**Author's response to reviews**

**Title:** Use of Imaging Biomarkers to Assess Perfusion and Glucose Metabolism in the Skeletal Muscle of Dystrophic Mice

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**Author's response to reviews:** see over
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Dear Editor-In-Chief, _BMC Musculoskeletal Disorders:_

We thank you for your interest in reconsidering our manuscript “Use of Imaging Biomarkers to Assess Perfusion and Glucose Metabolism in the Skeletal Muscle of Dystrophic Mice” for publication in BMC Musculoskeletal Disorders. Many important points were raised by yourself and by the reviewers, and are greatly appreciated. We believe that we have adequately addressed these comments, concerns and criticisms in a revised manuscript, and have included a point-by-point response to them, specifically indicating how we have revised the manuscript (as requested, changes have been tracked throughout the revised manuscript). We have, in addition, included an Acknowledgements section between the Authors contributions section and Reference list. Here we acknowledge anyone who contributed towards the study by making substantial contributions. We have also listed our sources of funding for the study.

_In response to:_

**Reviewer 1:**

1. In our original manuscript, we explained that the transient increase in perfusion and metabolism reflected the regenerative process. It was felt that this advocacy should be confirmed with data or evidences. In response to this, we have toned down our interpretation considerably, suggesting that the transient increases in perfusion and metabolism in both non-exercised and exercised mdx groups reflect active cycles of degeneration/regeneration and a moderate exacerbation of muscle pathogenesis, respectively. We believe that the greatest increase in blood flow, blood volume and glucose metabolism observed in udx mice is likely due to the severity of pathogenesis and inflammation in these mice. These conclusions are supported by histological findings. Given reports that cycles of degeneration/regeneration wane at ~ 3-4 months of age in mdx mice, we are not surprised to have observed a subsequent progressive decrease in blood flow, blood volume and metabolism. Importantly, our imaging of biomarkers enables us in the present study to detect differences between exercised mdx mice and their non-exercised littermates, with the former exhibiting a greater decline in each biomarker, differences not readily detected using conventional histological techniques. Not surprisingly, still greater declines in all imaging biomarkers was observed in severely-affected udx mice, findings that were, again, supported by our histological findings. These findings could have enormous implications for studies examining the mechanisms of DMD pathology that may improve or worsen as a result of exercise, etc, and will form the basis of subsequent studies; we believe, however, that in toning down our original interpretation, we have now sufficiently addressed Reviewer 1’s concerns.

2. We assessed changes in perfusion and metabolism using normalized data with respect to baseline values. As outlined in our Methods, our rationale for analyzing our data this way is that there is considerable heterogeneity in baseline values for perfusion and metabolic activity between each animal in a group. Thus, we normalize to baseline values to minimize this heterogeneity and demonstrate longitudinal changes.

More specifically,

A) It was suggested that assessing perfusion and metabolism per unit muscle volume or cross-sectional area might be more convenient – we see the Reviewer’s point, but don’t believe that this holds true in our case, that normalizing our data to baseline values as we have done is the most appropriate way for
us to effectively assess longitudinal changes in perfusion and metabolism, particularly given the heterogeneity between animals as noted above.

B) There were indeed significant differences in the baseline values as listed in Table 1. As discussed in our Methods, for both perfusion and metabolic rate analyses, data was normalized by the baseline value for each mouse, and the normalized values then averaged respective to week and group. This was, again, conducted to minimize biological variation between mice, and is an acceptable means of presenting such data.

C) It was stated that the data acquired from udx mice might reflect muscle atrophy – we expected that it would, being an accurate means to assess the disease state. Less muscle SHOULD be reflected by lower perfusion and metabolic rate values!

**Reviewer 2:**

1. It was stated that by measuring only the gastroc muscle, we might be seeing changes in only fibre type and missing other changes. We should clarify…while we attempted to measure perfusion and metabolic changes in as specified a ROI as possible (ie.,GM), neither CT nor PET has sufficient resolution to allow us to definitively distinguish the GM. As such, it is more correct for us to state that we, in fact, likely measured changes in a number of muscles in the posterior compartment of the hind limb – these muscles have now collectively been referred to as HL musculature throughout the revised manuscript.

2. It was pointed out that conclusions/literature regarding exercise improving pathogenesis were not explored, discussed, or referenced – this issue has now been addressed, and was an excellent point by the reviewer.

3. The last point made by Reviewer 2 was, in our minds, very similar to the 1st point made by Reviewer 1. We believed we have sufficiently addressed this concern in our modified Discussion (changes were tracked throughout).

Lastly, we have done our best to address the referees’ other minor points and suggestions, ie., to correct the mismatches of marks between Figure 3, 4 and their legends.

It is our hope that you now find this manuscript acceptable for publication. We thank you for your time and consideration.

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Sincerely,

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