

## **Endurance training and MitoQ supplementation improve spatial memory, VEGF expression, and neurogenic factors in hippocampal tissue of rats**

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Review timeline:

Received: 28 September, 2022  
Editorial decision: 28 September, 2022  
Revision received: 16 October, 2022  
Editorial decision: 21 October, 2022  
Revision received: 4 November, 2022  
Editorial decision: 4 November, 2022  
Revision received: 6 November, 2022  
Editorial decision: 8 November, 2022  
Revision received: 17 November, 2022  
Editorial decision: 19 November, 2022  
Published online: 13 December, 2022

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1<sup>st</sup> Editorial decision

28-Sept-2022

Ref.: Ms. No. JCTRes-D-22-00145

The effect of endurance training with MitoQ supplementation on improving spatial learning and gene expression of VEGF, BDNF and Sestrin2 in hippocampal tissue of Male Wistar Rats  
Journal of Clinical and Translational Research

Dear Dr. Aminizadeh,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Oct 28, 2022.

To submit a revision, go to <https://www.editorialmanager.com/jctres/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger  
Editor-in-Chief  
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: Dear authors,

Thank you for submitting this interesting study to JCTR.

I have carefully studied the manuscript and have the following comments:

MAJOR:

1) The authors measured transcript levels of several relevant genes involved in regulating various compartments of brain function. However, as meticulously explained and demonstrated in this article (<https://bmcbgenomics.biomedcentral.com/articles/10.1186/s12864-017-3683-9>), mRNA levels show poor or even inverse correlation with protein expression. Inasmuch as transcripts serve no biological function other than being a blueprint for protein synthesis, whereas proteins de facto account for all the biochemical and biological regulation in an organism, the study should measure protein levels associated with the genes of interest.

2) Based on the transcriptomic data, the authors draw all sorts of conclusions that are not rooted in actual empirical evidence but solely on known connections between protein and function. However, no functional parameters were monitored - no data on mitochondrial function, no data on oxidative stress in the brain, no data on the extent of vascularization, etc. Although some leeway can be provided for speculation, the current data sets fall short of such leeway and warrant additional experiments to be conducted as mentioned in this point.

3) In order for MitoQ to exert an effect in the brain, it must target to the brain. Consequently, the authors should demonstrate the accumulation of MitoQ in the brain using quantitative analysis (e.g., HPLC determination on brain tissue lysates).

MINOR:

- 1) The manuscript is in dire need of language polishing.
  - 2) The authors should highlight the beneficial effects in humans offered by MitoQ, which can be extrapolated from here:  
<https://pubmed.ncbi.nlm.nih.gov/?term=mitoQ&filter=pubt.clinicaltrial&sort=date&size=200>.  
The current data (after modifications as indicated in the "MAJOR" section) should subsequently be translated to a human setting, given that MitoQ is already being sold as a human food supplement. The data could therefore be reduced to practice, which makes the modified study valuable.
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Authors' response

Dear Editor and Reviewers,

Hope you are doing well.

We want to thank you for your valuable comments. I and my colleagues checked the comments, and we prepared the MS based on the comments, and resubmitted it to the journal.

Thanks you so much,

Cheers

Reviewers' comments:

Reviewer #1: Dear authors,

Thank you for submitting this interesting study to JCTR.

I have carefully studied the manuscript and have the following comments:

MAJOR:

1) The authors measured transcript levels of several relevant genes involved in regulating various compartments of brain function. However, as meticulously explained and demonstrated in this article (<https://bmcbgenomics.biomedcentral.com/articles/10.1186/s12864-017-3683-9>), mRNA levels show poor or even inverse correlation with protein expression. Inasmuch as transcripts serve no biological function other than being a blueprint for protein synthesis, whereas proteins de facto account for all the biochemical and biological regulation in an organism, the study should measure protein levels associated with the genes of interest.

We have measured the protein expression of VEGF in brain tissue, and we added the data in the MS. Unfortunately, because of the time and limitations in fund source for our project, we could not measure other factors in protein levels.

2) Based on the transcriptomic data, the authors draw all sorts of conclusions that are not

rooted in actual empirical evidence but solely on known connections between protein and function. However, no functional parameters were monitored - no data on mitochondrial function, no data on oxidative stress in the brain, no data on the extent of vascularization, etc. Although some leeway can be provided for speculation, the current data sets fall short of such leeway and warrant additional experiments to be conducted as mentioned in this point.

We have measured the tissue levels of GPx and SOD in hippocampal tissue, and reported them in MS.

3) In order for MitoQ to exert an effect in the brain, it must target to the brain. Consequently, the authors should demonstrate the accumulation of MitoQ in the brain using quantitative analysis (e.g., HPLC determination on brain tissue lysates).

We have measured the MitoQ in brain by HPLC method, and we have added the more information in the MS.

MINOR:

1) The manuscript is in dire need of language polishing.

The MS was checked grammatically carefully.

2) The authors should highlight the beneficial effects in humans offered by MitoQ, which can be extrapolated from here: <https://pubmed.ncbi.nlm.nih.gov/?term=mitoQ&filter=pubt.clinicaltrial&sort=date&size=200>. The current data (after modifications as indicated in the "MAJOR" section) should subsequently be translated to a human setting, given that MitoQ is already being sold as a human food supplement. The data could therefore be reduced to practice, which makes the modified study valuable.

We modified the MS based on the comment.

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2<sup>nd</sup> Editorial decision  
21-Oct-2022

Ref.: Ms. No. JCTRes-D-22-00145R1

The effect of endurance training with MitoQ supplementation on improving spatial learning and gene expression of VEGF, BDNF and Sestrin2 in hippocampal tissue of Male Wistar Rats  
Journal of Clinical and Translational Research

Dear Dr. Aminizadeh,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be

pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Nov 20, 2022.

To submit a revision, go to <https://www.editorialmanager.com/jctres/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger  
Editor-in-Chief  
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: Dear authors,

Thank you for submitting a revised draft of your MitoQ paper and for providing the raw data that was requested in a separate email from you.

Some concerns remain, while others still must be addressed:

1) The language needs additional polishing. Please engage a native speaker or contract a third-party service to help out. For example, Figure 1 y-axis title reads "retio", there are numerous instances of unjustifiable capitalization of words, there are no spaces between a word/value and symbol (e.g., mean±SD instead of mean ± SD, 95°C instead of 95 °C) and so forth. Your text must be in pristine shape before we can proceed with its publication. Do not use &. Moreover, do not report values to a decimal place that infers a precision not supported by the actual standard deviation. For example, the values in Table 1 do not require any decimal places in the most left outcome parameter and 1 decimal place in the central and right outcome parameters. Be consistent with notation, so 6 should read 6.0 if for all other values a single decimal point is used. There are also syntax errors, such as ameliorate used in the first sentence of Discussion should read improve.

2) The HPLC-MS data on hippocampal MitoQ concentration should be moved to the Results section as this constitutes a result. Why are no standard deviations provided? Please elaborate on the methods and provide me with the raw data.

3) Please indicate in each figure legend the sample size of each group.

4) Switch the presentation of Figure 1 and Figure 2 to make it biologically chronological (mRNA comes before protein).

5) Most importantly, the conclusions should follow the data. These are essentially as follows:

#### ET

No change relative to control: time in target quarter, BDNF, sestrin 2, SOD

Improvement relative to control: distance in target quarter, number of passes, VEGF (mRNA), VEGF (protein), GPx

#### MitoQ:

No change relative to control: BDNF, SOD

Improvement relative to control: distance in target quarter, time in target quarter, number of passes, VEGF (mRNA), VEGF (protein), sestrin 2, GPx

#### ET + MitoQ

No change relative to ET: distance in target quarter, time in target quarter, number of passes, VEGF (mRNA), VEGF (protein), BDNF, sestrin 2, GPx, SOD

Improvement relative to ET:

Ergo, your conclusion that endurance training could ameliorate spatial memory indicators is based on what? Five out of 9 indicators improved, whereas 4 of 9 did not. This is stretching it. To state that "researchers showed the effect of endurance training on improvements memory and the expression of genes involved in neurogenesis and memory (11, 28, 29)" demonstrates that your model was not entirely reproducible compared to other studies. You must present a realistic picture of your research data.

Furthermore, the statement that "our results showed that ET can increase the gene expression of BDNF in brain" is completely in conflict with the results. ET and MitoQ together did, but not ET alone. Your conclusion that "exercise training may control synaptogenesis, plasticity and neurogenesis in hippocampal tissue (40)" is a fallacy based on your data.

Finally, the last paragraph about oxidative stress is also replete with faulty reasoning. ET did not induce any oxidative stress, which would have been revealed by elevated SOD levels to counter such oxidative stress. To conclude that "in our study, MitoQ, by regulating the autophagy (22) and reducing the ROS production (43), and increasing GPx in brain can increase the Setrin2 expression, and those effects can be additive by ET, and other research showed the same increasement in antioxidant defense in hypertensive patients (36)" is really out of bounds. You did not show any data on autophagy nor ROS production, so that reference to literature is invalid. You also failed to show that, albeit ET increases GPx, ET did not increase sestrin 2 levels, so the link you are trying to establish is not based on valid and sound empirical premises.

Please modify the text so as to streamline conclusions with the data because this is unacceptable and rather surprising that scientists reason in this manner.

Thank you and the best of luck,

Michal Heger  
Editor

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Authors' response

Dear Editor and Reviewers:

Thank you so much for your valuable comments. I and my colleagues revised the MS carefully.

Please let me know if you need any further information.

Thank you so much,

Cheers,

Soheil

Reviewer #1: Dear authors,

Thank you for submitting a revised draft of your MitoQ paper and for providing the raw data that was requested in a separate email from you.

Some concerns remain, while others still must be addressed:

1) The language needs additional polishing. Please engage a native speaker or contract a third-party service to help out. For example, Figure 1 y-axis title reads "retio" (corrected), there are numerous instances of unjustifiable capitalization of words, there are no spaces between a word/value and symbol (e.g., mean±SD instead of mean ± SD, 95°C instead of 95 °C (corrected)) and so forth. Your text must be in pristine shape before we can proceed with its publication. Do not use & (corrected). Moreover, do not report values to a decimal place that infers a precision not supported by the actual standard deviation (corrected). For example, the values in Table 1 do not require any decimal places in the most left outcome parameter and 1 decimal place in the central and right outcome parameters. Be consistent with notation, so 6 should read 6.0 if for all other values a single decimal point is used (Corrected). There are also syntax errors, such as ameliorate used in the first sentence of Discussion should read improve (Corrected).

We have checked the errors and we have corrected them.

2) The HPLC-MS data on hippocampal MitoQ concentration should be moved to the Results

section as this constitutes a result. Why are no standard deviations provided?  
Please elaborate on the methods and provide me with the raw data.

We have changed the place of HPLC-MS data to results and we have added the SD to that.

In addition, I have sent the raw data (JUST MitoQ group for proving the load of MitoQ in Brain tissue) of HPLC to your email. Indeed, we do not have enough fund for measuring the MitoQ in other groups.

3) Please indicate in each figure legend the sample size of each group.

We have added the sample size of each group.

4) Switch the presentation of Figure 1 and Figure 2 to make it biologically chronological (mRNA comes before protein).

We have switched the figures.

5) Most importantly, the conclusions should follow the data. These are essentially as follows:

ET

No change relative to control: time in target quarter, BDNF, sestrin 2, SOD

Improvement relative to control: distance in target quarter, number of passes, VEGF (mRNA), VEGF (protein), GPx

MitoQ:

No change relative to control: BDNF, SOD

Improvement relative to control: distance in target quarter, time in target quarter, number of passes, VEGF (mRNA), VEGF (protein), sestrin 2, GPx

ET + MitoQ

No change relative to ET: distance in target quarter, time in target quarter, number of passes, VEGF (mRNA), VEGF (protein), BDNF, sestrin 2, GPx, SOD

Improvement relative to ET:

Corrected based on the comments.

Ergo, your conclusion that endurance training could ameliorate spatial memory indicators is based on what? Five out of 9 indicators improved, whereas 4 of 9 did not. This is stretching it. To state that "researchers showed the effect of endurance training on improvements memory and the expression of genes involved in neurogenesis and memory (11, 28, 29)" demonstrates that your model was not entirely reproducible compared to other studies. You must present a realistic picture of your research data.



Furthermore, the statement that "our results showed that ET can increase the gene expression of BDNF in brain" is completely in conflict with the results. ET and MitoQ together did, but not ET alone. Your conclusion that "exercise training may control synaptogenesis, plasticity and neurogenesis in hippocampal tissue (40)" is a fallacy based on your data.

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**Corrected.**

Please modify the text so as to streamline conclusions with the data because this is unacceptable and rather surprising that scientists reason in this manner.

Thank you and the best of luck,

Michal Heger  
Editor

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3<sup>rd</sup> Editorial decision  
04-Nov-2022

Ref.: Ms. No. JCTRes-D-22-00145R2

The effect of endurance training with MitoQ supplementation on improving spatial learning and gene expression of VEGF, BDNF and Sestrin2 in hippocampal tissue of Male Wistar Rats  
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at <http://www.jctres.com/en/author-guidelines/> under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Dec 04, 2022.

To submit a revision, go to <https://www.editorialmanager.com/jctres/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger  
Editor-in-Chief  
Journal of Clinical and Translational Research

Reviewers' comments:

Dear authors,

Please see my previous comment regarding language editing.

Note again that I will not accept poorly written manuscripts or manuscripts that require language editing for valid reasons.

We are trying to manage a professional, high-standard journal.

Michal Heger  
Editor

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4th Editorial decision  
08-Nov-2022

Ref.: Ms. No. JCTRes-D-22-00145R3

The effect of endurance training with MitoQ supplementation on improving spatial memory, protein expression of VEGF, and gene expression of some factors involved in neurogenesis in hippocampal tissue of Male Wistar Rats  
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at <http://www.jctres.com/en/author-guidelines/> under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Dec 08, 2022.

To submit a revision, go to <https://www.editorialmanager.com/jctres/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger  
Editor-in-Chief  
Journal of Clinical and Translational Research

Reviewers' comments:

please engage a native speaker

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5th Editorial decision  
19-Nov-2022

Ref.: Ms. No. JCTRes-D-22-00145R4  
Endurance training and MitoQ supplementation improve spatial memory, VEGF expression, and neurogenic factors in hippocampal tissue of rats  
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Please notify our assistant editor/production editor when you receive the proofs if your article should belong to a special issue specifying the issue's title.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger  
Editor-in-Chief  
Journal of Clinical and Translational Research

Comments from the editors and reviewers: