Author's response to reviews

Title: Oxidative stress in skin fibroblasts cultures from patients with Parkinson's disease

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Author's response to reviews: see over
Dear Prof. Norton:

Thank you very much for your letter regarding our article titled OXIDATIVE STRESS IN SKIN FIBROBLASTS CULTURES FROM PATIENTS WITH PARKINSON'S DISEASE.

We enclose a revised version taking in account the comments of the reviewers. In summary, we have made the following changes (highlighted in red colour):

**Reviewer's report. Carlo Ferrarese**

1) Please specify how mitochondrial proteins are extracted, since you go from total homogenates to mitochondrial proteins.

There was a typographical error in the paragraph "Respiratory chain enzymes and citrate synthase activity were measured in a DU-68 spectrophotometer (Beckman), applying 35-150 µg mitochondrial protein per 1 ml test volume." This paragraph was changed to: "Respiratory chain enzymes and citrate synthase activity were measured in a DU-68 spectrophotometer (Beckman), applying 35-150 µg of total protein per 1 ml test volume in every complex enzyme assay." This has been corrected in the text.

The mitochondrial proteins were not extracted or separated specifically from whole cell proteins. In every assay we have just measured every enzyme or molecule in the homogenate coming from a sonication process (and a previous frozen unfrozen process).

2) Please specify if all the determinations are performed within the range of linearity. 2bis) Related issue, Page 7: 35-150 ug proteins. Do you mean that you performed the experiments at different concentrations? Was it always within the linear range?

The range of linearity is defined in all our determinations in a wide range of protein concentration. So we decided to use the homogenates in order to have a total protein concentration of 35-150 micrograms per milliliter of final test
volume. This range was inside the linearity to all the activities of the OXPHOS complexes. These issues have been specified in the text.

3) Data are expressed both in Table and in Figures. Could you trim the redundancy?
Figure 1 only represents full data regarding complex V, while the tables summarized the mean ± SD of all the values included into the study. Despite we have maintained this figure in the revised version, it could be removed from the paper according to the editor’s criterion.

4) Page 4: references 34 and 35 have been inverted.
Thank you for the observation. This has been corrected.

Reviewer: Mark Cookson
1) The statistical methods should be clarified. The methods say “one-factor ANOVA test to look for significant differences and then post-hoc analyses (Bonferroni’s and Tukey’s honest test) were carried out. The results of each table were corrected for multiple comparisons by the use of Bonferroni’s correction. Correlation analyses were performed by using Pearson’s correlation coefficient.”
This is not a powerful approach. ANOVA for a single factor is usually equivalent to a straightforward group test if there is only one factor to be compared; here PD vs controls, for each enzyme measure. If the data are normally distributed, or if this appears to be a reasonable assumption then t-tests would be appropriate; if not then a non-parametric test could be used, such as Mann-Whitney.
Thank you for the comment. We checked the normality distribution of every variable and then we used the Students’s t-test for variables that followed a normal distribution and the Mann-Whitney test for the rest of variables. The manuscript was modified with the new information. The P values for COMPLEX V/CS, and COMPLEX V/protein in Table 2 were a little lower, but overall results did not change significantly.

2) I don’t see that multiple comparisons were used; each data point was used once in one comparison and it seems excessive to use a Bonferroni correction. The authors are free to disagree and use a strong correction, but it seems that this is likely to promote the rejection of significant differences. Also, I did not see any regression analyses but perhaps I missed them.
With “corrected for multiple comparisons” we meant the use of Bonferroni’s correction. We think that such a strong correction is adequate because we analyzed several factors. In addition, besides the parameters COMPLEX V/CS, and COMPLEX V/protein, none was statistically significant even before correction. Regression was not used in the study. Correlation analyses were used to analyze whether the values for enzyme activities in the PD group correlated with age at onset, duration, scores of the Unified Parkinson’s Disease Rating scales and Hoehn-Yahr staging (see the last paragraph in the results section).
3) I would suggest the discussion and results should be separated.
OK. Done.

4) Also in the introduction, some more context as to why mitochondrial function might be important in PD and where it has been measured previously would be helpful for the reader.
OK. Done.

We hope that, provided these changes, the paper would be suitable for publication in BMC Neurology

Sincerely yours:

Félix Javier Jiménez-Jiménez