Reviewer's report

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

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Reviewer: Junichi Sadoshima

Reviewer's report:

Using AMPD1 knock-out (KO) mice, the authors demonstrated the role of AMPD1 in diet-induced insulin resistance. AMPD1 is predominantly expressed in skeletal muscle and thus its genetic deletion provides them important information on the function of AMPD1 in skeletal muscle. In this manuscript, they clearly demonstrated that while at baseline AMPD1 KO mice had similar glucose tolerance and insulin sensitivity as wild-type (WT) control mice, AMPD1 KO mice ameliorated diet-induced glucose intolerance and insulin resistance compared with WT mice. Although diet-induced adiposity was not altered in AMPD1 KO mice, AMPK, which is usually downregulated in response to high-fat diet (HFD), was significantly activated in KO mice. They suggest that the increased activity of AMPK even under HFD confers insulin sensitivity and glucose tolerance in AMPD1 KO mice. Their findings are significant and interesting.

This reviewer has several comments that the authors should address, which are listed below.

Major

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.

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.

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.

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.

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, adipsin, glut4, and leptin.

Minor

1. In Page 8 line 16, “Table 3” may be a typo. May it be “Table 2”?

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

NO competing interest