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Single Muscle Fibre Contractile Function with Ageing

Greg Grosicki, Carlos S. Zepeda, and Christopher W. Sundberg
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The referees have opted to remain anonymous.

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

Dear Christopher,

Re: JP-TR-2022-282298 "Single Muscle Fibre Contractile Function with Ageing" by Greg Grosicki, Carlos S. Zepeda, and Christopher W. Sundberg

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

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

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

I hope you will find the comments helpful and have no difficulty in revising your manuscript within 4 weeks.

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I look forward to receiving your revised submission.

Best wishes

Professor Laura Bennet
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EDITOR COMMENTS

Reviewing Editor:

Thank you for submitting your review to the Journal of Physiology. Two expert reviewers have provided their assessment of your review. Whilst both found merit to your review, reviewer 2 in particular felt the organization could be improved substantially and that this would increase the impact of the work. That reviewer has provided very specific recommendations for revision. On the basis of this, we would like to give you the opportunity to revise your work to address the comments/suggestions of both reviewers.

Senior Editor:

Thank you for your review submission. Please evaluate the reviewers comments carefully and follow their advice as you revise your manuscript. Both reviewers have indicated a substantive revision is required
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REFEREE COMMENTS

Referee #1:

This review examines the effects of aging on single skeletal muscle fiber contractile function in humans, encompassing muscle fiber size, contractile force, velocity, power, as well as kinetic and calcium sensitivity of the constituent myofilaments. The work is well-referenced and needed in the field. The authors conclude from their review of the literature that there is evidence for profound age-related decrements in fiber size and contractility primarily in fast-twitch, myosin heavy chain (MHC) II-expressing fibers, but not slow-twitch, MHC I-expressing fibers. There are several modifications that are recommended to improve the impact of this work.

Comments:

1) Muscle fiber size: The authors rely on assessments of single muscle fiber size from diameter assessments on single muscle fibers that have been manually dissected from muscle and are being evaluated for contractile parameters. While this is logical given the focus of the review, the authors should probably survey the literature on aging effects on muscle fiber size from more rigorous, state-of-the-art techniques that provide a larger sample size to make conclusions.

2) Statistical analysis of pooled data: There are p-values provided for comparisons across age and sex for single fiber size and contractility parameters. It is not clear from what analyses these p-values are based upon. If the authors are taking mean values from studies and summing across age groups or across age groups in each sex, these mean values should be weighted based on the sample size for any statistical comparisons. While use of meta-analytical techniques are beyond the scope of this review, if the authors wish to make such comparisons, sample sizes should be considered to provide more valid comparisons.

3) Nature of the review: With reviews now becoming a field of science, it would be helpful if the authors would better describe how they went about conducting their review of the literature and describe what type of review they have undertaken (ie, systematic, scoping, etc) and its primary purpose. If the authors followed a systematic review process, this would be viewed as a stronger, more rigorous synthesis of the literature vs. a scoping review. Regardless of assigning a type to the review, the authors should at least describe their process of identifying and grading the quality of research evidence.

Minor comments:

Abstract - the Abstract states that "...lifelong aerobic exercise training is unable to prevent, or even attenuate, decrements in fast fiber function,..." and advocates for examining other nutritional, pharmaceutical and exercise strategies to preserve muscle fiber function. However, this review does not consider the effects of exercise on single fiber function. Such statements are inappropriate given the focus on this review and would be best left to the section on Future Work.

Page 4, Size-Cross-sectional Area (CSA) section: The authors must consider literature from other techniques regarding how aging affects muscle fiber size. The # of fibers analyzed for mechanical parameters are far too few and the assessments (ie, a single diameter or top and side diameters) are insufficient to draw conclusions. The authors can survey results from CSA assessments on single fibers used or mechanical analysis, but they must at least briefly discuss more rigorous and comprehensive work from other techniques to inform their conclusions.

Page 5, 1st paragraph: The authors correctly note that training status affects muscle fiber size when discussing the work of Korhonen et al. and later in the review discuss how daily activity levels may modify muscle fiber size and function with aging. Here again, consideration of CSA data from other studies using more rigorous techniques would be helpful here, as many of these have made comparisons across groups differing by training status or activity levels.

Page 6, first paragraph: Discussion of the number of failed fibers and the fiber type specificity should include the excellent work of Yu et al. 2007, which represents the first data to comprehensively quantify failure of fibers during mechanical analyses.

Page 6, Peak Isometric Tension (Po) section: The classical biophysical definition of "tension" is the pulling force exerted on or by a biological material relative to its cross sectional area. A more correct term would be "force" instead of tension for this title if the authors are surveying effects of age on raw force values. This is generally standard convention in the field (eg, Trappe et al. J Physiol 2003) and 2nd paragraph page 10.

Page 10, 2nd paragraph: If the authors wish to discuss how fiber size dictates contractile properties, they should more fully consider the excellent work by van Wessel et al. 2010, which defines the properties of fibers in the context of size in relation to oxygen diffusion limitations. In this biophysiological model, the size dependency is parsed in terms of force production vs. fatigue resistance, which also has relevance to aging and the oft described fatigue resistance of muscle with aging.

Page 11, Rate of Force Development section: The authors argue that the rate of force development during contraction is an important factor in physical function, using examples such as rapid movements to prevent falls. They then suggest that the muscle fiber kinetic parameter, ktr, is a valuable tool to assess muscle fiber function as it relates to whole muscle rate of force development. However, the assessment of ktr is essentially under unloaded conditions. Thus, how ktr relates to rate of force development from whole muscle assessments (as the rate of increase of force under loaded conditions) and its relationship to physical function is questionable. Put another way, the conditions under which ktr is measured are really not any less an extreme than those of isometric tension or unloaded shortening velocity. The authors may want to reconsider their rationale for use of ktr as a more physiologically relevant assessment.

Page 11, Rate of Force Development section: The authors seek to define the effects of aging on the kinetics of muscle contraction by reviewing data on ktr. If the goal is to examine effects of age on muscle contractile kinetics, they should also consider other work assessing these parameters using other systems. For example, the excellent work of Hook et al. 2001, Canepari et al. 2005 and Li et al. 2015 using single muscle fibers within the in vitro motility assay or the work of Miller et al. 2013 using sinusoidal length perturbation analysis. Work from all of these studies suggest that there are impairments in cross-bridge kinetic parameter with age, contrary to the authors' conclusion at the end of the section on ktr.

Page 13-14, statement spanning these pages: Several studies have attempted to assess fibers under more physiologically relevant temperatures and at in vivo Pi levels. The authors may want to note this throughout the review of work in this field, as this lends further weight to specific studies as being truer representations of the in vivo environment and, as such, likely more valid conclusions regarding age-related effects.

Figures 1-4: The Figures have a number of issues that should be resolved. It is unclear what the numbers on the x-axis represent and there is no discussion of this in the legends. The text on top of the x-axis pertaining to each study is difficult to see and will be more difficult when graphs are reproduced in journal format. The Y an O texts used to denote greater in young or old, respectively, is apparent from differences in mean values and it would be better if a larger "Y" or "O" over top of the mean values would be more visually apparent for the reader. The authors need to better define the basis of their

statistical comparisons for "mean values" derived by the authors. For example, were the final calculated mean values weighted for the sample size of each study? If not, simple unpaired t-tests are not the appropriate statistic for these comparisons, as taking the mean value of mean values from various studies does not fall within the realm of parametric statistical procedures, but instead fall within the confines of meta-analytical statistical approaches.

Referee #2:

General comment:

This manuscript summarizes a multitude of studies from the past 25 years, which investigated age-related changes in single fiber contractility from human biopsies. The number of studies and the integration of the data from the studies are noted as a tour de force. The following are suggestions that would greatly enhance the impact of the review article and likely provide explanations or thoughts considering the complex nature or limited complexity for the current findings.

1. Overall organization-experimental technique. Currently the manuscript identifies strengths of the permeabilized fiber experimentation early in the manuscript. The weaknesses, assumptions and caveats of the permeabilized fiber experimentation are noted within each of the contractile properties. With this current organization, the reader must be intimately involved with the experimental technique. Suggestion: Early in the manuscript discuss the strengths and weaknesses (assumptions, caveats) of the technique (include the importance of temperature, ionic strength, storage, and length of storage.) Although it is easy to do a single fiber experiment, to do the experiment well requires careful attention to details and to great knowledge of the preparation. A figure would be helpful to bring these concepts together. Also, the mechanically peeled fiber preparation is very different than the chemically permeabilized preparation. Since the review is focused on the permeabilized fiber preparation, consider removing any reference to the mechanically peeled fiber experimentation. Also, note if the fibers were identified by MHC via gel (%) and the range of fibers per muscle by fiber type.

2. Overall organization-ages, muscles, biopsy size. Currently the manuscript identifies young adult and old with limited or no mention of the specific ages. A discussion early in the review of the specific "ages" within the studies would be valuable along with a discussion on how the field has changed over time regarding the perspective of what age is considered "old." Consider adding a table with the ages used in each study, muscle biopsied, and fiber number by MHC type. Also, note what are the strengths or weaknesses of using only two age groups versus three (any?) and a cross-sectional research design versus a longitudinal research design. The characterization and importance of the muscle biopsied.

3. Overall organization- As above, a discussion earlier in the review focused on the role of physical activity and the impact of inclusion criteria.

4. Muscle or fiber quality. Mechanisms for impaired contractility. Consider adding a discussion of post-translational modifications of key proteins in respect to quality, a discussion focused on the proteins of ECC (DHPR/RyR), and de-differentiation resulting in both increased adiposity and fibrosis.

5. Characterizing or referencing of single fibers. Currently the review is combining the contractile characteristics of speed (fast and slow) with the MHC isoform (I and II) present when reviewing the studies. If the studies fiber typed each individual fiber as MHC I or MHC II (inclusive all MHC II isoforms or even separately), refer to the fibers as MHC I or MHC II (IIA etc.).

6. The term "size-specific tension", what is the definition of specific tension?

7. Lastly, the review is focused on "data mean" without any reference to potential alterations in distributions.

REQUIRED ITEMS:

-Please include an Abstract Figure. The Abstract Figure is a piece of artwork designed to give readers an immediate understanding of the Review Article and should summarise the main conclusions. If possible, the image should be easily 'readable' from left to right or top to bottom. It should show the physiological relevance of the Review so readers can assess the importance and content of the article. Abstract Figures should not merely recapitulate other figures in the Review. Please try to keep the diagram as simple as possible and without superfluous information that may distract from the main conclusion of the Review. Abstract Figures must be provided by authors no later than the revised manuscript stage and should be uploaded as a separate file during online submission labelled as File Type 'Abstract Figure'. Please ensure that you include the figure legend in the main article file. All Abstract Figures will be sent to a professional illustrator for redrawing and you may be asked to approve the redrawn figure before your paper is accepted.

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END OF COMMENTS

Confidential Review **17-May-2022**

EDITOR COMMENTS

Reviewing Editor:

Thank you for submitting your review to the Journal of Physiology. Two expert reviewers have provided their assessment of your review. Whilst both found merit to your review, reviewer 2 in particular felt the organization could be improved substantially and that this would increase the impact of the work. That reviewer has provided very specific recommendations for revision. On the basis of this, we would like to give you the opportunity to revise your work to address the comments/suggestions of both reviewers.

Senior Editor:

Thank you for your review submission. Please evaluate the reviewers' comments carefully and follow their advice as you revise your manuscript. Both reviewers have indicated a substantive revision is required.

Response: *We thank the editors for their investment in our work. The positive and constructive nature of the reviewers' criticisms enabled us to make what we believe are notable improvements to the manuscript.*

REFEREE COMMENTS

Referee #1:

This review examines the effects of aging on single skeletal muscle fiber contractile function in humans, encompassing muscle fiber size, contractile force, velocity, power, as well as kinetic and calcium sensitivity of the constituent myofilaments. The work is well-referenced and needed in the field. The authors conclude from their review of the literature that there is evidence for profound age-related decrements in fiber size and contractility primarily in fast-twitch, myosin heavy chain (MHC) II-expressing fibers, but not slow-twitch, MHC I-expressing fibers. There are several modifications that are recommended to improve the impact of this work.

Response: *We thank the reviewer for their positive comments and investment in our work.*

Comments:

1) Muscle fiber size: The authors rely on assessments of single muscle fiber size from diameter assessments on single muscle fibers that have been manually dissected from muscle and are being evaluated for contractile parameters. While this is logical given the focus of the review, the authors should probably survey the literature on aging effects on muscle fiber size from more rigorous, state-of-the-art techniques that provide a larger sample size to make conclusions.

Response: *We have incorporated some of what we feel to be the most relevant literature on fiber-type specific CSA measurements from studies using immunohistochemistry. Specific excerpts with corresponding page numbers integrating these studies are provided below:*

Page 5: Importantly, the observation that MHC I fibre size does not differ with age is also commonly reported by studies that use immunohistochemistry (IHC) of muscle cross-sections for the measurement of fibre size (Nilwik *et al.*, 2013; Callahan *et al.*, 2014; Verdijk *et al.*, 2014; Murgia *et al.*, 2017; Kelly *et al.*, 2018).

Page 6: Moreover, studies using IHC of muscle cross-sections have also reported age-related atrophy of the MHC II fibres in groups of lifelong recreationally active adults (Soendenbroe *et al.*, 2022), sprint-

trained athletes (Korhonen *et al.*, 2006), and world-class masters athletes (Sonjak *et al.*, 2019), suggesting that the fast fibre atrophy is not solely due to age differences in physical activity levels or training status.

Page 6: In support of this hypothesis, prolonged resistance exercise training (i.e., 24-weeks) has been shown to increase MHC II fibre size by an average of 24-29% in healthy older men and women when measured with IHC (Leenders *et al.*, 2013; Nilwik *et al.*, 2013).
The inclusion of the literature in this area, however, was not comprehensive. The primary reason for including the separate section on fibre CSA in this review is because of the importance of this fibre parameter to absolute force and power and evaluating whether intrinsic contractile function is altered by age (i.e., normalized power and specific tension).

2) Statistical analysis of pooled data: There are p-values provided for comparisons across age and sex for single fiber size and contractility parameters. It is not clear from what analyses these p-values are based upon. If the authors are taking mean values from studies and summing across age groups or across age groups in each sex, these mean values should be weighted based on the sample size for any statistical comparisons. While use of meta-analytical techniques are beyond the scope of this review, if the authors wish to make such comparisons, sample sizes should be considered to provide more valid comparisons.

Response: *We appreciate the reviewer's suggestion to weight the contribution of each studies data to the collective mean based on the sample sizes. We had several discussions about using this approach prior to the initial submission and believe the approach we have taken provides the most accurate representation of the collective literature for the following reasons: First, some studies do not report the fiber n for all the variables and different fiber types (see new table 2), and thus, we would have to exclude these studies and/or make assumptions about the n for each variable. Second, none of the conclusions change when we use the weighted means versus use the approach where each study contributes equally to the overall mean (see example figures below). Third, weighting the means based on sample size assumes that everything else is equal between studies, and the only factor that needs to be considered in the contribution of data to the overall mean is the sample size. Finally, we consulted with a biostatistician, Dr. Mehdi Maadooliat, and he confirmed that the approach we used is one of several valid approaches that can be used to combine data from multiple studies when the sample size is not available for all the studies. We have clarified in the text where the p-values for the collective means come from and why we have chosen not to weight the contribution of each study to the overall means based on the sample sizes.*

3) Nature of the review: With reviews now becoming a field of science, it would be helpful if the authors would better describe how they went about conducting their review of the literature and describe what type of review they have undertaken (ie, systematic, scoping, etc) and its primary purpose. If the authors followed a systematic review process, this would be viewed as a stronger, more rigorous synthesis of the literature vs. a scoping review. Regardless of assigning a type to the review, the authors should at least describe their process of identifying and grading the quality of research evidence.

Response: *The Journal of Physiology does not publish systematic reviews or meta-analyses. We clarify at the end of the introduction on page 4 that this is a narrative review. In response to a comment made by Reviewer 2, we also now include two tables that report the subject characteristics, number of fibres studied, and several parameters that are important to the outcomes of single fiber contractile function experiments. Within the legend of this table, we also describe the process used to identify the studies to be incorporated in our narrative review. It should also be noted that the number of laboratories capable of conducting single fibre experiments is small, and thus, we are confident that we have not excluded major studies that are pertinent to the conclusions made in this review.*

Minor comments:

Abstract - the Abstract states that "...lifelong aerobic exercise training is unable to prevent, or even attenuate, decrements in fast fiber function,..." and advocates for examining other nutritional, pharmaceutical and exercise strategies to preserve muscle fiber function. However, this review does not consider the effects of exercise on single fiber function. Such statements are inappropriate given the focus on this review and would be best left to the section on Future Work.

Response: *We report the findings from the two most comprehensive studies conducted on the effects of lifelong aerobic exercise on single fibre size and contractile function throughout the review on pages 5, 6, 7, 8, 9, and 10 (Gries et al. 2019, Grosicki et al. 2021). We have clarified in the abstract, however, that we are referring to contractile function, as lifelong aerobic exercise may have important effects on other functional cellular parameters. We acknowledge that a discussion on lifelong aerobic exercise was missing in our fiber power section and have added to the discussion in this section. Below we provide the text where we highlight the findings from the two lifelong aerobic exercise studies:*

Page 5: Intriguingly, cross-sectional comparisons of MHC I fibre size in endurance trained older and younger individuals suggest that habitual physical activity and lifelong aerobic exercise may augment this hypertrophic response (Coggan *et al.*, 1990; Grosicki *et al.*, 2021)…
Page 6: We speculate that the limited myocellular growth potential may contribute, at least in part, to the lack of an apparent benefit for MHC II fibre size observed with lifelong aerobic exercise in older men (Grosicki *et al.*, 2021) and women (Gries *et al.*, 2019).
Page 7: … and lifelong aerobic exercise training appears unable to attenuate the deficits in absolute P_o in older men or women (Gries *et al.*, 2019; Grosicki *et al.*, 2021).
Pages 8-9: For example, lifelong participation in aerobic exercise training may increase single fibre shortening velocity in older adults (Gries *et al.*, 2019; Grosicki *et al.*, 2021), leaving open the possibility that different physical activity levels and/or training status among the older cohorts may explain some of the inter-study differences (D'Antona *et al.*, 2007).
Pages 9-10: An important observation, however, is that the absolute power of the MHC I fibres from older adults who participated in lifelong aerobic exercise training were greater than that of healthy active younger men and women (Gries *et al.*, 2019; Grosicki *et al.*, 2021), providing further evidence that MHC I fibres of older adults likely have a preserved ability to adapt to aerobic exercise training.
Page 10: … it is interesting to note that the three studies observing higher normalized power also reported smaller CSA of the MHC II fibres from both healthy older adults and those who performed lifelong aerobic exercise training (Sundberg *et al.* 2018, Gries *et al.* 2019, Grosicki *et al.* 2021).

Page 4, Size-Cross-sectional Area (CSA) section: The authors must consider literature from other techniques regarding how aging affects muscle fiber size. The # of fibers analyzed for mechanical parameters are far too few and the assessments (ie, a single diameter or top and side diameters) are insufficient to draw conclusions. The authors can survey results from CSA assessments on single fibers used or mechanical analysis, but they must at least briefly discuss more rigorous and comprehensive work from other techniques to inform their conclusions.

Response: *We have added several references on pages 5 and 6 that used immunohistochemical staining of biopsy cross-sections. These studies support the early seminal findings by Lexell and colleagues that further strengthen the conclusion that MHC I fibre size is well-preserved with ageing, but that MHC II fibre atrophy is present even in individuals who are recreationally active, sprint-trained athletes, and world-class masters athletes.*

Page 5, 1st paragraph: The authors correctly note that training status affects muscle fiber size when discussing the work of Korhonen et al. and later in the review discuss how daily activity levels may

modify muscle fiber size and function with aging. Here again, consideration of CSA data from other studies using more rigorous techniques would be helpful here, as many of these have made comparisons across groups differing by training status or activity levels.

Response: *Similar to the previous comment, we have added data from immunohistochemical studies to expand the discussion on fiber CSA.*

Page 6, first paragraph: Discussion of the number of failed fibers and the fiber type specificity should include the excellent work of Yu et al. 2007, which represents the first data to comprehensively quantify failure of fibers during mechanical analyses.

Response: *We have referenced Yu et al. 2007 as one of the studies reporting failure of fibers during mechanical analyses.*

Page 6, Peak Isometric Tension (Po) section: The classical biophysical definition of "tension" is the pulling force exerted on or by a biological material relative to its cross-sectional area. A more correct term would be "force" instead of tension for this title if the authors are surveying effects of age on raw force values. This is generally standard convention in the field (eg, Trappe et al. J Physiol 2003) and 2nd paragraph page 10.

Response: *We now use the conventional nomenclature and refer to the data as force, instead of tension. When the force values are expressed relative to the fibre cross-sectional area, we refer to the data as tension. All substitutions have been highlighted.*

Page 10, 2nd paragraph: If the authors wish to discuss how fiber size dictates contractile properties, they should more fully consider the excellent work by van Wessel et al. 2010, which defines the properties of fibers in the context of size in relation to oxygen diffusion limitations. In this biophysiological model, the size dependency is parsed in terms of force production vs. fatigue resistance, which also has relevance to aging and the oft described fatigue resistance of muscle with aging.

Response: *While we agree that the fiber type-fiber size paradox outlined by van Wessel is intriguing and likely bears relevance to aging, we are hesitant to veer too far from the focus of the paper, which is on permeabilized single muscle fibre contractile function. Moreover, the seminal paper by Hickson (1980), upon which the van Wessel paper greatly relies, is subject to scrutiny (e.g., Murach and Bagley, 2016).*

Page 11, Rate of Force Development section: The authors argue that the rate of force development during contraction is an important factor in physical function, using examples such as rapid movements to prevent falls. They then suggest that the muscle fiber kinetic parameter, ktr, is a valuable tool to assess muscle fiber function as it relates to whole muscle rate of force development. However, the assessment of ktr is essentially under unloaded conditions. Thus, how ktr relates to rate of force development from whole muscle assessments (as the rate of increase of force under loaded conditions) and its relationship to physical function is questionable. Put another way, the conditions under which ktr is measured are really not any less an extreme than those of isometric tension or unloaded shortening velocity. The authors may want to reconsider their rationale for use of ktr as a more physiologically relevant assessment.

Response: *We had no intention of suggesting that k_{tr} is a more physiologically relevant assessment than the other parameters presented in the manuscript and have rephrased the first sentence of this section to prevent the readers from coming to a similar interpretation. Additionally, the k_{tr} assessment is made following a slack-reextension maneuver of a Ca^{2+} activated fibre, thus the fibre is contracting isometrically, which is a loaded condition.*

Page 11, Rate of Force Development section: The authors seek to define the effects of aging on the kinetics of muscle contraction by reviewing data on ktr. If the goal is to examine effects of age on muscle contractile kinetics, they should also consider other work assessing these parameters using other systems. For example, the excellent work of Hook et al. 2001, Canepari et al. 2005 and Li et al. 2015 using single muscle fibers within the in vitro motility assay or the work of Miller et al. 2013 using sinusoidal length perturbation analysis. Work from all of these studies suggest that there are impairments in cross-bridge kinetic parameter with age, contrary to the authors' conclusion at the end of the section on ktr.

Response: *The goal of this section is to present the data that investigates the rate of force development at the single fiber level. The gold standard approach is the k_{tr} assessment, which is determined by the kinetics of the low- to high-force state of the cross-bridge cycle (Metzger and Moss, 1990a, b). In vitro motility assay experiments, while providing valuable information about cross-bridge kinetics, are more analogous to the experiments assessing shortening velocity at the single fiber level. The data presented in figure 3 show that aging has little to no effect on single fiber shortening velocity in either fiber type. The reasons for the discrepancies between the fiber shortening velocity data and in vitro motility assay data are interesting but is beyond the scope of this review. How the sinusoidal length perturbation analysis translates to rates of force development is unknown. In addition, of all the parameters derived from the sinusoidal analysis in Miller et al. 2013, few were observed to differ with age, the differences that were observed were restricted to fibers from only women, and the findings have yet to be replicated by another study.*

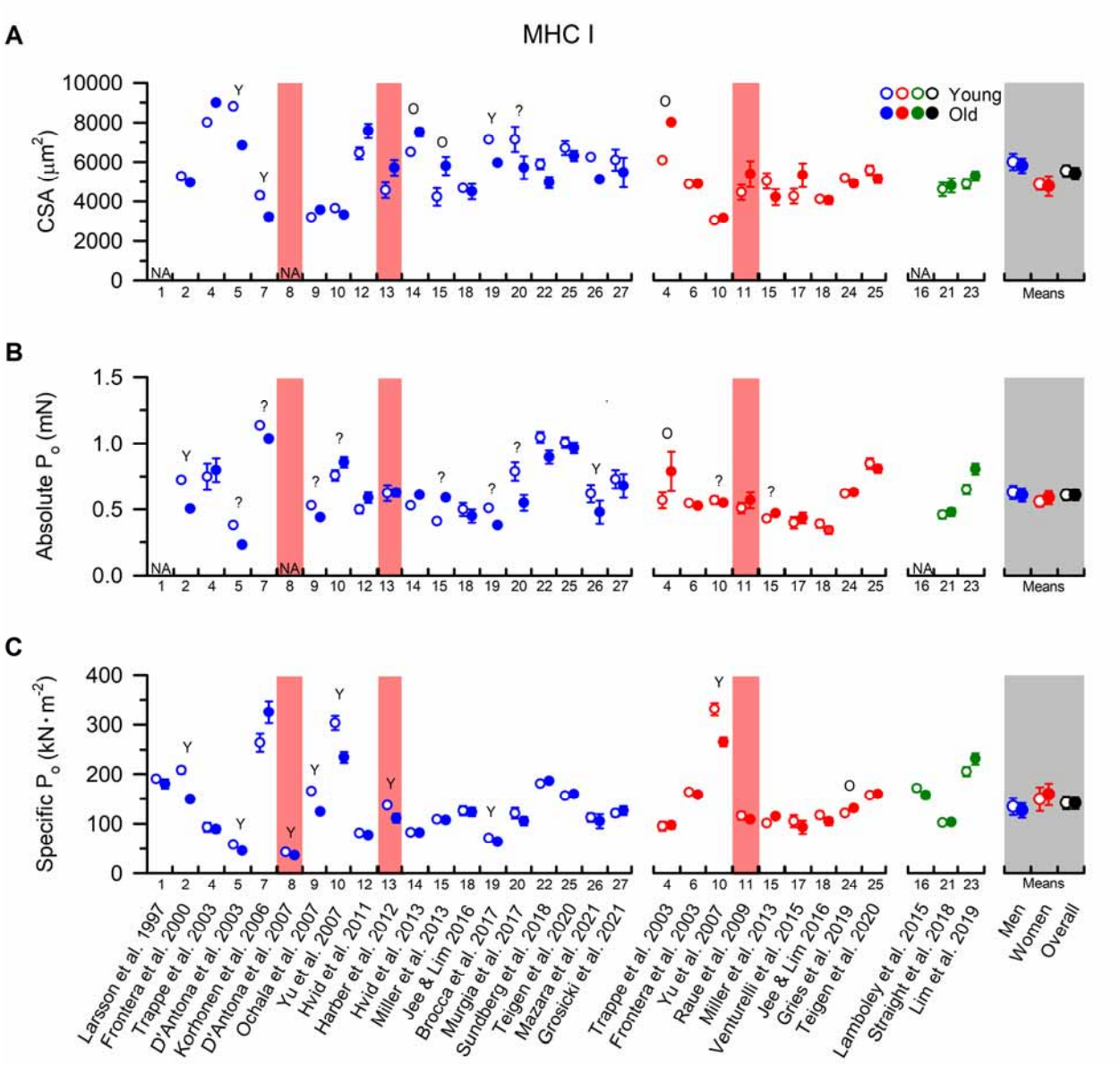
Page 13-14, statement spanning these pages: Several studies have attempted to assess fibers under more physiologically relevant temperatures and at in vivo Pi levels. The authors may want to note this throughout the review of work in this field, as this lends further weight to specific studies as being truer representations of the in vivo environment and, as such, likely more valid conclusions regarding age-related effects.

Response: *We added a table that includes the temperature and solution composition used in each of the studies. This table reveals to the readers that only two studies have investigated single fibre contractile function with age under warmer temperatures (i.e., above room temperature) and with concentrations of Pi that are closer to physiological (Miller et al., 2013 and Sundberg et al., 2018). Because of the low number of studies, we have elected to not add additional emphasis to the conclusions from these studies, although we agree with the reviewer that these findings are likely more directly translatable to what is occurring in vivo.*

Figures 1-4: The Figures have a number of issues that should be resolved. It is unclear what the numbers on the x-axis represent and there is no discussion of this in the legends. The text on top of the x-axis pertaining to each study is difficult to see and will be more difficult when graphs are reproduced in journal format. The Y an O texts used to denote greater in young or old, respectively, is apparent from differences in mean values and it would be better if a larger "Y" or "O" over top of the mean values would be more visually apparent for the reader. The authors need to better define the basis of their statistical comparisons for "mean values" derived by the authors. For example, were the final calculated mean values weighted for the sample size of each study? If not, simple unpaired t-tests are not the appropriate statistic for these comparisons, as taking the mean value of mean values from various studies does not fall within the realm of parametric statistical procedures, but instead fall within the confines of meta-analytical statistical approaches.

Response: *The numbering of the studies in the figures and tables is now clarified in the captions. We increased the font size for the text on top of the x-axis to as large as possible without going into the*

column of the next study. We attempted to move the Y and O used to denote studies with statistical significance overtop the symbols as the reviewer suggested. This made it difficult to identify the number of studies observing statistical differences (see sample figure below), and thus, we elected to keep the figures as originally designed. As mentioned above, several studies did not include the number of fibers or we were required to make assumptions about the number of fibers used for each variable. As a result, we have elected to keep our original approach, and equally weight the contribution of each study to the overall means, which allowed us to include the data from all the studies (the red in the figures indicates the studies that could not be included in the pooled data if we weighted based on fibre numbers). We consulted with biostatistician, Dr. Mehdi Maadooliat, and this approach is one of several valid approaches that can be used to combine data from multiple studies. We did run statistical tests to assess for normality and homogeneity of variance, which are assumptions that need to be met for parametric statistical procedures. We also ran weighted regression analyses, and none of the conclusions changed using the alternative statistical approach. We have now included the description of these statistical tests in the figure captions.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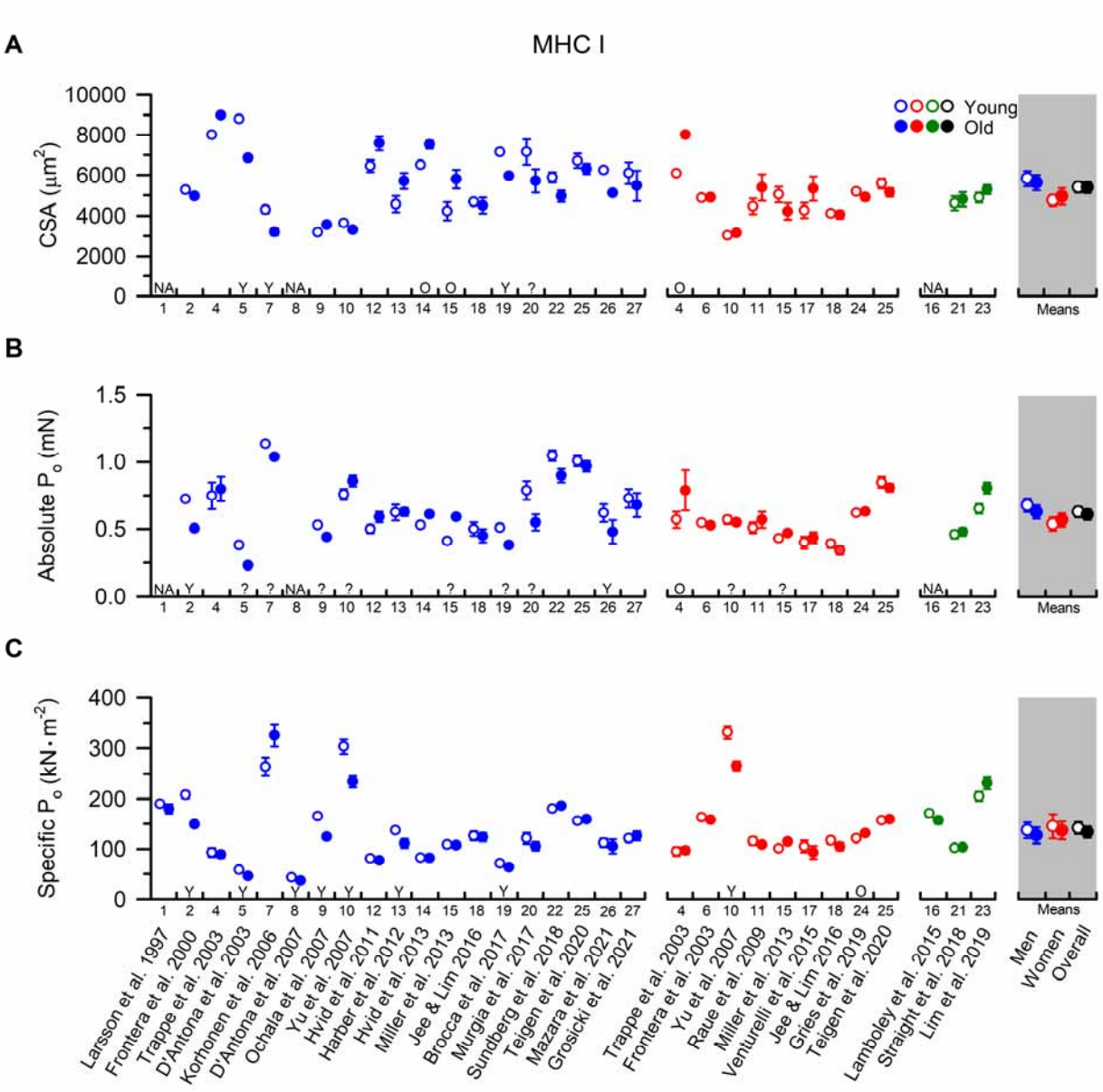
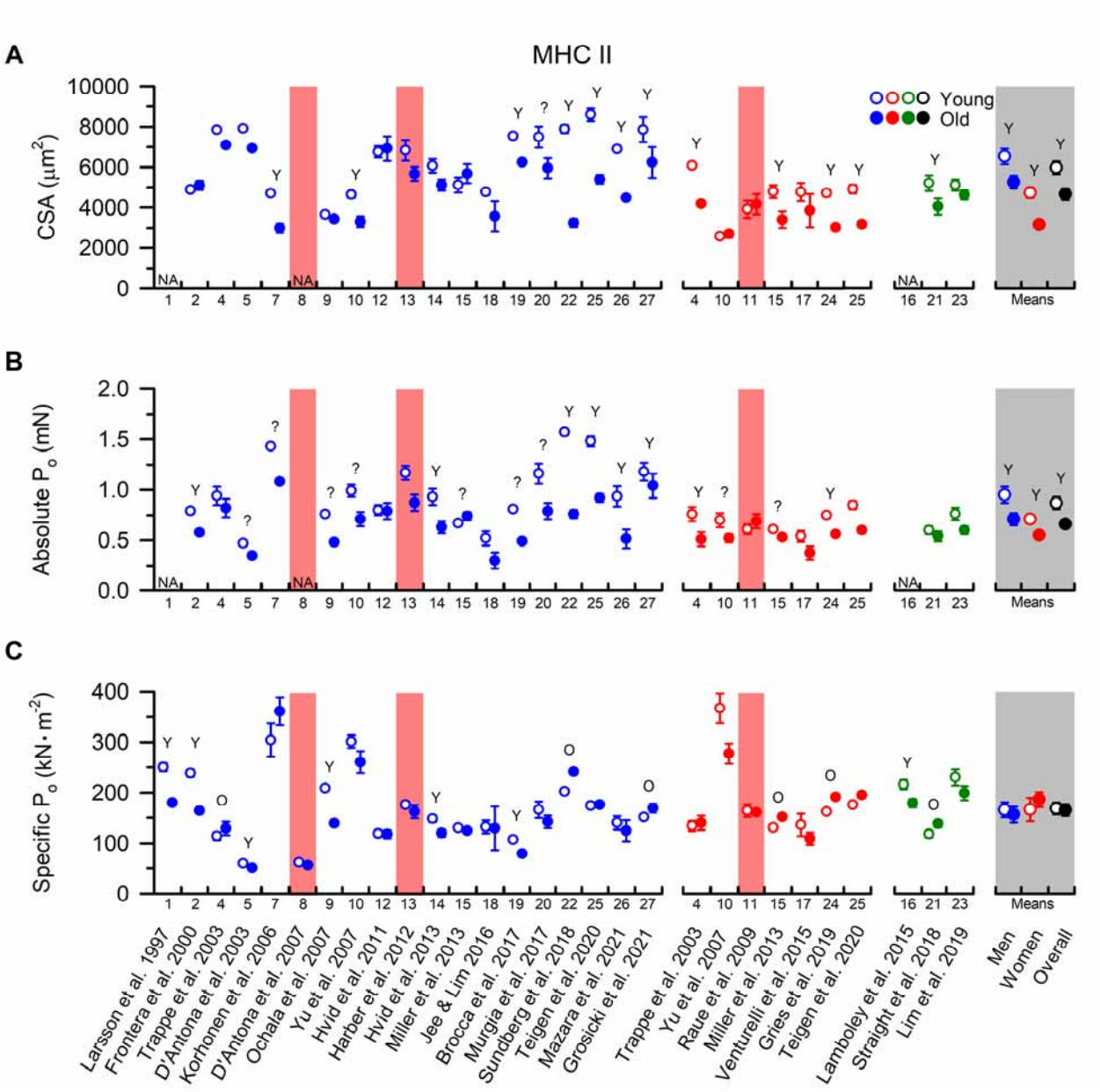
Overall
Larsson et al. 1997
Frontera et al. 2000
Trappe et al. 2003
D'Antona et al. 2003
Korhonen et al. 2006
D'Antona et al. 2007
Ochala et al. 2007
Yu et al. 2007
Hvid et al. 2011
Haber et al. 2012
Hvid et al. 2013
Miller et al. 2013
Jee & Lim 2016
Brocca et al. 2017
Murgia et al. 2017
Sundberg et al. 2018
Teigen et al. 2020
Mazara et al. 2021
Grosicki et al. 2021
Trappe et al. 2003
Frontera et al. 2003
Yu et al. 2007
Raue et al. 2009
Miller et al. 2013
Venturelli et al. 2015
Jee & Lim 2016
Gries et al. 2019
Teigen et al. 2020
Lamboley et al. 2015
Straight et al. 2018
Lim et al. 2019


A
MHC I
CSA (μm²)
Young
Old
Means
B
Absolute P_o (mN)
C
Specific P_o (kN · m⁻²)
Larsson et al. 1997
Frontera et al. 2000
Trappe et al. 2003
D'Antona et al. 2003
Korhonen et al. 2006
D'Antona et al. 2007
Ochala et al. 2007
Yu et al. 2007
Hvid et al. 2011
Harber et al. 2012
Hvid et al. 2013
Miller et al. 2013
Jee & Lim 2016
Brocca et al. 2017
Murgia et al. 2017
Sundberg et al. 2018
Teigen et al. 2020
Mazara et al. 2021
Grosicki et al. 2021
Trappe et al. 2003
Frontera et al. 2003
Yu et al. 2007
Raue et al. 2009
Miller et al. 2013
Venturelli et al. 2015
Jee & Lim 2016
Gries et al. 2019
Teigen et al. 2020
Lamboley et al. 2015
Straight et al. 2018
Lim et al. 2019
Men
Women
Overall


A
MHC II
CSA (μm²)
Young
Old
B
Absolute P₀ (mN)
C
Specific P₀ (kN·m⁻²)
Means
Larsson et al. 1997
Frontera et al. 2000
Trappe et al. 2003
D'Antona et al. 2003
Korhonen et al. 2006
D'Antona et al. 2007
Ochala et al. 2007
Yu et al. 2007
Hvid et al. 2011
Harber et al. 2012
Hvid et al. 2013
Miller et al. 2013
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Brocca et al. 2017
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Teigen et al. 2020
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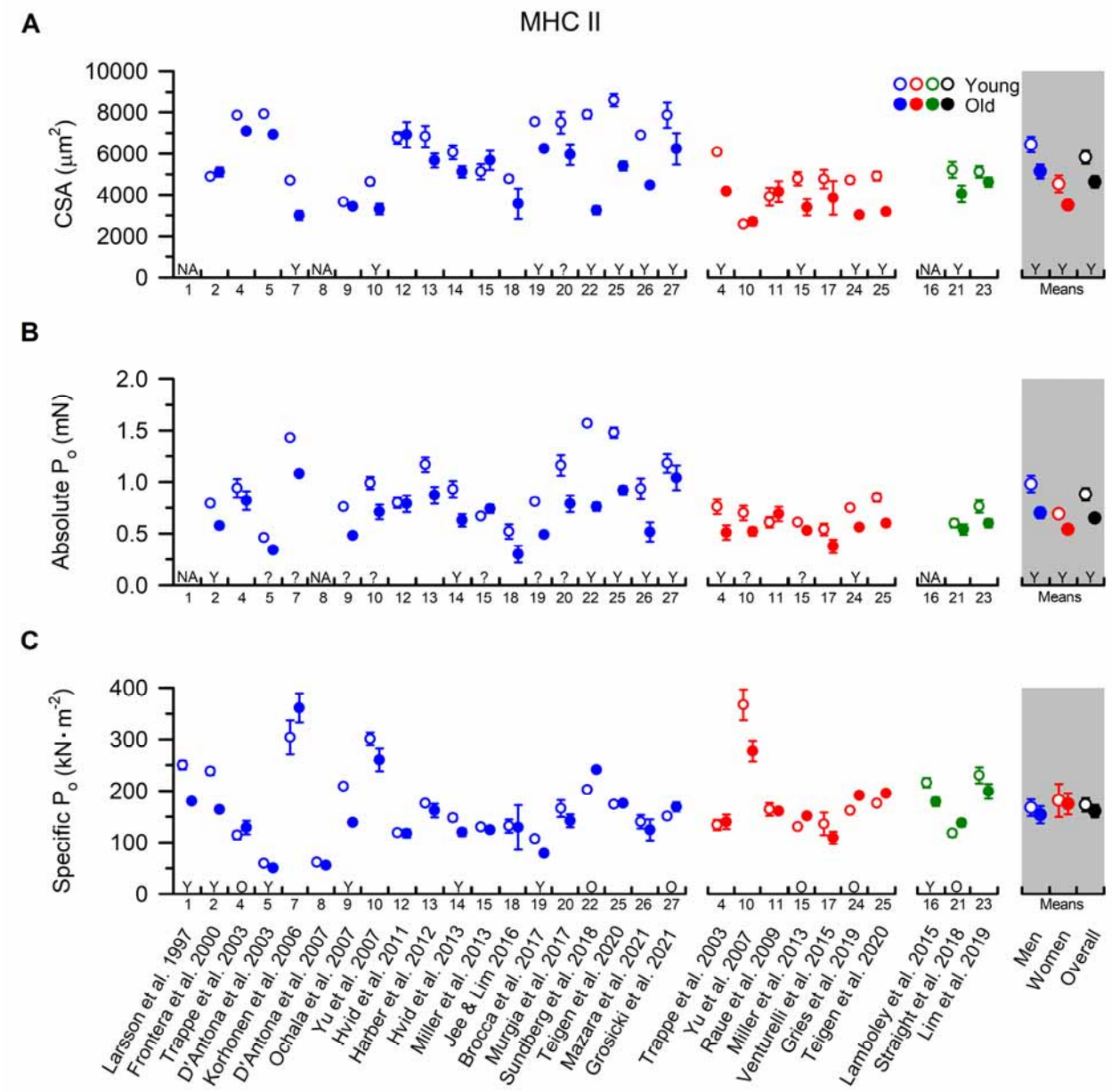
Lamboley et al. 2015
Straight et al. 2018
Lim et al. 2019
Men
Women
Overall



Referee #2:

General comment:

This manuscript summarizes a multitude of studies from the past 25 years, which investigated age-related changes in single fiber contractility from human biopsies. The number of studies and the integration of the data from the studies are noted as a tour de force. The following are suggestions that would greatly enhance the impact of the review article and likely provide explanations or thoughts considering the complex nature or limited complexity for the current findings.

Response: *We are honored that the reviewer recognizes our review as a "tour de force" and thank them for their investment in our work.*

1. Overall organization-experimental technique. Currently the manuscript identifies strengths of the permeabilized fiber experimentation early in the manuscript. The weaknesses, assumptions and caveats of the permeabilized fiber experimentation are noted within each of the contractile properties. With this current organization, the reader must be intimately involved with the experimental technique. Suggestion: Early in the manuscript discuss the strengths and weaknesses (assumptions, caveats) of the technique (include the importance of temperature, ionic strength, storage, and length of storage.) Although it is easy to do a single fiber experiment, to do the experiment well requires careful attention to details and to great knowledge of the preparation. A figure would be helpful to bring these concepts together. Also, the mechanically peeled fiber preparation is very different than the chemically permeabilized preparation. Since the review is focused on the permeabilized fiber preparation, consider removing any reference to the mechanically peeled fiber experimentation. Also, note if the fibers were identified by MHC via gel (%) and the range of fibers per muscle by fiber type.

Response: *We agree with the reviewer that conducting a quality single fiber study requires careful attention to several critical steps. However, to provide detail of all the steps necessary for a well-controlled single fiber study is outside the scope of this review and likely warrants a separate reproducibility and methodology manuscript. We have now included a table that highlights the experimental procedures used in the included single fiber studies. We refer to this table throughout the review when discussing that differences in methodology may be contributing to the discrepancies between the studies. The single study that used the mechanically skinned fiber preparation happens to be one of only six studies to evaluate Ca^{2+} sensitivity, so we have elected to still include this study in our review. All studies included in the review identified the fibre type either via SDS-PAGE or western blotting.*

2. Overall organization-ages, muscles, biopsy size. Currently the manuscript identifies young adult and old with limited or no mention of the specific ages. A discussion early in the review of the specific "ages" within the studies would be valuable along with a discussion on how the field has changed over time regarding the perspective of what age is considered "old." Consider adding a table with the ages used in each study, muscle biopsied, and fiber number by MHC type. Also, note what are the strengths or weaknesses of using only two age groups versus three (any?) and a cross-sectional research design versus a longitudinal research design. The characterization and importance of the muscle biopsied.

Response: *We have made a table that identifies the subject characteristics, number of fibers studied, the activity/training status of the participants, the muscle biopsied, etc. The rationale for including a discussion on what is characterized as 'old', whether the definition of 'old' has changed over time, and whether studies should include several age groups is not clear, because it does not appear to have any major influence over the conclusions made in the review and would detract from the major findings. We recognize the limitations of employing cross-sectional study designs in aging research on page 10 and highlight that few longitudinal studies have been conducted in the concluding remarks. We chose not to expand on the discussion of the few longitudinal studies, because the findings are difficult to reconcile with the prevailing literature using cross-sectional studies.*

3. Overall organization- As above, a discussion earlier in the review focused on the role of physical activity and the impact of inclusion criteria.

Response: *This information is now included in Table 1.*

4. Muscle or fiber quality. Mechanisms for impaired contractility. Consider adding a discussion of post-translational modifications of key proteins in respect to quality, a discussion focused on the proteins of ECC (DHPR/RyR), and de-differentiation resulting in both increased adiposity and fibrosis.

Response: *This review is focused on the permeabilized single fibre contractile function changes with ageing, which does not investigate the potential alterations in EC Coupling. As a result, post-translational modifications that may alter EC coupling are considered outside the scope of this review. To our knowledge skeletal muscle cells, which are post-mitotic, are unable to de-differentiate so the comment about adiposity and fibrosis is not applicable.*

5. Characterizing or referencing of single fibers. Currently the review is combining the contractile characteristics of speed (fast and slow) with the MHC isoform (I and II) present when reviewing the studies. If the studies fiber typed each individual fiber as MHC I or MHC II (inclusive all MHC II isoforms or even separately), refer to the fibers as MHC I or MHC II (IIA etc.).

Response: *We have gone through the text and removed any reference to contractile speed (fast and slow) that preceded MHC classification (i.e., "slow MHC I" has now been changed to "MHC I"). Additionally, we have changed the headings in the figures to only refer to the myosin heavy chain isoform expressed and not the contractile speed. We have also clarified that only studies that determined the myosin heavy chain isoforms were included in the data sets for the figures.*

6. The term "size-specific tension", what is the definition of specific tension?

Response: *We have defined specific tension on page 7 as the isometric force normalized to cross-sectional area (P_o/CSA) and have removed all text that referred to this measurement as size-specific tension.*

7. Lastly, the review is focused on "data mean" without any reference to potential alterations in distributions.

Response: *For each single fibre parameter, we report the number of studies that did versus did not observe an age difference and depict the distribution of the findings from all the studies in each of the figures. We believe this is the most transparent approach to depict the distribution of the data.*

REQUIRED ITEMS:

-Please include an Abstract Figure. The Abstract Figure is a piece of artwork designed to give readers an immediate understanding of the Review Article and should summarise the main conclusions. If possible, the image should be easily 'readable' from left to right or top to bottom. It should show the physiological relevance of the Review so readers can assess the importance and content of the article. Abstract Figures should not merely recapitulate other figures in the Review. Please try to keep the diagram as simple as possible and without superfluous information that may distract from the main conclusion of the Review. Abstract Figures must be provided by authors no later than the revised manuscript stage and should be uploaded as a separate file during online submission labelled as File Type 'Abstract Figure'. Please ensure that you include the figure legend in the main article file. All Abstract Figures will be sent to a professional illustrator for redrawing and you may be asked to approve the redrawn figure before your paper is accepted.

-Please upload separate high quality figure files via the submission form.

-Author profile(s) must be uploaded via the submission form. Authors should submit a short biography (no more than 100 words for one author or 150 words in total for two authors) and a portrait photograph of the two leading authors on the paper. These should be uploaded, clearly labelled, with the manuscript submission. Any standard image format for the photograph is acceptable, but the resolution should be at

least 300 dpi and preferably more. A group photograph of all authors is also acceptable, providing the biography for the whole group does not exceed 150 words.

Dear Chris,

Re: JP-TR-2022-282298R1 "Single Muscle Fibre Contractile Function with Ageing" by Greg Grosicki, Carlos S. Zepeda, and Christopher W. Sundberg

I am pleased to tell you that your Topical Review article has been accepted for publication in The Journal of Physiology, subject to any modifications to the text that may be required by the Journal Office to conform to House rules.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

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Best wishes,

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EDITOR COMMENTS

Reviewing Editor:

Thank you for your thorough revision of your manuscript. All prior concerns have been addressed adequately.

Senior Editor:

Thank you for your excellent review, and many apologies for the delay, which resulted from matters beyond our control

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REFEREE COMMENTS

Referee #1:

The authors have addressed all of the issues in my review. This is an excellent review and should have sustained impact on the field.

1st Confidential Review **22-Aug-2022**