

Dear Editors and Reviewers

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Aerobic exercise alleviates cardiac dysfunction correlated with lipidomics and mitochondrial quality control" (ID: antioxidants-3589087). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Our changes/additions to the manuscript are given in red text. Furthermore, we would like to show the details as follows.

Reviewer #2:

Q1: *How is aerobic exercise clinically relevant in Heart failure (HF)? Many HF patients are exercise intolerant. As such, improvement observed in cardiac remodeling, structure and function might not be applicable or relevant to many patients. The authors need to comment about that and also mention it in their discussion as a potential caveat.*

Response: It is the benefits and risks of exercise for patients with heart failure that need to be added to the text, as suggested by the reviewers, and we have placed comments in this regard on lines 405 through 415 of the discussion section of the manuscript.

Q2: *The conclusions drawn in Lines 211-227 that mitochondrial dynamics in HF is disrupted is far-fetched without any morphological data to support it. Decrease in OPA1 (fusion, cristae morphology regulator), increase in MFN2 (fusion) and decrease in Drp1 (fission) protein content alone is not sufficient to draw that conclusion which forms a major part of the mechanism that the authors mention. Further, in lines 295-298 the authors mention that HF indirectly exacerbates mitochondrial fragmentation (more fission then?) though decrease in OPA1 is not sufficient to come to this conclusion. As such, the authors need to measure fission/fusion and look at cristae morphology as well.*

Response: We are sorry for our oversight in the presentation of the statement. We have made changes in lines 339 to 343 in the discussion section of the manuscript. Meanwhile, since previous laboratory studies have been reported about mitochondrial cristae morphology disorders in heart failure, such as reference 27, about the morphology part we did not perform in this experiment.

Q3: *There is discrepancy in how the authors present their conclusion regarding mitochondrial dynamics as well. In lines 224-227, they mention that, "mitochondrial division in the myocardium of heart failure mice was downregulated" meaning less fission. Further, in their discussion in lines 295-296 they mention, "heart failure-induced myocardial dysfunction indirectly exacerbates mitochondrial fragmentation" meaning more fission. The authors need to explain this apparent contradiction.*

Response: We apologize very much for our inaccurate presentation, which has been reworked in accordance with the reviewer's suggestions, and the changes are highlighted in red text on lines 339 to 342 of the manuscript.

Q4: *In Figure 5, the authors figure heading reads, "Aerobic exercise restores lipidomic disturbances and cardiolipin acyl composition in left heart cardiomyocytes of heart failure mice". However, none of the presented results show any sort of significance in the data to warrant this strong conclusion. The authors need to revisit this finding to make sure that the extrapolated results support their conclusion.*

Response: We set the test period at a 10-week period in order to approximate the symptoms of heart

failure patients. This longer trial period may have caused the lipidomic metrics in Figure 5 to miss the period of significant difference. However, our Figure 4 illustrates significant differences in the phospholipids that play a key role in the improvement of heart failure with exercise, such as cardiolipin.

Q5: *What is Pyridostigmine? What is the rationale behind using it in the study and in their HF animal model? The authors need to mention this as part of their introduction rather than introducing it pretty late in the discussion.*

Response: We apologize for our missing explanations on PYR drugs. We have provided additional explanations in lines 104-107 and 448-450 of the manuscript.

Q6: *Please mention what kind of statistical measurement were used to identify significance between groups in each figure legend as well. For example, what kind of stats were used in Fig 4G to see the observed strong significance between S and HF (<0.001), HF and HP for an $n=4$? Also, please explain figure 4m-n in the text as well and elaborate a bit about the mechanism behind PG, CL, lipid peroxidation in the result section so that it is easier for reader to follow and maintain the flow of the manuscript.*

Response: We have made changes to the relevant sections.

6.1 We apologize for our statistical oversight in Figure 4G. And we have re-uploaded Figure 4.

6.2 In this statistics, we used one-way ANOVA and two-way ANOVA as our main statistical methods, with a sample size of $n=4$ for the S and HF groups in Figure 4G.

6.3 We have explained figure 4m and figure 4n in lines 202 to 203 of the manuscript and explained multiple lipids in lines 172 to 186 for better understanding.

Q7: *For section 2.4, the authors need to define a little bit about the relevance of SM, PS, PE, PC and the rationale behind assessing it should also be in the results before the discussion (322-324).*

Response: Based on the reviewer's suggestion, we have revised part 2.4 of the manuscript which is indicated in red text in lines 172 to 186 of the manuscript accordingly.

Reviewer #2:

Responses to the detailed comments are provided as follows:

Q1: *Line 56- first should be mitochondria and not mitochondrial.*

Response: We are very sorry for our incorrect writing and we have corrected it in line 64 of the manuscript.

Q2: *Fig 2 legend random B and also the authors do not have any info about F-H.*

Response: We explain Figures 2F and 2H in line 130 of the manuscript.

Q3: *Fig 3: Reorder HE, Masson and WGA so that respectively makes sense. Right now, it reads like WGA was used to measure fibrosis.*

Response: To provide a more accurate account of WGA's experimental results, we have re-explained the additional description in line 166 of the manuscript.

Q4: *Lines 272 beneficial spacing.*

Response: We appreciate your suggestion and we've redone the changes here.

Q5: *Lines 275-277 is weak and very broad. Need to be more specific about lipids and proteins.*

Response: Considering the reviewer's suggestion, we have provided a more detailed explanation in the first paragraph of the Discussion section, which is marked in red text on lines 293 to 295 and 299 to 307 of the manuscript.

Q6: *Line 356 should read modulates.*

Response: We have made changes in line 404 of the manuscript as suggested by the reviewers.