason to NAFLD, and also the dysbiosis of intestinal microbiota could be another important factor to induce NAFLD. In our study, after feeding a restricted calorie diet of high-sugar and high-fat diets, the rats showed a liver lipid deposition trend. Although the changes of restrictive high-fat diet group and high-sugar diet group were very small as compared with FFAT group; however, the changes were significant as compared with high-protein diet group. As restrictive high-fat diet group, high-sugar diet group and high-protein diet group had same caloric intake, we concluded that compared with the high-protein diet, the NAFLD-inducing effects of high-fat and high-sugar diets are independent from calories. In addition, the results still showed us a very interesting relationship between the diet composition and intestinal microbiota independent of calorie intake.

Comment 2: The changes of microbita were expected results. However, the meaning and scientific reason should be discussed.

Response: Thanks for your very thoughtful suggestion. We have added following sentences in discussion section to discuss the meaning and scientific reason for the changes of microbita.

“In the present study, the RSUG group showed increased abundance of Bacteroidetes. Some species of bacteria in this phylum, such as *Bacteroides thetaiotaomicron*, can encode adequate carbohydrate active enzymes for carbohydrate metabolism of food[39].

This enables the host to extract more energy from the diet, which will be deposited in liver in the form of triglycerides. Unlike the RSUG group, the RFAT group showed an increased abundance of Firmicutes; however, how the bacteria in this phylum affect NAFLD development is not clear. The effect of a high-protein diet on the composition of the intestinal microbiota is not well studied. We found that the PRO group had increased abundance of Bacteroidetes and *Sutterella* bacteria, and decreased abundance of Firmicutes. This change in intestinal microbiota was similar to that in the RSUG group; however, the PRO group also had increased abundance of *Prevotella* and *Oscillospira*. Kovatcheva-Datchary *et al* [40] found that *Prevotella* is associated with improvement in glucose metabolism. *Oscillospira* has never been cultured, so it is an enigmatic bacterial genus and little is known of it role in the intestinal tract. However, recent studies found that *Oscillospira* is positively associated with leanness, and is reduced in pediatric NASH[41,42]. We conclude that the beneficial effects of high-protein diet on NAFLD may be closely associated with *Prevotella* and *Oscillospira*, and requires further study."

(Page14 line 24 to page 15 line 12)

Reviewer's code: 02155326

Comment 1: There exists a great deal of grammar and spelling errors.

Response: Thank you for your comments. The manuscript has been checked and revised the mistakes with the help of a native English speaking medical editor.

Comment 2: It is difficult to distinguish different groups in figure 1B.

Response: Thanks very much for your suggestion. We have added different colors for different groups in Figure 1 B.

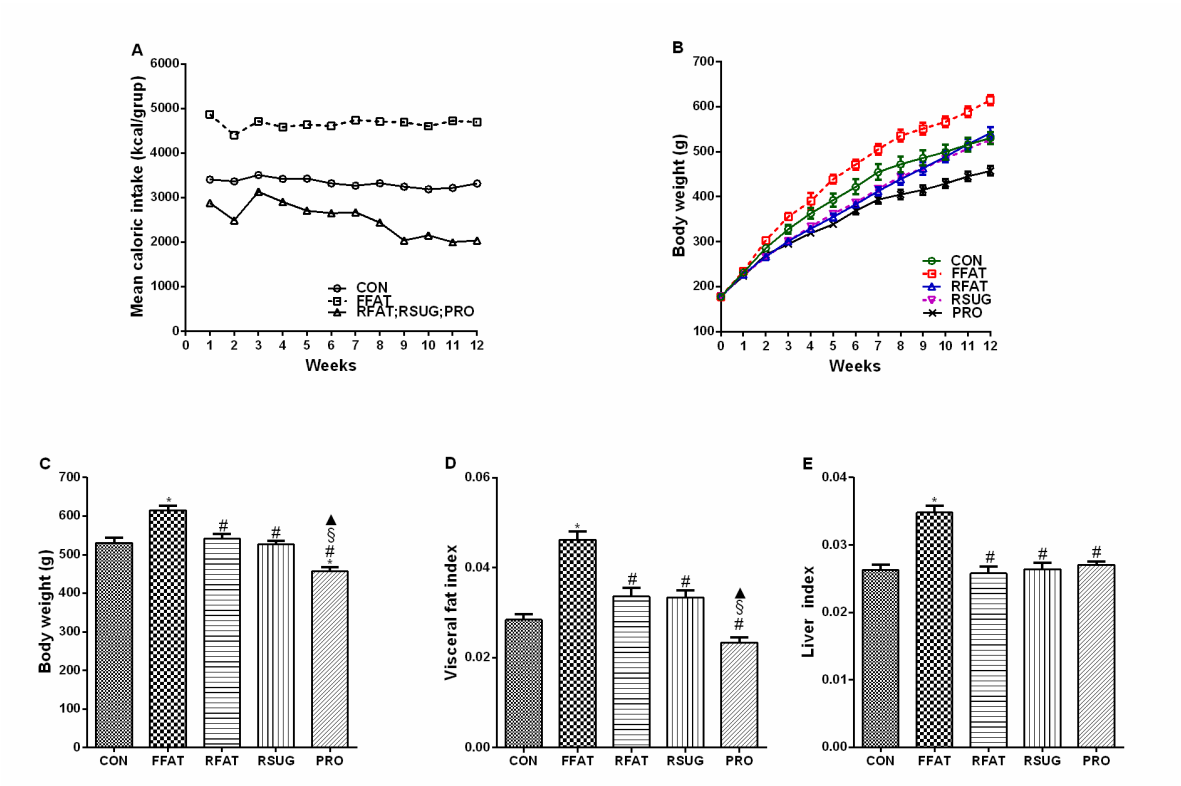

Figure 1 Caloric intake, body weight, visceral fat index, and liver index in rats. (A) Mean caloric intake; (B) Body weight at different experimental period; (C) Body weight at 12 wk; (D) Visceral fat index, calculated as visceral fat weight/body weight; (E) Liver index, calculated as liver weight/body weight. Differences were denoted as follows: *P < 0.05 *vs* CON group rats, #P < 0.05 *vs* FFAT group rats, §P < 0.05 *versus* RFAT group rats, ▲P < 0.05 *vs* RSUG group rats.

Comment 3: The introduction about intestinal microbiota, for example the association of changes in the composition of intestinal microbiota with NAFLD development, is inadequate.

Response: Thanks for your very thoughtful suggestion. We have rewrited the association of intestinal microbiota with NAFLD development as following sentence in introduction section.

“Germ-free mice are resistant to NAFLD induced by high-fat diet[14]. However, when the intestinal microbiota was introduced into germ-free mice, the mice showed a rapid increase in body fat content and liver triglycerides. When a high-fat diet induces NAFLD, it also causes dysbiosis of the intestinal microbiota[15]. Moreover, small bowel bacterial overgrowth is associated with NAFLD, and patients with NASH have a lower percentage of Bacteroidetes[16,17]. These studies show the close links between the intestinal microbiota and NAFLD.”

(Page 5 line 24 to line 30)

Comment 4: More in-depth discussion is needed on the finding related to changes in intestinal microbiota.

Response: Thanks for your very thoughtful suggestion. We have added following sentences in discussion section to further discuss the finding related to changes in intestinal microbiota.

“In the present study, the RSUG group showed increased abundance of Bacteroidetes. Some species of bacteria in this phylum, such as *Bacteroides thetaiotaomicron*, can encode adequate carbohydrate active enzymes for carbohydrate metabolism of food[39]. This enables the host to extract more energy from the diet, which will be deposited in liver in the form of triglycerides. Unlike the RSUG group, the RFAT group showed an increased abundance of Firmicutes; however, how the bacteria in this phylum affect NAFLD development is not clear. The effect of a high-protein diet on the composition of the intestinal microbiota is not well studied. We found that the PRO group had increased abundance of Bacteroidetes and *Sutterella* bacteria, and decreased abundance of Firmicutes. This change in intestinal microbiota was similar to that in the RSUG group; however, the PRO group also had increased abundance of *Prevotella* and *Oscillospira*. Kovatcheva-Datchary *et al* [40] found that *Prevotella* is associated with improvement in glucose metabolism. *Oscillospira* has never been cultured, so it is an enigmatic bacterial genus and little is known of it role in the intestinal tract. However, recent studies found that *Oscillospira* is positively associated with leanness, and is reduced in pediatric NASH[41,42]. We conclude that the beneficial effects of high-protein diet on NAFLD may be closely associated with *Prevotella* and *Oscillospira*, and requires further study.”

(Page14 line 24 to page 15 line 12)

Reviewer’s code: 00189256

We are very appreciated for your comment.