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Cyclically producing the Same average muscle-tendon force with a smaller duty increases metabolic rate

Owen N. Beck, Jonathan Gosyne, Jason R. Franz and Gregory S. Sawicki

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Review timeline

Original submission:	25 February 2020
1st revised submission:	1 June 2020
2nd revised submission:	22 July 2020
Final acceptance:	23 July 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2020-0431.R0 (Original submission)

Review form: Reviewer 1 (Natalie Holt)

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Acceptable

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
Yes

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
No

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
Please see attached file. (See Appendix A)

Review form: Reviewer 2 (J Donelan)

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Max Donelan
END

Decision letter (RSPB-2020-0431.R0)

03-Apr-2020

Dear Dr Beck:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

The quite constructive reviewers' comments are mainly presentational/stylistic rather than deeply scientific but nonetheless substantial improvements to the MS are needed to make it more accessible to the broad audience of Proc B.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:

If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr John Hutchinson, Editor
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
Associate Editor: Doug Altshuler
This is an interesting manuscript containing creative experiments that examine the interface between muscle force dynamics and energetic cost. It has broad appeal potentially, but in its current form, the manuscript suffers from organization and writing issues that will make it difficult for most readers to appreciate. I would like to see if the authors can revise the work so that the presentation matches the quality of the science.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
Please see attached file

Referee: 2

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Author's Response to Decision Letter for (RSPB-2020-0431.R0)

See Appendix B.

RSPB-2020-0431.R1 (Revision)

Review form: Reviewer 1 (Natalie Holt)

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
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Quality of the paper: Is the overall quality of the paper suitable?
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Is the length of the paper justified?
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Should the paper be seen by a specialist statistical reviewer?
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Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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Is it accessible?
N/A

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Is it adequate?
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Do you have any ethical concerns with this paper?
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Comments to the Author
I found this revised manuscript to be a major improvement over the first submission. I still have some substantial suggestions for revision, but would like to leave it up to you (the authors) as to whether you feel these revisions are necessary. I do think that they will increase the readability of the paper, and ultimately its impact on the field. So on behalf of future readers, please consider making them.

GENERAL COMMENTS

1. You make an interesting point about muscle mechanics or fibre type explaining the increase in metabolic cost with the shortening of duty factor. And you write that accounting for activity duration (a proxy for fibre type) doesn't improve your ability to explain increases in metabolic cost, which ran against your hypothesis. This is all fine but I want to propose a different way to frame your experiment as it relates to this question. I suggest a) proposing both mechanics and fibre type as possible contributors to increase in cost with decrease in duty factor, and then b) proposing to study soleus in isolation as a way to control for fibre type (because it is homogeneously slow twitch), thus focusing on just mechanics. In this way, the focus on soleus becomes a creative part of the study design that allows you to more effectively study muscle mechanics in isolation. Sure, you are doing this after the fact but from the readers point of view, it will help understand that your results are about mechanics and not fibre type. It will also help avoid the conclusion that fibre type doesn't matter as that is outside of your ability to test in this experimental design.

2. I found the introduction pretty unreadable with its many equations. While I love my math, this particular flow of equations didn't help with explaining your central points. I suggest you put them in the supp material except for the ones that you actually need and use later on (which by my count was very few of them). In its place, I suggest you put supplementary Figure 2 into the main paper as it is more effective at doing what the equations are trying to do. I would include in that figure an indication of increased active muscle volume and then how muscle volume maps on to met cost. It is OK for it to be conceptual rather than driven by calculations. And then in the intro text, I would describe in a single paragraph what the equations try to do. I think it is something like this: "To achieve the same average force over a complete cycle, but with shorter duty factors, muscles have to generate greater forces. But these muscles have a tendon in series--the tendon applies the forces to the bones so muscle has to stretch the tendon to longer lengths to get the higher tendon forces required to generate higher external forces. Greater tendon stretch means greater muscle fibre shortening. This fibre shortening reduces the ability of the muscle fibres to generate force requiring more muscle fibres--or active muscle volume--to achieve the same instantaneous level of force. It reduces the force generation ability of muscle fibres for two reasons. The first is due to the force-length relationship. Assuming that fibres are at their optimal length for force generation when the tendon is slack, great fibre shortening moves them leftward on their force-length relationship and away from their optimal length--more active fibres are now required to achieve the same force. The second is due to the force-velocity relationship. A greater distance of fibre shortening and over a shorter period of time (because duty factor is shorter) results in faster shortening velocities. Muscles are weaker at faster velocities requiring more active fibres to achieve the same force." And so on. One thing about this that I still wonder about in this logic is that while it is clear that this increases the active muscle volume instantaneously, it is harder to see that when integrated over a cycle it will still result in greater time-integrated active muscle volume. But I do think this to be true because without these effects, decreasing duty factor results in increased external force during the duty and thus increased active muscle volume during the duty. But then after the duty you have no active muscle volume for longer

and I think everything mathematically cancels to have no effect on active muscle volume unless you include the effects of length and velocity on force potential (I hope that this is clear).

3. That LG is not contributing to the measured metabolic cost is still not convincing.

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SPECIFIC COMMENTS
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L31: I always report the p-value and not just that it is less than some value. $p<0.0X$ is hold over from when we had to look up the values in the backs of textbooks.

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L259: 80 cm?

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L330: Great topic sentence. I do wonder why we should ever care about isometric active muscle volume in your work.

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L362: Not a constant vertical GRF.

L407: "Healthy" is not a good choice of word here.

END

Max Donelan

Decision letter (RSPB-2020-0431.R1)

01-Jul-2020

Dear Dr Beck

I am pleased to inform you that your manuscript RSPB-2020-0431.R1 entitled "Duty factor affects muscle contractile mechanics, active muscle volume, and metabolic cost during cyclic muscle-tendon force generation" has been accepted for publication in Proceedings B. Congratulations!!

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To revise your manuscript, log into https://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as ‘tracked changes’ to be included in the ‘response to referees’ document.

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a ‘data accessibility’ section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

6) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Dr John Hutchinson
Editor, Proceedings B
mailto:proceedingsb@royalsociety.org

Associate Editor:
Board Member: 1
Comments to Author:
Associate Editor: Doug Altshuler

The referees and I agree that this manuscript provides new insight about the metabolic consequences of cyclic force generation. This work will be of broad interest to the readership at Proceedings B. Both referees have provided some additional suggestions that I ask you to consider carefully as you prepare the final document for us to send to the press.

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s)
I found this revised manuscript to be a major improvement over the first submission. I still have some substantial suggestions for revision, but would like to leave it up to you (the authors) as to whether you feel these revisions are necessary. I do think that they will increase the readability of the paper, and ultimately its impact on the field. So on behalf of future readers, please consider making them.

GENERAL COMMENTS

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END

Max Donelan

Referee: 1

Comments to the Author(s)
Please see attached

Author's Response to Decision Letter for (RSPB-2020-0431.R1)

See Appendix D.

Decision letter (RSPB-2020-0431.R2)

23-Jul-2020

Dear Dr Beck

I am pleased to inform you that your manuscript entitled "Cyclically Producing the Same Average Muscle-Tendon Force with a Smaller Duty Increases Metabolic Rate" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

You are allowed to post any version of your manuscript on a personal website, repository or preprint server. However, the work remains under media embargo and you should not discuss it with the press until the date of publication. Please visit https://royalsociety.org/journals/ethics-policies/media-embargo for more information.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

This study addresses the question of whether the way in which a given force-time integral is generated by muscle influences the metabolic cost incurred. This question is critical to understanding the mechanics and energetics of terrestrial locomotion. This study focuses on how the time course of a given impulse (and therefore the peak force) alters the metabolic cost incurred and demonstrates that, at least for higher forces, a shorter duration and higher peak force incurs a greater metabolic cost. This is a beautifully designed experiment and potentially an interesting result. However, I have some experimental and rhetorical concerns that should be addressed.

The cost of force vs work – the authors pitch their argument in terms of the cost of generating force to support bodyweight. While I don't necessarily disagree with this, it might be more representative of the field (and the findings of this paper) to bring in a discussion of work earlier in the paper. I leave it to the author's discretion as to how this is achieved but I might recommend things like:

- A brief study of the energetics literature highlighting those focusing on force (largely Kram et al) and work (mostly everyone else) in the paragraph starting line 49. This could certainly end with the conclusion that force may be the dominant determinant of cost (Arellano and Kram, 2014 - partitioning the metabolic cost of human running)
- Including a calculation of work, and its role in cost, in the results rather than just as part of the discussion. I appreciate the way in which the authors ultimately handled the unavoidable greater fascicle shortening in brief contractions, but I'd totally written off the main result as being confounded by this until I got to the very end. It would seem preferable to me to have this more upfront and to directly quantify work in the results section.

Contributions of synergistic muscles – while this is unavoidable, I find it to be somewhat concerning. The authors suggest that the lack of variation in ankle joint moment over a range of knee angles means that the biarticular muscles are not contributing, and so everything can be attributed to soleus. While this may be valid for force, it seems less valid for energetics. LG is certainly being activated and so consuming some metabolic energy in calcium handing and doing internal crossbridge work, even if force is not being generated at the ankle joint. The authors attempt to deal with this by showing a lack of a significant difference in LG activation across soleus activation durations. However, there appears to be a suggestion of a trend towards greater LG activation during brief soleus activations ($p=0.069$; Fig. 4d). This could certainly contribute to the increased cost, particularly as it would likely reflect recruitment of more expensive faster fibers.

- I would suggest that the authors acknowledge this as a potential contributor to their main finding. I do not feel this detracts from the message of the paper. If anything, it lends some qualitative support to the rate of force generation hypothesis proposed by Kram and Taylor (1990) and somewhat reiterated here.

The mechanistic rationale – Throughout the paper, the authors raise interesting and convincing reasons for why cost would change with activation duration. However, I find them to be a little scattered and not well related to what is being tested in this system. Mechanisms need to more thoroughly described in relation to this study throughout. A few examples:

- Line 50-51 Given the later discussion of Ca^{2+} costs, it would seem valuable to mention it here (Homsher et al., 1972 etc).

- Line 60-62 I think there's maybe a distinction to be made between the rate at which muscle needs to be turned on and off to achieve the required force in the available time (which is largely a fiber type, and maybe a Ca^{2+}, argument) and the steady state cost of force at different activation levels. I think the arguments here largely pertain to the former, in which case the paper might benefit from a more explicit and consistent discussion of these factors throughout.
- If one of the goals of this paper is to test the hypothesis that faster fibers being recruited in brief contractions increases cost, and that the soleus really is the dominant force producer and energy consumer, to what extent does its relatively slow fiber type composition of this muscle limit the findings of the paper?

Terminology – I initially had a really hard time trying to figure out what was being varied. I find the description of a shorter activation time with a constant activation-deactivation time to be really confusing. I would refer to activation-deactivation time as the time the muscle takes to be turned on and then off (which is variable across conditions). Whereas here the authors seem to be using it to describe the whole contraction cycle? Given that keeping the force-time integral and contraction frequency constant whilst varying the amount of time the muscle is on is such as nice controlled replica of terrestrial locomotion with varying duty factors, it might be helpful to draw parallels to, and terminology from, that? I would certainly replace activation-deactivation time with something more like contraction frequency.

Figures/ stats – the *in vivo* nature of these experiments makes it hard to control all the variables. Hence, there is a lot of sorting through effects in the statistics. I think this is largely done well. However, I'm having a bit of a hard time following what the * is indicating in figures. It might be helpful to more explicitly include models and p values in figure legends? Or be clearer about this somewhere.

Line 45 - 'separate their independent contributions on metabolism' typo?
Line 294 – 'combining the results from our study to that of the literature' typo?

Appendix B

We thank Drs. Doug Altshuler, Max Donelan, and Referee 1 for their positive comments and exquisite suggestions that helped us improve our manuscript. Following their questions, comments, and suggestions, we heavily updated the manuscript's story to better communicate our science. Notably, we heavily re-organized the introduction, added many equations and figures to explain our rationale, contextualized the results section text, and focused our story more heavily on duty factor and active muscle volume vs. metabolic energy expenditure. Also noteworthy, our manuscript now leans on the terminology and theory of the 'cost of generating force' hypothesis [Kram & Taylor; *Nature* 1990]. For example, we now use 'cycle average force' rather than 'force-time integral' and our results/figures reflect these changes. We feel that our new terminology is more familiar with our readership. Please note that due to length constraints of the journal, we now have a supplementary material section that includes two discussion sections, 3 supplementary figures, and 1 supplementary table.

We responded to each comment below via underlined text.

Associate Editor: Doug Altshuler
This is an interesting manuscript containing creative experiments that examine the interface between muscle force dynamics and energetic cost. It has broad appeal potentially, but in its current form, the manuscript suffers from organization and writing issues that will make it difficult for most readers to appreciate. I would like to see if the authors can revise the work so that the presentation matches the quality of the science.

Thank you for the positive comments.

We updated the manuscript to better communicate the science.

Referee 1

This study addresses the question of whether the way in which a given force-time integral is generated by muscle influences the metabolic cost incurred. This question is critical to understanding the mechanics and energetics of terrestrial locomotion. This study focuses on how the time course of a given impulse (and therefore the peak force) alters the metabolic cost incurred and demonstrates that, at least for higher forces, a shorter duration and higher peak force incurs a greater metabolic cost. This is a beautifully designed experiment and potentially an interesting result. However, I have some experimental and rhetorical concerns that should be addressed.

Thank you. We hope that we addressed your experimental and rhetorical concerns below.

The cost of force vs work – the authors pitch their argument in terms of the cost of generating force to support bodyweight. While I don't necessarily disagree with this, it might be more representative of the field (and the findings of this paper) to bring in a discussion of work earlier in the paper. I leave it to the author's discretion as to how this is achieved but I might recommend things like:

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To focus our manuscript on the metabolic cost of generating force, we followed your advice and introducing the relevant energetics literature. But, rather than diving into the mechanical work's association to metabolic energy expenditure, we expanded on the importance of considering the body's net mechanical work during locomotion (*e.g.*, ~0 during level ground steady-state locomotion). Then, we dove into the influence of how muscle operating length and contraction velocity affects active muscle volume and metabolic energy expenditure. Notably, we still have a supplementary material discussion paragraph that links muscle mechanical work to metabolic energy expenditure.

- Including a calculation of work, and its role in cost, in the results rather than just as part of the discussion. I appreciate the way in which the authors ultimately handled the unavoidable greater fascicle shortening in brief contractions, but I'd totally written off the main result as being confounded by this until I got to the very end. It would seem preferable to me to have this more upfront and to directly quantify work in the results section.

We agree that only highlighting average force and the duration of active force generation's influence on metabolic energy expenditure in our intro-results is an incomplete story. As you mentioned, shorter duty factors incur greater peak forces, which cause tendons to stretch more and cause the muscles to operate at shorter lengths and faster velocities. To reconcile how muscle fascicle length change affects our hypotheses, we reorganized our manuscript to highlight how duty factor, muscle operating length, and contraction velocity affect the volume of muscle activated to generate force. We now include muscle length and velocity's influence on the volume of muscle activated to generate force and its yielding metabolic rate throughout the introduction, methods, results, and conclusion.

Due to the flow and length of our new story, we put the muscle mechanical work paragraph in the supplementary material discussion. However, because we now highlight muscle length and velocities changes throughout the manuscript, we are upfront about how changing fascicle length may affect metabolic power.

Contributions of synergistic muscles – while this is unavoidable, I find it to be somewhat concerning. The authors suggest that the lack of variation in ankle joint moment over a range of knee angles means that the biarticular muscles are not contributing, and so everything can be attributed to soleus. While this may be valid for force, it seems less valid for energetics. LG is certainly being activated and so consuming some metabolic energy in calcium handing and doing internal crossbridge work, even if force is not being generated at the ankle joint. The authors attempt to deal with this by showing a lack of a significant difference in LG activation across soleus activation durations. However, there appears to be a suggestion of a trend towards greater LG activation during brief soleus activations ($p=0.069$; Fig. 4d). This could certainly contribute to the increased cost, particularly as it would likely reflect recruitment of more expensive faster fibers.

• I would suggest that the authors acknowledge this as a potential contributor to their main finding. I do not feel this detracts from the message of the paper. If anything, it lends some qualitative support to the rate of force generation hypothesis proposed by Kram and Taylor (1990) and somewhat reiterated here.

We agree that it is important to acknowledge the potential contributor of synergistic muscles (such as the gastrocnemius) on metabolic energy expenditure. In our updated manuscript, dividing total active muscle volume by the duration of active force production did not improve the relationship between biomechanics and metabolic energy expenditure. We discussed this finding below:

Lines 405-416: In our study, the duration of active force generation (*i.e.*, an approximation of muscle fiber economy [9, 10, 23, 26, 27]) may not have improved the relationship between biomechanics to metabolic energy expenditure because our studied muscle, the soleus, has a relatively homogenous muscle fiber composition [57] – one of our many potential limitations (see supplementary material for additional potential limitations). Thus, decreasing the duration of active force production may only recruit slightly less economical soleus muscle fibers and not elicit a measureable metabolic effect. Yet, decreasing the duration of active force production increased lateral gastrocnemius activation (Fig. 6), which has a healthy mixture of slower/more economical and faster/less economical muscle fibers [57]. Therefore, the short durations of active force production likely required multiple triceps surae muscles to activate a greater quantity of less economical muscle fibers compared to the long durations of active force production.

The mechanistic rationale – Throughout the paper, the authors raise interesting and convincing reasons for why cost would change with activation duration. However, I find them to be a little scattered and not well related to what is being tested in this system. Mechanisms need to more thoroughly described in relation to this study throughout. A few examples:

We agree and feel that our updated manuscript better communicates the mechanisms between biomechanical changes and the resulting metabolic energy expenditure.

• Line 50-51 Given the later discussion of Ca2+ costs, it would seem valuable to mention it here (Homsher et al., 1972 etc).

We now focus our story on the duration of active force production and duty factor, and we removed the explicit discussions of how cyclically activating-deactivating muscles at faster rates affects metabolic energy expenditure. Still, we added a sentence to our introduction stating that that faster muscle fibers may be less economical than slower muscle fibers partly due to greater calcium pumping.

Lines 84-86 Faster muscle fibers are presumably less economical than slower fibers due to their greater ATP utilization for calcium pumping [32] and cross-bridge cycling [31].

• Line 60-62 I think there's maybe a distinction to be made between the rate at which muscle needs to be turned on and off to achieve the required force in the available time (which is largely a fiber type, and maybe a Ca2+, argument) and the steady state cost of force at different activation levels. I think the arguments here largely pertain to the former, in which case the paper might benefit from a more explicit and consistent discussion of these factors throughout.

This is a great point. Accordingly, we updated our wording to clarify that we studied the effects of the duration of active force production rather than 'muscle activation time' throughout the manuscript.

• If one of the goals of this paper is to test the hypothesis that faster fibers being recruited in brief contractions increases cost, and that the soleus really is the dominant force producer and energy consumer, to what extent does its relatively slow fiber type composition of this muscle limit the findings of the paper?

Similar to a previous comment, we now addressed the soleus fiber composition in our manuscript using the following passage:

Lines 405-416: In our study, the duration of active force generation (*i.e.*, an approximation of muscle fiber economy [9, 10, 23, 26, 27]) may not have improved the relationship between biomechanics to metabolic energy expenditure because our studied muscle, the soleus, has a relatively homogenous muscle fiber composition [57] – one of our many potential limitations (see supplementary material for additional potential limitations). Thus, decreasing the duration of active force production may only recruit slightly less economical soleus muscle fibers and not elicit a measureable metabolic effect. Yet, decreasing the duration of active force production increased lateral gastrocnemius activation (Fig. 6), which has a healthy mixture of slower/more economical and faster/less economical muscle fibers [57]. Therefore, the short durations of active force production likely required multiple triceps surae muscles to activate a greater quantity of less economical muscle fibers compared to the long durations of active force production.

Terminology – I initially had a really hard time trying to figure out what was being varied. I find the description of a shorter activation time with a constant activation-deactivation time to be really confusing. I would refer to activation-deactivation time as the time the muscle takes to be turned on and then off (which is variable across conditions). Whereas here the authors seem to be using it to describe the whole contraction cycle? Given that keeping the force-time integral and contraction frequency constant whilst varying the amount of time the muscle is on is such as nice controlled replica of terrestrial locomotion with varying duty factors, it might be helpful to draw parallels to, and terminology from, that? I would certainly replace activation-deactivation time with something more like contraction frequency.

We agree and updated our terminology.

- The duration of active force production – refers to the time-course that the soleus is generating force (as measured on the dynamometer).
- The force generation cycle – refers to a bout where the soleus generates force and then undergoes a relaxation (none force producing) period.
- Duty factor: The duration of active force production divided by the force generation cycle duration.

- We also now refer to cycle average force rather than force-time integral.

Figures/ stats – the in vivo nature of these experiments makes it hard to control all the variables. Hence, there is a lot of sorting through effects in the statistics. I think this is largely done well. However, I'm having a bit of a hard time following what the * is indicating in figures. It might be helpful to more explicitly include models and p values in figure legends? Or be clearer about this somewhere.

We made an effort to be more specific in our results section and figures. For instance, in the results section, we now interpret our data to guide the reader through the progression of our stats in the context of our hypotheses. In our updated manuscript, we also interpret our lower and higher cycle average ankle moment trials separately, which we signify in our figures with asterisks that have a corresponding color.

Line 45 - 'separate their independent contributions on metabolism' typo?

We deleted this sentence.

Line 294 – 'combining the results from our study to that of the literature' typo?

We deleted this sentence.

Referee: 2

Comments to the Author(s)
This research has promise. I really like the study design, and I think it is carried out with care. The results are convincing, and I think they are a meaningful contribution to our field's scientific knowledge base. But the manuscript is difficult to champion in its current form. Below I lay out my rationale first with some general comments, and then with some line-by-line specific comments.

Thank you for the positive feedback.

GENERAL COMMENTS

The manuscript struggles with its clarity. I requested and rapidly received some answers to clarification questions, which helped in my review. I suggest that this information should be included for subsequent reviews by all reviewers. Here are some of the issues I have regarding clarity.

To improve the manuscript's clarity we updated our wording, added equations, figures, tables, and text.

First, some definitions. I think that "muscle activation time" is not properly defined within the manuscript. The definition I received in the clarifying document was helpful, but the use of "activation" still suffers from being different than how it is normally used. In my experience, it is normally used to refer to the period of time when the muscle transitions from not being active to being active and does not include the period of time after that when the muscle remains active. Similarly, “deactivation” refers to the period of time when the muscle activity transitions from being active to not being active and does not include the period of time after that when the muscle is not active. “Time” is also misleading as one can interpret it as a point in time. For example, “muscle activation time” could be interpreted as the point in time where the muscle activity crossed some threshold. Here is terminology that I think is more accurate and will work better for readers: “duration of muscle activity”.

We agree that we used ambiguous terminology. To improve our manuscript’s clarity, we now exclusively use ‘duration’ when describing an event’s time-course. Also, because our study focuses on the metabolic cost of generating force, we now focus on the ‘duration of active force generation’. The duration of active force generation refers to the time-course that the soleus is actively generating force. Rather than using ‘deactivation’ we use ‘relaxation’ to indicate that the muscle is no longer actively generating force. We also use the force generation cycle to indicate a bout of active muscle force production and subsequent relaxation.

I think the manuscript’s clarity would benefit from leveraging the established terminology of duty factors and duty cycles. Here you controlled for the same cycle period, with the same average muscle force, but varied the fraction of the cycle over which the muscle generated force (the duty factor). A more informative title becomes: “Generating the same average muscle force with shorter duty factors increases the metabolic rate of cyclic force generation”.

We agree and now use the term ‘duty factor’ regularly in our manuscript.

As suggested, we updated our title using duty factor terminology:

Title: Duty factor affects muscle contractile mechanics, active muscle volume, and metabolic cost during cyclic muscle-tendon force generation

One of the challenging parts of the paper for me regarding clarity was the results section. It read as a large collection of single sentence comparisons without a framework to help understand why the reader should care about these particular comparisons. I strongly recommend that each paragraph have a topic sentence that summarizes the point of the comparisons that follow. For example, a topic sentence for the first paragraph of the results might be something like: “Our study design was successful at creating conditions that differed in duty cycle while controlling for average cycle force”.

We updated our results section with better topic sentences to help guide the reader and contextualize our statistical comparisons. In doing so, we added the following topic sentences:

Lines 321: The participants well-performed the protocol.

Lines 336-337: Despite not affecting isometric active muscle volume, duty factor influenced total active muscle volume by modulating soleus fascicle force-length and force-velocity potential.

Lines 350: Overall, greater total soleus active muscle volume increased net metabolic power

Lines 358-359: Based on muscle activation patterns, we presume that co-activation did not affect the relationships between duty factor, total soleus active muscle volume, and net metabolic power.

Shifting from clarity to the manuscript's focus. You have chosen to focus on the duration of muscle activity and compare that to changes in metabolic power. I recommend instead that you focus on the duration of muscle force generation. This is functionally what will matter to a running animal, and much closer to what you controlled in the experiment. I waffled a bit on whether you should focus on ankle torque as this is even closer to what you controlled, but I think your efforts to isolate soleus justify a focus on muscle. This simplifies the manuscript as you can then remove the muscle activity comparisons except to support your argument that you effectively isolated soleus. It also more tightly integrates your measurements on fibre kinematics and kinetics as everything will be about force generation.

We agree and focused on the duration of active force production due to its relevance to walking and running animals.

A final general comment is that the novelty of this work should be better emphasized. There have been a number of previous papers that have studied the main question posed in the present manuscript. Many of these are mentioned in the Discussion section (refs 49-51, 60) but perhaps the most important one is not: Hogan MC, Ingham E, Kurdak SS. Contraction duration affects metabolic energy cost and fatigue in skeletal muscle. Am J Physiol. 1998 Mar 1;274(3 Pt 1):E397-402. I recommend a new introduction paragraph that puts the present work in the context of the work already done by Hogan and others. This paragraph should answer the question of how this new work adds to the knowledge that already exists from prior work. I suspect that the answer might be that the novelty of the present work is mainly on the insights provided by the fibre-level measurements. However, as written, this is not how the manuscript is currently pitched (c.f. last paragraph of introduction).

With our introduction and new story line (closely following the cost of generating force hypothesis - Kram & Taylor *Nature* 1990), the novelty of our manuscript is separating the independent influence of the duration of active force production and duty factor on metabolic energy expenditure during locomotion-like contractions.

In our introduction, we note that there are disagreements among scientists regarding whether the duration of active force production affects metabolic rate due to alternative findings and other correlated parameters (*e.g.* duty factor and stride frequency).

Lines 90-92: Despite these compelling results, alternative findings have caused some scientists to question whether shorter ground contact durations increase the metabolic energy expenditure of locomotion [12, 18, 33-37].

Lines 108-110: Thus, duty factor and/or stride frequency, not ground contact duration, may underlie the changing metabolic energy expenditure across walking and running speeds [4, 34, 40-42].

Because we are trying to improve the clarity of our story and focus on the metabolic cost of force generation, its duration, duty factor, active muscle volume, etc. We are less directly addressing the potential metabolic influence on force production cycle frequency. Hogan et al. (1998) maintained a constant duty factor and had muscle-tendons vary the duration of active force production and cycle frequency. They were unable to maintain constant average force (a requirement of locomotion) but did suggest that shorter durations of active force production and faster frequencies incur more muscle fatigue (less force per activation) and greater metabolic rates. They primarily attributed the increased metabolic rate to faster frequencies, rather than shorter durations of active force production. In our study, we highlight how changing duty factor in a muscle-tendon affects active muscle volume and how the duration of active force production links active muscle volume to metabolic energy expenditure. We commented on our versus Hogan's results in our discussion:

Lines 433-440: For example, Hogan et al. [58] measured the metabolic energy expenditure of cyclic muscle-tendon force production using a consistent nerve stimulation and duty factor while varying the duration of active force production and cycle frequency. In that study [58], Hogan et al. were unable to establish whether increased metabolic energy expenditure were due to shorter durations of active force production and/or faster cycle frequencies. combining Hogan et al.'s [58] results with the current study, active muscle volume and activation-deactivation frequency may be two primary biomechanics factors driving metabolic energy expenditure during cyclic muscle-tendon contractions.

SPECIFIC COMMENTS

Apologies in advance for comments that are terse. I don't often ask for changes to be made in the line by line comments below. I leave it to the authors' judgement as to how to address the concern (i.e. either by a response to me or also to a change in the manuscript.)

L22/L23: Metabolism is vague. Metabolic energetics?

We changed 'metabolism' to metabolic energy expenditure.

L33: Doesn't make sense to read.

We deleted the sentence.

L60: "Despite this realization, scientists have not established whether generating muscle force over shorter or longer times increases metabolic energy expenditure during locomotion [7, 27, 28]." I don't think this is accurate. What about the above Hogan ref or refs 49-51, 60? Barclay or Rall might also have claims about this.

We agree that some scientists think that shorter durations of active force production incur a greater metabolic energy expenditure, but this is not universally agreed upon.

To provide a few examples regarding the disagreement, Hogan et al. (1988) primarily attributed their increased metabolic rate to calcium pumping associated with more rapid muscle on-off frequencies rather than the shorter durations of force production. Gutmann & Bertram (2017a) argued that 'the apparent relationship between metabolic rate and force rate observed in treadmill running is likely not a fundamental characteristic of muscle physiology' and later (2017b) '[Our] result contrasts with studies that suggest that muscle force rate or muscle force rate per time determines the metabolic cost per time of force production in other bouncing gaits such as running'. Further, Moore (Sports Med 2016) reviewed the literature and concluded that running with shorter ground-contact times is more economical versus running with longer ground contact times 'Considering the empirical evidence, one economical running strategy could be aiming to shorten ground-contact times whilst maintaining stride frequency'. These are a few examples of the lack of consensus regarding ground contact time and metabolic energy expenditure during locomotion.

Further, our new introduction lays the argument that the concurrent changes to duty factor and stride frequency make in difficult to unravel the duration of active force generation's metabolic cost.

Line 103: $\frac{1}{t_c} = \frac{Freq_{stride}}{DF}$ [Eq. 4]

L63: Are fast fibres less economical? What about Ca+ costs?

Faster muscle fibers expend more metabolic energy (split ATP at faster rates) than slower muscle fibers when generating force and performing cross-bridge work (Muscle and Exercise Physiology, Publisher: Academic Press, Chp 6 – Efficiency of Skeletal Muscle – Chris J. Barclay).

Calcium release is greater in faster versus slower muscle fibers. Thus faster fibers likely utilize more ATP for calcium shuttling than slower fibers when generating a unit force (Barclay *J Physiol* 2012). We updated our manuscript to specify why faster muscle fibers may be less economical than slower muscle fibers.

Lines 82-86: decreasing ground contact duration involves the recruitment of faster muscle fibers that utilize more ATP per unit of active muscle volume (less economical muscle fibers) [23, 26, 29-32]. Faster muscle fibers are presumably less economical than slower fibers due to their greater ATP utilization for calcium pumping [32] and cross-bridge cycling [31].

L64: "and/or by operating muscles at decreased lengths with faster shortening velocities [9, 30, 31]." Why would this increase cost?

Decreased lengths and faster shortening velocities reduce muscle force output per unit activation/active muscle volume. Thus, operating muscles at decreased lengths and faster shortening velocities increases metabolic energy expenditure because the animal needs to activate a greater volume of muscle to generate the required force.

L68: What does "fundamentally" mean here? Should this be "increase"?

We deleted 'fundamentally'

L85: "Regulate" is a poor word choice.

We deleted 'regulate'

L85: Have to be clear that this EMA is a simplification that helps with understanding.

We restructured the introduction and added simple equations to help readers succinctly understand EMA's relevance to our hypotheses.

Lines 123-126: Greater GRFs increase the forces required by the leg extensor muscle-tendons (F_{mts}) as long as there is not a large increase in the leg joint's effective mechanical advantage (EMA). EMA is the ratio of the muscle-tendon and the leg-joint axis of rotation's moment arm length (r) and the GRF vector to leg-joint axis of rotation's moment arm length (R) ($EMA = \frac{r}{R}$) [9, 24, 25].

L81 paragraph: This paragraph has a lot of jargon and implicit simplifications that obscure what I think is a straightforward point - stride averaged ground reaction force is equal to body weight but stance averaged ground reaction force is not. You can accomplish the same stride average force with high force and short duration impulses, or the opposite. I suggest re-writing this paragraph.

We deleted this paragraph.

L113 - so N=8?

Nope, N=11. We re-wrote this section for clarity.

Lines 189-195: Eleven of the fourteen volunteers who enrolled in our study completed the protocol (resulting sample size: 11 participants; average ± SD; age: 24.5 ± 3.5 yrs; height: 1.78 ± 0.06 m; mass: 74.8 ± 10.7 kg; Achilles tendon moment arm: 5.0 ± 0.8 cm; optimal soleus fascicle length: 3.86 ± 0.7 cm; maximum soleus fascicle shortening velocity: 26.1 ± 4.6 cm/s; and resting metabolic power 80 ± 11 W). The three volunteers who enrolled but did not complete the protocol were removed from the analyses because they were unable to achieve the targeted muscle-tendon mechanical output and yield serviceable metabolic data for at least half of their trials.

L142 - need angle convention.

Line 203-204: 90º indicates perpendicular segments and more acute angles indicates joint flexion.

L153 - strange horizontal bar over numbers.

Strange horizontal bar over numbers indicate a repeating decimal.

L163: a) vs. Time not vs. Muscle activation time. B) integral is spelled incorrectly on b) y axis.

Good call, we updated our figures to indicate time & we no longer refer to integral.

L166: "* indicates that…" As written, it is not clear what is being compared. What is the "time-course of muscle activation"?

We updated our asterisks and figure legends to be more specific.

Also, we no longer use 'time-course' in our manuscript.

L168: do participants have to learn to do this? Why only 1 min averages? That's atypical.

We did not assess adaptation or learning throughout this study. Randomizing trial order mitigates the potential for participant learning to affect our results. From pilot testing 2 people, this task was seemed fairly simple, straightforward, and required minimal learning.

Due to the time-delays in oxygen uptake kinetics, we analyzed metabolic data following minute 4 & 9 (for the resting trial) and averaged over the last minute of a metabolic trial. Both averaging metabolic data after minute four and during the last 1–3 min of a task is the standard approach for analyzing metabolic data (Brooks et al., 1996; Gottschall and Kram, 2003; Grabowski et al., 2005; Houdijk et al., 2009; Arellano and Kram, 2011; Farris and Sawicki, 2011; Snyder and Farley, 2011). Also, for metabolic tasks that incur less energy expenditure than locomotion, longer resting trials are typical to ensure a truer resting value (Huang & Ahmed 2014 *J Neurophysiol*; Huang et al. 2012 *J Neuroscience*).

L174: I think the idea here is that either RER was below 0.7 which doesn't make sense or above 1.0 which means that energy was coming from non-oxidative sources so you weren't able to measure it with this technique.

Correct, there are multiple ways for RER to be disconnected from RQ (*e.g.* hyperventilation, food, and alcohol consumption).

New Methods: Why doesn't the activation time and the deactivation time add up to 1.33?

All of our targeted durations of active force production plus the subsequent relaxation phase equaled 1.33 seconds (0.88+0.44 s; 0.66+0.66 s; 0.44+0.88 s all equal 1.33 s)

Lines 232-236: We systematically varied the duration between the downbeats and upbeats to alter the duration of active force production (three targeted active force production−force offset conditions: $0.\bar{8} - 0.\bar{4}$ s, $0.\bar{6} - 0.\bar{6}$ s, $0.\bar{4} - 0.\bar{8}$ s) while always targeting a total muscle-tendon force generation cycle that equaled $1.\bar{3}$ s (Suppl Table 1 and Fig. 1).

New Methods - It is still not clear how soleus activation time is being defined. Would help to have it included in the graphic.

We now highlight the duration of active force production rather than activation time. And as suggested, we indicate this duration in our new methods figure (Fig. 1)

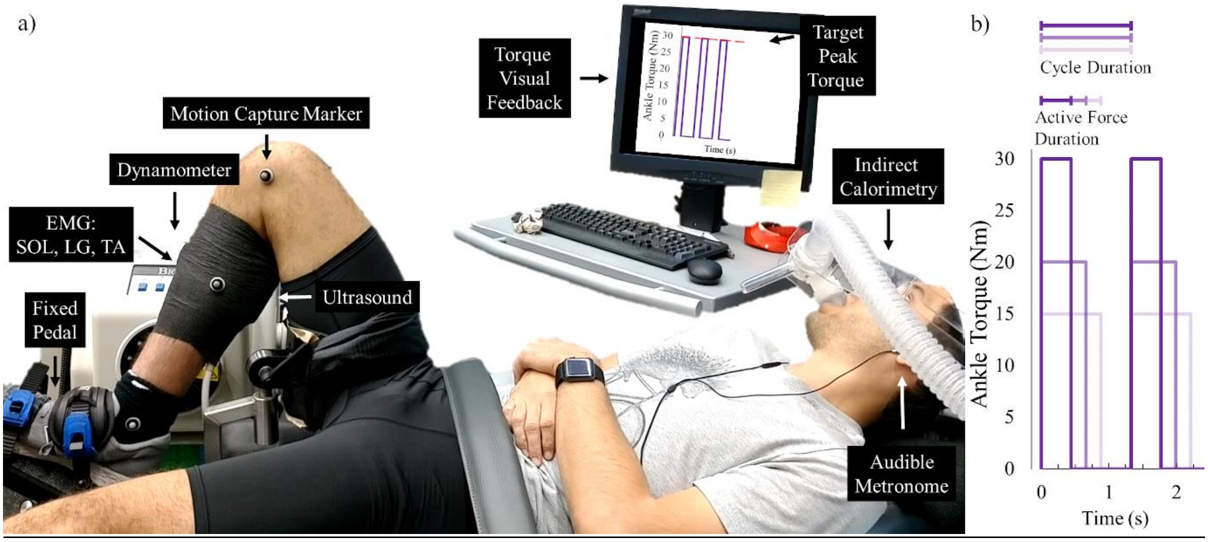


Figure 1. a) Experimental setup of a participant cyclically generating soleus muscle-tendon force to produce a plantar flexor moment that exerts an external torque on a fixed-dynamometer pedal following the cues of an audible metronome and visual feedback. EMG is electromyography, SOL is soleus, LG is lateral gastrocnemius, and TA is tibialis anterior. b) Ankle torque versus time for three conceptual trials that yield the same average ankle torque.

New Table 1: Why are these tact (e.g. 0.4s) so different from that reported in Fig 1 (e.g. 0.75 s). If this is the difference between targeted and measured, this should be explained and a different variable name should be given to each.

We now prefaced our desired protocol instructions as the 'targeted' durations and torque value to differentiate the desired parameters versus the measured parameters.

The difference between the targeted and measured duration of active force production is likely due to delays between the nervous system and muscle force generation, as well as the time delay between muscle onset/offset and the targeted peak force (*e.g.* muscle force production is not a square wave like we show in Fig. 1).

L180: "activation-relaxation" cycle. Here is a new definition of activation not consistent with other definitions. (e.g. L193: "activation-deactivation cycles").

We no longer use 'activation-relaxation' terminology and now choose to use 'force generation cycle'

L195: "We used a 1 Nm threshold to determine ankle-joint biomechanics". This statement does not make sense.

We updated this passage for clarity.

274-276: Due to small fluctuations in dynamometer torque, we implemented a 1 Nm dynamometer torque threshold to decipher the duration of active force production.

L195: So is this a definition of activation and deactivation that is based on torque and not EMG?

We now focus on the duration of force production, not the soleus activation signal.

L205: maybe "estimate" rather than "determine" as there could be other contributors.

We changed determine to estimate

Lines 283-285: We calculated soleus muscle-tendon force (F_{mt}) by dividing net ankle moment (M_{ank}) by the Achilles tendon moment arm length (r_{AT}), and then we divided soleus muscle-tendon force by fascicle pennation angle to estimate soleus fascicle force (F_{sol}).

L215: "total activation-deactivation cycle time". Not sure if this based on torque or EMG or something else.

All durations in the manuscript refer to the duration when we measure dynamometer torque >1 Nm threshold.

228: "Overall, soleus activation time affected the ankle-joint's moment-time integral (p=0.008) (Fig. 1)" I don't understand what is being compared here. Is this a comparison with the green, and within the purple, in Fig 1b? L230 suggests not as it appears that this is the comparison being presented in L230. So what is the comparison in L228?

We updated our results section. We wanted to communicate that the duration that participants were generating torque with their soleus was independent of the total force production cycle's average torque. Re-written sentence below:

Lines 324-326: Importantly, both the duration of active force production and duty factor were independent of cycle average ankle moment (all $p \geq 0.699$) (Fig. 2) and cycle frequency (all $p \geq 0.175$) within each moment level (Table 1).

229: "…but not the time-integrated soleus force ($p=0.483$)" How is this possible? Equation 1 suggests that it must be due to changes in the pennation angle?

Correct, pennation angle can decoupled ankle moment from soleus fascicle force. However, upon double checking our pennation values, average ankle moment and average fascicle force remained independent from the duration of active force production and duty factor. See updated results passage below:

Lines 330-334: Moreover, because the ankle's effective mechanical advantage was constant and duty factor did not affect the average ($p \geq 0.240$) or maximum ($p \geq 0.091$) soleus fascicle pennation angle within each moment level, cycle average fascicle force was independent of duty factor (Fig. 2). Consequently, isometric active muscle volume was independent of duty factor within each moment level ($p \geq 0.252$).

230: It is not helpful to use two different terms to refer to the same quantity: "ankle-joint's moment-time integral" and "time-integrated ankle-joint moment".
This is a good suggestion and we improved the consistency of our wording throughout, we discussed the two cycle average ankle torque levels throughout our results as lower and high moment levels.

231: "Additionally…" I don't really understand this comparison either, and it is not grammatically clear whether the "which" statement refers to the soleus activation time or the total activation-deactivation cycle. Nor is it clear how the total activation-deactivation cycle is defined.

This sentence tried to highlight our result that the duration of active force production was statistically independent of the duration of the force generation cycle. Here is our updated sentence:

Lines 324-326: Importantly, both the duration of active force production and duty factor were independent of cycle average ankle moment (all $p \geq 0.699$) (Fig. 2) and cycle frequency (all $p \geq 0.175$) within each moment level (Table 1).

L228-L233: I think the idea with this paragraph is that your protocol design worked to produce conditions where soleus was active for different durations but had the same impulse? Can you make something like that your topic sentence? It currently reads like a collection of comparisons for which the reader is unclear why they are being made.

We updated our paragraph and topic sentence to indicate that our participants achieved the protocol.

Lines 315-324: The participants well-performed the protocol. Namely, participants performed two distinct cycle average ankle moment levels (lower and higher moment levels) (Fig. 2). The higher moment level elicited a 48% greater cycle average ankle moment ($p<0.001$) and non-different cycle duration ($p=0.141$) compared to the lower moment level (Table 1). Importantly, both the duration of active force production and duty factor were independent of cycle average ankle moment (all $p\geq0.699$) (Fig. 2) and cycle frequency (all $p\geq0.175$) within each moment level (Table 1). Thus, even though participants did not perfectly achieve the targeted dynamometer torque and duration values (Supplementary Table 1), they did successfully vary their duration of active force production and duty factor while maintaining a constant total cycle average ankle moment and cycle frequency.

It would also help the reader if in the intro, or methods, you explained why it is necessary to have to jump through these hoops to produce controlled conditions of this nature. That is, why can't one just change the duration of muscle activity while keeping impulse constant?

We agree with this comment. Accordingly, we restructured our introduction and added 14 simple equations to help describe how the constraining physics of locomotion cause some biomechanical parameters to be related.

L235: This is a good topic sentence, but it seems to bury the more interesting point that activity duration has an effect on met cost independent of force impulse.

With our new topic sentence (see below) we want to highlight the relationship between active muscle volume and metabolic power before diving into the subtleties.

Line 350: Overall, greater total soleus active muscle volume increased net metabolic power.

L241: rather than present how a 0.4 s decrease effects met power in watts, I suggest something like the following: "Based on these relationships, a doubling of the force impulse results in a 2.1x increase in metabolic power, whereas halving the force duration results in a 1.3x increase in met power". This will help the reader understand the magnitude of the effect of decreasing duration. If not something like this, then definitely something like L237 where you include percent changes and not just absolute changes.

We now explain the effects of biomechanics on net metabolic power using the coefficient of determination.

Lines 350-351: Across both moment levels, increasing participant total active muscle volume explained 72% of the increased net metabolic power ($r = 0.845$; $p<0.001$).

Further, we link biomechanical parameters to other biomechanical parameters using percent changes:

Lines 361-363: Moreover, both decreasing duty factor and increasing total soleus active muscle volume yielded greater soleus (all $p \leq 0.010$) and lateral gastrocnemius (all $p \leq 0.033$) activation (Fig. 6).

L249 and L264: These paragraphs would benefit from topic sentences that clarified for the reader what the main take-home point is and how it relates to the purpose of the experiment. As a reader, it is hard to keep engaged about why I should care about another comparison.

As aforementioned, we added topic sentences to help keep the reader engaged in our results.

280: Why proof-of-concept?

We deleted this passage.
292: So what is the mechanism for what you find here?

We deleted this sentence.

300: First introduction of "less economical muscle dynamics" and introduced without definition.

We now define 'less economical muscle dynamics' at their first introduction.

Lines 83-84: the recruitment of faster muscle fibers that utilize more ATP per unit of active muscle volume (less economical muscle fibers)

300: I feel like it is unnecessary to compete shorter durations/higher force of muscle activity against increased muscle volume. Instead, it feels to me like the increased muscle volume explains why the shorter duration/higher force increases cost.

In our updated manuscript we argue that duty factor affects active muscle volume and the duration of force generation affects the metabolic energy expenditure per unit volume (see introduction for rationale).

300: One more comment about this sentence: it first read to me like you were questioning whether or not met cost actually increased. It made me doubt whether I understand what you actually found.

We deleted this sentence.

303: Shorter than optimal muscle lengths? Is there an assumption here about at what length at which the muscle is acting?

We now state:

Lines 288-290: Next, we calculated isometric soleus active muscle volume using soleus muscle-tendon force and Equation 2, where optimal fascicle length equals participant resting soleus fascicle length and stress equals 20 N/cm^2 [54].

317: Is Fact a new variable? Is it the same as Fsol defined earlier?

We no longer use Fact

Fsol is soleus fascicle total muscle force (active force + passive force) [Beck et al 2019 *ESSR*]

317: It would help the reader if you explained how Eq 2 works.

We now develop the rationale behind the active muscle volume calculation throughout the introduction with multiple paragraphs and equations.

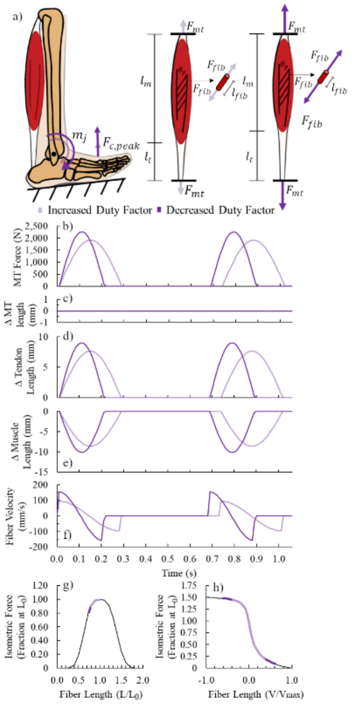
343: This is statement is misleading. Were you to not have a tendon in series and compare two conditions where in one you had +5 and -5 Joules of work (net=0) and another where you had +50 and -50 J, clearly the latter would have higher cost.

We deleted the sentence.

For what it is worth, data from Holt et al. *J Exp Biol* 2014 may disagree with your example. Based on Holt et al. an isometric muscle contraction (0 net and gross mechanical work) had the same 'cost of force production' (Joules per Newton Sec) as a muscle performing net 0 mechanical work but undergoing cyclic stretch-shorten cycles (non-zero gross mechanical work).

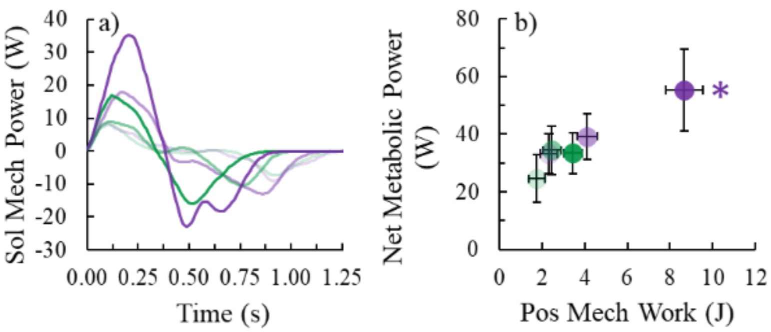
336: What is missing from this paragraph is an explanation of what the muscle is performing work on. I suppose it must be the tendon, but this should be explained to the reader. It would also be good to explain why the shorter durations/higher forces result in more work. Finally, it would be better to have the x axis of Fig 6 be average muscle power, rather than work, and the slope of the line reported so that we can tell what the efficiency is.

We agree and use our introduction text, supplemental figures 1 and 2, as well as multiple equations to describe how tendon excursion affects muscle length changes.



Supplementary Figure 2. Duty factor influences muscle-tendon mechanics during cyclic contractions**.** Schematic showing a) stance leg with a peak ground reaction force ($F_{c,peak}$) and plantar flexor joint moment (m_j) during hypothetical conditions with a larger (lighter purple) and smaller (darker purple) duty factor. Graphical depictions of how we expect duty factor to affect the corresponding muscle-tendon force (F_{mt}), whole muscle length (l_m), tendon length (l_t), muscle fiber force (F_{fib}), and muscle fiber length (l_{fib}). Time-series graphs of b) muscle-tendon force, c) muscle-tendon length change, d) tendon length change, e) muscle fiber length change, and f) muscle fiber velocity for the respective larger and smaller duty factor conditions. Expected normalised muscle fiber operating range depicted on Hill-type g) force-length (FL) and h) force-velocity (FV) curve for the respective larger and smaller duty factor conditions. L_0 is optimal muscle fiber length.

Regarding our fascicle mechanical work figure – we chose to show mechanical power time-series and positive mechanical work, to be consistent with many of the manuscript's preceding figures.



Supplementary Figure 3. a) Soleus (Sol) mechanical power versus time and b) net metabolic power versus positive mechanical work. Lighter to darker colour indicates longer to shorter duration of active force production per ankle moment level. Green and purple asterisks (*) indicate that the corresponding average moment level's positive mechanical work affects indicated dependent variable ($p<0.05$).

358: I don't see this. The pattern of LG activity in Fig 4 looks just like the pattern of soleus activity.

Good catch. We had an averaging error and now report that duty factor affects both Soleus and LG.

Lines 385-387: Moreover, both decreasing duty factor and increasing total soleus active muscle volume yielded greater soleus (all $p\leq0.010$) and lateral gastrocnemius (all $p\leq0.033$) activation (Fig. 8).

Max Donelan

Hi Max

Appendix C

I am still very much in favor of publishing this method and result, but am unfortunately still struggling with the presentation, particularly in the background section. I have included some comments here that might help guide changes.

Line 48 – I have some concerns about the idea of average net work as a concept. It doesn't seem totally analogous to force to me as there are many combinations of positive and negative work that give the same net work. I think my preference would still be to pitch this based on the ongoing argument as to whether force or work determines cost. Relatedly, I'm not convinced that this 'leveraging of physical constraints' argument doesn't diminish the significance of the work i.e. making it seems to make it more about the approach rather than the direct significance for locomotion. Maybe it would be better as a methods side note to justify the constant force-time integral?

Lines 90-92 – This feels weak. Maybe giving more concrete examples would help?

Line 107-110 – I'm a bit lost here. There is no mechanistic reasoning why changing these variables would explain the changing cost. This might be my personal biases, but I feel like if we're trying to link mechanics and energetics, we have to be discussing the mechanistic muscle-level reasons as to why cost would change.

Lines 150-161 - I think I'm in favor of the force-length-velocity potential argument. But it seems like it would be better placed in the same section as the fiber type argument. I would like to see a concise summary of all the reasons metabolic cost might vary with the locomotor parameters investigated.

Lines 162-165 – It seems to me that the purpose of this study is to investigate the mechanistic underpinnings of the cost coefficient. But that idea seems to get lost.

Lines 172-186 – I like the idea of these predictions, but I'm finding it a little hard to link it to the previous information

Line 232 – upbeat force-offset?

Lined 399-416 – I think this section highlights my confusion throughout about why active force duration might be an important factor. I would assume that any effects of active force duration are driven by duty factor. I fail to see why, and no mechanistic reason is given, as to why active force duration would have a separate effect on metabolic cost.

Fig 3 – should the force-length relationship be 'shorter' and 'longer' rather than shortening and lengthening

I'm not particularly in favor of the limitations and discussion of muscle work being in the supplementary material. To address space concerns, I think I would prefer to see a condensing of some of the equation heavy sections of the introduction (the readership should be sufficiently familiar to not require all of these equations?) to make space for this in the manuscript itself

Appendix D

A media summary: a short non-technical summary (**up to 100 words**) of the key findings/importance of your manuscript.

To better link walking and running mechanics to energy expenditure, this investigation studied participants as they cyclically contracted their calf muscle in a manner that mimics key aspects of locomotion: constant cycle-average force and work. Overall, decreasing the fraction of time that the calf muscle is producing force per each force-production cycle (*i.e.*, duty factor) caused greater tendon stretch, increased muscle fascicle shortening, and consequently increased energy expenditure. Thus, by accounting for structural (*e.g.*, tendon stiffness) and functional (*e.g.*, duty factors) parameters during walking and running, researchers may better explain metabolic energy changes across locomotor tasks and animal species.

Comments to Reviewers:

We thank the handling editor and reviewers for many positive remarks and useful feedback. Based on the feedback, we updated our manuscript and responded to each comment below using underlined text (see below).

Reviewer(s)' Comments to Author:

I am still very much in favor of publishing this method and result, but am unfortunately still struggling with the presentation, particularly in the background section. I have included some comments here that might help guide changes.

Line 48 – I have some concerns about the idea of average net work as a concept. It doesn't seem totally analogous to force to me as there are many combinations of positive and negative work that give the same net work. I think my preference would still be to pitch this based on the ongoing argument as to whether force or work determines cost. Relatedly, I'm not convinced that this 'leveraging of physical constraints' argument doesn't diminish the significance of the work i.e. making it seems to make it more about the approach rather than the direct significance for locomotion. Maybe it would be better as a methods side note to justify the constant force-time integral?

During steady-state walking and running, cycle-average vertical ground reaction force always equals body weight and net mechanical work always equals a constant number. Participants can generate vertical force on the ground using many different force profiles, and similarly, they can perform mechanical work using many different mechanical energy profiles. We agree that these two metrics don't perfectly capture animal movement or the demands from each muscle, but we do feel that both metrics are useful to guide our protocol towards emulating steady-state walking and running.

We apologize for over emphasizing our approach, but we are concerned that many readers will not understand how our protocol emulates locomotion biomechanics if we do not restate how we leveraged the physical constraints of locomotion in our dynamometer protocol.

Again, we apologize regarding the differences in desired introduction. Rather than pitting force vs. work, we highlight active muscle volume as a driving force behind metabolic energy expenditure. While traditionally active muscle volume is a force-based model, the updated equations also use force-length-velocity potential, which may correlate with mechanical work. We prefer to highlight the differences between stride frequency, ground contact duration, and duty factor in our introduction, address mechanical work in our discussion, and further compare force vs. work in future studies.

Lines 90-92 – This feels weak. Maybe giving more concrete examples would help?

We deleted this weak sentence.

Line 107-110 – I'm a bit lost here. There is no mechanistic reasoning why changing these variables would explain the changing cost. This might be my personal biases, but I feel like if we're trying to link mechanics and energetics, we have to be discussing the mechanistic muscle-level reasons as to why cost would change.

We agree that changing ground contact duration, stride frequency, and duty factor only help us estimate how muscle contractions change, and in turn how much energy they expend. Accordingly, we updated our introduction to explicitly state how each kinematic parameter serves as a proxy for aspects of muscle contractions that affect metabolic energy expenditure.

Lines 71-78: Two such parameters that are easily measureable and likely serve as proxies for muscle ATP utilisation are ground contact duration [18] and stride frequency [4, 10]. Ground contact duration serves as the duration of active muscle force production, and decreasing it likely involves the activation of muscle fibres that utilise more ATP per unit active muscle volume (less economical muscle fibres) [12, 18, 26-29]. Stride frequency serves as a muscle's active force production cycle frequency, and increasing it likely increases ATP utilisation primarily due to transporting ions (Ca^{2+} and Na^{+}-K^{+}) across cell membranes at faster rates [14, 30, 31].

Lines 87-100 explain how duty factor may alter muscle mechanics to alter metabolic energy expenditure.

Lines 150-161 - I think I'm in favor of the force-length-velocity potential argument. But it seems like it would be better placed in the same section as the fiber type argument. I would like to see a concise summary of all the reasons metabolic cost might vary with the locomotor parameters investigated.

We re-wrote a shorter introduction to improve its readability. We now discuss the potential metabolic influence of ground contact duration (fibre type recruitment) and stride frequency (ion shuttling) (see above comment) prior to explaining how duty factor affects force-length-velocity potential. Further, we only use a couple equations in our introduction, and relegate the rest to the supplementary material.

Lines 162-165 – It seems to me that the purpose of this study is to investigate the mechanistic underpinnings of the cost coefficient. But that idea seems to get lost.

We agree with this comment. In previous manuscript versions we argued that we were studying the effects of ground contact duration vs. duty factor. And that ground contact duration would affect the cost coefficient by yielding more metabolic power per unit volume. In the current manuscript, we pivoted a bit and now reason that the cost coefficient should not change due to the soleus' homogenous fibre type composition. With this new logic, we did not reinsert detail on how the cost coefficient changed in our study.

Lines 172-186 – I like the idea of these predictions, but I'm finding it a little hard to link it to the previous information

We updated this section to better link these predictions to previous information.

Lines 115-120: By studying the soleus, which has a relatively homogenous fibre type composition [32], the greater metabolic energy expenditure that is associated with activing less economical muscle fibres over shorter durations of active force production should be trivial. This enabled us to investigate how duty factor affects metabolic energy expenditure, independent from the metabolic influence of active force production duration (*e.g.*, fibre type recruitment) and cycle frequency (*e.g.*, ion pumping).

Line 232 – upbeat force-offset?

We altered our wording from 'offset' to 'no force production'

Lined 399-416 – I think this section highlights my confusion throughout about why active force duration might be an important factor. I would assume that any effects of active force duration are driven by duty factor. I fail to see why, and no mechanistic reason is given, as to why active force duration would have a separate effect on metabolic cost.

We re-wrote many paragraphs to try to improve the manuscript's readability and logic. Now, in our introduction, we try to more clearly and concisely describe how duty factor affects muscle force-length-velocity potential (and total active muscle volume) whereas the duration of active force production affects energy utilization per unit muscle volume (cost coefficient). Then, we argue that the metabolic influence of the duration of active force production is minimal in the current study, and thus changes is duration of active force production *per se* do not affect metabolic energy expenditure. Rather, changing duty factor does affect metabolic energy expenditure due to altered muscle force potential. We also highlight the potential limitations of our assumption that the duration of active force production does not affect metabolic energy expenditure in our study.

Lines 338-356: Because the soleus, but not the gastrocnemius muscles, cyclically produced force that generated a plantar flexion moment, we deemed the gastrocnemius muscles to elicit a fairly constant and small metabolic energy expenditure across conditions. As such, we attributed the change in metabolic energy expenditure across conditions to the soleus. Producing the same cycle-average force over shorter durations typically increases metabolic energy expenditure due to the activation of faster, less economical muscle fibres [12, 17, 18]. However, given soleus' relatively homogeneous muscle fibre composition [32], it likely yields similar rates of metabolic energy per unit active muscle volume ($\dot{\rho}_p$ in Eq. 5). Therefore, we reasoned that the metabolic influence of the duration of active force production was likely minimal in our study. To ensure that the activation of different muscle fibre types (from the soleus and gastrocnemius muscles) did not affect our conclusions, we performed post-hoc analyses which revealed that scaling total active muscle volume by the rate of active force production (1/ground contact duration in [17, 18]) did not improve the correlation between participant total active muscle volume and net metabolic power (r=0.840 versus 0.845). In other words, assuming that shorter durations of active force production recruited less economical muscle fibres did not improve the correlation between total active muscle volume and net metabolic power. Therefore, the increased metabolic energy expenditure typically associated with a shorter duration of active force production [17, 18] was likely not present in our study.

Fig 3 – should the force-length relationship be ‘shorter’ and ‘longer’ rather than shortening and lengthening

We agree and updated Figure 3 to state ‘shorter’ and ‘longer’.

I’m not particularly in favor of the limitations and discussion of muscle work being in the supplementary material. To address space concerns, I think I would prefer to see a condensing of some of the equation heavy sections of the introduction (the readership should be sufficiently familiar to not require all of these equations?) to make space for this in the manuscript itself

We followed this suggestion. We condensed our background and added the work paragraph and limitations sections into the discussion.

Referee: 2

Comments to the Author(s)
I found this revised manuscript to be a major improvement over the first submission. I still have some substantial suggestions for revision, but would like to leave it up to you (the authors) as to whether you feel these revisions are necessary. I do think that they will increase the readability of the paper, and ultimately its impact on the field. So on behalf of future readers, please consider making them.

GENERAL COMMENTS

1. You make an interesting point about muscle mechanics or fibre type explaining the increase in metabolic cost with the shortening of duty factor. And you write that accounting for activity duration (a proxy for fibre type) doesn’t improve your ability to explain increases in metabolic cost, which ran against your hypothesis. This is all fine but I want to propose a different way to frame your experiment as it relates to this question. I suggest a) proposing both mechanics and fibre type as possible contributors to increase in cost with decrease in duty factor, and then b) proposing to study soleus in isolation as a way to control for fibre type (because it is homogeneously slow twitch), thus focusing on just mechanics. In this way, the focus on soleus becomes a creative part of the study design that allows you to more effectively study muscle mechanics in isolation. Sure, you are doing this after the fact but from the readers point of view, it will help understand that your results are about mechanics and not fibre type. It will also help

avoid the conclusion that fibre type doesn't matter as that is outside of your ability to test in this experimental design.

We took your suggestions and now propose that both fibre type and muscle force potential could affect metabolic energy expenditure by holding frequency constant and decreasing duty factor. Then, by studying the soleus (controlling for fibre type) we were able to isolate the metabolic influence of muscle force potential vs fibre type in our protocol.

Lines 115-122: By studying the soleus, which has a relatively homogenous fibre type composition [32], the greater metabolic energy expenditure that is associated with activing less economical muscle fibres over shorter durations of active force production should be trivial. This enabled us to investigate how duty factor affects metabolic energy expenditure, independent from the metabolic influence of active force production duration (*e.g.*, fibre type recruitment) and cycle frequency (*e.g.*, ion pumping). Because we expected duty factor to affect muscle contractile mechanics and total active muscle volume (Eq. 3, 4 and Suppl. Material), we hypothesized that decreasing duty factor would increase metabolic energy expenditure.

2. I found the introduction pretty unreadable with its many equations. While I love my math, this particular flow of equations didn't help with explaining your central points. I suggest you put them in the supp material except for the ones that you actually need and use later on (which by my count was very few of them).

We agree and now only kept a few important equations in our introduction. We moved the rest to the supplementary material.

In its place, I suggest you put supplementary Figure 2 into the main paper as it is more effective at doing what the equations are trying to do. I would include in that figure an indication of increased active muscle volume and then how muscle volume maps on to met cost.

We agree and added the indicated figure back into the introduction (now labeled Fig. 1). We chose to keep the figure as is, which highlights how duty factor may affect force-length and force-velocity potential, but does not bridge to active muscle volume or metabolic cost. Conceptually going from force-length and force-velocity potential to active muscle volume to metabolic energy expenditure is hopefully straight forward for our readership with our updated introduction and wealth of related literature.

It is OK for it to be conceptual rather than driven by calculations. And then in the intro text, I would describe in a single paragraph what the equations try to do. I think it is something like this: "To achieve the same average force over a complete cycle, but with shorter duty factors, muscles have to generate greater forces. But these muscles have a tendon in series--the tendon applies the forces to the bones so muscle has to stretch the tendon to longer lengths to get the higher tendon forces required to generate higher external forces. Greater tendon stretch means greater muscle fibre shortening. This fibre shortening reduces the ability of the muscle fibres to generate force requiring more muscle fibres--or active muscle volume--to achieve the same instantaneous level of force. It reduces the force generation ability of muscle fibres for two reasons. The first is due to the force-length relationship. Assuming that fibres are at their optimal length for force generation when the tendon is slack, great fibre shortening moves them leftward on their force-length relationship and away from their optimal length--more active fibres are now required to achieve the same force. The second is due to the force-velocity relationship. A greater distance of fibre shortening and over a shorter period of time (because duty factor is shorter) results in faster shortening velocities. Muscles are weaker at faster velocities requiring more active fibres to achieve the same force." And so on.

We added a conceptual paragraph similar to what you described to help link duty factor to metabolic energy expenditure.

Lines 87-107: To briefly explain, while producing the same cycle-average force, decreasing duty factor requires animals to produce greater peak muscle forces (Fig. 1). Greater peak muscle forces further stretch in-series tendons, yielding greater muscle fibre shortening. In turn, greater muscle fibre shortening decreases the muscle's potential to produce force due to shorter operating lengths and faster shortening velocities (Fig. 1). Based on this framework, muscle force-length (FL) and force-velocity (FV) potential may be a function of duty factor (Eq. 3).

$$FL, FV = f(DF) \qquad \text{[Eq. 3]}$$

If so, smaller duty factors would require animals to activate a greater volume of muscle to continue producing the same cycle-average force. This notion can be formalised by incorporating Equation 3 into the calculation of *total* active muscle volume (Eq. 4), which updates the traditional isometric active muscle volume equation (Eq. 1) by using active muscle fibre force (F_{act}) and muscle fibre force-length-velocity potential instead of muscle-tendon force and isometric force production, respectively [11].

$$V_{act,tot} = \frac{F_{act} \cdot L_0}{\sigma \cdot FL \cdot FV} = \frac{F_{act} \cdot L_0}{\sigma \cdot f(DF)} \qquad \text{[Eq. 4]}$$

Subsequently, by accounting for the rate of metabolic energy expenditure per unit active muscle volume ($\dot{\rho}_p$), changes in total active muscle volume theoretically mimic changes in metabolic energy expenditure ($\dot{E}_{met}$).

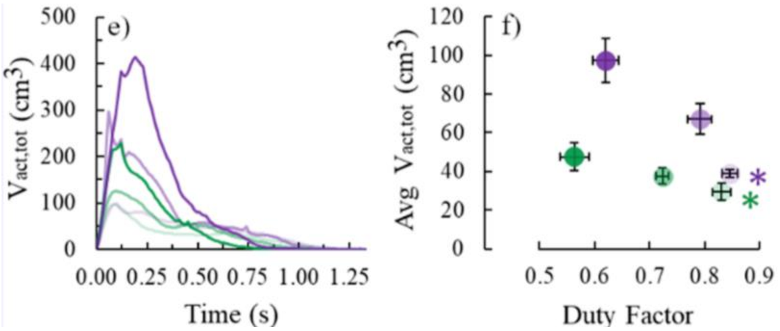
$$\dot{E}_{met} = \dot{\rho}_p \cdot V_{act,tot} \qquad \text{[Eq. 5]}$$

Altogether, walking and running with a smaller duty factor may increase total active muscle volume (Eq. 4) and metabolic energy expenditure (Eq. 5) by decreasing muscle fibre force-length and force-velocity potential (Eq. 3).

One thing about this that I still wonder about in this logic is that while it is clear that this increases the active muscle volume instantaneously, it is harder to see that when integrated over a cycle it will still result in greater time-integrated active muscle volume. But I do think this to be true because without these effects, decreasing duty factor results in increased external force during the duty and thus increased active muscle volume during the duty. But then after the duty you have no active muscle volume for longer and I think everything mathematically cancels to have no effect on active muscle volume unless you include the effects of length and velocity on force potential (I hope that this is clear).

We show that decreasing duty factor did worsen force-length and force-velocity potential and increase cycle average total active muscle volume. Theoretically, if force-velocity potentials cancel each other out (balanced decrease and increase in potential with shortening and lengthening, respectively), decreased duty factor would cause greater muscle shortening, shorter lengths, and worse cycle average force-length potential, which increases total active muscle volume.

Figures from paper:



3. That LG is not contributing to the measured metabolic cost is still not convincing.

We agree that LG may have contributed to metabolic energy expenditure. We acknowledged this in our discussion section (see below). The muscle activity of the LG changed very similar to that of the SOL and the duration of ground contact did not improve the relationship between SOL total active muscle volume and metabolic rate. Thus, it could be that LG active muscle volume changes were in the same manner as SOL active muscle volume changes, thereby our conclusions remain the same.

Lines 338-356: Because the soleus, but not the gastrocnemius muscles, cyclically produced force that generated a plantar flexion moment, we deemed the gastrocnemius muscles to elicit a fairly constant and small metabolic energy expenditure across conditions. As such, we attributed the change in metabolic energy expenditure across conditions to the soleus. Producing the same cycle-average force over shorter durations typically increases metabolic energy expenditure due to the activation of faster, less economical muscle fibres [12, 17, 18]. However, given soleus' relatively homogeneous muscle fibre composition [32], it likely yields similar rates of metabolic energy per unit active muscle volume ($\dot{\rho}_p$ in Eq. 5). Therefore, we reasoned that the metabolic influence of the duration of active force production was likely minimal in our study. To ensure that the activation of different muscle fibre types (from the soleus and gastrocnemius muscles) did not affect our conclusions, we performed post-hoc analyses which revealed that scaling total active muscle volume by the rate of active force production (1/ground contact duration in [17, 18]) did not improve the correlation between participant total active muscle volume and net metabolic power (r=0.840 versus 0.845). In other words, assuming that shorter durations of active force production recruited less economical muscle fibres did not improve the correlation between total active muscle volume and net metabolic power. Therefore, the increased metabolic energy expenditure typically associated with a shorter duration of active force production [17, 18] was likely not present in our study.

4. Try to always be clear that the active muscle volume is integrated over the time of the full cycle. This is not always clear in the text and figure labels.

We agree and added 'cycle-average' in many places in the new manuscript.

5. The results section could use some examples of the magnitudes of differences in addition to the p-values you provide. With only p-values, a reader can't tell if differences are meaningful. Also, I personally treat the p-value as only a single indicator of the importance of a finding and also like to know effect size and confidence intervals. With that said, I don't want this for all comparisons as it will become unreadable. My recommendation is to not rely solely on p-values.

We agree that p-values do not fully explain our results. To keep the readability and length of our manuscript, we present 25 results figure panels (with means and SE), a results table, present correlations (r-values), interpret percentage differences, and present publically available data to help inform the reader about our study's findings.

SPECIFIC COMMENTS

As I wrote last time, please accept my apologies if these comments come across as terse.

L31: I always report the p-value and not just that it is less than some value. p<0.0X is hold over from when we had to look up the values in the backs of textbooks.

We reported the actual p-value when it is equal to or above 0.001. If the p-value is less than 0.001, we feel that it is satisfactory to put $p<0.001$ due to such a small number. Rather than stating the p-value for every comparison (*e.g.*, both lower and higher moment levels) we only report less than or equal to the highest p-values if significant (*e.g.*, both $p\leq0.033$ if the values are $p=0.033$ and $p=0.002$) or greater than or equal to the lowest number if both p-values are not significant (*e.g.*, both $p\geq0.123$ if the values are $p=0.123$ and $p=0.932$). If one moment level has a significant p-value and the other moment level has a non-significant p-value, we report the p-values separately.

We not added a sentence to the manuscript that address this comment:

Line 261-262: Unless otherwise specified, each p-value covers both moment levels for the respective comparison.

L33 ";" is mis-used.

Thanks, we fixed our grammar mistake.

L35: I suggest that "however" should start a new sentence.

We deleted the indicated sentence.

L40: "essential" is too strong. Lots of animal behaviour and evolutionary hypotheses have nothing to do with energetics.

We agree and changed 'essential' to 'important'.

L50: stride-averaged vertical ground reaction force must be equal to body weight during all steady locomotion, including when walking on a slope.

We updated the sentence from 'on level ground' to locomotion in general (e.g., including uphill).

L81: I don't think "theoretically" is needed.

We deleted 'theoretically'

L85: Is "presumably" needed? Is this as much of a fact as much of what we know in physiology?

We deleted 'presumably'

L99: contact duration of a single leg.
We updated the sentence.

Line 82-83: decreasing the product of single leg ground contact duration…

L107: AND duty factor,

Some combination for duty factor, ground contact duration, and stride frequency may affect walking and running economy. We cannot rule out the possibility for one, two, or all three of these parameters does not actually affect muscle mechanics and metabolic energy expenditure. Accordingly, we kept and/or

L112: At this point, I sure would have preferred the pitch being about muscle mechanics independent of fibre type rather than duty factor independent of stance duration.

We agree and updated this sentence as you suggest.

L128: Equations 6 and 7?

We deleted the indicated section

L143: One assumption that you make that I wonder if it is well justified is that slack length is the optimal length.

Good question. Based on Rubenson et al. 2012 (*J Exp Biol*) when the ankle is at 80- 84 degrees the end of a stride during walking with minimal soleus muscle activation, soleus fascicle length was ~0.9 to 1 L/L_0. While we cannot confidently extrapolate their locomotion data to our dynamometer data, it seems feasible that when our participant's ankle is at 90 degrees with no muscle activation, our assumption that their soleus fascicle length is roughly at its optimal length may be appropriate.

Hopefully, future studies will answer this question by mapping soleus force length curves to ankle angle.

We also acknowledged this assumption in our limitations section.

L142: This paragraph is good and possibly all that is needed instead of the equations.

Thank you. We removed most of the background's equations and condensed the section's text.

L156: Nice! Get to here more quickly in this introduction.

Vrooooom

L161: Is this peak active volume? Or a function of time? If the latter, could you show which variables are a function of time?

This is active muscle volume as a function of time. For digestibility, we prefer to present this equation as stride-average active muscle volume to be consistent with the literature (Kram & Taylor 1990; Taylor 1994; Kipp et al. 2018, Griffin et al. 2003). If people go to the reference that shows its derivation (Beck et al. 2019 ESSR), they will get a better idea of the equation.

L177: consider short, medium, and long for variables related to time.

We used short and long when referring to time and fascicle length.

L183: recruitment OF muscle fibres

We deleted the sentence.

L229: remove the x in 6 x 5-minute. Also, consider spelling out numbers less than ten.

We deleted 'x' and now spell out numbers less than ten.

Line 165: Next, participants performed six, five-minute trials with least five minutes of rest…

L234: You mix _ with – in this nomenclature.

We updated this passage to the following:

Lines 170-171: (three targeted active force production durations: $0.\overline{8}, 0.\overline{6}, 0.\overline{4}$ s)

L251: For your future methodology, you shouldn't average breath by breath rates of oxygen (or CO_2). You should first figure out total volume or risk introducing an error.

We agree. Also, we averaged four 15 second O2 & CO2 averages in this study.

L259: 80 cm?

Thank you for catching this typo. 8 cm.

L287: I wonder about how good all these assumptions are in this paragraph.

We feel that these assumptions are reasonable based on previous research, but there is definitely room for future studies to test assumptions that are critical to our results and active muscle volume calculations.

L330: Great topic sentence. I do wonder why we should ever care about isometric active muscle volume in your work.

We use isometric active muscle volume to highlight the independent influence of force-length-velocity potential on the total active muscle volume calculation. *i.e.*, readers can visualize difference between isometric vs. total active muscle volume, knowing the difference between the two parameters is only due to decreased force-length-velocity potential.

L346: Remind reader why this is unexpected. Also, you should justify the order of adding in the explanatory variables. You could have first done tc and then added in active muscle volume and concluded that active muscle volume wasn't necessary. So why this particular order?

We now address this comment in a discussion paragraph.

Lines 334-356: In the present study, we sought to decouple the metabolic influence of the duration of active force production and duty factor while muscle-tendons cyclically produced the same cycle-average force using a constant cycle frequency. To do so, we set each participant's knee angle to 50º, which likely placed slack in bi-articular gastrocnemius muscle-tendons and yielded the soleus as the primary contributor to the plantar flexion moment [33, 35]. Because the soleus, but not the gastrocnemius muscles, cyclically produced force that generated a plantar flexion moment, we deemed the gastrocnemius muscles to elicit a fairly constant and small metabolic energy expenditure across conditions. As such, we attributed the change in metabolic energy expenditure across conditions to the soleus. Producing the same cycle-average force over shorter durations typically increases metabolic energy expenditure due to the activation of faster, less economical muscle fibres [12, 17, 18]. However, given soleus' relatively homogeneous muscle fibre composition [32], it likely yields similar rates of metabolic energy per unit active muscle volume ($\dot{\rho}_p$ in Eq. 5). Therefore, we reasoned that the metabolic influence of the duration of active force production was likely minimal in our study. To ensure that the activation of different muscle fibre types (from the soleus and gastrocnemius muscles) did not affect our conclusions, we performed post-hoc analyses which revealed that scaling total active muscle volume by the rate of active force production (1/ground contact duration in [17, 18]) did not improve the correlation between participant total active muscle volume and net metabolic power (r=0.840 versus 0.845). In other words, assuming that shorter durations of active force production recruited less economical muscle fibres did not improve the correlation between total active muscle volume and net metabolic power. Therefore, the increased metabolic energy expenditure typically associated with a shorter duration of active force production [17, 18] was likely not present in our study.

L362: Not a constant vertical GRF.

We now clarify that we are referring to a constant cycle-average force.

L407: “Healthy” is not a good choice of word here.

We deleted healthy.

END

Max Donelan