The Journal of Physiology

Exercise training remodels subcutaneous adipose tissue in adults with obesity even without weight loss

Cheehoon Ahn, Benjamin J. Ryan, Michael W Schleh, Pallavi Varshney, Alison C Ludzki, Jenna B Gillen, Douglas W. Van Pelt, Lisa M Pitchford, Suzette M Howton, Thomas Rode, Scott L Hummel, Charles F. Burant, Jonathan P Little, and Jeffrey F. Horowitz
DOI: 10.1113/JP282371

Corresponding author(s): Jeffrey Horowitz (jeffhoro@umich.edu)

The referees have opted to remain anonymous.

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Transaction Report:

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

Dear Dr Horowitz,

Re: JP-RP-2021-282371 "Exercise training remodels subcutaneous adipose tissue in adults with obesity even without weight loss" by Cheehoon Ahn, Benjamin J. Ryan, Michael W Schleh, Pallavi Varshney, Alison C Ludzki, Jenna B Gillen, Douglas W. Van Pelt, Lisa M Pitchford, Suzette M Howton, Thomas Rode, Scott L Hummel, Charles F. Burant, Jonathan P Little, and Jeffrey F. Horowitz

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

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

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.

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I hope you will find the comments helpful and have no difficulty returning revisions within 4 weeks.

If you need to check to make sure that your Methods section conforms to the principles of UK regulations, you may wish to refer to Grundy (2015):
Grundy (2015) J. Physiol. 2015 Jun 15;593(12):2547-9 https://doi.org/10.1113/JP270818

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Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

Thank you for submitting the results of this clinical trial to the Journal of Physiology. Although the manuscript has received favorable feedback, the reviewers have also brought up several points that need to be addressed to improve the clarity and the scientific value of the manuscript. Along with the updated version of this article, please prepare and upload the statistical summary document and make the necessary changes to the study figures/tables to comply with the result reporting guidelines of the journal.

REFEREE COMMENTS

Referee #1:

Ahn et al. have examined the effects of 12 weeks of MICT or HIIT on indices of adipose tissue remodelling and metabolism in individuals with obesity. Importantly, body weight was "clamped" and consequently the effects of the exercise training interventions were independent of weight loss. The authors provide evidence of subtle reductions in abdominal subcutaneous adipose tissue adipocyte size in parallel with changes in markers of capillarization, extra cellular matrix remodelling and lipolytic and mitochondrial proteins. While the reported effects of exercise were modest there are several important strengths of this study that need to be highlighted. These include: 1) a comparison of two different modes of exercise in human participants with obesity, 2) a comparison between two time points following exercise, 1 and 4-days, 3) the use of state-of the -art in vivo tracer methodology to asses whole body fatty acid metabolism and 4) controlling for exercise-induced weight loss. These points notwithstanding there are several issues that need further clarification.

1. The authors argue that the current study design allows for an elucidation of the "direct" effects of exercise on adipose tissue (e.g. line 73 amongst others in the paper). This is likely overstated as changes in adipose tissue could be secondary to any number of alterations due to exercise training, not related to body weight. In this regard the authors are encouraged to be careful with their wording. Perhaps "independent of weight loss" is more appropriate to state than "direct effects of exercise."

2. The authors should strongly consider discussing their data within the context of what has already been reported from this trial in relation to insulin sensitivity. In this regard it would appear that improvements in insulin sensitivity in these subjects do not require large and robust increases in adipose tissue remodelling. This would be informative to discuss from the standpoint of mechanisms mediating the beneficial effects of exercise on systemic glucose homeostasis.

3. The authors report that prior exercise training did not improve the ability of insulin to suppress lipolysis. Could this be due, in part, to the high levels of insulin that were infused? Would reducing the amount of insulin infuse reveal an effect of training on adipose tissue insulin sensitivity? This could be discussed.

4. The authors report changes in the protein content of some metabolic proteins such as FAT/CD36. They suggest that since this protein is increased on day 1 but not 4 following training that this speaks to an acute adaptive response to training and not a chronic adaptation (line 565). It could very well be that this adaptation requires a chronic training stimulus, that once removed results in a fairly rapid degradation in the protein. Within this light I'm not certain that these changes should be referred to as acute, unless the authors can demonstrate increases in protein content following a single bout of exercise.

5. The authors attempt to discuss their findings of alterations in adipose tissue fibrosis in comparison to work completed using rodents (line 512). A key point that should likely be taken into consideration is that the rodent work was conducted in animals housed at room temperature and thus the animals were under a degree of thermal stress and the results of their findings likely reflects an interaction between chronic thermal stress and exercise.

6. Please express all data as means + SD. It would seem the figures are mean + SEM

7. Whenever possible, individual data points should be shown

8. For all blots please indicate the approximate apparent molecular weight

9. Please provide a representative loading control image. Given the large number of blots, perhaps one "representative" image per panel could be shown and this indicated in the figure legends.

Referee #2:

The authors have conducted a study comparing moderate intensity continuous exercise training vs. high intensity interval training on sub cutaneous in obese adults. In the current manuscript, the authors report some indices of metabolic health, fatty acid metabolism, and adipocyte morphology and protein expression.

In the absence of weight loss both exercise modalities reduced adipocyte size and increased the proportion of smaller

adipocytes, increased capillary number, and modestly altered the abundance of select proteins involved in adipocyte remodeling, lipid turnover and oxidation, and the MAPK pathway. Whole body lipolysis in the basal state and in response to an insulin clamp were largely unaltered be either training modalities. Interestingly, both exercise modalities had similar effects on adipocyte morphology and protein expression.

Major comment:

While the current report provides some interesting and novel data - it seems a little limited in terms of its scope. Indeed, at first it seemed odd to me that the authors would go to the trouble of doing clamps and stable isotope infusions only to measure the Ra of palmitate. However, having read their recently published paper in JCEM (ref 13 in the current paper), i can see that most of the clamp data was included there. Since it is necessary to report some of the same data in the current manuscript (i.e. peak VO2) - I would suggest that some additional clamp data could be included in this paper. For example, it would be interesting to know if glucose infusion rate or Rd was related to palmitate Ra either prior to or after both exercise interventions. Also, was glucose Ra related to palmitate Ra pre/post exercise? Finally - I note you used glycerol Ra as a measure of adipocyte lipolysis and insulin sensitivity in your JCEM paper - and used palmitate Ra in the current manuscript. While you did not see much of an effect of exercise of whole body ffa turnover or adipose tissue insulin sensitivity, since you saw some histological evidence of altered adipocyte lipid turnover, it may be worth calculating adipocyte fatty acid recycling rates pre and post exercise from glycerol and palmitate Ra's.

Other comments:

1. Key points 1 and 2 are just bullet point descriptions of the background and approach. I think key points should focus on the key take homes from the study data. The fourth key point is rather speculative and again doesn't really convey an important take home from the study data.

2. In my opinion the introduction is far too long. I believe this should be re-written with brevity in mind.

3. Since you include DEXA data it may be useful to present VO2peak as a function of lean mass.

4. Since you see the COX IV expression is elevated with both training modalities - it make be worth while blotting for some additional mito proteins to be a little more confident that there is greater WAT mito protein content with training.

5. You discuss the difference in energy expenditure (EE) with the two modalities (lines 582-583 and line 610-613) as being marked, arguing that it is perhaps surprising that some of the effects of exercise were so similar between the MICT and HIIT groups. I would argue the the differences in EE you see between the two exercise modalities is actually rather minor and perhaps of limited physiological significance - particularly with regards to WAT lipid turnover. I think the metabolic impact of the marked differences in exercise intensities between MICT and HITT are likely more acutely seen at the level of muscle (at least with regards to fuel use).

END OF COMMENTS

Confidential Review **13-Sep-2021**

We greatly appreciate the reviewers and editor for their very favorable evaluation and comments on our manuscript. We have revised the manuscript to address all concerns, and we believe these revisions have greatly improved our manuscript. We have highlighted our changes in the revised manuscript. Additionally, we have provided a point-by-point response to all the reviewers' specific comments below (our responses are in bold).

REFEREE COMMENTS

Referee #1:

1. The authors argue that the current study design allows for an elucidation of the "direct" effects of exercise on adipose tissue (e.g. line 73 amongst others in the paper). This is likely overstated as changes in adipose tissue could be secondary to any number of alterations due to exercise training, not related to body weight. In this regard the authors are encouraged to be careful with their wording. Perhaps "independent of weight loss" is more appropriate to state than "direct effects of exercise."

We have now modified the wording throughout our manuscript to avoid the notion that we were testing "direct effects".

2. The authors should strongly consider discussing their data within the context of what has already been reported from this trial in relation to insulin sensitivity. In this regard it would appear that improvements in insulin sensitivity in these subjects do not require large and robust increases in adipose tissue remodeling. This would be informative to discuss from the standpoint of mechanisms mediating the beneficial effects of exercise on systemic glucose homeostasis.

We agree with the reviewer that including comments about our present findings in the context of insulin sensitivity in our previous publication would be advantageous. We have now modified our discussion to discuss our findings in the context of insulin sensitivity that we previously reported (Lines 641-647). Importantly, however, in our previous study, we found insulin sensitivity to improve only in the several hours after the most recent session of exercise – whereas insulin sensitivity essentially returned to pre-training levels 4 days later (without an acute session of exercise). The robust improvement in response to acute exercise is well-known – and this transient response appears to be related to the exercise-induced transient reduction in muscle glycogen and short-lived changes in enzyme/metabolic pathway activity – and not to longer-lasting structural changes in tissues. Therefore, the newly added statements to our revised manuscript address only the chronic/persistent responses to training. More specifically, we now address how our present findings indicate that training induced only relatively modest changes in adipose tissue structure, which aligned with our previous findings that exercise training did not evoke a persistent improvement in insulin sensitivity (4 days after exercise).

3. The authors report that prior exercise training did not improve the ability of insulin to suppress

lipolysis. Could this be due, in part, to the high levels of insulin that were infused? Would reducing the amount of insulin infuse reveal an effect of training on adipose tissue insulin sensitivity? This could be discussed.

The reviewer makes an excellent point, and we have now incorporated discussion about this into our revised manuscript (Lines 535-543). Based on previous work (e.g., Shojaee-Moradie, et al., Diabetologia. 50(2), 2007) in which exercise training without weight loss did not change the anti-lipolytic response to a low dose of insulin (0.3mU/kg/min which approximates ~12mU/m^2/min), we do not believe that our insulin dose was too high to mask potential training-induced improvements in the anti-lipolytic sensitivity to insulin. But we agree that expanding our discussion to address this issue is an important addition.

4. The authors report changes in the protein content of some metabolic proteins such as FAT/CD36. They suggest that since this protein is increased on day 1 but not 4 following training that this speaks to an acute adaptive response to training and not a chronic adaptation (line 565). It could very well be that this adaptation requires a chronic training stimulus, that once removed results in a fairly rapid degradation in the protein. Within this light I'm not certain that these changes should be referred to as acute, unless the authors can demonstrate increases in protein content following a single bout of exercise.

We have modified our discussion to more appropriately interpret our findings regarding the increase of CD36 (Lines 551-556).

5. The authors attempt to discuss their findings of alterations in adipose tissue fibrosis in comparison to work completed using rodents (line 512). A key point that should likely be taken into consideration is that the rodent work was conducted in animals housed at room temperature and thus the animals were under a degree of thermal stress and the results of their findings likely reflects an interaction between chronic thermal stress and exercise.

The reviewer raises an interesting point regarding the potential for thermal stress to influence the lack increase in adipose tissue fibrosis in the exercising rodents in the study by Kawanishi, et al. However, it is important to note that the non-exercising animals in their study were also housed at the same temperature and adipose tissue fibrosis did increase in these animals. So it is not clear whether the housing temperature in Kawanishi et al's influenced the exercise-induced prevention of fibrosis in their study. Given we could only crudely postulate about the potential role of thermal stress, we would prefer not expand our discussion to include this speculation on our part.

6. Please express all data as means + SD. It would seem the figures are mean + SEM

We have modified our data presentations. Now all data are presented as mean ± SD.

7. Whenever possible, individual data points should be shown

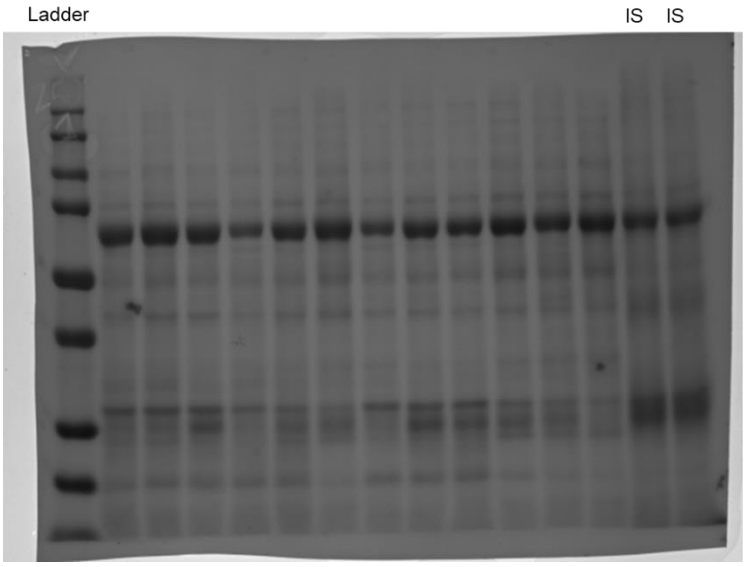
We have now added individual data points and lines connecting the repeated measures data for each subject in Figure 2, 3, 4. We also added individual data points in Figure 6. However, we did not add lines to connect the repeated measures for each subjects because we felt this created "congestion" of the figure that may distract from the readers ability to decipher the figure. We did not add individual data points to the immunoblot figures (Figure 5,7, and 8) for the same reason.

8. For all blots please indicate the approximate apparent molecular weight

We have now included approximate molecular weights on all blots.

9. Please provide a representative loading control image. Given the large number of blots, perhaps one "representative" image per panel could be shown and this indicated in the figure legends.

We used 'Memcode' staining as a loading control (which stains the full lane) and because all the blots were normalized to the corresponding lane of Memcode staining, we think it may not benefit to include Memcode images given the large size of the image and the number of the bands. Instead, we are attaching an example of Memcode staining image here for the reviewers.



Example of Memcode staining.

Additionally, we have modified our blot images in Figures 5, 7, and 8 to now include internal standard that we used to account for gel-to-gel variability (labeled in the blots as "IS" for internal standard)

Referee #2:

Major comments:

at first it seemed odd to me that the authors would go to the trouble of doing clamps and stable isotope infusions only to measure the Ra of palmitate. However, having read their recently published paper in JCEM (ref 13 in the current paper), i can see that most of the clamp data was included there. Since it is necessary to report some of the same data in the current manuscript (i.e. peak VO2) - I would suggest that some additional clamp data could be included in this paper. For example, it would be interesting to know if glucose infusion rate or Rd was related to palmitate Ra either prior to or after both exercise interventions. Also, was glucose Ra related to palmitate Ra pre/post exercise?

The reviewer raises interesting points regarding the potential interaction between glucose infusion rate or Rd to palmitate Ra. Accordingly, we conducted correlational analyses to assess potential relationships between palmitate Ra (both basal and insulin-stimulated) and measures of basal and insulin-stimulated glucose metabolism (e.g., basal glucose Ra, clamp glucose infusion rate, glucose Rd) before and after exercise training. However, there were no significant relationships between any of these variables (all $-0.15 \leq R^2 \leq 0.3$ without any significant p-values; $0.45 \leq p \leq 0.95$). Because these correlational analyses do not add to the interpretation from our recently published JCEM paper (Ryan et al, JCEM vol. 105, 2020) regarding the relationships between lipolytic rate and measures of glucose metabolism – we have not added this to the revised manuscript.

I note you used glycerol Ra as a measure of adipocyte lipolysis and insulin sensitivity in your JCEM paper - and used palmitate Ra in the current manuscript. While you did not see much of an effect of exercise of whole body ffa turnover or adipose tissue insulin sensitivity, since you saw some histological evidence of altered adipocyte lipid turnover, it may be worth calculating adipocyte fatty acid recycling rates pre and post exercise from glycerol and palmitate Ra's.

The reviewer raises another interesting point here. However, when we calculated adipose tissue fatty acid recycling rate either in absolute terms (i.e., [3 x Ra glycerol] - Ra FFA) or expressed as a percentage of total lipolytic rate - we found no effect of training (or training group). Therefore, we do not believe these fatty acid recycling data add meaningfully to the interpretation of the present study – and we would prefer to avoid the redundancy of including these previously published glycerol Ra data here in this publication.

Other comments:

1. Key points 1 and 2 are just bullet point descriptions of the background and approach. I think key points should focus on the key take homes from the study data. The fourth key point is rather speculative and again doesn't really convey an important take home from the study data.

We have modified the key points to convey our findings and the take-home messages more effectively.

2. In my opinion the introduction is far too long. I believe this should be re-written with brevity in mind.

We have now shortened the introduction considerably.

3. Since you include DEXA data it may be useful to present VO2peak as a function of lean mass.

We have added VO_2peak normalized by fat free mass (ml/kgFFM/min) in Table 1.

4. Since you see the COX IV expression is elevated with both training modalities - it make be worth while blotting for some additional mito proteins to be a little more confident that there is greater WAT mito protein content with training.

We respect the opinion of this reviewer regarding the addition of other mitochondrial proteins, but we are uncertain whether adding more mitochondrial proteins would strengthen our findings given the discordance between the protein expression of COXIV and SDHA that we report and some other recent findings from others that reported similar findings. For example, Riis et al (J. Appl. Physiol. vol. 126, 2019) have reported increased protein expression of SDHA, but no changes in VDAC (voltage-dependent anion channel) or PDH (pyruvate dehydrogenase) in aSAT from healthy males in response to 10 weeks of endurance training. More recently, Mendham et al (Scientific Reports, vol 10, 2020) reported increased mitochondrial function (measured by high-resolution respirometry and fluorometry), but no apparent changes in mitochondrial DNA content in aSAT from women with obesity after 12 weeks of exercise training. These findings, including ours, support the notion that exercise training may increase mitochondrial content in human aSAT, but it is unclear why only some of mitochondrial proteins were increased by training. We speculate this could largely be attributed to the technical limitations (i.e. whole-adipose tissue immunoblot or mtDNA extraction) and we acknowledge the need for more in-depth analysis to test this hypothesis (i.e. high-resolution respirometry, single-cell RNAseq, spatial transcriptomics, etc). Therefore, we think it may not benefit to add more mitochondrial proteins in our immunoblot data. Instead, we have modified our discussion to point out the limitation (Lines 568-571).

5. You discuss the difference in energy expenditure (EE) with the two modalities (lines 582-583 and line 610-613) as being marked, arguing that it is perhaps surprising that some of the effects of exercise were so similar between the MICT and HIIT groups. I would argue the the differences in EE you see between the two exercise modalities is actually rather minor and perhaps of limited physiological significance - particularly with regards to WAT lipid turnover. I think the metabolic impact of the marked differences in exercise intensities between MICT and HITT are likely more acutely seen at the level of muscle (at least with regards to fuel use).

We agree with this excellent point made by the reviewer. We have now deleted our discussion regarding the difference in energy expenditure to avoid potential confusion.

Additional modifications

We have now added scale bars in microscopic images.

We have added corresponding legend for the graphical abstract in the manuscript.

Dear Dr Horowitz,

Re: JP-RP-2022-282371R1 "Exercise training remodels subcutaneous adipose tissue in adults with obesity even without weight loss" by Cheehoon Ahn, Benjamin J. Ryan, Michael W Schleh, Pallavi Varshney, Alison C Ludzki, Jenna B Gillen, Douglas W. Van Pelt, Lisa M Pitchford, Suzette M Howton, Thomas Rode, Scott L Hummel, Charles F. Burant, Jonathan P Little, and Jeffrey F. Horowitz

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

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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.

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I hope you will find the comments helpful and have no difficulty returning your revisions within 4 weeks.

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I look forward to receiving your revised submission.

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

Yours sincerely,

Michael C. Hogan
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-You must upload original, uncropped western blot/gel images (including controls) if they are not included in the manuscript. This is to confirm that no inappropriate, unethical or misleading image manipulation has occurred https://physoc.onlinelibrary.wiley.com/hub/journal-policies#imagmanip These should be uploaded as 'Supporting information for review process only'. Please label/highlight the original gels so that we can clearly see which sections/lanes have been used in the manuscript figures.

EDITOR COMMENTS

Reviewing Editor:

Comments to the Author (Required):
Congratulation on this very interesting study and successfully addressing the comments of the reviewers. Prior to accepting this manuscript for publication to the Journal of Physiology, please revise the references to match the guidelines of the journal. Since the sample size is less than 30 per group, all figures should present the individual points. Finally, please consider to "break" figures 7A and 8A to multiple smaller figures (e.g., one for each protein presented) to improve the readability of those figures.

Individual point need to be included in Figure 7A and 8A. Precise p-value are included only in the text of the document but not figures.

Senior Editor:

Please also attend to all points in 'Required Items' above.

REFEREE COMMENTS

Referee #1:

The authors have done a good job in addressing my previous comments. I have no further points that need to be addressed.

Referee #2:

I appreciate the time and effort taken by the authors in providing thorough responses to my original comments. I have no further comments.

These are difficult studies to do - I congratulate the research team on their interesting data.

END OF COMMENTS

1st Confidential Review **11-Jan-2022**

We appreciate Reviewing and Senior editors for their comments on our manuscript. We have revised the manuscript to address all comments. We have highlighted our changes in the revised manuscript. Additionally, we have provided a point-by-point response to all editors' specific comments below (our responses are in bold).

Editors

Reviewing Editor:

Comments to the Author (Required):
Congratulation on this very interesting study and successfully addressing the comments of the reviewers. Prior to accepting this manuscript for publication to the Journal of Physiology, please revise the references to match the guidelines of the journal. Since the sample size is less than 30 per group, all figures should present the individual points. Finally, please consider to "break" figures 7A and 8A to multiple smaller figures (e.g., one for each protein presented) to improve the readability of those figures.

Individual point need to be included in Figure 7A and 8A. Precise p-value are included only in the text of the document but not figures.

We have now modified Figure 7 and 8 into multiple smaller figures and have added individual points and lines. To make all western blot data consistent, although it was not mentioned by the editor, we also modified Figure 5 into multiple smaller figures with individual points and lines. Accordingly, we have added specific figure numbers in the Result section (Lines 391-449) and modified the figure legends (Figure 5, 7, and 8).

Senior Editor:

Please also attend to all points in 'Required Items' above.

- **We have modified the Reference List to the journal's format.**
- **We believe the Additional Information section is now complete. We added 'Author Contribution' (Lines 682-689).**
- **We are uploading a high-resolution schematic figure through the journal's premium BioRender site.**
- **We are uploading full western blot images including controls (i.e., Memcode) that were used as the representatives in the manuscript.**

Dear Dr Horowitz,

Re: JP-RP-2022-282371R2 "Exercise training remodels subcutaneous adipose tissue in adults with obesity even without weight loss" by Cheehoon Ahn, Benjamin J. Ryan, Michael W Schleh, Pallavi Varshney, Alison C Ludzki, Jenna B Gillen, Douglas W. Van Pelt, Lisa M Pitchford, Suzette M Howton, Thomas Rode, Scott L Hummel, Charles F. Burant, Jonathan P Little, and Jeffrey F. Horowitz

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

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