Response to Peer-reviewers's Comments

The main corrections in the paper and the responds to the peer-reviewers' comments are as follows:

Reviewer #1: The aim of this study was to examine the effect of Polygonatum kingianum rhizome extract (PK) on lipid and metabolomic profiles in high fat diet-fed rats. Rats were fed regular or high-fat diet for 14 weeks; separate groups received simultaneously simvastatin or PK extract intragastrically. It is demonstrated that high fat diet increased serum total cholesterol but not triglycerides whereas both cholesterol and triglycerides increased in the liver. PK corrected these abnormalities similarly to simvastatin. In addition, serum, liver and urine samples were subjected to HPLC-MS based untargeted metabolomics profiling in both positive and negative ionization modes. The results indicate that all samples clearly differ between normal and HFD groups. Both PK and simvastatin significantly affected metabolomics profiles with PK generally restoring it more closely to normal groups. Potential biomarkers of the PK activity were identified. In the positive ionization mode, 15, 17 and 18 biomarker candidates were identified in serum, urine and liver samples, respectively, whereas in the negative ionization mode 4, 7 and 22 biomarker candidates were identified in these samples, respectively. Metabolic pathways altered by HFD and PK were identified by KEGG database. The results suggest that PK may be useful in the treatment of dyslipidemia. The topic and the findings are of interest. Sample preparation, HPLC-MS metabolomics profiling and data analysis were performed by modern sophisticated methods and are described in detail. However, there are also some important concerns to be addressed.

1) Caloric composition of both diets (% of calories provided from carbohydrates, proteins and fat) should be presented.

Response: Calories provided from carbohydrates, proteins and fat were 62%, 26% and 12%, respectively, which has been presented in the manuscript (lines 219–220).

2) Due to fundamental differences in plasma lipoprotein metabolism between rats and humans, the rat is not an optimal model to study the effects of lipid-lowering medications. This issue should be discussed.

Response: We agree with the reviewer that plasma lipoprotein metabolism between rats and humans have fundamental differences. However, to the best of our knowledge, the rat model is high-frequently used to preliminarily evaluate the effects of lipid-lowering medications due to the easy-manipulation and low-cost. Moreover, the hepatic lipid parameters were also evaluated in our study. The preliminary discussion of this issue has been presented in our manuscript (lines 483–485).

3) Only very basic lipid parameters were measured in serum (total cholesterol and triglycerides). It would be of interest to present lipoprotein fractions (HDL, LDL) as well as major apolipoproteins (B, A-I).

Response: We agree with the reviewer that more lipid parameters should be measured. However, the remaining samples in the present study were not enough for assaying the lipoprotein fractions (HDL, LDL) as well as major apolipoproteins (B, A-I) which will be systemataciallly evaluated in the further study.

4) Triglyceride and cholesterol concentrations in the liver should be better presented per mg protein rather than per ml of homogenate.

Response: Triglyceride and cholesterol concentrations in the liver have been presented per g protein.

5) To get more insight into the mechanism of PK activity, it would be of interest to include the additional group of rats fed the normal diet and receiving PK.

Response: We agree with what the reviewer described. It is valuable to include the additional group of rats fed the normal diet and receiving PK, which will be met in the further investigation. However, this was not included in the present study, because it was not synchronously performed with the other groups.

6) It would be of interest to present data such as body weight, serum glucose and insulin concentrations as well as markers of insulin sensitivity/resistance such as HOMA-IR. Did PK have any effect on food intake and body weight or improved lipid metabolism irrespectively of body weight?

Response: The effects of PK on body weight and food intake have been presented in Table 1 in our manuscript. As shown in Table 1, the HFD slightly increased body weight, while PK and simvastatin showed non-significant affection on body weight and food intake. In addition, the data on serum glucose, insulin concentrations and markers of insulin sensitivity/resistance was not provided, due to the non-enough samples for determining these parameters. They will be met in the further research.

7) Some of the altered metabolic pathways are associated with branched-chain aminoacid (BCA) metabolism. The role of BCA in cardiometabolic diseases such as obesity, metabolic syndrome and dyslipidemia has been extensively studies. The results should be discussed in this context.

Response: We agree with the reviewer that the role of BCA in cardiometabolic diseases such as obesity, metabolic syndrome and dyslipidemia has been extensively studies. However, the BCA metabolism was not found by metabolic pathway analysis in the present study. This may be attributed to un-affection (or non-significant affection) of PK extract on BCA. Thus, the BCA metabolism was not involved in this manuscript.

8) What molecular mechanism of PK activity could be suggested?

Response: Our results indicated that PK might regulate phenylalanine, tyrosine, tryptophan, valine, leucine and isoleucine biosynthesis, and tryptophan, tyrosine, phenylalanine, starch, sucrose, glycerophospholipid, arachidonic acid, linoleic acid, nicotinate, nicotinamide and sphingolipid metabolism. The suggested molecular mechanism of PK activity was presented in the conclusion of our manuscript.

9) The dose of PK extract vs. simvastatin was relatively high. Thus, the conclusion that PK restored metabolomics profiles more closely to normal than simvastatin is the over-interpretation of the data. In addition, how relevant could be this dose regarding humans?

Response: We agree with what the reviewer described. The conclusion that PK

restored metabolomics profiles more closely to normal than simvastatin in the manuscript had been deleted. In addition, the PK dose of rat experiments was calculated according to the PK dose of humans with the following formula that is high-frequently used to relate the rat's dose with human's dose:

PK dose of rat experiments/day/rat = 12 g/day/person ÷ 70 kg × 6.3 × dose times,

12 g/day/person is the PK dose of humans, which is recorded in the Chinese Pharmacopoeia (2015 edition),

70 kg is the human middleweight,

6.3 is the conversion coefficient,

Dose times is no more than ten in rat experiment, and four times was used in the present study.

10) Tables 1 and 2/statistical analysis: high fat group should be first compared to normal group and then treated groups to high fat untreated group. Using high fat group as a reference is not appropriate because normally fed group represents the control in this experiment.

Response: Statistical analysis in Tables 1 and 2 had been performed according to what the reviewer described.

11) Line 72: the sentence that "statins stimulate GI tract" needs clarification.

Response: The sentence that "statins stimulate GI tract" had been deleted from the manuscript.

12) Methods: it is stated that rats were kept in metabolic cages for 3 weeks (line 168). Is it correct? Why so long?

Response: The state that “rats were kept in metabolic cages for 3 weeks” is correct. Rats were kept in metabolic cages for 3 weeks to collect enough urine samples for detecting more metabolites, which may found more potential biomarkers.

13) Line 355: the conclusion about effects of PK on starch and sucrose metabolism is confusing; starch and sucrose are hydrolyzed in the GI tract and strictly speaking are not components of metabolic pathways in humans. This sentence represents the misinterpretation of KEGG including all metabolic pathways irrespectively of species.

Response: We agree with the reviewer that starch and sucrose are hydrolyzed in the GI tract. Both sucrose and starch can be broken down into glucose by digestive juice. Glucose can be degraded to dihydroxyacetone phosphate by glycolysis. Dihydroxyacetone phosphate can be reduced to glycerol and can also be converted to pyruvate through glycolysis. Pyruvate is converted by oxidation and decarboxylation to acetyl-CoA, which can be used to synthesize fatty acids and further synthesize fat with glycerol.

In the present study, we found that glucose content in serum samples was significantly reduced after PK administration, which involved starch and sucrose metabolism found by metabolic pathway analysis. Thus, we suggested that PK extract regulated the starch and sucrose metabolism to reduce glucose content in HFD-feeding rats, which further reduced lipid synthesis and treat dyslipidemia.

These results and explains have been presented in the discussion of our manuscript (lines 534–544).