We thank the reviewers for their constructive comments and suggestions on the manuscript. Below is our point-to-point response to their comments.

Reviewer #1 (03699916)

1. *"The result section in the abstract is needed to provide the results in little bit detail, for example the P values should be shown to see if the effects is significant."*

We have enhanced the result section of the abstract according to the reviewers' suggestions. However, due to the word limit, we could not include too much detail in the abstract.

2. *"Some parts in the Result section should belong to Introduction or Discussion, it is suggested to move it to the Introduction or discussion. For example: …"*

We have carefully revised the manuscript according the reviewer's comments. The parts were moved to either introduction or discussion, where they fit better.

3. *"The repetition should be avoided. For example, the description of "we previously reported that LZD, a traditional Chinese herbal medicine…""*

We apologize for the mistakes. We carefully checked the manuscript and revised it where necessary. Repetitions have now been avoided. We also rewrote sentences when necessary to make the manuscript reads more smoothly.

4. *"Normally, the references should not be cited in the Result section. There are several places in the result section have cited references, please check."*

We agree with the reviewer that the Result section should not contain references, under normal circumstances. Thus, we moved the parts explaining the rationale why choosing SREBP-1c, FASN, ACCα, SCD-1 and PPARγ for testing to Methods section. This improves the readability of the manuscript. After the revision, the Result section no longer contains any reference.

Reviewer#2 (03811054)

1. *"What are the LD50 of LZD and CR?"*

We apologize for not making it clear to the readers. CR stands for caloric restriction. Thus, no LD50 for CR can be provided. LZD stands for Lingguizhugan decoction, which are water extracts of 12-traditionally used Chinese herbal plants. There are little, if none, reports on the toxicity of LZD. Additionally, LZD has widely been used in clinic, and generally believed to be safe. Furthermore, we found that the dose used in our study, i.e. 4.14 g/kg body weight, comparable to the dose used in clinic, did not cause any disturbance in liver and renal functions, arguing that LZD is not toxic for liver and kidney. Perhaps because it is not toxic, LD50 of LZD is not studied till now. It is interestingly to explore this in the future study, but it is not within the immediate scope of the current study to show that LZD exerts its effect via gut microbiota.

2. *"Authors collected blood samples from tail vein of mice at 0, 15, 30, 60, 120 min after glucose injection…What is the amount of blood that was taken each time in the experiment?"*

The blood samples were drawn to measure blood glucose levels. At each time point, around 10-20 μL blood was collected.

3. *"H&E staining and Oil Red O staining…. is there a reference to this method?"*

We have used the H&E and ORO staining method in our recent publication (Ren et.al., Circulation Research, 2018). We have revised the manuscript and included the reference for better clarity.

Reviewer#3 (00597793)

1. *"Why did you not examine the effect of LZD on the flora of the rodents and see if it directly changed the GI microbiome and metabolic/weight factors? You could then do all of the studies reported here to confirm your results and to study the mechanism of effects?"*

We understand the concern of the reviewer on the difference between gut microbiome and fecal microbiome. Yet, current methods to collect gut microbiome are not problem free. For instance, flushing the microbiome out of the gut using PBS or other buffers are often less efficient. Thus, gut microbiome measured using this method is often less representative than fecal microbiota. It is also possible to directly isolate DNA from the gut, which would then contain both DNA from gut microbiome and the host, and performing the sequencing. The problem is that this would require much more data to be acquired, and less abundant microbes would be difficult to detect. We agree that fecal microbiota is perhaps not a perfect representative of GI microbiome. But we demonstrated that fecal microbiota transplantation can improve the metabolic parameters of HFD-fed mice. This provides an direct and important evidence to show that CR&LZD exerts its effect via gut microbiota.

2. *"METHODS-what is MQ? OCT?"*

We have changed MQ to ultrapure water to make it clear to the reader. We also replaced OCT by optimal-cutting temperature compound for better clarity.

3. *"METHODS, Immunoblotting – explain to the reader why you choose these genes? You do so later in the DISCUSSION section. I suggest you move the section from the DISCUSSION to here."*

We fully agree with the reviewer that explaining the rationale why choosing the lipogenic genes for testing is more appropriate in the Methods section. Thus, we have revised the manuscript accordingly.

4. *"RESULTS- remove the first few lines of the RESULTS. You have said it already. Begin with "We first tested…""*

The manuscript has been revised according to the reviewer's comment.

5. *"RESULTS-Chart 2E-the 2 bars appear to me to be equal, not different?"*

We apologize for the confusion caused. We indeed found that CR&LZD treatment did not alter plasma cholesterol and triglycerides levels of ND-fed mice. We have corrected for the mistakes in stating the effects of CR&LZD in the Results section.

6. *"RESULTS-Figure 3. Put a color scheme above as you do in Fig 2."*

We now included a color scheme as we did in Fig 2, following the reviewer's suggestion.

7. *"DISCUSSION- please discuss weight loss with the LZD"*

We have now included few sentences in the Discussion section to discuss the effect of LZD on body weight reduction, which were quoted here "Yet, it is not clear how does LZD reduces body weight. Perhaps, as illustrated in HFD-fed mice receiving FMT of CR&LZD treated mice, CR&LZD may increase OCR of the mice by promoting FA oxidation."

Reviewer#4 (00053493)

1. *"what is the composition of LZD? What are the properties of these compounds? Are they antioxidants, anti-inflammatory? Why not utilizing a pure compound instead of an extract?"*

LZD are composed of Radix Codonopsis, Cortex Poria, Rhizoma Atractyldis Macrocephalae, Ramulus Cinnamomi, Radix Astragali, Rhizoma Dioscoreae, Pericarpium Citri Reticulatae, Rhizoma Pinelliae, Semen Coicis, Radix Morindae Officinalis, Epimedium Folium, and Radix Glycyrrhizae. All are well-accepted and widely used in traditional Chinese medicine. As the reviewer pointed out, LZD is not a pure compound thus it has diverse functions, including antioxidant and anti-inflammatory functions. Using a pure compound may on one hand make it easier to understand its pharmacological mechanism, but on the other hand miss the effective component(s) of the drug, especially in case of herbal medicines. Also, the aim of the study was to explore how LZD improves metabolic parameters in obese patients.

2. *"Did you characterized the composition of FMT? Please insert a table with the probiotics of FMT. Why not utilizing a well-characterized preparation of probiotics?"*

We performed fecal microbiota transplantation (FMT) by gavage the mice with fecal homogenates prepared using feces and PBS, on a regular basis (every 3 days). The fecal microbiota profile can alter frequently, thus it would be interesting to characterize the fecal microbiota profile every time giving the fecal homogenates. However, the cost required for such experimental plan will be shocking. Thus, we only characterized the microbiota profile of the donor mice, i.e. saline treated ND-fed mice and LZD-treated ND-fed mice, at the last FMT, which has been shown in Figure 5-8. Since the fecal homogenates contained all kinds of microbes, we thus think preparing a table with probiotics of FMT is less necessary. We appreciate the reviewer's suggestion to use a well-characterized preparation of probiotics. Yet,

identifying the potential microbe(s), regardless whether its probiotics or pathogenic, mediating the effects of LZD, is the core question of the current study.

3. *"In your model. Did you find inflammation or fibrosis?"*

We agree it would be interesting to measure inflammation status of the mice, but this is not within the immediate scope of the present study. We thus did not measure inflammatory factors. Moreover, it is well known that 16-weeks on HFD only induces hepatosteatosis in C57BL6 mice. Additionally, C57BL6 mice are insensitive to HFD-induced inflammation and fibrosis (Hebbard *et al.*, Nature Review Gastroenterology & Hepatology, 2011). Thus, we did not indend to explore inflammation or fibrosis status of the mice.

4. *"Explain the molecular mechanism by which FMT decreased lipogenic factors in livers"*

We found that the abundance of butyrate-producing bacteria has been increased by FMT. Butyrate has been reported to increase cellular oxygen consumption rate, inhibiting hepatic lipid biosynthesis. We speculate that increased butyrate levels maybe the mechanism for decreased lipogenic factors in the liver. We discussed this in the last part of the discussion, which was quoted here "In the current study, we found that HFD decreased the abundance of phyla Firmicutes and Bacteroidetes, but increased the abundance of Proteobacteria, differing with previous report. It is possible that the origin of the mice and the housing environment may have had an impact on the gut microbiota profile. Yet, we found that FMT from CR+LZD-treated mice increased the abundance of species Alistipes finegoldii, Alistipes putredinis and Bacteroides coprophilus, which belong to the phyla Bacteroidetes. Of note, a decreased abundance of Bacteroides coprophilus has been reported in MetS patients[35]. Alistipes putredinis has recently been identified as a butyrate producer in the gut[36]. Butyrate is a short-chain FA and plays an important role in maintaining the health of the colon. The main butyrate-producing bacteria are within the phylum of Firmicutes, but members of the Actinobacteria, Bacteroidetes, Fusobacteria, Proteobacteria, Spirochaetes and Thermotogae also have butyrate-synthesis pathways[36]. It is worth noting that FMT from mice treated with CR+LZD strongly increased the abundance of Thermotogae. Dietary butyrate supplementation can attenuate diet-induced obesity, insulin resistance, and hyperlipidemia in mice by activating mitochondrial functions[37,38]. Additionally, butyrate is also capable of reducing lipid secretion and lipoprotein biosynthesis in hepatic cells[39]. Interestingly, butyrate also increases OCR of epithelial cells[40]. Taken together, FMT from CR+LZD-treated mice could increase FA oxidation and limit lipid biosynthesis, by increasing the abundance of butyrate-producing bacteria in the gut. In conclusion, we report that CR in combination with LZD attenuates diet-induced obesity and hepatosteatosis by modulating the gut microbiota."

5. *"Does a leaky gut was involved in the pathogenesis? Was it a mechanism?"*

It is unlikely that under the current experimental procedure, i.e 16-weeks HFD feeding and regular intragastric gavage, would cause a leaky gut. Similar procedure and mice (C57BL6) used have previously been used in testing the effects of polysaccharides isolated from cordyceps sinensis on HFD-induced obesity (Wu et.al., Gut, 2018). No gut leaky has been noticed in the study or similar studies.

6. *"Did you make any attempt to measure inflammation (TRLs, NF-kappaB, TNF-alpha, Il-1) ? "*

We did not measure inflammation factors as the reasons stated to answer the reviewer's question #3.

7. *"The way in which results are presented is confusing (at least to me) because groups are missing in histograms and histologies. I cannot see the control (NORMAL) group, for instance, in histologies, a slice showing the normal parenchyma or normal biochemical values to compare with are missing"*

We apologize if the data presentation confused the reviewer. We did not include the histological results of the liver for ND-fed mice because under normal diet feeding these mice will not have abnormal lipid deposit. We want to focus on displaying the dramatic effects of FMT on hepatosteatosis. This way of presenting data is well accepted (please see our recent publication: Ren *et.al.*, Circulation Research, 2018).

8. *"Medicinal plants or LZD may act as prebiotics ? "*

We fully agree with the reviewer that medical plants or medicines made of medical plants, may act as prebiotics.

9. *"Despite that manuscript was edited, it contains some typos, please see the introduction section "has been show", please use abbreviations through the manuscript after defining once.*

We apologize for the type errors. We have now carefully checked the manuscript and revised accordingly. Now, the manuscript should not contain such mistakes.