We thank the reviewers for their review of our recent submission to the *Journal of Visualized Experiments.* We have listed the comments and our responses below. The manuscript has been modified in response to the comments and to include red text where significant sections of text were modified. Also, there are comment balloons that correspond to each comment/ response.

**Editorial comments:**

Please reply to the following comments at your earliest as this manuscript is on priority for us.

E-1: References 15 and 41 are incomplete.

E-2: Reference 21 lists the issue number as 2011, which is more likely the year.

*Response:* We have proofread the reference section.

E-3: Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

*Response:* We have proofread the manuscript thoroughly.

E-4: Often reviewers request the addition of a large amount of details or explanations. We realize that, especially in the protocol section, brevity and clarity are important for a JoVE publication and expect the focus to be on providing a framework for the method presented rather than a comprehensive review of the research field. Please address each comment in your rebuttal and note if you choose not to include the requested information in the text and the reasoning behind this decision.

**Reviewer #1:**

*Major Concerns:*

R/1-1: In the Introduction and throughout the manuscript it is assumed that that reader is familiar with neuromuscular diseases and what the various motivations might be for imaging these. Although the common underlying pathological processes (targeted here by imaging) and concept of biomarkers are mentioned, there is no wider context given for this work. It would be appropriate to include some sentences or a paragraph and references that introduce neuromuscular disease from the patient or clinical perspective (including, for example, indication of symptoms such as weakness and disability, characteristics such as progression and prevalence). Polymyositis and and dermatomyositis are studied here (although in fact only data from DM is presented) with the implication that the work describes a protocol that is potentially more widely applicable to other conditions. Some justification and further background about neuromuscular disease needs to be given for this point to be made convincingly.

*Response:* We have expanded the discussion of this point in the leading paragraph to the Discussion section (page 18).

R/1-2: In the Protocol section, most necessary steps are described in detail. However, there are several areas where further detail would be helpful. In section 2.3.2 very little information is given about considerations relating to coil selection. This is an important aspect (particularity in relation to optimizing signal for the qualitative imaging) and an area where most users would have considerable choice available. Some comment on the relative merits of surface coils, arrays and transmit-receive coils would be appropriate here.

*Response:* We have expanded the presentation of these important points in the Discussion (page 21).

R/1-3: Similarly, in section 3.1.3 only cursory mention is made of choosing 'slice position relative to reproducible anatomical landmarks'. This is a critical area for achieving reproducibly in serial scanning and this work could make a useful contribution here by commenting further. Both these aspects are relatively visual, practical activities which may provide added value to the video script.

*Response:* We have described this procedure in the Discussion (page 20), and will illustrate it in the video.

R/1-4: There is a considerable jump between the end of section 3 and section 4. Making the transition from scanner acquired images to data that can be processed offline can be major undertaking and the utility of this protocol is weakened by omitting to make reference to this. Strategies vary of course, but at least the transfer approach and data format used in this work should be specified as an example to make the protocol description more complete.

*Response:* We have described this procedure in the Protocol (step 3.6.4, page 11).

R/1-5: Similarly, the ROI drawing strategy and example software could be mentioned in further detail in section 4.2. Examples in 4.2.3 could be useful.

*Response:* All of our analyses are conducted using custom-written codes in MATLAB. We have included a sample ROI in Figure 1 and have included examples of data to exclude. Please see page 16.

R/1-6: In the descriptions of fitting to the qMT and diffusion models a certain level of detail is given but often less than is required to perform a full analysis. It may suffice in these sections to refer to the relevant literature to avoid confusion. For example, there is insufficient detail to define ω1CWPE in equation [3], ω-bar and u in equation [4] or how the integration is performed. In section 4.1.4 the lambda terminology is never specifically defined as the eigenvalues and the ADC is included in equation [7] without previous definition.

*Response:* As suggested by the reviewer, we have reduced the scope of this discussion and refer instead to the original publications.

R/1-7: In general, clearer distinction should be made between which sequences and fat suppression strategies are implementable using generic readily available modules on clinical scanners (ie T1 and T2-weighted FSE), and which require specialist pulse programming so the reader/viewer can make judgments about the consequences of omitting certain components.

*Response:* In the Discussion, we have noted which components of the protocol have been specifically programmed on our system. Please see pages 18, 23, and 24.

*Minor Concerns:*

*R/1-8:* In 4.1.2 it may be worth mentioning there are other approaches beyond fitting to a purely mono- exponential function.

*Response:* We have added this point to item 4.1.3. Please see page 12.

R/1-9: Line 4.6.1 - further detail of the masking approach would be useful.

*Response:* We have added detail to this point. Please see step 4.1.1 on page 11.

R/1-10: This work was conducted with Philips hardware. Throughout there is mention that 'many systems' take different approaches to the sequence options available. In an ideal situation, specific recommendations would be made for the several alternative systems (at lease for the T1 and T2 weighted components) to make this optimally useful protocol for the novice user.

*Response:* We recognize the value of doing this. Unfortunately, without access to other vendors’ systems, we are unable to do so.

R/1-11: Only data from the pool size ratio is presented. It would be helpful if discussion touched on the relative merit of other parameters available in the fitting process.

*Response:* We have added these points to the Discussion section. Please see page 24.

R/1-12: Please clarify the term 'classical units' (line 119).

*Response:* We have changed this description from “classical units” to “physical units of measurement,” which we believe to be more standard usage. Please see page 4.

R/1-13: References describing the NNLS approach would be appropriate (line 164)

*Response:* We have included the original citation for this approach. Please see page 5.

R/1-14: Line 294 may read better as 'with the help of MRI software'

*Response:* This phrase appears to have been an editorial addition made without the authors’ knowledge. We have corrected it. Please see page 10.

R/1-15: Line 305 - specific suggestions of alternative shimming/placement strategies could be more helpful here

*Response:* We have presented further details in the Discussion. Please see pages 20-21.

R/1-16: Is it appropriate to mix units of Tesla and Gauss (line 525)?

*Response:* Both of these quantities are now described in units of Tesla. Please see page 18.

R/1-17: line 515 typo

*Response:* We have corrected this error. Please see page 18.

R/1-18: EPI abbreviation in tables not formally defined elsewhere.

*Response:* We have corrected this error. Please see page 17.

R/1-19: Table 1 is never labeled as such - line 233

*Response:* We have corrected this error. Please see Table 1.

R/1-20: The importance of fitting R2\* maps is mentioned in the discussion of fat-water imaging, an example map here could be helpful.

*Response:* Given the limitations of space, we are only able to include the parameter maps that provide quantitative data used to characterize muscle disease.

**Reviewer #2:**

*Major Concerns:*

R/2-1: My major concern is that the protocol is quite long as it is, and while this might be feasible for some neuromuscular disease patients, a number of them suffer from contractures and/or weakness and atrophy of the muscles which makes it very uncomfortable for them to lie on their back for long. If all the sequences described in the protocol serve a specific purpose this is acceptable, but I feel that there are some sequences that could be superfluous. For instance, the inclusion of a fat suppressed and non-fat suppressed sequence for assessment of T2 seems too much for me. With recent multi-exponential fitting approaches the water T2 and fat T2 can be obtained from a single non FS measurement, instead of acquiring both FS and non-FS, this adds 12 minutes to the protocol. Along similar lines, why is a quantitative T1, a qMT and a qT2 measurement needed if they all assess edema? I think the added value of including all different techniques in the protocol should be explained more clearly. Along with this, the exclusion of other sequences, like 1H MRS for a true measurement of water T2, or 31P MRS could be added to the manuscript.

*Response:* We agree with the reviewer that some of the sequences contain overlapping pathological sensitivities. However, we disagree that *T1*, *T2*, and qMT all assess edema exclusively. *T1* and *T2* are also sensitive to fat infiltration; *T2* may be sensitive to membrane permeability changes; qMT is also sensitive to fibrosis. We have a new section to the discussion that discusses this issue (page 25). We have also included a discussion of MR spectroscopy (page 23). Regarding the value of FS and non-FS *T2* measurements: while this is not the place to debate the merits of another article, we respectfully disagree with the approach that uses multiexponential to attempt to characterize the muscle-only *T2*, in the presence of a non-FS dataset.

R/2-2: Secondly, the manuscript does not refer to a previous article in this field ("Towards harmonization of protocols for MRI outcome measures in skeletal muscle studies: Consensus recommendations from two TREAT-NMD NMR workshops, 2 May 2010, Stockholm, Sweden, 1-2 October 2009, Paris, France"). There are some differences in the current protocol compared to that protocol, and the rationale should be explained. In line with this, I think it is worth mentioning that the parameters used in this protocol are a guideline, but not carved in stone. There will be authors who will disagree. This should be made clear.

*Response:* We regret not having discussed this important contribution to the literature. We have cited and discussed it in the revised manuscript. We have also emphasized that the protocol presented is the one that we consider optimal for our experimental goals, and that the reader may choose to make other decisions concerning the experimental protocol that s/he chooses to adopt. Please see pages 18 and 23.

R/2-3: Finally, the authors limit their scanning to a small section of the upper leg, without explaining why this region was chosen, this should be explained, especially as the authors mention themselves that muscular diseases are spatially varying. A field of view of ~10 cm seems a bit small, especially as no whole leg scans are in the protocol to guide where the most interesting pathologies could be.

*Response:* We presume that the reviewer is referring to the foot-head of the slice packet, not the field of view. Our protocol includes several large field of view pilot scans to localize pathology. We have ensured that this information is present in the revised manuscript. Please see page 17.

*Minor Concerns:*

R/2-4: P4, lines 129-131. There is probably a reference to the term biomarker somewhere, I think it is worth it to include it.

*Response:* We first used this term in line 124; the paragraph is written to define the term biomarker as well as the properties of a good biomarker. Please see page 4.

R/2-5: P6, line 234. This sentence is now very strong, the authors should consider changing it to something less directive.

*Response:* We have changed this sentence to read, “Define an MRI protocol in advance; suggested imaging parameters are found in Tables 2-6.” Please see page 7.

R/2-6: P7, line 256-260. Why choose the thigh?

*Response:* We have added text to page 25 to define the rationale for imaging the thigh.

R/2-7: P8, line 301. The authors should consider changing the order of the scans, as the sequence for fat/water imaging can also provide a B0 map, which would avoid acquiring a separate B0 map.

*Response:* We prefer to acquire a separate B0 map so that shimming can be assessed quantitatively prior to acquiring any of the qMRI data.

R/2-8: P8, line 311. What is the 30% based on? A recent article (Azzabou et al, JMRI 2014) shows that with 30% deviation T2 values can already change up to 4 ms, at least with their qT2 measurement.

*Response:* The value of 30% is based on our own pilot studies. We are using extremely robust refocusing pulses and find that the *T2* values are unaffected by this degree of *B1* inhomogeneity. Azzabou et al did not specify the type of refocusing pulse that they used, and so we are unable to explain the difference between our experience and theirs.

R/2-9: P10, line 355. I suggest to change this to "MERA toolbox or similar software", and the recent approaches for multi-exponential fitting of T2 data of muscle should be included in the text in the discussion.

*Response:* We have rewritten this section; please see page 12. However, as noted, we do not think that the multiexponential approach described by Azzabou is appropriate as a way to separate the water and fat *T2* values, and we do not recommend its use.

R/2-10: P11, line 381. The description for the fat/water analysis is very limited compared to the other methods, please elaborate.

*Response:* Because the entire algorithm is available for free download and does not need to be implemented by an individual user, we have limited our discussion of the computational details of this algorithm; further information is available, however, in the Discussion (page). We have also noted the possibility of using other algorithms (please see page 13).

R/2-11: P11, line 4.2.2. Can the different image times not be co-registered so that adaptation of the ROIs is not necessary anymore?

*Response:* In practice, we find that there will almost always be subject motion between individual image acquisitions and so this procedure will typically be necessary.

R/2-12: P14, line 515. There is two times the word "these" here

*Response:* We have corrected this error. Please see page 18.

R/2-13: P15, line 560 - 567. There is actually a study where this was tested in boys with Duchenne, Garrood P. et al, JMRI 2009)

*Response:* We have discussed this work. Please see page 22.

R/2-14: P15, line 574. I think it should be added that the type of disease studied will also affect data quality, in muscle diseases with purely inflammation, FS techniques will not be as needed.

*Response:* We have added this point to the discussion section. Please see pages 19 and 25.

R/2-15: P16, line 602. What are the tolerances in B0 and B1 based on? Why are they 'acceptable'?

*Response:* These tolerances are based on pilot studies in which we observed that field deviations outside of these limits create errors in the quantitative measurements. Please see page 20.

R/2-16: P17, line 639. There are some references that exploit this heterogeneity in muscles diseases, including Willcocks et al, Neuromuscular disorders 2014 and references therein.

*Response:* We have included a discussion of this work. Please see page 22.

R/2-17: P17, line 660-666. I do not agree that T2 should be performed with and without FS, as explained above.

*Response:* Please see our response to R/2-1.

R/2-18: P19, lines 731-735. This paragraph assumes that T1 values do not change in disease, while it is mentioned earlier by the authors that T1s of water change in inflammation, and the T1 of water could also change due to the presence of fat. I would suggest to increase the TR, similar to suggest in the article by Hollingsworth et al mentioned by major concerns, to avoid this issue.

*Response:* We have re-written this sentence to include mention of using measured *T1* values. Please see page 25.

R/2-19: Fig 2. Please add a caption in the figure itself (Bo and B1). If the scaling for the B1 map is adjest to start from 50%, differences are more clearly visible

*Response:* We have added this notation. We have also adjusted the scaling in the B1 map.

R/2-20: Fig 3. Please add a caption FS and non-FS in the figure itself

*Response:* We have added this notation.

R/2-21: Fig 3. Please add a caption FS and non-FS in the figure itself

*Response:* We have added this notation.

R/2-22: Fig 6. Please add a caption FA and MD in the figure itself

*Response:* We have added this notation.

R/2-23: Table 3. It is stated that B1 map has 55 slices, which I think is incorrect

*Response:* This is correct.

R/2-24: Table 5. The SPAIR FS is missing from the DTI part

*Response:* SPAIR was not used in this acquisition.

R/2-25: Table 6. Number of slices is mentioned twice

*Response:* We have removed one of these instances.

**Reviewer #3:**   
I am happy to note that authors have done a good job in the advancement of neuromuscular diseases by using the Quantitative MRI methods .The abstract is well specified .the method is also well written and explanation through MATLAB (The MathWorks Inc, Natick MA) is generously . I have noted the following lines and very much is appreciable at this juncture . It will be better to take more samples for study .

Neuromuscular diseases present with a temporally and spatially heterogeneous, multi-faceted pathology. Quantitative MRI methods are presented that are capable of characterizing many aspects of this pathology.

All of the analyses described below are performed using custom-written codes for MATLAB (The MathWorks Inc, Natick MA). All data are processed on a voxelbasis.

To reduce computational time, the analyses below are conducted only for those voxels that meet an image-specific signal intensity threshold.

ROIs are initially specified on the anatomical images by defining the boundaries of each muscle of interest.

Each ROI is visually examined and pixels are removed to avoid inclusion of partial volume artifacts, non-contractile tissue, and flow artifacts

Many neuromuscular diseases exhibit pathological signs of atrophy, inflammation, fat infiltration, and fibrosis. QMRI is able to characterize these many of these pathophysiological changes non-invasively. However, each qMRI method described here has associated sources of error and many have a complex interpretation. For obvious reasons, it is important to understand and recognize these errors. In the remainder of this section, we discuss thetechnical aspects of the protocol that we feel reduce the errors. We also discuss the interpretation of the data.

I strongly recommend for the publication as a full article in the journal . No need to take more care regarding samples at this time .

*Response:* Thank you.