T cells mediate cell non-autonomous arterial aging in mice

Daniel W Trott, Daniel R Machin, Tam TT Phuong, Adelola O Adeyemo, Samuel I Bloom, R Colton Bramwell, Eric Sorensen, Lisa A Lesniewski, and Anthony J Donato
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The following individual(s) involved in review of this submission have agreed to reveal their identity: Tom Longden (Referee #2); Calum Wilson (Referee #3)

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Senior Editor: Harold Schultz

Reviewing Editor: Calum Wilson

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 19-Apr-2021

Dear Dr Trott,

Re: JP-RP-2021-281698X "T cells mediate cell non-autonomous arterial aging in mice" by Daniel W Trott, Daniel R Machin, Tam TT Phuong, Adelola O Adeyemo, Samuel I Bloom, R Colton Bramwell, Eric Sorensen, Lisa A Lesniewski, and Anthony J Donato

Thank you for resubmitting your revised Research Paper to The Journal of Physiology. It has been assessed by the original Reviewing Editor and Referees and has been well received. Some final revisions have been requested.

Please advise your co-authors of this decision as soon as possible.

The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made.

I hope you will find the comments helpful and have no difficulty returning your revisions within one week.

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I look forward to receiving your revised submission.

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Yours sincerely,

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-If n ÃƒÆ'Ã‚Â¢ÃƒÂ¢Ã¢â€šÂ¬Ã‚Â{degree sign}Ãƒâ€šÃ‚Â¤ 30, all data points must be plotted in the figure in a way that reveals their range and distribution. A bar graph with data points overlaid, a box and whisker plot or a violin plot (preferably with data points included) are acceptable formats.

-If n > 30, then the entire raw dataset must be made available either as supporting information, or hosted on a not-for-profit repository e.g. FigShare, with access details provided in the manuscript.

-'n' clearly defined (e.g. x cells from y slices in z animals) in the Methods. Authors should be mindful of pseudoreplication.

-All relevant 'n' values must be clearly stated in the main text, figures and tables, and the Statistical Summary Document (required upon revision)

-The most appropriate summary statistic (e.g. mean or median and standard deviation) must be used. Standard Error of the Mean (SEM) alone is not permitted.

-Exact p values must be stated. Authors must not use 'greater than' or 'less than'. Exact p values must be stated to three significant figures even when 'no statistical significance' is claimed.

-Statistics Summary Document completed appropriately upon revision

-A Data Availability Statement is required for all papers reporting original data. This must be in the Additional Information section of the manuscript itself. It must have the paragraph heading "Data Availability Statement". All data supporting the results in the paper must be either: in the paper itself; uploaded as Supporting Information for Online Publication; or archived in an appropriate public repository. The statement needs to describe the availability or the absence of shared data. Authors must include in their Statement: a link to the repository they have used, or a statement that it is available as Supporting Information; reference the data in the appropriate sections(s) of their manuscript; and cite the data they have shared in the References section. Whenever possible the scripts and other artefacts used to generate the analyses presented in the paper should also be publicly archived. If sharing data compromises ethical standards or legal requirements then authors are not expected to share it, but must note this in their Statement. For more information, see our Statistics Policy.

EDITOR COMMENTS

Reviewing Editor:

Ethics Concerns:
Authors need to ensure that the paper complies with the animal guidelines and have been instructed to do so.

Comments to the Author:
Trott et al present a revision of their manuscript reporting on the involvement of immune responses in the vascular dysfunction that accompanies old age. In this work, the authors demonstrate increased T cell accumulation and vascular dysfunction in the conduit and resistance arteries of aged mice (increased artery stiffness and impaired vasodilation). The authors go on to present data showing that the impairment of artery function is absent after pharmacological of T cells, and confirm some of the findings is a genetic model (RAG-1-/- mice). Whilst the exact nature of T-cell mediated vascular dysfunction are not elucidated, the authors hint that this may be due to inflammatory insult altering artery structure and reducing nitric oxide bioavailability.

The authors have addressed the majority of previous concerns listed by both reviewers. However, there is still a lingering concern regarding the pressure myography data. The authors will be aware that the method use to constrict isolated blood vessels critically determines the contribution of various endothelial cell vasodilator pathways (e.g., see 10.1113/JP274797). However, as noted by referee 1, it appears from the text that the authors have grouped together both myogenically and non-myogenically active vessels. Usually, vascular responses are assessed separately in vessels that develop spontaneous tone or where tone is induced with a smooth muscle cell agonist. The author's must satisfactorily address this important point, and this may require additional experimentation.

The authors must also provide ensure that their paper is in full compliance with the Journal of Physiology Animal Ethics policy (https://physoc.onlinelibrary.wiley.com/hub/animal-experiments). As an example, no information is given regarding their animal's access to food and water, euthanasia

Senior Editor:

Comments for Authors to ensure the paper complies with the Statistics Policy:

Authors must define what sample size n represents in the methods text, tables, and figures legends. Tables and figures need to report sample sizes. Sample size should represent an independent sampling of animals, not within-animals replications. In addition, methods need to explicitly state that variance is the standard deviation of the mean.

Figures 1-6 need to show the measure of variance, along with the data points. Either as SD in a bar graph or interquartile ranges in box whisker plots.

If the statistical summary document has errors please describe what is incorrect:
Authors need to define 'n' in the table

Comments to the Author:

The authors will need to provide a proper definition of sample size throughout, fully address all journal animal ethics policies (e.g. we could find no description of euthanasia!), and provide the measure of variance in the figures. The authors will also need to address the grouping of the isolated blood vessels. This would entail a scientificly valid reason to group both myogenically and non-myogenically active vessels (which appears to be counter to published standards) or split these datasets so that myogenically active vessels and those constricted with phenylephrine are analyzed separately. This may require additional experimentation.

REFEREE COMMENTS

Referee #2:

Thank you for taking the time to address previous comments.

There remains a lingering concern over the myography data. If I interpret the new text correctly, the authors are mixing data from vessels that developed myogenic tone with those that did not develop tone so instead they were preconstricted with phenylephrine. These two different states of the vessel have the potential for the balance of a number of signaling pathways in the smooth muscle and endothelium to be markedly different. Given that aged mice have a blunted response, my interpretation of the new description is that the authors used this maneuver more in old mice than in young mice, but still compare the responses of these two groups in Figure 7.

If this is correct, this seems like an unfair comparison to make, as the starting conditions are not the same in each group. Please further clarify exactly what the basis of constriction are for these groups. If they are mixed as I interpret above, please repeat these experiments with either preconstricted vessels only, or with those that developed tone - the latter being much preferred.

END OF COMMENTS

We appreciate the general enthusiasm of the editors and reviewers for our manuscript and we have revised the manuscript to address their remaining critiques. Our specific responses are described in detail below.

EDITOR COMMENTS

The authors have addressed the majority of previous concerns listed by both reviewers. However, there is still a lingering concern regarding the pressure myography data. The authors will be aware that the method use to constrict isolated blood vessels critically determines the contribution of various endothelial cell vasodilator pathways (e.g., see 10.1113/JP274797). However, as noted by referee 1, it appears from the text that the authors have grouped together both myogenically and non-myogenically active vessels. Usually, vascular responses are assessed separately in vessels that develop spontaneous tone or where tone is induced with a smooth muscle cell agonist. The author's must satisfactorily address this important point, and this may require additional experimentation.

The editors and reviewers raise an important point. All arteries were preconstricted with phenylephrine with the exception of two old wild type arteries which exhibited greater than 20% myogenic tone prior to the sodium nitroprusside dose response shown in Fig 9E. The SNP dose response was not different from those in the group that did not exhibit myogenic tone and removal of these two arteries from the dataset did not alter the conclusion that there were no group differences between young wild type, old wild type, young Rag-1-/- mice and old Rag-1-/- mice. Further because all arteries were preconstricted with phenylephrine prior to Acetylcholine both with and without L-NAME or TEMPOL the conclusions drawn from these data are not altered either. We have revised the methods to read, "unless noted in the results, arteries were preconstricted using phenylephrine (≤ 2 μM)" and indicated in the text of the results the arteries which were not preconstricted.

The authors must also provide ensure that their paper is in full compliance with the Journal of Physiology Animal Ethics policy (https://physoc.onlinelibrary.wiley.com/hub/animal-experiments). As an example, no information is given regarding their animal's access to food and water, euthanasia

We have added information regarding food, water and euthanasia to the methods section.

Senior Editor:

Comments for Authors to ensure the paper complies with the Statistics Policy:

Authors must define what sample size n represents in the methods text, tables, and figures legends. Tables and figures need to report sample sizes. Sample size should represent an independent sampling of animals, not within-animals replications. In addition, methods need to explicitly state that variance is the standard deviation of the mean.

We have revised the text of the manuscript and figure legends accordingly.

Figures 1-6 need to show the measure of variance, along with the data points. Either as SD in a bar graph or interquartile ranges in box whisker plots.

We have added error bars indicating standard deviation to all panels of figures 1-6.

If the statistical summary document has errors please describe what is incorrect:

Authors need to define 'n' in the table

We have revised the statistical summary document to indicate that n is the number of animals for each experiment unless otherwise noted.

Comments to the Author:

The authors will need to provide a proper definition of sample size throughout, fully address all journal animal ethics policies (e.g. we could find no description of euthanasia!), and provide the measure of variance in the figures. The authors will also need to address the grouping of the isolated blood vessels. This would entail a scientificly valid reason to group both myogenically and non-myogenically active vessels (which appears to be counter to published standards) or split these datasets so that myogenically active vessels and those constricted with phenylephrine are analyzed separately. This may require additional experimentation.

We have revised the manuscript according to the comments above addressing each of these issues.

REFEREE COMMENTS

Referee #2:

Thank you for taking the time to address previous comments.

There remains a lingering concern over the myography data. If I interpret the new text correctly, the authors are mixing data from vessels that developed myogenic tone with those that did not develop tone so instead they were preconstricted with phenylephrine. These two different states of the vessel have the potential for the balance of a number of signaling pathways in the smooth muscle and endothelium to be markedly different. Given that aged mice have a blunted response, my interpretation of the new description is that the authors used this maneuver more in old mice than in young mice, but still compare the responses of these two groups in Figure 7.

If this is correct, this seems like an unfair comparison to make, as the starting conditions are not the same in each group. Please further clarify exactly what the basis of constriction are for these groups. If they are mixed as I interpret above, please repeat these experiments with either preconstricted vessels only, or with those that developed tone - the latter being much preferred.

The reviewer raises an important point. Please see our response to the editor above. We have also revised the methods to indicate that arteries that did not exhibit ≥ 20% constriction from their peak diameter in response to phenylephrine were discarded. The mean and SD of preconstriction percentages are reported in Tables 2 & 3.

END OF COMMENTS

1st Revision - Editorial Decision 26-May-2021

Dear Dr Trott,

Re: JP-RP-2021-281698XR1 "T cells mediate cell non-autonomous arterial aging in mice" by Daniel W Trott, Daniel R Machin, Tam TT Phuong, Adelola O Adeyemo, Samuel I Bloom, R Colton Bramwell, Eric Sorensen, Lisa A Lesniewski, and Anthony J Donato

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert Referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

Please advise your co-authors of this decision as soon as possible.

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I look forward to receiving your revised submission.

If you have any queries please reply to this email and staff will be happy to assist.

Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

See comments from referees below. For consistency, the authors are asked to remove the two data points corresponding to the arteries that developed myogenic tone.

REFEREE COMMENTS

Referee #2:

Thank you for addressing this comment. I suggest removal of the two data points in question entirely for consistency between experiments and removal of the final sentence from the Results referring to this.

Referee #3:

The authors have clarified the methods used to constrict the blood vessels and point out that only two vessels developed myogenic tone (shown in Figure 9E). Removal of these datapoints did not affect the outcome of the statistical analyses. However, the authors have chosen to keep the results in the datasets.

To avoid confusion, I believe it would be simpler if these datapoints were simply removed from the manuscript. As it stands, the inclusion of this data means the authors have grouped data looking at two very different methods of artery constriction.

END OF COMMENTS

Response to reviewers: **In response to the editor and reviewer comments, we have removed the data points and reference to them from the manuscript. We appreciate the reviewers' and editor's time and careful consideration of our manuscript.**

EDITOR COMMENTS

Reviewing Editor:

See comments from referees below. For consistency, the authors are asked to remove the two data points corresponding to the arteries that developed myogenic tone.

REFEREE COMMENTS

Referee #2:

Thank you for addressing this comment. I suggest removal of the two data points in question entirely for consistency between experiments and removal of the final sentence from the Results referring to this.

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The authors have clarified the methods used to constrict the blood vessels and point out that only two vessels developed myogenic tone (shown in Figure 9E). Removal of these datapoints did not affect the outcome of the statistical analyses. However, the authors have chosen to keep the results in the datasets.

To avoid confusion, I believe it would be simpler if these datapoints were simply removed from the manuscript. As it stands, the inclusion of this data means the authors have grouped data looking at two very different methods of artery constriction.

2nd Revision - Editorial Decision 21-Jun-2021

Dear Dr Trott,

Re: JP-RP-2021-281698XR2 "T cells mediate cell non-autonomous arterial aging in mice" by Daniel W Trott, Daniel R Machin, Tam TT Phuong, Adelola O Adeyemo, Samuel I Bloom, R Colton Bramwell, Eric Sorensen, Lisa A Lesniewski, and Anthony J Donato

I am pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

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EDITOR COMMENTS

Reviewing Editor:

In previous review, author's were asked to remove two data points and this change has now been made and the manuscript updated. It is this editor's opinion that the manuscript is suitable for publication in The Journal of Physiology.
