Author's response to reviews

Title: Functional overload attenuates plantaris atrophy in tumor-bearing rats.

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Author's response to reviews: see over
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Dear BioMed Central Editorial Team,

We are happy to have an opportunity to resubmit our revised manuscript to BMC Cancer. The panel of reviewers called this manuscript “well written” and “a topic of great interest”. That stated, they also made many important suggestions, which we have incorporated into this revised submission. We are grateful for their efforts and now present an improved paper for re-review. Please find our comments below that address the reviewers’ specific concerns.

Thank you for your time and consideration,

Jeffrey S. Otis, PhD
Simon J. Lees, PhD
Jay H. Williams, PhD
Note to all reviewers

We greatly appreciate your comments about our manuscript. In response to your concerns, we have attempted to clarify our results and conclusions. Drs. Otis and Lees are no longer at Virginia Tech, both having left to pursue post-doctoral studies. Thus, we no longer have access to the animals, MH-7777 cells, reagents or equipment necessary to provide additional data not already supplied within this resubmission (e.g., ATP-independent proteasome activity in FO groups). Nevertheless, we contend that this paper has significant scientific merit and, now that we have addressed your concerns, feel it positively contributes to the current knowledge base of cancer-induced skeletal muscle derangements and potential treatment options to attenuate myopathies.

Reviewer #1

Major Compulsory Revisions

(1) The reviewer makes an excellent point in that monitoring the daily food intakes of tumor-bearing rats that were housed in pairs may not be reliable. The average total food consumption/day/2 rats was recorded and compared to all other cages. No difference was detected. Further, no animal displayed physiological signs commonly associated with loss of appetite or anorexia (e.g., failure to groom).\(^1\) Finally, this model has been previously used to study the influence of cancer cachexia.\(^2\) However, we have attempted to clarify our presumption in the Methods section (Tumor model subsection).

(2) Tumor-bearing rats had significantly smaller muscles, including gastrocnemius, plantaris, extensor digitorum longus, tibialis anterior and diaphragm (Figure 2). As stated in the statistics subsection of the Methods and in the second paragraph of the results section, this significance was determined by ANOVA followed by SNK post-hoc tests.

Further, to identify the effect of FO surgeries on plantaris mass, data in Figure 4 are presented as the ratio of both plantaris muscles from the same animal, as we
have previously published\(^3\). Due to systemic atrophy in TB rats (Figure 2), we are not able to normalize FO plantaris mass to a muscle that itself failed to atrophy. Therefore, this ratio in TB rats (sham/contralateral, third bar) should be close to 1.0, and should not appear any different from the ratio from healthy controls (sham/contralateral, first bar). As reported in the statistics section, the lack of significance between these values was confirmed with a 2-way ANOVA followed by an SNK post-hoc test.

This concern is related to the concern made by reviewer #2 (Discretionary Revisions #2). Our data make it difficult to distinguish between hypertrophy and how much can be considered resistance to atrophy. Either way, plantaris loading secondary to FO does result in increased relative muscle mass.

**Minor Essential Revisions**

1. We have defined the correlation calculation as ‘r’ and apologize for the oversight. Further, we have flipped our references to Figures 1A and B in the text of the results section to read correctly.

2. As requested, we have reported our statistics on page 13.

3. As requested, the group N has been included in each figure legend.

**Discretionary Revisions**

1. We agree that the paragraph discussing TNF-α would be more relevant had cytokine levels been measured. This was obviously beyond the scope of the current study and this paragraph has been deleted.

2. The paper by Al-Majid is a significant investigation into the influence of increased electrical activity in the setting of cancer cachexia. We have included this work in
our discussion section (Influence of FO interventions on plantaris mass subsection).

**Reviewer #2**

*Major Compulsory Revisions*

None

*Minor Essential Revisions*

(1) In this study, ATP-independent proteasome activity was determined solely to identify the influence of MH-7777 cell implantation and resultant tumor formation on degradative pathways in host skeletal muscle. We avoided taking a measurement of protein degradation in FO animals because ATP-independent proteasome activity is likely highly variable in skeletal muscles actively engaged in hypertrophy. In general, resistance training decreases protein turnover\(^4\,^5\), and while we agree that the combined influence of FO and cancer on markers of protein degradation is an important question, these questions were beyond the scope of this current work.

(2) The reviewer makes a sound point in that alterations to skeletal muscle myosin heavy chain isoforms may occur in complete absence of concomitant changes to metabolic capacities. In fact, we have published such a phenomenon in spinal cord transected rats.\(^6\) Our original statement about the potential of a more fatigable state was used merely to speculate about tumor-induced ATP-independent degradation of myoglobin. Nevertheless, we have minimized the assertiveness of our original statement (page 17, paragraph 1).

*Discretionary Revisions*

(1) We have amended our statement appearing on page 4 that concerned the effects of cancer on protein degradation and synthesis. The statement is now
less general and speaks more to the effects of cancer on protein degradation—which is more relevant to the current work.

(2) We have previously published the data in Figure 4 in the same format. Please see our responses to Major Compulsory Revisions #2 from reviewer #1.

Reviewer #3

Major Compulsory Revisions

(1) The MH-7777 cell line has been used previously to study the resultant effects of cancer cachexia. However, the reviewer is correct in that no published work is available that details the influence of MH-7777 cell implantation and subsequent tumor development on ATP-dependent proteasome activity in skeletal muscle. Here, we provide two novel results: (1) ATP-independent activity is induced in rats bearing a tumor derived from MH-7777 cells, and (2) these rats experience significant, systemic skeletal muscle atrophy. Together, we feel that the paper by Bland and colleagues and these two pieces of evidence adequately establish tumor-induced skeletal muscle derangements. While potential alterations to ATP-dependent proteasome activity secondary to MH-7777 cell implantation would be intriguing, it is beyond the scope of the current work.

(2) The reviewer is correct about Figure 3B. The data appearing in the results section are accurate and the accompanying figure has been adjusted accordingly. We apologize for the oversight.

(3) As pointed out by the reviewer in point #1, MH7777 cells are not routinely used to study cancer cachexia. Our statements appearing on page 15, paragraph 2 were made to show that tumors derived from MH7777 cells produce the same systemic atrophic effects as previously published in other cell lines (e.g., Yoshida AH-130 rat ascites hepatoma or C26 adenocarcinoma cell lines). We appreciate the numerous works detailing the systemic effects due to cancer cachexia and our intentions were certainly not to declare our work as seminal.
(4) The reviewer contends that the lack of soleus atrophy in TB rats should not be surprising. However, a report by Diffee\textsuperscript{8} showed significant atrophy in mouse soleus muscles only 21 days after tumor cell implantation. Because our model failed to produce soleus atrophy, we believe that one possible explanation may be that the tumor load may have spared this muscle. Certainly, a longer duration for tumor growth may have affected soleus mass and this supposition has been added to the discussion. This notion is supported when one considers that the percentage of slow MHC fibers in a rat soleus is greater than that from the mouse. Therefore, the point made by reviewer #3 that a slower muscle is more resistant to cancer-induced atrophy may be correct in our model (with a longer duration), but was not witnessed due to the species difference between rat and mouse models. We have amended the discussion section to address this possibility.

(5) In general, our data suggest that MHC isoforms transition from fast-to-slow in plantaris muscles from TB rats. Because we did not see tumor-induced soleus atrophy, we neglected to analyze the MHC composition of soleus muscles. However, Diffee and colleagues\textsuperscript{8} have shown alterations to MHC isoform composition in atrophied soleus muscles from TB mice. Further, FO surgeries stimulate plantaris muscle hypertrophy, therefore, to identify the MHC composition of the gastrocnemius or EDL muscles from control or TB rats was beyond the scope of the current work.

\textit{Minor Compulsory Revisions}

(1) We have deleted this sentence from the abstract.

(2) Please see comments made to Reviewer #2 in the \textit{Minor Essential Revisions} section, point #1.

(3) We have amended the methods section that describes the ATP-independent proteasome assay.
We agree that these sentences were confusing and misleading. We appreciate the editorial advice and have made the appropriate clarifications.

References


