Author's response to reviews

Title: AMPD1: A Novel Therapeutic Target for Reversing Insulin Resistance

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Author's response to reviews: see over
Dear Sir

Thank you very much for your kindly reviewing our manuscript for BMC Endocrine Disorders. We revised our manuscript according to the reviewers’ comments. Also, we considered the following aspect as well.

Regarding the most recent report on AMPD1 deficient mice and insulin metabolism, we added the following sentences in the discussion.

During the revision of this report, other reports indicated that AMPD1 deficient mice showed only moderate increase in AMPK activation despite increase in AMP levels and AMP/ATP ratio but no favorable metabolic phenotype in HFD challenge. We think that different protocol for HFD challenge created different results and that further study is awaited for delineating the importance of AMPD1 on insulin metabolism.

Thank you very much for your kind consideration about these matters.

Best regards,

Takayuki Morisaki
Response to the reviewer #1

Thank you very much for your kindly reviewing our manuscript.

Comment 1. Although data presented clearly indicate that p-AMPK levels are higher in skeletal muscles of A1(-/-) mice, do the authors have any other data to indicate that AMPK activity is also elevated? A corollary to this comment is to ask for more information about the specificity of the reagent that was used to generate the phosphorylation data. I feel that this may be important information for the reader because For example, while it is well established that phosphorylation at alpha-Thr(172) is linked to AMPK activation, phosphorylation at alpha-Ser(485/491) has been shown to antagonize AMPK activation in the rat heart.

>Regarding the comment, we added ACC and p-ACC levels to indicate the change of AMPK activity in the revised Figure 5. Regarding the phosphorylated position of AMPK, we showed the one at alpha-Thr(172) since antibody against p-AMPK at alpha-Thr172 was used. Although we did not test the status of AMPK phosphorylation at alpha-Ser(485/491), we thought that the up-regulation of p-AMPK at alpha-Thr172 indeed triggered the increase of p-ACC.

Also, thank you very much for your kind suggestion on our careless mistakes
2. Methods, under “Glucose and insulin tolerance,” first paragraph: “administered” is mis-spelled.
>According to the comment, the correction was made in the revised manuscript.
3. “Results and discussion” should be changed to “Results,” as the authors later preface the Discussion section with “Discussion.”
>According to the comment, the correction was made in the revised manuscript.
4. Third paragraph under Discussion: A comma is needed between “As expected” and “fat content” Also, the sentence beginning with this text is a bit long with many commas. Perhaps it could be broken up into two sentences.
>According to the comment, a comma is put between “As expected” and “fat content”.
Also, this sentence was divided into two sentences in the revision.
5. Fourth paragraph under Discussion: “faction” should be changed to “fraction”.
>According to the comment, the correction was made in the revision.
6. First sentence under Conclusions does not make sense and should be re-written for clarity:
“In conclusion, the results of this study validate AMP deaminase as potential new drug
target for the amelioration of insulin resistance treat one of the underlying causes of the metabolic syndrome and Type II diabetes.”

According to the comment, the correction was made as the following in the revision.

‘In conclusion, the results of this study validate AMP deaminase as potential new drug target for the amelioration of insulin resistance, which is one of the underlying causes of the metabolic syndrome and Type II diabetes.’

Regarding any isoform-specific inhibitor, we of course would like test such one on the effect of metabolic changes when it would be available.
Response to the reviewer #2

1. AMPD1 is inactivated only in skeletal muscle, while AMPD1 KO mice ameliorated diet-induced systemic insulin resistance. The authors mentioned in the discussion that it is unclear which organs are responsible for the improved glucose metabolism in AMPD1 KO mice. Although it is clear that skeletal muscle is one of the important insulin-targeting organs for glucose metabolism, information regarding the AMPK activity at baseline and after HFD in other organs, such as liver as liver and adipose tissue would help understanding the mechanisms by which AMPD1 KO mice improved systemic glucose metabolism. The information on glucose metabolism, such as glucose uptake and fatty acid/glucose oxidation, in each organ at baseline and after HFD would further support your findings. 

>Thank you very much for your important comments. We understand that not only skeletal muscle but liver, adipose tissue, and others have an important role for regulating glucose metabolism at baseline and after HFD. However, we found that no difference of AMPD2 mRNA expression was observed in liver between WT and AMPD1 KO mice at baseline or after HFD. Therefore, we think that the AMPK activity at baseline and after HFD in other organs is not appreciably affected by AMPD1 deficiency.

Therefore, the following discussion was added in the revised manuscript.

‘Also, besides the skeletal muscle, liver, adipose tissue, and others have an important role in regulating glucose metabolism at baseline and after HFD challenge. However, we found that no difference of AMPD2 mRNA expression was observed in liver between Wt and A1(-/-) mice at base line or after HFD challenge (supplemental Figure 2), while AMPD2 mRNA expression was slightly increased in liver of both mice after HFD. Therefore, we thought that the AMPK activity at baseline or after HFD challenge in other organs was not appreciably affected by AMPD1 deficiency.’

2. It is unclear whether endogenous AMPD1 is activated or downregulated in response to HFD. Please show the activity and expression of endogenous AMPD1 at baseline and after 12-week HFD in WT and AMPD1 KO mice.

> Thank you very much for your valuable comment. Although we thought that endogenous AMPD1 might be changed in response to HFD, AMPD1 KO mice showed very low AMPD activity and no compensatory upregulation of AMPD2 or AMPD3 mRNA was observed in muscles (data not shown). Therefore, we think that the activity and expression of endogenous AMPD1 at baseline and after 12-week HFD will not add
the important information.

However, we added the following discussion in the revised manuscript.
‘Decreased levels of IMP in skeletal muscles in A1(-/-) mice indicated that other AMPD genes were not upregulated in muscles. In fact, total AMPD activity was quite low in muscles of A1(-/-) mice as reported before [7]. Therefore, skeletal muscles in A1(-/-) mice should have striking difference in metabolic conditions if there were any change of endogenous AMPD1 at baseline and after 12-week HFD in Wt mice.’

3. Although the authors showed that IMP is significantly decreased in AMPD1 KO mice, the mechanisms of AMPK upregulation is unclear, since ATP, AMP and the ratio seem not to be altered in KO mice compared to WT mice. Does IMP directly inhibit AMPK activity? AMPD1 product, uric acid, has been reported to inhibit AMPK activity. Please discuss the mechanisms by which AMPK is activated in AMPD1 KO mice in your setting.

>Thank you very much for your valuable suggestion. We add the discussion on the possible effect for insulin signaling by the change of IMP and down-stream metabolite uric acid as the following.
‘Regarding AMPK activation in A1(-/-) mice, it could be possible that other metabolites including IMP, hypoxanthine, or uric acid in the pathway downstream to AMPD might play a role for it. Further investigation is awaited for delineation of precise mechanisms for AMPK activation in AMPD1 deficiency.’

4. The authors mentioned that AMPK activity is increased by deletion of AMPD1. Please show ACC and pACC expression levels by western blotting to support the authors' finding.

>Regarding the comment, we added ACC and p-ACC levels to indicate the change of AMPK activity in the revised Figure 5.

5. The mechanism by which the genetic deletion of AMPD1 increase leptin receptor is unclear. The authors mentioned that the enhanced activity of the leptin pathway contributes to the improvement in insulin signaling in AMPD1 KO mice. If so, the beneficial role of AMPD1 KO in insulin sensitivity would be abrogated by crossing AMPD1 KO mice with db/db mice. Please discuss this. If the result of mRNA expressions is available, also please show the mRNA expressions of other insulin signaling-related proteins, such as adiponectin, adipisin, glut4, and leptin.

>Thank you very much for your valuable suggestion. Unfortunately, we did not have
mRNA expression results on adiponectin, adipsin, glut4, or leptin, and could not show them at present. However, we added the discussion on the importance of additional experiment by crossing AMPD1 KO mice with db/db mice as the following.
‘In this regards, the crossing A1(-/-) mice with db/db mice will provide the further evidence for the interaction between AMPD activity and the leptin receptor.’

Minor
1. In Page 8 line 16, “Table 3” may be a typo. May it be “Table 2”?
> Thank you very much for your finding a typo in our manuscript. The amendment was made in the revised one.