Author’s response to reviews

Title: Musculoskeletal application and validation of speckle-tracking ultrasonography

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Author’s response to reviews:

Dear Editor

BMC Musculoskeletal Disorders

Re: BMSD-D-18-01312

Enclosed please find a revised version of our manuscript “Musculo-skeletal application and validation of speckle tracking ultrasonography” by Frich et al. We have carefully considered and addressed all points raised by the reviewers and believe this has significantly improved the overall quality of the manuscript. A detailed point-by-point list of our replies to the issues raised and the corresponding changes made in the manuscript are provided below. All parts of the text that have been modified are given under “Author action” in the point-by-point response and underlined in the revised manuscript.

Point-by-point reply:

Response to reviewer #1:
Joseph J. Sarver (Reviewer 1): Introduction:

1. The authors have done an adequate job reviewing the literature to justify the need for their study.

Methods:

The methods have several sections that are inadequate and need SIGNIFICANT revision before this manuscript can be published. Specifically:

1. Strain

a) The authors refer to sarcomere strain, but such structures are not in the same scale as the US measurements used to determine strain. At best, the authors are measuring tissue strain.

Author reply: Strain is a unitless measurement of dimensional or deformational change. Imaging-based techniques have been derived and refined in order to quantify myocardial strain in clinical practice (D’Hooge et al, Regional strain and strain rate measurements by cardiac ultrasound, Eur J Echocardiogr, 1, 154-70, 2000; Dandel & Hetzer, Echocardiographic strain and strain rate imaging, Int J Cardiol, 132, 11-24, 2009; Mor-Avi et al, Current and evolving echocardiographic techniques for the quantitative evaluation of cardiac mechanics, J Am Soc Echocardiogr, 24, 277-313, 2011). This technique is being embraced and increasingly adopted in many echocardiography laboratories worldwide (Collier et al, A Test in Context: Myocardial Strain Measured by Speckle-Tracking Echocardiography, J Am College Cardiol, 69, 8, 2017) and has recently been applied in healthy and surgically repaired human achilles tendons (Beyer et al, Speckle tracking in healthy and surgically repaired human Achilles tendons at different knee angles, Transl Sports Med 1, 79-88, 2018) and in deep dorsal neck muscles in individuals experiencing whiplash-associated disorders (Rahnama et al, Alterations in the Mechanical Response of Deep Dorsal Neck Muscles in Individuals Experiencing Whiplash-Associated Disorders Compared to Healthy Controls, Am J Phys Med Rehabil, 97, 75-82, 2018). The above references use ‘tissue deformation’ and consequently we agree that sarcomere strain should be avoided.

Author action: Sarcomere strength has been deleted and replaced with ‘tissue/muscle strain’ or ‘tissue/muscle deformation’ throughout the entire manuscript. Furthermore, to acknowledge that the methodology has been applied in echocardiography and recently tested in deep dorsal neck muscles, the following has been added to the muscle strain analysis section (Line 203-204): ”Strain, which is a unit-less measurement of dimensional or deformational change of the tissue, was analyzed offline and blinded to the examiner.” Furthermore, we added to Lines 207-215 the
following: “The software and technique utilized in our study have been studied extensively in the field of cardiology and is today recognized as an important clinical tool and used on a daily basis. In theory, the software detects reflected scattered signals (speckles) within muscle tissue. Based on the unique movement of these speckles, it is possible to calculate deformation as the absolute shortening between 2 speckles divided by the distance between these. The EchoPac speckle-tracking software, provided by GE Medical, automatically detects the movement of these. For analysis of loops (i.e. video recorded muscle contraction), an elongated tracking region of interest (ROI) was placed manually along the central raphe within the muscle and frame-to-frame displacement was estimated using multiple overlapping small kernels.”

b) The authors description of how strain was determined was wholly inadequate. To begin with, muscle is a very complex tissue, with 3D strain. The impact of their simplification to 1D strain was not discussed in sufficient detail. Next the authors given no description of how the software determines strain. At a minimum, strain is a change in length over initial length. What initial length? What length change was determined? Without this background information, it is impossible to assess the quality of the results. While the authors do describe that the strain measures are proprietary from the device used, this is insufficient explanation for a scientific paper. Without knowing those details, it is the duty of the authors to evaluate the strain accuracy, which could be done with phantoms.

Author reply: We thank the reviewer for his insightful comments and have tried to give a more comprehensive explanation on how to measure strain in the manuscript. The software and technique utilized in our study have been studied extensively in the field of cardiology and are today recognized as important clinical tools and used on a daily basis. In theory, the software detects reflected scattered signals (speckles) that are unique for each signal, and based on the movement of these is able to calculate the deformation of the individual parts of the muscle. Strain is a dimensionless variable describing the absolute shortening between 2 speckles divided by the distance between these. However, the software automatically detecting the movement of these is provided by GE Medical and is trademark. Other vendors as Philips and Siemens are also able to calculate strain although using different algorithms.

Evaluation of new diagnostic tools is difficult when there is no gold standard, and criterion validity may then be evaluated using a widely accepted reference standard from another construct of outcome measures (Measurement in Medicine: A Practical Guide. De Wet et al, Cambridge University Press 2011)). Thus, as no ‘gold standard’ exists of speckle tracking ultrasonography within skeletal muscles we chose isometric sub-maximal and maximal voluntary contractions as a reference standard representing a validated outcome measure of a very similar/parallel construct. Thus, we believe that the use of phantoms is superfluous in the present manuscript.
Furthermore, strain gauge technique as a measure of external muscle tension was employed to control for pre-tension of the investigated muscles. The instruction (as written in the initial manuscript) for the subjects was to tighten up the slack of the test setting (non-resilient straps and muscle-tendon system of the investigated muscle) requiring a pretension of approximately 5% MVC. Thus, and according to the length-tension relationship of skeletal muscles, we expect that the measurements represent the strain (i.e. active muscle deformation) from an almost relaxed muscle to the pre-defined sub-maximal contractions.

Author action: To acknowledge the valued critics by the reviewer, we have (as also listed above) added the following to the muscle strain analysis section (Line 203-217): "Strain, which is a unitless measurement of dimensional or deformational change of the tissue, was analyzed offline and blinded to the examiner. To track muscle strain, a multi-kernel block-matching scheme devised specifically for tracking muscle movement was used. In practice we used the Q-analysis function of the EchoPac speckle-tracking software developed by GE Healthcare (version BT 12). The software and technique utilized in our study have been studied extensively in the field of cardiology 14 and is today recognized as an important clinical tool and used on a daily basis. In theory the software detects reflected scattered signals (speckles) within muscle tissue. Based on the unique movement of these speckles it is possible to calculate deformation as the absolute shortening between 2 speckles divided by the distance between these. The EchoPac speckle-tracking software, provided by GE Medical, automatically detects the movement of these.

For analysis of loops (i.e. video recorded muscle contraction), an elongated tracking region of interest (ROI) was placed manually along the central raphe within the muscle and frame-to-frame displacement was estimated using multiple overlapping small kernels. The quality of the recorded loops varied in some volunteers, depending on the external load and general movement of the arm, especially in high-loading contractions."

c) Furthermore, given that motion of the probe relative to the muscle, or the scapula relative to the probe would dramatically affect the results, the authors need to state how such potential noise values were taken into account. That is, from an image analysis point of view there is no different between the muscle moving relative to the probe (either due to contraction, or scapular motion), or the probe moving relative to the muscle.) Without accounting for such 'artifact' motion, it is not possible to assess the accuracy of the results.

Author reply: We acknowledge that the description of this potential bias can be elaborated more closely. However, as strain is a deformational change/absolute shortening between 2 speckles divided by the distance of the potential longitudinally displacement (i.e. the probe moving relative to the muscle), this should not bias the analysis.
In case of displacement of the probe relative to the muscle or speckles sliding out of the ROI, the integrated quality assurance in the EchoPac software led us to discard the particular loop if tracking was not possible in at least 2 of the 3 zones of the ROI panel.

Author action: In addition to the initial description that the ultrasound probe was kept in the same position, we have added the following to the revised manuscript:

(Line 182-183):” The ultrasound probe was kept in the same position relative to the muscle during recordings of voluntary muscle contractions under load.”

(Line 218-221):” A software built-in quality assurance implied that low-quality loops were discarded from the analysis if i) displacement of the total muscle was larger than the image size, ii) if the probe was moved relative to the muscle resulting in defined speckles sliding out of the ROI or iii) if tracking was not possible in at least 2 of the 3 zones of the ROI panels”.

Results:

The results were presented clearly presented, captions were appropriate, figures were clear and presented in a clear manner

Discussion:

1. Paragraph 1

a) With high degree of accuracy is incorrect. Accuracy would imply they compared their measurements of strain to a gold standard, which they have not. They found correlations between MVC and measured strain, further work is necessary to consider these strains 'accurate'.

Author reply: As stated in the purpose, the study reports on a novel, direct, and non-invasive assessment of muscle deformation patterns. We measured the contractile properties (strain) of two shoulder muscles by speckle tracking ultrasonography (STU) and validated strain against sub-maximal, isometric muscle contractions in healthy individuals. We acknowledge that the observed correlations between isometric MVC and measured strain do not necessarily prove that the methodology is accurate.

Validation is a term that has become synonymous with “accuracy.” To measure validity, or accuracy, is to assess how closely the results from a new diagnostic method approximate the current gold standard ("Precision" and "accuracy": two terms that are neither. Streiner &Norman, J Clin Epidemiol. 2006 Apr; 59(4):327-30.). As also stated above, evaluation of a new diagnostic tool is difficult when there is no gold standard. Criterion validity may then be evaluated using a
widely accepted reference standard from another construct of outcome measures (Measurement in Medicine: A Practical Guide. De Wet et al, Cambridge University Press 2011)). Thus, as no ‘gold standard’ exists of speckle tracking ultrasonography within skeletal muscles, the validation was performed using reference standards from a very similar and most probably dependent validated construct (i.e. isometric MVC). Consequently, we agree that an alternative wording than ‘accuracy’ is a better choice.

Author action: We have revised the first paragraph of the Discussion (Line 258-262) to the following: "The STU modality revealed intramuscular strain variations from approximately 10%-20% during varying sub-maximal isometric loading of the SS and BB muscles. Based on moderate to strong correlations between strain and isometric muscle contractions our data suggest the first steps towards validating the technique and advancing speckle technology as a promising clinical tool for measurement of skeletal muscle function.”

Furthermore, we have added a separate section on limitations to the Discussion stating that criterion validity was achieved by comparing strain with isometric MVC as no gold standard exists within the construct (Line 338-243):” In lack of a ‘gold standard’, the current criterion validation was performed using a widely accepted reference standard from a very similar and most probably dependent validated construct (i.e. isometric muscle contractions). Consequently. The observed correlations between muscle strain and isometric muscle force are indicative of but does not necessarily prove that the methodology is accurate.”

2. Paragraph 2

a) The authors state they "measure tension directly". This is incorrect. If accurate, which remains to be seen, at best their method could measure muscle strain, which is not the same as muscle tension. The relationship between a strain and tension (or stress), in a dynamic, non-linear, viscoelastic, an-isotropic materials such as muscle is extremely complicated to suggest otherwise is simply incorrect

Author reply: We thank the reviewer for the comment and we acknowledge that muscle tension is not measured directly by muscle strain. However, the current study demonstrates correlations between external muscle tension (evaluated by isometric muscle force) and muscle strain (evaluated by STU). Furthermore, we agree that the association between tension and strain is extremely complicated and, as demonstrated by the current strong associations (r = 0.60-0.76), the simple linear regressions do not explain the entire variation.

Author action: To acknowledge that tension is not measured directly, we have revised the sentence to the following (Line 263-265): ”The proposed modality is non-invasive and provides information about the deformation of the structural elements of the human skeletal muscle during isometric contractions in healthy individuals.”
3. Paragraph 4

a) Strong correlation does not, as the authors suggest, validation was achieved successfully. At best the correlation, which was for limited range of MVC, supports the hypothesis that the, for a given arm orientation, and under isometric conditions, as the force produced at the hand increased, the tissue displacement increased. This is by no means, validation.

Author reply: As also stated above, the current study is the first attempt to validate this novel technique on human skeletal muscles. Evaluation of new diagnostic tools is difficult when there is no gold standard and criterion validity may then be evaluated using a widely accepted reference standard from another construct of outcome measures (Measurement in Medicine: A Practical Guide. De Wet et al, Cambridge University Press 2011)). Consequently, the current study partly investigated criterion validity but we do agree that the current analysis does not justify that the methodology is fully validated and does not provide accurate measures of strain.

Author action: To avoid confusion regarding the definition of validation we have in the abstract replaced “…validates strain against sub-maximal…” with “…correlating peak strain against standardized sub-maximal…”. Later in the abstract we have clearly stated that validation was performed by correlating strain against sub-maximal isometric load conditions. Finally, the conclusion (in the abstract) has been totally revised to acknowledge the reviewers’ critics:”We demonstrate that STU can be applied on healthy skeletal musculature (SS and BB muscles). The observed correlations between strain and isometric contractions suggest a valid technique. However, the concept of measuring muscle deformation non-invasively needs further investigation on validity, accuracy, responsiveness, and reliability before its therapeutic and research potential will be triggered.”

In main text, we have added the following paragraph to the Discussion (Line 282-2294):” The current data supports the hypothesis that ultrasonic speckle patterns can be tracked in vivo in human skeletal muscle and that measured muscle strain values correlate with sub-maximal isometric muscle contractions. Lopata et al 12 demonstrated increasing strain values in relation to external force outputs of the BB in five healthy individuals using a former ultrasonography technology with approximately half the sampling frequency (38-50Hz) as the current (>140Hz). Lately, Rahnama et al 10 used custom-made software that processed the unique speckle pattern formed by the acoustic ultrasound waves that were scattered and reflected upon hitting the muscles. They measured strain values for healthy dorsal neck muscles (11-34%) that are very similar to the current study. The present study is, however, the first attempt to validate this novel technique in human skeletal muscles. Despite lack of a gold standard, criterion validity may be obtained under reasonable conditions using a widely accepted reference standard from another construct of outcome measures.19 The current study therefore does not justify a full validation of STU nor that ultrasonic speckle patterns provide accurate measures of strain.”
4. Paragraph 5

a) The authors describe many of the limitations in existing techniques but fail to address the many limitations of their study within the same context. For example, they point out some of the limitations in shear wave elastography (SWE), another ultrasound technique, but fail to mention that probe orientation, probe motion and operator experience are also a limitation in their technique. At no point, did they repeat their experiments to see if they have the same findings on a different day, or with different people, or under a myriad of other conditions that would affect the repeatability and reliability of their technique.

Author reply: We have added the following section to the new Limitation section:

Author action: “In lack of a ‘gold standard’, the current criterion validation was performed using a widely accepted reference standard from a very similar and most probably dependent validated construct (i.e. isometric muscle contractions). Consequently. The observed correlations between muscle strain and isometric muscle force are indicative of but do not necessarily prove that the methodology is accurate.

Of notice, operator experience including careful control of the transducer's position and orientation along muscle fibers may influence the quality of the recorded loops. Furthermore, improved visual control of the applied force (%MVC) by the subject during testing may affect accuracies of the external force measurements and corresponding speckle analysis. Finally, no inter- and/or intra-operator reliability was provided for the present setup, which should be conducted before implementing the technique in research or clinic.”

5. Conclusion:

1. The authors state that measuring muscle deformation 'will' influence future studies, but they provide no evidence that this will be true. They show no difference between groups (healthy and control, or fresh vs fatigued muscle, or …). While their results are interesting, this is only a first step in a long road toward that goal, and their wording should make it clear that this is well understood.

Author reply: We certainly agree and have removed the speculative parts of the conclusion and added what needs to be established in the future

Author action: The conclusion has been revised to the following: "STU was applied on healthy SS and BB muscles and showed intramuscular strain variations from approximately 10%-20% during varying sub-maximal isometric conditions. Moderate to strong correlations between strain
and isometric contractions suggest a valid technique. However, this concept of identifying muscle deformation non-invasively needs further investigation before unfolding of its clinical potential.”

Response to reviewer #2:

John Drazan, Ph.D (Reviewer 2): Review:

Notes to author:

The purpose of this study was to develop a new modality for characterizing muscle function using ultrasound speckle tracking in the biceps and supraspinatus. The test cohort was a convenience sample of 10 subjects with no history of neuromuscular disorder. Muscle deformation was characterized using ultrasound speckle tracking while subjects performed cyclic isometric contractions against a custom dynamometer at several magnitudes normalized relative to a subject’s maximal voluntary contraction. The correlation between muscle strain and force generated was tested using a one way ANOVA. Results showed that observed strain characterized using ultrasound speckle tracking was correlated with contraction magnitude.

This study is interesting because it demonstrates the feasibility of characterizing muscle function using clinically available equipment, however there are extensive issues with word choice throughout the paper (e.g. incorrect definition of strain and changing between the word cycles in Line 148 and loops in 167 among others) that make the paper difficult to understand. Additionally, there are sections of the methods that are not reported in the results, for example retesting five days later.

Author reply: Thanks for the valued comments. We agree that issues with word choice were present in the initial manuscript and have made numerous revisions accordingly, which we believe have improved the readability.

Author action: Specifically, we have removed the initial sentence regarding ‘cine loops’ as pointed out by the reviewer. Furthermore, the sentence regarding test-retest has been deleted since we did not conduct these tests. This is now clearly mentioned in the new Limitations section (Line 347-349):”Finally, no inter- and/or intra-operator reliability was provided for the present setup, which should be conducted before implementing the technique in research or clinic” Moreover, we have gone through the manuscript and made sure that muscle strain is defined as ‘muscle deformation’ or ‘tissue deformation.’”
Introduction:

The introduction is OK, however there are several issues regarding word choice and framing the study. If the SEMG is the gold standard for evaluating muscular function, why wasn't SEMG used as the comparison rather than isometric dynamometry? Why would isometric dynamometry be used as a comparison at all? The value of isometric dynamometry is not discussed until the discussion section.

Author reply: We agree that the link between Speckle, SEMG and isometric dynamometry can be improved.

Author action: The sentence regarding SEMG has been revised to the following (Line 93-96):”To date, electromyography is used as a pseudo outcome for the assessment of muscle performance by measuring the sum of active motor units in the vicinity of the electrodes, however it may be biased and is not a direct measure of muscle strain.” Furthermore, the initial sentence regarding potential bias of SEMG has been deleted. Finally, to link speckle with isometric dynamometry the following sentence has been added to the Introduction (Line 107-108):” So far it remains unclear, however, if muscle strain values of different muscles are associated with the intensity of voluntary muscle contractions.”

Also, sarcomeres are too small to be evaluated using ultrasound. Strain, deformation, and tension seem to be used interchangeably and this is confusing. What is actually important and what is being measured? Ultrasound speckle tracking has already been used for skeletal muscle as early as 1989. Therefore, the hypothesis of "ultrasonic speckle patterns can be tracked in vivo in skeletal muscle" has already been demonstrated. Instead it seems like you hypothesized and tested that strain measure using ultrasonic speckle tracking is correlated with submaximal isometric contractions.

Author reply: As also stated above, we have gone through the manuscript and made sure that muscle strain is defined as ‘muscle deformation’ or ‘tissue deformation’. We also acknowledge that STU has already been used for skeletal muscle, which now is included in the Introduction.

Author action: The Introduction now states (Line 76-78):”Currently, few in vivo imaging techniques are able to characterize the contractile properties of muscle tissue. Among these, modern ultrasound apparatus equipped with automatic tracking of clusters of speckles has been used for functional assessment of the heart 7 and the diaphragm 8.”

Finally, the Objective has been revised (Line 109-113):” The objective of this study was therefore to address the clinical and technological research gaps within the field of in vivo muscle contraction advancing speckle technology towards validation as a clinical tool for non-invasive measures of muscle deformation. Thus, we aimed to test the hypothesis that the
displacement of ultrasonic speckle patterns during isometric muscle contractions in upper extremity skeletal muscles correlates with muscle strain.”

Methods:

The methods are well described; however, they are confusing. A figure or picture of the custom dynamometer would be helpful to visualize how this testing was performed. Is STU a real time process where it takes place while each ultrasound frame is captured? Or is the ultrasound data captured, and later analyzed? It is unclear to me whether the described "STU" refers to the speckle tracking analysis or the collection of data. For data analysis, what is Q-analysis? Is this a software package? Also, the methods switched from cycles to loops at Line 166. If these are different things, I have no idea what a loop is. Overall, what values are established a priori to indicate an accurate test?

Author reply: A figure of the test setup is partially provided in figure 1. We used a strap-on dynamometer not shown due to its position much to the side of the test person.

Author action: None

Author reply: STU refers to data collection and processing of data. Q-analysis' is a function of the EchoPac software.

Author action: We have revised the sentence (Line 201-205):” Strain, which is a unit-less measurement of dimensional or deformational change of the tissue, was analyzed offline and blinded to the examiner. To track muscle strain, a multi-kernel block-matching scheme devised specifically for tracking muscle movement was used. In practice we used the Q-analysis function of the EchoPac speckle-tracking software developed by GE Healthcare (version BT 12)”.

Results:

You mentioned doing test-retest repeatability. Where did this go?

Author reply: The sentence regarding test-retest has been deleted since we did not conduct these tests.

Author action: This is now clearly mentioned in the new Limitations section (Line 342-344): ”Finally, no inter- and/or intra-operator reliability was provided for the present setup, which should be conducted before implementing the technique in research or clinic.”
Discussion:

The discussion seems to draw conclusions not supported by the data collected. How can you say, "high accuracy?" How does this approach compare to previous studies using SEMG or other modalities? In line 201, you use the word invented. I suggest "proposed" instead because ultrasound speckle tracking has been used for decades. Overall, the words deformation, strain, and tension are used seemingly interchangeably. Each of these terms have specific, technical definitions which while have similarities, are unique. How are STU measurements independent of probe pressure?

Author reply: We appreciate the comments and have made several adjustments and additions to the Discussion.

Author action: The following paragraph has been added that explains why isometric dynamometry was chosen and also why ‘accuracy’ should be avoided (Line 279-291): ”The current data supports the hypothesis that ultrasonic speckle patterns can be tracked in vivo in human skeletal muscle and that measured muscle strain values correlate with sub-maximal isometric muscle contractions. Lopata et al 12 demonstrated increasing strain values in relation to external force outputs of the BB in five healthy individuals using a former ultrasonography technology with approximately half the sampling frequency (38-50Hz) as the current (>140Hz). Lately, Rahnama et al 10 used custom-made software that processed the unique speckle pattern formed by the acoustic ultrasound waves that were scattered and reflected upon hitting the muscles. They measured strain values for healthy dorsal neck muscles (11-34%) that are very similar to the current study. The present study is, however, the first attempt to validate this novel technique in human skeletal muscles. Despite lack of a gold standard, criterion validity may be obtained under reasonable conditions using a widely accepted reference standard from another construct of outcome measures.19 The current study therefore does not justify a full validation of STU nor that ultrasonic speckle patterns provide accurate measures of strain.”

The following paragraph has been added to the Limitations section (line 334-344): “In lack of a ‘gold standard’, the current criterion validation was performed using a widely accepted reference standard from a very similar and most probably dependent validated construct (i.e. isometric muscle contractions). Consequently. The observed correlations between muscle strain and isometric muscle force are indicative of but do not necessarily prove that the methodology is accurate.

Of notice, operator experience including careful control of the transducer's position and orientation along muscle fibers may influence the quality of the recorded loops. Furthermore, improved visual control of the applied force (%MVC) by the subject during testing may affect accuracies of the external force measurements and corresponding speckle analysis. Finally, no
inter- and/or intra-operator reliability was provided for the present setup, which should be conducted before implementing the technique in research or clinic.”

The word ‘invented’ has been replaced by ‘proposed’ (Line 260).

Muscle strain is now defined as ‘muscle deformation’ or ‘tissue deformation’.

General notes:

* The purpose of this study was to analyze muscle contractile properties using ultrasound speckle tracking.

Author reply: The purpose has been revised and now focuses on the potential correlation between strain and isometric dynamometry

Author action: Purpose revised (Line 109-113): “The objective of this study was therefore to address the clinical and technological research gaps within the field of in vivo muscle contraction advancing speckle technology towards validation as a clinical tool for non-invasive measures of muscle deformation. Thus, we aimed to test the hypothesis that the displacement of ultrasonic speckle patterns during isometric muscle contractions in upper extremity skeletal muscles correlates with muscle strain.”

* Is electromyography the gold stand for assessment of muscle performance? Line 65

Author reply: We acknowledge that defining SEMG as a gold standard for muscle performance is misleading.

Author action: The sentence in the Introduction has been revised (line 93-95):” To date, electromyography is used as a pseudo outcome for the assessment of muscle performance by measuring the sum of active motor units in the vicinity of the electrodes, however it may be biased and is not a direct measure of muscle strain.”

* Sarcomere tension vs fascicle tension. Line 65

Author reply: To avoid confusion the specific sentence has been deleted and the Introduction has been revised.
Author action: The revised Introduction now states the following (Line 89-91):” Skeletal musculature contains bundles of muscle fibers called myofibrils and each myofibril is a chain of sarcomeres, which are the smallest repeating functional units in the muscle.”

* Tension vs contractility Line 74

Author reply: ‘Tension’ is usually associated with sarcomere length (Gordon et al, 1966) and as a consequence we use ‘tension’ to describe the mechanical function following sarcomere shortening. ‘Contractility’ is a broader terminology of muscle function that may not be (directly) related with sarcomere tension

Author action: Consequently ‘contractility’ is only used 3 times within the revised manuscript when muscle function as a non-specified function is described/discussed.

* Empirical evidence of what?

Author reply: The sentence has been modified

Author action: Empirical has been deleted

* Awkward sentence at 105. What is happening here?

Author reply: We have revised the awkward sentence.

Author action: (Line 148-150):” The STU (see below) was performed under controlled conditions by measuring the external force by custom made dynamometry (Force Transducer, U9C, ≥2kN, Hottinger Baldwin Messtechnik GmbH, Darmstadt, Germany).”

* GE Software? Line 143

Author reply: Thanks for pointing out the mistake.

Author action: ‘GE software’ has been replaced with ‘ultrasound machine’ (Line 163)

* Cine loop? Line 144
Author reply: ‘Loops’ refers to the video recording representing one muscle contraction. The ‘cine’ has been deleted.

Author action: ‘Loops’ is now defined in the Method section (Line 211-213):” For analysis of loops (i.e. video recorded muscle contraction), an elongated tracking region of interest (ROI) was placed manually along the central raphe within the muscle and frame-to-frame displacement was estimated using multiple overlapping small kernels”.

* Why wasn't STU performed during the MVC 119?

Author reply: The current set-up of stabilizing the test person, ultra sound transducer and muscle dynamometer did not allow measurements at or above 80%. This was initially explained in Method section (Line 196-197).

Author action: None

* What is Q-analysis? Line 164

Author reply: ‘Q-analysis’ is a function of the EchoPac software, which now has been clarified.

Author action: We have revised the sentence (Line 201-205): "Strain, which is a unit-less measurement of dimensional or deformational change of the tissue, was analyzed offline and blinded to the examiner. To track muscle strain, a multi-kernel block-matching scheme devised specifically for tracking muscle movement was used. In practice we used the Q-analysis function of the EchoPac speckle-tracking software developed by GE Healthcare (version BT 12).”

* What are the properties of the custom made dynamometer?

Author reply: We are not sure what the reviewer exactly is asking for regarding ‘properties’ but we have added the capacity of ≥2kN in the technical description.

Author action: (Line 148-150):”The STU (see below) was performed under controlled conditions by measuring the external force by custom made dynamometry (Force Transducer, U9C, ≥2kN, Hottinger Baldwin Messtechnik GmbH, Darmstadt, Germany)”

* What is a cycle, and what is a loop? Line 166
Author reply: We agree that the two terminologies may have been used interchangeably. In the current revised manuscript ‘cycles’ refers to the isometric test contraction at the different submaximal intensities whereas ‘loop’ refers to the video recordings of the test cycles.

Author action: To make this clear the following definition has been added (Line 211):” For analysis of loops (i.e. video recorded muscle contraction)…”

* For results, what about the retest protocol mentioned in Line 161

Author reply: As also mentioned above the retest was not conducted

Author action: To acknowledge this the following has been added to the Limitations section (Line 342-344):” Finally, no inter- and/or intra-operator reliability was provided for the present setup which should be conducted before implementing the technique in research or clinic.”

* Line 210, if isometric dynamometry is bad, why are you using it to validate your system.

Author reply: We admit that the reason why isometric dynamometry is not a sufficient measure of muscle contractility but still used as an accepted reference value was not described satisfactorily.

Author action: To address this issue the following sections have been added:

(Line 266-268):” Although reliable, isometric dynamometry is a different construct and does not provide information about the quality of the muscle fibers being tested, the method qualifies as a reference for the present validation analyses.”

(Line 279-291):” The current data supports the hypothesis that ultrasonic speckle patterns can be tracked in vivo in human skeletal muscle and that measured muscle strain values correlate with sub-maximal isometric muscle contractions. Lopata et al 12 demonstrated increasing strain values in relation to external force outputs of the BB in five healthy individuals using a former ultrasonography technology with approximately half the sampling frequency (38-50Hz) as the current (>140Hz). Lately, Rahnama et al 10 used costum-made software that processed the unique speckle pattern formed by the acoustic ultrasound waves that were scattered and reflected upon hitting the muscles. They measured strain values for healthy dorsal neck muscles (11-34%) that are very similar to the current study. The present study is, however, the first attempt to validate this novel technique in human skeletal muscles. Despite lack of a gold standard, criterion validity may be obtained under reasonable conditions using a widely accepted reference standard from another construct of outcome measures.19 The current study therefore does not justify a full validation of STU nor that ultrasonic speckle patterns provide accurate measures of strain.”
In lack of a ‘gold standard’, the current criterion validation was performed using a widely accepted reference standard from a very similar and most probably dependent validated construct (i.e. isometric muscle contractions). Consequently, the observed correlations between muscle strain and isometric muscle force are indicative of but do not necessarily prove that the methodology is accurate.”

* Line 201: Speckle tracking using ultrasound is a well documented technique within the field of biomechanics as early as 1989. The word invention is most likely not appropriate to describe the work done here.

Author reply: We agree and the current revision reflects an altered purpose where the invention of the technology has been deleted.

Author action: Several changes have made to acknowledge this issue – for instance the Introduction, Purpose and Conclusion:

(Line 100-102):” Currently, few in vivo imaging techniques are able to characterize the contractile properties of muscle tissue. Among these, modern ultrasound aperture equipped with automatic tracking of clusters of speckles has been used for functional assessment of the heart 7 and the diaphragm 8“

(Line 109-113):” The objective of this study was therefore to address the clinical and technological research gaps within the field of in vivo muscle contraction advancing speckle technology towards validation as a clinical tool for non-invasive measures of muscle deformation. Thus, we aimed to test the hypothesis that the displacement of ultrasonic speckle patterns during isometric muscle contractions in upper extremity skeletal muscles correlates with muscle strain.

(Line 247-351):” STU was applied on healthy SS and BB muscles and showed intramuscular strain variations from approximately 10%-20% during varying sub-maximal isometric conditions. Moderate to strong correlations between strain and isometric contractions suggest a valid technique. However, this concept of identifying muscle deformation non-invasively needs further investigation before unfolding of its clinical potential.”

* If EMG is presented as the gold standard, why isn't that the comparison?

Author reply: The revised Introduction deals with the issue of SEMG. Furthermore, it is not possible to perform STU on the same position/vicinity as the EMG electrode positions since the two methods will ‘steal’ each other’s location and/or provide movement artefacts.
In skeletal muscles, contraction is stimulated by action potentials transmitted by motor neurons. To date, electromyography is used as a pseudo outcome for the assessment of muscle performance by measuring the sum of active motor units in the vicinity of the electrodes, however it may be biased and is not a direct measure of muscle strain.

With the current changes we hope that manuscript is now acceptable for publication in BMC Musculoskeletal Disorders.

Yours Lars Henrik Frich

MD PhD