Response to Reviewer 1 Comments

**Major points:**

**Point 1:** While AMPK is an important regulator of energy homeostasis, I am not convinced that it is the only player mediating the effects of soy isoflavones in adipose tissue. The same group recently published in the same journal that soy isoflavones regulated lipid metabolism in the liver via Akt/mTOR pathway. Have they checked this pathway also in this study? To further prove that AMPK is relevant in this context they could also use an AMPK inhibitor in vitro to show that this would inhibit the effects of the treatment.

**Response 1:** We appreciate the reviewer’s concerns and suggestions. We have previous shown that soy isoflavones regulate liver fatty acid metabolism through the AKT/mTOR pathway. However, we found that soy isoflavones did not appear to have an effect on AKT activity in the 3T3-L1 cells (Attached figure. 1). Besides, cording to your suggestion, we have used an AMPK-inhibitor, Compound C, to further prove that AMPK is relevant in this context, and the data are included in the revised manuscript.

![Attached figure.1 Effects of daidzein and genistein on the ATK pathway in adipocytes.](image-url)
**Point 2:** Soy isoflavones have been shown to improve lipid metabolism via AMPK in other publications (e.g. Cederroth et al. Diabetes 2008, Hwang et al. BBRC 2008). What is the novelty in their findings? Maybe this can be more pronounced in the discussion.

**Response 2:** We appreciate the reviewer’s concern, and we have already added more discussion about the novelty in the revised manuscript.

**Point 3:** Introduction, page 2: “…, the role of AMPK in adipose tissue, an important component of metabolism, is unclear.” Please be more precise – unclear in which aspect?

**Response 3:** We appreciate the reviewer’s concern, we have made this part more clearer.

**Point 4:** Intro page 2 “…, the exact mechanisms of soy isoflavones on lipid metabolism in male animals is not clear.” Why do the authors pronounce male animals? Is there a known gender differences regarding the effect of soy isoflavones?

**Response 4:** We appreciate the reviewer’s concern. The high fat diet induced male obesity model and the female ovariectomized obesity model are commonly used models for studying obesity. Female obesity models involve more complex physiological processes that challenge the successful establishment of obesity models, such as adding less fat compared to male animals [1]. Furthermore, higher estrogen levels in females and abnormal changes in estradiol levels during menstruation pose additional challenges to obesity models [2-3]. Soy isoflavones, a phytoestrogen, have a structure similar to that of estradiol [4], and have more uncertainty in the female animal obesity model. Therefore, it is more reasonable to use a male animal model.

**Point 5:** Figures in general: Information on the number of independent experiments or significance thresholds are missing in several figures.

**Response 5:** We appreciate the reviewer’s concern, and we have added relevant information in all the figures.
**Point 6:** Fig. 1C: labelling of group significance values is not intuitive

**Response 6:** We appreciate the reviewer’s concern, we have described the labeling of group significance values more clearly in the legend that “a” represents the control group, “b” represents the obesity group, “c” represents the low-dose soy isoflavones group, “d” represents the middle-dose soy isoflavones group, and “e” represents the high doses of soy isoflavones.

**Point 7:** Fig. 2D: The quality of the ORO stainings are not sufficient as most of the adipocytes are not stained at all. I would be easier to show triglyceride content of the tissues instead.

**Response 7:** We appreciate the reviewer’s concern. First, our staining results are consistent with most of the reported studies, in which a large number of fat cells are also not been stained [5-8].

**Point 8:** Fig. 3C: The control group is missing.

**Response 8:** We appreciate the reviewer’s concern. We have made this more clearer in the revised legend that the discontinue line in the graphs represent the control group.

**Minor points**

**Point 1:** Abstract page 1 line 27 might read “We also found that common components…”

**Response 1:** We appreciate the reviewer’s concern, and we have made revision.

**Point 2:** page 1 line 29: “further shown…”

**Response 2:** We appreciate the reviewer’s concern, and we have made revision.

**Point 3:** 2.2 it is not clear from the text how cell viability was measured
Response 3: We appreciate the reviewer’s concern, and we have describe the cell viability assay in more detail in Revised Manuscript 4.5.

Point 4: page 3 line 99 might read: “…in all fat depots investigated as compared to DIO rats.”

Response 4: We appreciate the reviewer’s concern, and we have made revision.

Point 5: Fig 2 legend: there are two bar sized given – which one is correct?

Response 5: We appreciate the reviewer’s concern. The area of mesenteric fat cells is relatively small, and we used a 400x image to obtain a more intuitive cell area. In addition, we used a 200x image to obtain a more intuitive epididymal fat and mesenteric fat cell area. Therefore, there are two bars for different images.

Point 6: Fig 3B: check legends: bands are either labelled with b-actin or the adipose tissue. Please label the band with the protein name.

Response 6: We appreciate the reviewer’s concern, and we have made revision.

Point 7: page 7 line 151: “…homeostasis, and has a negative…”

Response 7: We appreciate the reviewer’s concern, and we have made revision.

Point 8: Fig 4 legend: use “treatment” instead of “intervention”

Response 8: We appreciate the reviewer’s concern, and we have made revision.

Point 9: page 7, lines 169/170: check grammar

Response 9: We appreciate the reviewer’s concern, and we have made revision.

Point 10: page 6 line 194: should read SREP-1?

Response 10: We appreciate the reviewer’s concern, and we have made revision.

Point 11: page 8 line 194: There is only one study cites, not studies

Response 11: We appreciate the reviewer’s concern, and we have made revision.

Point 12: table 1: Sodium chloride

Response 12: We appreciate the reviewer’s concern, and we have made revision.
References


