Author's response to reviews

Title: Increased expressions and levels of tachykinin both ipsilaterally and contralaterally in response to unilateral rabbit muscle overuse leading to muscle and nerve affection and inflammation

Authors:

Sture K Forsgren (sture.forsgren@anatomy.umu.se)
Per S Stål (per.stal@anatomy.umu.se)
Yafeng Song (yafeng.song@anatomy.umu.se)
Jiguo Yu (jiguo.yu@idrott.umu.se)

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Author's response to reviews: see over
Dear Professor Sean McGee/Mr Reynaldo Aldea Jr.

Dear representatives of BMC Muskuloskeletal Disorders Editorial Team

Many thanks for the e-mail related to our paper submitted for BMC Muskuloskeletal Disorders (MS 4221022959008416: Y Song, P Stål, J Yu, S Forsgren). It was nice to see that both referees found that the paper was very interesting. We have now gone through the paper extensively and revised it. We here send the revised manuscript. We send it in two versions: One that is "clean" (i.e. "Finally revised") and one where the changes we have done are shown in red colour ("Revised with markings"). The line/page references that are given below relates to the "Finally revised" version.

We much thank the reviewers for their comments and suggestions. We also much thank the Associate Editor for comments. We here describe the changes that have been done:

A) With respect to comment from the Associate Editor (relates to the English of the paper)

Yes, we agree, the English needed to be improved. It was thus useful with English editing. We have used the company suggested in the e-mail from you, i.e. Edanz. We got very good (and extensive) comments from them. Their suggestions were really very good.

The parts where the most extensive changes concerning the English language are made are the Abstract, the Introduction (Background) and the Discussion. As you will see, a very large number of changes have been made.

B) With respect to the comments by reviewer Zong Mei:

Concerning Major revisions:

We see the point. However, we found it very important to make evaluations for correlations for the various parameters (Tachykinin concentrations, Intensity of reactions in blood vessel walls, vascular density) between the two sides (the experimental side and the contralateral side). Thus, a very interesting observation is the finding that there are positive correlations between the two sides, showing that the tachykinin upregulation not only occurs for the experimental (exercised) side but also for the non-exercised (contralateral) side (cross-over effects).

Concerning Minor revisions:

1) The spelling of "expression" is corrected.
2) We gave injections in the ordinary i.m. way to get systemic effects. We used muscles that are customary for i.m. injections for rabbits. We never injected into the experimental muscle (m. triceps surae).
3) We have now inserted label in Figure 1.
4) Based on the comment we realize that we more clearly should write what is the main point with the figure in the figure legend (that one here can compare the situation for the experimental side with that in the contralateral side at the various time points). This has thus been done (bottom part, page 57). We also now clarify that the resting (control) value is shown in the figure.
5) We see the point. We had actually been thinking of that ourselves. However, we considered that it was very important to show not only the specific stainings but also the control stainings on the same montage, and in certain cases, to clarify the morphologic characteristics (so the specificity and locations of the reactions could be clarified). That relates to Figs 3-6 concerning in situ hybridization results. We think that is very important for the reader to really see that the reactions are specific (i.e. to be able compare antisense with sense stainings). It would also be redundant to frequently show features for exercised and non-exercised sides on the same montages, as the results for the non-exercised were similar as those for the exercised side.

The same reasoning concerning choosing of figures relates to the preabsorption stainings. Thus, if we had shown these features for both sides, the pictures would also in this case have been redundant. Instead, the montages are shown to clearly depict that reactions are specific for the various structures (Fig 7e,f, 9a,b, 12).

On the other hand, we found it relevant and important to show certain features for the control tissue (tissue from completely non-exercised animals) on the same montage as features from experimental animals (Fig 2, 7). Thus, it was between the muscles of completely non-exercised animals and those of experimental animals that differences were observable.

Nevertheless, we have, where it was appropriate, shown features for both sides of the experimental animals in some of the figure montages (Fig 2, 10, 11).

The results from the important double-stainings (Fig 8, 9c,d, 10, 13) are not shown for both sides as the information would be similar.

In conclusion, concerning (5), we see the point raised by the reviewer. However, having had the same thoughts in mind, we finally concluded that it was most appropriate to construct the montages in the way we did. It is also a fact that the other reviewer did not comment on the grouping of the figures.

6) We agree with the reviewer. Thus, Fig 4 has been reduced (the C-picture is withdrawn). Concerning vessel reactions: One of the figures in Fig montage 11 has been withdrawn. We still want to show Fig 3 as the pattern shown here is not shown in any other montage.

7) We see the point. Fig 9 has therefore been reduced. Thus, two of the previous figures in Fig montage 9 are deleted.

We much thank the referee for the constructive comments.

C) With respect to comments by reviewer Mary F Barbe

It is nice to read that the referee answers questions 1-8 with Yes. However, there were certain points that were commented on and that required revision (see below).

Comment to point 5: See below (under a, Results). Concerning 6 (see under c, Results). Concerning 9: The English writing/grammar is corrected for. Edanz has gone through the writing. Typos are corrected for.

Concerning Minor essential revisions:

a) The Methods section is shortened. It is 1.5 pages shortened.

b) The number of animals per group is now better described (lines 13,14, page 7). Description of numbers per group is also given on lines 9, 10 from the bottom, page 6.
c) We see the point. The addition has been made (lines 1 and 2 from the bottom, page 9).
d) We have changed in accordance with the suggestion by the reviewer (line 13 from the bottom, page 5).
e) We have changed as the reviewer suggests (line 6 from the bottom, page 7).
f) Change has been made; line 6, page 8.
g) Yes, it is possible to reduce the Materials & Methods part. It has been reduced by 1.5 pages.
h) OK, we see the comment about Discussion.
i) Yes, as seen by ourselves, and as seen via the English linguistic revision made by Edanz, spelling errors were noted. These have been adjusted.
j) Yes, we have merged the subparts for legends to Figs 10 and 11 into one legend per figure.
k) We have now better clarified what we mean (lines 6, 7 from the bottom, page 28 and lines 2-5, page 29).
l) This part has been changed in accordance with the suggestion by the reviewer (lines 8,9, page 33).

Major essential revisions

a) It was indeed so that T cells did not express tachykinin-like immunoreactivity (as it was written on page 21 in the original manuscript). Also in Discussion (page 31) we described that it was two other white blood cells that displayed tachykinin-like immunoreactions. Nevertheless, we can understand why the referee gave the comment, and it is presumably related to the fact that we highlighted that cells demarcated by the T-cell antibody indeed are immunoreactive (Fig 10). However they do not show co-localization with tachykinin immunoreactivity (as seen in Fig 10). Due to the revision of the legend for Fig 10 (c.f. above), the reaction patterns are now more clearly described.
b) This paragraph (lines 3-8, page 26) has now been partly reorganized and is to some extent rewritten.
c) Discussion: It should be clarified that the "low number" only relates to the in situ hybridization. For the immunohistochemical stainings, the htx-eosin stainings and the EIA analyses, samples from all animals were examined for. We had apparently not described that clearly enough. This fact is now better clarified (line 8, page 9 concerning the immunohistochemical stainings; line 7, page 19 concerning the EIA procedure). It was already in the original ms written that sections of all specimens were processed for morphology (end part of page 8 in the previous version of the manuscript). Nevertheless, reinforced by the comment from the reviewer, we have now included a comment on the fact that specimens from all animals were not evaluated with in situ hybridization (bottom line page 15 and top line, page 16). Nevertheless, as we wrote in the original manuscript, we had a clear basis for the choosing of samples and we consider that the samples that were utilized were representative samples (lines 1,2, page 16).

We much thank the reviewer for the constructive criticism.

In conclusion
In conclusion, we find that the paper has markedly improved by the revision. Based on the comments and suggestions by Edanz, we realize that an English editing was necessary. The comments by the reviewers were valuable.

Please note that, besides the two new versions of the manuscript, new figure montages related to Figs 1,4,9 and 11 are sent (for explanations, see above). Based on comments we got, the title has been changed.

We hope that the paper now is found acceptable for BMC Musculoskeletal Disorders.