Combined magnetic resonance and diffusion tensor imaging analyses provide a powerful tool for in vivo assessment of deformation along human muscle fibers

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\textbf{A B S T R A C T}

Muscle fiber direction strain provides invaluable information for characterizing muscle function. However, methods to study this for human muscles in vivo are lacking. Using magnetic resonance (MR) imaging based deformation analyses and diffusion tensor (DT) imaging based tractography combined, we aimed to assess muscle fiber direction local tissue deformations within the human medial gastrocnemius (GM) muscle. Healthy female subjects (n = 5, age = 27 ± 1 years) were positioned prone within the MR scanner in a relaxed state with the ankle angle fixed at 90°. The knee was brought to flexion (140.8 ± 3.0°) (undeformed state). Sets of 3D high resolution MR, and DT images were acquired. This protocol was repeated at extended knee joint position (177.0 ± 1.0°) (deformed state). Tractography and Demons nonrigid registration algorithm was utilized to calculate local deformations along muscle fascicles. Undeformed state images were also transformed by a synthetic rigid body motion to calculate strain errors. Mean strain errors were significantly smaller than mean fiber direction strains (lengthening: 0.2 ± 0.1% vs. 8.7 ± 8.5%; shortening: 3.3 ± 0.9% vs. 7.5 ± 4.6%). Shortening and lengthening (up to 23.3% and 116.7%, respectively) occurs simultaneously along individual fascicles despite imposed GM lengthening. Along-fiber shear strains confirm the presence of much shearing between fascicles. Mean fiber direction strains of different tracts also show non-uniform distribution. Inhomogeneity of fiber strain indicates epimuscular myofascial force transmission. We conclude that MR and DT imaging analyses combined provide a powerful tool for quantifying deformation along human muscle fibers in vivo. This can help substantially achieving a better understanding of normal and pathological muscle function and mechanisms of treatment techniques.

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1. Introduction

Arranged in series within muscle fibers, sarcomere is the basic functional element of skeletal muscle. Force production capacity of sarcomere depends on its length. Therefore, quantification of length changes along fibers of human muscles, in vivo bears great importance for characterizing muscle’s function.

Magnetic resonance imaging (MRI) analyses are highly suitable for quantifying local tissue deformations in human muscles. Demons’ registration (Huijing et al., 2011; Yaman et al., 2013), phase-contrast (Kinugasa et al., 2008; Pappas et al., 2002; Zhou and Novotny, 2007), displacement encoding with stimulated echoes (Fiorentino et al., 2012) and spatial-tagging (Englund et al., 2011) were used previously. However, these techniques alone are insufficient for determining deformation along muscle fibers.

Diffusion tensor imaging (DTI) is an MRI methodology that detects diffusivity of water within biological tissues by applying diffusion sensitizing gradients in multiple directions. Diffusion of free water molecules in tissue is limited by tissue structures such as cell membranes (Prompers et al., 2006). Therefore, tensors storing data characterizing diffusion of water molecules along each gradient direction identify tissue architecture. Animal (Van Donkelaar et al., 1999) and cadaver (Budzik et al., 2007) studies have shown that the first eigenvector of the diffusion tensor corresponds to the direction of muscle fibers. Damon et al. (2002) presented feasibility and validation of tractography for determining pennation angles using DTI. Repeatability of structural muscle measures such as pennation angle and fiber length has been demonstrated for humans in vivo (Heemskerk et al., 2010). Hence, DTI tractography allows for visualizing orientation of muscle fibers.

Using MRI based deformation analyses and DTI based tractography combined, we aimed to assess muscle fiber direction local tissue deformations within the human medial gastrocnemius (GM) muscle as caused by knee angle changes.

2. Materials and methods

Experimental procedures were in strict agreement with guidelines and regulations concerning human welfare and experimentation set forth by Turkish law, and approved by a Committee on Ethics of Human Experimentation at Istanbul University, Istanbul School of Medicine, Istanbul.

2.1. Subjects

Five healthy woman subjects ((mean±SD) age = 27±1 years, height = 159±4 cm, body mass = 50±4 kg) volunteered (Table 1). After a full explanation of the purpose and methodology, the subjects provided an informed consent.

2.2. Experimental protocol

Subjects were positioned prone on the MRI patient table. The left leg was brought to a reference position: (I) the ankle was fixed at 90° by using a custom made MRI compatible device. A piece of Velcro under the heel and straps over the ankle allowed joint position fixation. To locate the knee joint, a piece of Velcro was attached over the patella and also on the MRI table. The knee was brought to flexion by elevating the torso with support such that the trunk of the subject is brought as close as possible to the MRI bore wall in order to attain the maximal flexed knee position (Fig. 1A). The knee angle measured using a universal goniometer (Norkin and White, 1995) in this undeformed state equaled 140.8 ± 3.0°. After moving the patient table into the MRI bore, sets of 3D high-resolution MR and DT images were acquired at relaxed state.

Subsequently, the support was removed and the knee was brought to extension (Fig. 1B). In this deformed state, the knee angle equaled 177.0 ± 1.0°. Sets of 3D high-resolution MR and DT images were acquired with the subjects maintaining their relaxed state.

2.3. MR and DT image acquisition

3D locator imaging was performed to plan the subsequent imaging sequences. 3D turbo fast low-angle shot [Turbo FLASH] based (see Table 2 for MR imaging parameters) coronal MR image sets were collected using 3T MR scanner (Magnetom Trio; Siemens, Erlangen, Germany) with two 6-channel surface coils. Choices of high bandwidth and frequency encoding in proximo-distal direction (Weis et al., 1998) allowed minimizing potential chemical shift artifacts.

2D single shot echo planar imaging (ss-EPI) based axial DT image sets were collected with fat saturation and posterior-anterior direction frequency encoding to minimize potential chemical shift artifacts in the region of interest. Non-weighted b0 images and 12 diffusion-weighted gradient images with unique directions were acquired for eight signal averages (see Table 2 for DT imaging parameters).

3. Calculation

3.1. Calculation of deformations

MR images were rigidly aligned with the DT images. Starting from the proximal half of the imaged portion of the lower leg (corresponding to mid-GM belly), in each of 112 consecutive cross-sectional slices studied, the GM region was distinguished manually (Fig. 1C and D).

Deformations caused by knee extension were calculated by aligning MR images acquired in the deformed and undeformed states. Demons algorithm (Thirion, 1998), i.e., a nonrigid and nonparametric image analysis technique was applied. Utilizing arrays of voxel intensities, this algorithm relies on differences between grayscale values of consecutive images.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>Upper leg length (cm)</th>
<th>Lower leg length (cm)</th>
</tr>
</thead>
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<td>A</td>
<td>25</td>
<td>160</td>
<td>47</td>
<td>42.0</td>
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</tr>
<tr>
<td>B</td>
<td>27</td>
<td>154</td>
<td>45</td>
<td>49.0</td>
<td>36.0</td>
</tr>
<tr>
<td>C</td>
<td>29</td>
<td>160</td>
<td>52</td>
<td>43.0</td>
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<td>28</td>
<td>165</td>
<td>56</td>
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<td>36.0</td>
</tr>
<tr>
<td>E</td>
<td>27</td>
<td>158</td>
<td>52</td>
<td>44.0</td>
<td>38.0</td>
</tr>
</tbody>
</table>
voxels within each image and corresponding voxels in deformed and undeformed images. Image differences calculated iteratively are used to characterize displacement values for each voxel. During each iteration, updated displacement fields are smoothed by a Gaussian kernel for regularization of local displacements and global motion. Finally, after obtaining successful alignment of images by minimizing image differences, information on real deformation is available for each cubic shape comprised of four adjacent image voxels.

### 3.2. Determination of muscle fiber directions

Rician noise was eliminated (Gallichan et al., 2010) from raw diffusion weighted images (DWI). Subsequently, diffusion tensor of each voxel was calculated. To determine GM muscle fiber tracts, streamline tractography with 4th order Runge-Kutta numerical integration was performed on in-house software built on VAVframe framework1. Tract seed points were generated from voxels showing a minimum directionality of diffusion (FA ≥ 0.1) (Froeling et al., 2012). The seed points were bi-directionally tracked with 0.7 mm integration.
steps (half of the smallest voxel dimension (Descoteaux et al., 2009)). Each integration point forms a tract node. Tracking algorithms typically make use of the known muscle geometry including boundary (Bolsterlee et al., 2015; Lansdown et al., 2007) and tract length (Heemskerk et al., 2010) to filter out possible irrelevant tracts. Based on a review by Chow et al. (2000) reporting human GM fiber lengths, the criterion we implemented rejected tracts shorter than 30 mm, and longer than 50 mm. In addition, tracking was terminated if at least one of the following conditions was met: (i) The current tract node’s FA < 0.5 and (ii) tract curvature >5° per step (Froeling et al., 2012). Two polygonal regions of interests, marked manually on deformed state b0 images, were used to constrain the tracts to be in the GM. Tracts passing through both were accepted for further processing.

Fiber tract pennation angles (θ) were calculated based on Lansdown et al. (2007): deep GM aponeurosis was marked on the anatomic images, reconstructed in 3D and smoothed. Subsequently, unit normal vectors (n) were calculated for each node of the aponeurosis model. For each tract, r is defined as the vector between the first node of the tract on the aponeurosis and the subsequent nodes along the tract. For each node, the pennation angle is calculated as

\[ θ = \sin^{-1}(n \cdot r) \]

and their mean along each tract is considered as its pennation angle.

### 3.3. Calculation of strains

Displacement fields in the global coordinates were mapped on the deformed state b0 images and were linearly interpolated onto the nodes of the fiber tracts based on the weighted means of the grid neighbors of a given voxel in the displacement field.

Using the displacement fields obtained, deformation gradient matrix F, characterizing deformation at each fiber tract node, was calculated by using displacement gradient (Vu) in material coordinates:

\[ F = Vu + I \]  

(1)

Subsequently, the right Cauchy-Green strain tensor C

\[ C = F^T F \]  

(2)

and Green-Lagrange strain tensor E was calculated for each node in order to assess deformations within GM after knee extension

\[ E = \frac{1}{2} [F^T F - I] \]  

(3)

For each node the following were calculated:

1. The strain tensor was rotated to align with the unit tangent vector of the tract M, yielding fiber direction strain values.
2. In order to assess shearing between fascicles, along-fiber shear strain was determined based on (Blemker and Delp, 2005; Criscione et al., 2001). Strain invariants

\[ I_4 = J^{2/3} I_m^2 \]  

(4)

Nodal coordinates with corresponding fiber direction and along-fiber shear strain values were transferred to Slicer 4 (http://www.slicer.org) for visualization (Fedorov et al., 2012) of local muscle tissue deformations along the GM tracts per subject in order to interrogate their homogeneity. A serial distribution of fiber direction strain hence, inhomogeneity of those values along the GM tracts was considered as an indicator of altered force production potential of sarcomeres located at different parts of the tract. In addition, mean values of nodal fiber direction strains of each tract were calculated and visualized per subject. A parallel distribution of fiber direction strain hence, inhomogeneity of those values across different GM tracts was considered as an indicator of altered capacity of different tracts to the excursion of the muscle. Therefore, serial and parallel distributions were considered as metrics of high functional significance.

3. The determinant of F was used to assess volume change

\[ dV/dV_{0} \]

For voxels in the deep GM aponeurosis, eigenvalue analyses were done to obtain peak local lengthening (E_l) and shortening (E_s). The eigenvectors determine the direction of peak strains. The peak local deep GM aponeurosis strains in the proximo-distal direction, aligned with the Achilles tendon were distinguished.

### 3.4. Testing of MRI noise artifacts and repeatability

In order to quantify possible artifacts due to noise within the MRI scanner, two separate MR image sets of a subject were acquired in subsequent imaging performed in constant position. This assessment showed that the noise artifacts are small yielding strain values of 2.3 ± 1.2% and 2.0 ± 1.4% for lengthening and shortening, respectively.

Repeatability of fiber direction strain patterns was tested within one subject. After completing the original protocol, the repeat protocol was executed: (i) the subject’s torso was elevated again with the trunk support, while keeping the knee joint position consistent with the previous flexed position. Sets of 3D high-resolution MR and DT images were then acquired for the original protocol. (ii) Subsequently, the trunk support was removed, and care was taken to maintain identical extended knee joint position as in the original acquisition. Separate sets of 3D high-resolution MR and DT
images were acquired also for this repeat protocol. Testing was done while maintaining a relaxed state at all times. Deformation analyses and tractography were performed for the original protocol and its repetition. The original protocol yielded a general pattern of fiber direction strains, which indicates that the proximal track segments are lengthened, whereas the distal ones are shortened. The repeat protocol matched that quite well (Fig. 2).

3.5. Calculation of artifacts

The validity of Demons algorithm in quantifying tissue deformations was shown with vigorous testing (Yaman et al., 2013). Presently, image sets of the undeformed state were transformed by a "synthetic rigid body motion" imposed on the data: 10° rotation within the cross-sectional plane (representing endorotation of the knee during flexion (Moglo and Shirazi-Adl, 2005)), 3° rotations in the coronal and sagittal planes, and 4 mm translation axially. Subsequently, the undeformed state and these transformed image sets were compared. The displacement fields calculated using Demons algorithm were mapped onto the tracked fibers. Theoretically, imposed rigid body motion should cause no strains. Therefore, resulting strains represent strain errors and volume errors.

3.6. Statistics

Fiber direction strain distributions deviate from normal distributions. Therefore, for lengthening and shortening separately, nonparametric Wilcoxon rank sum tests were performed for differences between in vivo deformations and strain errors. Wilcoxon rank sum tests were also performed to assess any changes of volume. The level of significance was chosen at $p < 0.05$.

Pair-wise comparisons of mean fiber direction strain along individual tracts within each subject were done based on Kruskal–Wallis with Dunn’s post hoc test. Ratio of the number of fiber pairs with statistically different mean strains to the total number of fiber pair combinations quantify parallel distribution of strain per each subject.

4. Results

Pooled data show mean±SD of measurements from all subjects: (1) pennation angles equal $17.1 ± 10.4°$. (2) Strain errors ($0.2 ± 0.1\%$ and $3.3 ± 0.9\%$ for lengthening and shortening, respectively) are small and are significantly different from fiber direction strains ($8.7 ± 8.5\%$ and $7.5 ± 4.6\%$ for lengthening and shortening, respectively) occurring due to knee extension imposed (Fig. 3A). (3) Volume change ($0.059±0.005$ error, vs. $0.050±0.091$) was insignificant. Pooled strain data from all subjects (Fig. 3B), and strains visualized along the GM tracts for each subject (Fig. 4) indicate a serial distribution of fiber direction strains occurring within the GM muscle fibers. Fig. 4 shows that despite the globally lengthened condition of the GM, locally lengthened and shortened parts are simultaneously present within the same tracts. For all subjects, there is a general pattern indicating that the proximal track segments are lengthened, whereas the distal ones are shortened. However, intersubject variability exists: (i) for subjects A-D, approximately the proximal half of the tracts shows predominantly lengthening, whereas for subject E, this is limited to a much smaller portion. (ii) Amplitude of local shortening (10–12% for subjects A and B, 17% for subject D, whereas, 19% and even up to 23% for subjects C and E, respectively) and lengthening (14–15% for subjects A and B, whereas 26% and 35% for subjects E and D, respectively, and up to 116.7% subject C) varies with a gradual transition from lengthening to shortening in the proximo-distal direction. Certain tracts present an opposite deformation pattern with shortening at proximal and lengthening at distal ends (e.g., by 15.4% and 25.4%, respectively in medial fascicles of subject D).

Along-fiber shear strains visualized per subject (Fig. 5) confirm the presence of much shearing between fascicles. 

Distributions of mean fiber direction strains of different tracts for the pooled data from all subjects (Fig. 6) indicate a parallel distribution of strain occurring among muscle fibers of the GM. This is visualized for each subject in Fig. 7. Parallel distribution of strain quantified equals 18.3%, 26.4%, 14.4%, 4.6% and 17.0% for subjects A, B, C, D and E, respectively. For

Fig. 2 – Results of the repeatability test. Fiber direction strain distributions occurring due to the original (A), and the repeat (B) protocols match quite well. Note that the strain amplitudes are somewhat smaller in the repeat protocol, which is ascribed to predominantly to viscoelastic properties of muscle and connective tissues.
Fig. 3 – Effect of altered knee angle across all subjects. Green–Lagrange strain tensor of each node rotated to align with the unit tangent vector of the corresponding GM tract yields fiber direction strain values presented as local fiber direction lengthening and shortening. (A) Box and whisker plots: the horizontal line inside each box represents the median strain value; the upper and lower edges of each box itself represent upper and lower quartiles respectively (i.e., the 75th and 25th percentiles), and lines extending from each end of the box (whiskers) indicate the peak values of the fiber direction strains plotted. Left panel: strain errors. Right panel: strains along GM fibers due to imposed knee angle change. (B) Distribution of fiber direction strains of different nodes for the pooled data from all subjects.

Fig. 4 – Serial distribution of fiber direction strain. (A)-(E) The fiber direction strains are mapped on identified GM tracts separately for each subject. A cross-sectional slice within the distal half of the diffusion weighted image volume is used as a reference. Negative values show local shortening and positive values show local lengthening. Strain upper limits are not normalized to reflect individual serial distribution differences. However, to achieve a better presentation, peak values of local shortening and lengthening are excluded for subject C. These values are indicated separately in the figure.
subjects A, B and D, superficial tracts (corresponding to more distal muscle fibers) show a negative mean strain, whereas positive values are calculated for deeper tracts. A gradient of mean fiber direction strains also exists in subjects C and E. However, this shows a more complex characteristic indicating inter-individual differences.

Deep GM aponeurosis strains (mean $\pm$ SD of pooled data indicates 22.4 $\pm$ 13.5% and 17.2 $\pm$ 7.9% of peak local lengthening and shortening, respectively) show sizable inhomogeneity (Fig. 8). Note that for about a fourth of the aponeurosis surface, was the deformation aligned with the Achilles tendon (6.7% and 20% of the voxels showed lengthening and shortening, respectively in that direction).

5. Discussion

5.1. Methodology developed

Methods for quantifying muscle tissue deformation in the context of muscle architecture are lacking: (i) ultrasound studies are limited to either two dimensional deformation analyses (Bojsen-Moller et al., 2010) or three dimensional structural analyses (Rana et al., 2013). (ii) MRI analyses in a single slice (Fiorentino et al., 2012) or a three dimensional volume (Huijing et al., 2011; Pamuk and Yucesoy, 2015; Yaman et al., 2013), yield a good representation of muscle tissue deformation, however physiologically relevant fiber direction length changes remain unknown. (iii) Numerous studies utilizing DTI and tractography provide anatomical information for human muscle fibers in vivo (Bolsterlee et al., 2015; Froeling et al., 2012; Heemskerk et al., 2010; Sinha et al., 2011), but they provide no information regarding deformation. Among the limited number of studies combining DTI and deformation analyses, Englund et al. (2011) analyzed contraction-induced deformation within muscle volume. However, without doing fiber tractography, they made a voxel-wise comparison of the peak principal strain’s direction with the principal diffusion direction and showed no consistent match. Felton et al. (2008) did perform tractography and aligned the strain-rate tensor obtained during muscle activity with the resulting fiber tracts. However, imaging was confined to only two slices, thus not informative for the general muscle volume.

We have combined deformation analyses in three-dimensional muscle volume with DTI and tractography. This allows localizing muscle fascicles and mapping fiber direction deformations along them. This is a major step for
understanding muscle mechanics and physiology. Pennation angle of tracked fibers and standard deviation of pennation angles agree with previous reports (Chow et al., 2000; Lansdown et al., 2007) indicating anatomical representativeness of the method developed. Calculation of tissue deformations relies on Demons algorithm which, has been utilized and validated for calculating deformations in various tissues including pelvic floor (Wang et al., 2005), lung (Latifi et al., 2013) myocardium (Gao et al., 2014) and cortical bone (Christen et al., 2012). Deformation of human lower leg

Fig. 7 – Parallel distribution of fiber direction strain among different GM fiber tracts per subject. (A)-(E) Mean fiber direction strains of different tracts are mapped on a reference cross-sectional slice separately for each subject. The reference slice is chosen so as to maximize the fiber representation within the muscle cross section. Negative values represent shortening and positive values represent lengthening. Strain limits are not normalized to reflect individual parallel distribution differences.

Fig. 8 – Distribution of deep GM aponeurosis strains per subject. (A–E) The deformation fields are visualized using glyphs. Each glyph represents deformation of a voxel. The direction of each glyph is determined by the corresponding eigenvector. The length of each glyph is proportional to the size of local aponeurosis tissue lengthening or shortening. A cross-sectional slice within the distal half of the diffusion weighted image volume is used as a reference. Strain upper limits are not normalized to reflect individual serial distribution differences.
musculature was also assessed (Huijing et al., 2011; Yaman et al., 2013). A rigid body motion test was performed by imposing synthetic motion on image sets, which represents a much larger scale motion than possible subject movement during imaging. This should theoretically cause zero strain and indeed yielded very small error strains also presently. Yaman et al. (2013) conducted further critical testing of the reliability of Demons algorithm by imposing known deformations on the image sets. One key finding was that the strains calculated are conservative estimators of actual local deformations. To the best of our knowledge no data is available in the literature, which presents amplitudes of strain occurring along human GM fibers upon passive knee extension in vivo. Therefore, a direct comparison of the strains shown presently is not possible. Using ultrasonography in passive state, De Monte et al. (2006) measured the changes in GM fascicle lengths. Their data depicted at knee angles of 144° and 174° indicates that imposed knee movement at constant ankle position causes approximately 18% fascicle strain. Our findings show for similar test conditions that strain along the GM tracts will vary from approximately 8% shortening to 9% lengthening. Although the order of magnitude of strain values agrees, we don’t know the overall fascicle length change. Yet, we know from a combined cadaver and MRI assessment that global muscle length changes on imposed joint movement can be quite different from local ones (Huijing et al., 2011). Therefore, a match between fascicle length changes and local fiber direction strains is unlikely. On the other hand, for a comparable knee angle change imposed, Yaman et al. (2013) assessed principal strains within the lower leg muscles including m. gastrocnemius. Mean of peak local lengthening and shortening that they reported (10.3 ± 1% and 9.7 ± 1%, respectively) were indeed comparable to the present strain amplitudes. Note however that those peak strains were higher than the present fiber direction strains. This agrees with the conclusion of Zhong et al. (2008) and Englund et al. (2011) that the peak length changes within a muscle occur not necessarily along the muscle fibers. New studies are indicated to achieve a good understanding of gross muscle and fascicle length changes vs. local fiber direction strains as this has high functional relevance. The mechanism providing a biomechanical explanation to the heterogeneous nature of local strains shown presently is addressed below.

Overall, Demons algorithm is highly suitable for assessing muscle tissue deformations within a three dimensional volume with fairly good resolution. Note that no preprocessing was performed on the MRI data prior to the deformation analyses: (i) avoiding resolution matching between the MR and DT images prevents the data from plausible non-physiological outcome of related interpolations. (ii) Aligning MR image coordinates rigidly with DT image coordinates avoids synthetic deformation. The deformation vectors obtained were mapped to the correct coordinate system representing the deformed state fibers. In sum, we demonstrate a novel and reliable tool capable of assessing human muscle function in vivo, by utilizing physiologically relevant fiber direction strains.

5.1.1. Explanations for inhomogeneity of muscle fiber direction strains

A major finding is inhomogeneity of muscle fiber direction strains. This involves a general fiber direction strain pattern showing lengthened proximal and shortened distal track segments. Hence local lengthening can occur simultaneously with local shortening within different parts of the same tracts. The repeatability test conducted confirmed consistency of such general deformation pattern. Strain artifacts cannot explain this because the amplitudes of muscle fiber direction strain distribution are much higher. Intersarcomere dynamics (Julian and Morgan, 1979), or instability theories (Allinger et al., 1996; Zahalak, 1997) also cannot explain such inhomogeneity because of the inactive state of present testing. Note that earlier modeling (Blemker and Delp, 2005; Fernandez et al., 2005; Yucesoy et al., 2003) and MR imaging analyses (Zhong et al., 2008) did show inhomogeneity of strain within the muscle. Blemker et al. (2005) developed a model which incorporates skeletal muscle mechanical properties and the structural properties of biceps brachii muscle to assess the causes of non-uniform strains reported earlier in a dynamic imaging study by Pappas et al. (2002). They attributed distribution of length changes along the fascicles primarily to muscle architecture including fascicle length and curvature variations within the muscle. The authors also reported major along-fiber shear strains and indicated this as a factor affecting the potential for muscle fibers to transmit force laterally via intramuscular connective tissues. Particularly considering force transmission in series-fibered muscle, the concept of shear linkage between adjacent structures by intramuscular connective tissues was introduced (Purslow, 2002; Purslow and Trotter, 1994; Trotter and Purslow, 1992; Trotter et al., 1995). Our present findings also show substantial along-fiber shear strains representing shearing between GM fascicles. As muscle fibers are not directly connected to each other, such shearing presents evidence for major mechanical interaction occurring between the muscle fibers and the extracellular matrix (ECM). This mediates interaction between different muscle fibers and comprises a mechanism for muscle architecture to affect deformation along muscle fibers. Hence, muscle fiber-ECM mechanical interactions are central to the biomechanical explanation of the present findings. In general, deformation along any member is determined by the force equilibrium. Muscle fibers are widely considered as members under uniaxial loading because they are regarded as mechanically constrained only at their ends, where the myotendinous junctions are (Tidball, 1991). For such a member, the deformation should be uniform along its length, but the present findings do not confirm this. However, muscle fibers and the ECM have multi-molecular connections along the full peripheral length of the muscle fiber (Berthier and Blaineau, 1997). Therefore, additional to the forces acting on a muscle fiber at the myotendinous junctions, loads can act also along its full length. Accordingly, considering muscle fiber as a member under uniaxial loading is mechanically incomplete. In an earlier experiment, Street (1983) freed a muscle fiber from its surrounding fibers only in the middle and assessed its length changes on imposed passive stretch. This yielded different sarcomere lengths in the exposed fiber, indicating...
inhomogeneous deformation. This mechanism has been referred to as myofascial force transmission (Huijing, 1999), and the loads along the muscle fiber originating from the ECM and the neighboring muscle fibers have been referred to as myofascial loads (Yucesoy, 2010). Huijing et al. (1998) and Jaspers et al. (2001) showed that a discontinuity in the ECM affects functioning of muscle fibers differentially within the muscle and causes muscle force to change. The mechanism of effects of muscle fiber-ECM mechanical interactions has been studied extensively using finite element modeling, which confirmed theoretically that myofascial loads can cause inhomogeneity of fiber direction strains with major implications for muscle force production (Yucesoy and Huijing, 2012; Yucesoy et al., 2002) and outcome of treatments (Turkoglu and Yucesoy, 2016; Yucesoy and Huijing, 2009; Yucesoy et al., 2007, 2015). The present data show valuable evidence for this mechanism for human muscles in vivo.

Note the analogy between a muscle fiber and the whole muscle. Although myotendinous junctions are essential for bodily movement, they are not the exclusive sites for mechanical interaction between the muscle belly and its surroundings. Instead, direct collagenous linkages exist between the epimysia of adjacent muscles. Additionally, collagen reinforced neurovascular tracts provide indirect intermuscular connections between distant muscles (e.g., Yucesoy and Huijing, 2007). These connections are in continuity with other connective tissues such as intermuscular septum, interosseal membrane, and compartmental fascia and show complex mechanics stemming from nonlinear, inhomogeneous material properties and pre-strain (Yucesoy, 2010). Joint movement causes relative position of the muscle belly to change leading to stretching of those tissues connecting it to its surroundings (Maas et al., 2003). Consequently, epimuscular myofascial loads of varying magnitudes and directions can act along the muscle belly and further affect muscle’s mechanics via muscle fiber-ECM mechanical interactions (Yucesoy, 2010).

The present experiment involves knee extension. This means that the proximal end of the GM is stretched in the proximal direction because it spans the knee joint. Fascicle lengthening in the proximal GM regions agrees with the imposed muscle stretch by the tendon. However, variation of that along those fascicles and the shortening occurring in the distal GM regions cannot be explained solely by the imposed muscle stretch. Instead, the findings indicate the presence of distally directed epimuscular myofascial loads acting along the fascicles. Note that the rest of the lower leg muscles are mono-articular, spanning only the ankle joint. Therefore, on imposed knee extension the GM position relative to those muscles is changed. Hence, not only the muscle, but also its epimuscular connections are proximally stretched causing distally directed epimuscular myofascial loads to act on the GM belly. These loads affect mechanical equilibrium locally inside the muscle, which explains the diminished elongation in the proximal regions of GM fascicles towards the mid-GM belly and the compression in the distal regions. This is also reflected on the distribution of deep GM aponeurosis strains. However, inter-subject variability exists indicating that the magnitudes and directions of those loads should be more complex, ascribed to complex mechanical properties of the epimuscular connections. Flausibly, stiffer collagenous linkages between the GM and SOL muscles could contribute to the complexity in strain distributions, anchoring fascicle parts in more distal GM regions.

Bojsen-Moller et al. (2010) showed displacement gradients in the GM and SOL muscles upon passive knee extension, capable of imposing differential myofascial loading. Others presented heterogeneous local deformations (Huijing et al., 2011) and fascicle length changes (Tian et al., 2012) within the SOL despite being in global isometric condition ascribable to myofascial interaction mechanism. The contribution of the shared distal tendon providing mechanical coupling of GM and SOL muscles cannot be overlooked (Maas and Sandercock, 2008; Tian et al., 2012). However, intermuscular mechanical interaction via the shared tendon cannot be the dominant mechanism for the present findings since the length changes reported by e.g., Tian et al. (2012) for the Achilles tendon are much smaller than those shown locally within the muscle. Besides, interaction via the Achilles tendon is unlikely to lead to sizable fascicle shortening in passive test conditions. The present imaging did not involve the tendons. Hence, no deformation data is available for the Achilles tendon. However, the deep GM aponeurosis strains are heterogeneous over its surface. This supports the previously shown aponeurosis strain distributions (Blemker et al., 2005; Muraoka et al., 2003; Shin et al., 2009) in a three dimensional analysis. Moreover, the direction of the peak deep GM aponeurosis strains is not necessarily aligned with the Achilles tendon. These findings indicate that the myotendinous structures play a highly complex role in muscle functioning, which involves not only the transmission of muscle force to the tendon but also sustaining the mechanical equilibrium within the muscle by baring loads in various directions and amplitudes.

5.2. Limitations and implications

Tracts obtained are representative of muscle fascicles and not individual muscle fibers. This is bound by the resolution of the state of the art DTI acquisition methods for large enough muscle volumes. Wu et al. (2013) reached a finer, 0.125 mm isometric resolution for DTI acquisition. However, they worked on rat brain, which requires a much smaller field of view compared to human muscles. On the other hand, using a comparable field of view at the cost of assessing a local small muscle volume only, would still be insufficient for resolving individual sarcomeres. Therefore, the present methodology provides a superb tool for assessing physiologically relevant human muscle tissue deformations in vivo, but calculates length changes of only large groups of sarcomeres within a volume. Consequently, possible distribution of sarcomere length changes along several segments of fascicles can be determined objectively. Accordingly, the strain values obtained represent averages of those of numerous sarcomeres within a voxel. Hence, deformation heterogeneity along the tracked fascicles can be considered to underestimate the underlying heterogeneity at sarcomere level.

The present study shows serial and parallel distribution of strain along the GM fibers, which, bound to the limitations addressed may represent serial and parallel heterogeneity of
sarcomere lengths. Serial sarcomere length distributions were correlated to muscle’s length range of force exertion (Morgan et al., 2000; Wohlfart et al., 1977). Moreover, a shift of muscle optimum length to a longer length was shown with increasing parallel heterogeneity (Willems and Huijing, 1994). Previous modeling showed mechanism of that in healthy (e.g., Yucesoy and Huijing, 2012; Yucesoy et al., 2003) and treated muscle (Yucesoy and Huijing, 2009; Yucesoy et al., 2007), and how that relates to muscle length range of force exertion. Therefore, the metrics our methodology provides are relevant for assessing changes in joint range of motion of human subjects. This is a central parameter for human mobility, as decreased joint mobility is a common problem in e.g., cerebral palsy patients (Matthiasdottir et al., 2014). Pre- vs. post-treatment comparison of such patients using the present technique can help assess the outcomes of common interventions (e.g., aponeurotomy, botulinum toxin injections) objectively.

The present study was performed in passive conditions. However, the methods described here can also be applied to conditions involving submaximal effort. These conditions can be used to simulate postural demands of daily tasks such as driving, key pressing, cycling. Human muscle function can be assessed with potential implications also forergonomics. The acquisition time may limit the effort level, and lack of joint movement during acquisition could be considered a limitation. Yet, a single joint angle manipulation in active or passive state suffices for the assessment of targeted deformation along the fiber direction within a three-dimensional muscle volume. Other imaging methods with repetitive joint motion requirements could present a problem particularly for patient groups with movement disorders, such as cerebral palsy patients. However, an appropriate use of the present methodology can allow for a major improvement in our understanding of the condition of these patients’ muscles.

In conclusion, using advanced MRI-DTI techniques combined, a methodology was developed which yields physiologically relevant muscle fiber direction deformation information. Effects of knee movement assessed indicate major strain distribution along human GM muscle fibers.

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References


Pamuk, U., Yucesoy, C.A., 2015. MRI analyses show that kinesio taping affects much more than just the targeted superficial tissues and causes heterogeneous deformations within the whole limb. J Biomech. 48, 4262–4270.


