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Repeatability of DTI-based skeletal muscle fiber tracking

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Abstract

Diffusion tensor imaging (DTI)-based muscle fiber tracking enables the measurement of muscle architectural parameters, such as pennation angle (θ) and fiber tract length (L_{ft}), throughout the entire muscle. Little is known, however, about the repeatability of either the muscle architectural measures or the underlying diffusion measures. Therefore, the goal of this study was to investigate the repeatability of DTI fiber tracking-based measurements and θ and L_{ft} . Four DTI acquisitions were performed on two days that allowed for between acquisition, within day, and between day analyses. The eigenvalues and fractional anisotropy were calculated at the maximum cross-sectional area of, and fiber tracking was performed in, the tibialis anterior muscle of nine healthy subjects. The between acquisitions condition had the highest repeatability for the DTI indices and the architectural parameters. The overall inter class correlation coefficients (ICC's) were greater than 0.6 for both θ and L_{ft} and the repeatability coefficients were $\theta < 10.2^\circ$ and $L_{ft} < 50$ mm. In conclusion, under the experimental and data analysis conditions used, the repeatability of the diffusion measures is very good and repeatability of the architectural measurements is acceptable. Therefore, this study demonstrates the feasibility for longitudinal studies of alterations in muscle architecture using DTI-based fiber tracking, under similar noise conditions and with similar diffusion characteristics.

Keywords

tractography; ICC; pennation angle; fiber length

INTRODUCTION

Diffusion tensor imaging (DTI)-based fiber tracking is extensively used to reconstruct the trajectories of white matter tracts (1) and in recent years, of skeletal muscle fibers as well (2–6). DTI-based muscle fiber tracking is possible due to the anisotropic diffusion of water within muscle tissue. The direction of greatest water diffusion can be obtained by measuring the diffusion coefficient in at least six directions and estimating a diffusion tensor. The resulting principle eigenvector has been shown to be aligned with the longitudinal direction of the muscle fiber (7,8). Reconstruction of fiber tracts is accomplished by combining the principal eigenvector information for consecutive voxels. Previously, we have described a DTI fiber tracking procedure in which fibers are tracked from seed points along the aponeurosis (the tissue of muscle fiber insertion) to the muscle border (5). This method enables the reconstruction of the muscle architecture, which is an important determinant of the force and velocity potentials of a muscle (9–11). The architecture can be characterized by the fiber length (L_f), physiological cross-sectional area, and pennation angle (θ), where θ is the angle at which fibers insert into the aponeurosis or tendon.

The trajectory of the fiber tracts depends on the underlying anatomy, but also on the noise, image acquisition characteristics, artifact characteristics of the data, and the fiber tracking algorithm (12–16). The estimated eigenvalues, eigenvectors, and fractional anisotropy values are biased by a low signal to noise ratio (SNR) of the diffusion data (12,13,16). When muscle DTI data are acquired at SNR <60, there are b-value-dependent errors in estimating the tensor and its derived indices and the estimated principal eigenvector differs from the true direction (16). The gradient scheme used influences the accuracy of the DTI estimates, with evenly spatially distributed gradient sampling and a higher number of gradient directions both increasing the accuracy of the estimated direction of the eigenvectors (15). Partial volume effects diminish the accurate estimation of the DTI indices: neuronal fibers can kiss or cross and consequently average out (1), while adipose tissue infiltrates in muscle introduce a volume fraction with randomly distributed eigenvectors, lower true diffusivity, and lower true anisotropy. The effect of variation in the underlying DTI data on fiber tracking also has been the focus of several studies (17–20) by using computational methods with different noise levels, determining repeatability of mean DTI indices within the tracts, or repeated definition of the seed points. Ding *et al.* (21) developed a method to bundle fiber tracts based on similarities between tracts and illustrated the reliability of the algorithms used.

These studies provide great insight into the sensitivity to image acquisition and the repeatability of the DTI-indices. However, with respect to repeatability of fiber tracts there are still some unresolved issues. Among these are that while simulations provide a good starting point for understanding the effects of noise, their applicability is limited to the modeling assumptions; therefore, they should not replace experimental studies. In addition, noise sensitivity and reproducibility analyses are different questions, as reproducibility analysis involves questions not just of noise sensitivity but also normal physiologic variation, subject motion, et cetera that are not present in noise sensitivity analyses. Finally, to our knowledge, no previous study has reported in a systematic manner on the reproducibility of fiber tracts in any tissue (as opposed to only the DTI indices themselves).

The many additional steps required in a fiber tracking application merit further investigation with regard to reproducibility.

In addition, little is known about the reproducibility of DTI in skeletal muscle, for either the DTI indices or the fiber tracks. It can be expected that the repeatability of skeletal muscle DTI differs from that in brain. Skeletal muscle DTI presents unique challenges with regard to quality of shimming, low SNR, and higher SNR requirements brought about by factors such as low diffusion anisotropy. Knowing the amount of normal experimental and physiological variation will provide meaning to application studies, as it provides a framework to which future publications should be evaluated.

Biologically relevant physiological changes are relatively small in both the muscle architectural measures and the underlying diffusion measures. The DTI indices have shown to vary by a few percent in healthy muscle with gender (22), age (23), or muscle length (24). A higher relative change, on the order of 10–20%, in one or more of the DTI indices has been measured with muscle damage or disease (25–30). For muscle architectural studies, the alterations in pennation angles can be small. A transient increase of 1.5° in θ was measured immediately after exercise until exhaustion (31), whereas bed rest for 5 weeks caused a $2\text{--}4^\circ$ decrease in θ (32). In addition, elite sprinters showed a $2\text{--}5^\circ$ smaller θ than distance runners (33). In the tibialis anterior (TA) muscle, the pennation angle changes by 2° over a foot rotation of 15° (34).

Therefore, the goal of this study was to investigate the repeatability of DTI-based fiber tracking in skeletal muscle, including both the underlying DTI indices and the calculated muscle architectural parameters [θ and fiber tract length (L_{ft})]. A total of four DTI acquisitions were performed on two days that allowed for between acquisitions (identical position), within day (after repositioning), and between days analyses. We found that the between acquisitions had the highest repeatability for the DTI indices and the architectural parameters and that the overall repeatability of the method is sufficient to detect changes in muscle architecture.

METHODS

Experimental protocol

Four sets of measurements were obtained with the foot in 0 degrees of plantar flexion (the anatomical position). These measurements were: 1) a baseline measurement, 2) a measurement immediately following the baseline measurement without repositioning, 3) a measurement after complete repositioning, in which the subject stepped from the table and coils were removed, and 4) a measurement on another day. Measurements 1, 2, and 3 were performed at the same day. These three measurements were on either the first scan day ($n=5$) or the second scan day ($n=4$). The second scan day occurred, on average, 12 days later and in most cases ($n=8$) at the same time of day. The order of all the scans was assigned randomly. We performed both anatomical scans and DTI scans for measurements 1, 3, and 4, whereas for measurement 2 only the DTI scans were reacquired.

The four measurements enabled the analysis of the following conditions: between acquisitions, within day, and between days. The between acquisitions (BA) analysis was performed with measurements 1 and 2 and indicates the influence of acquisition noise and image registration. The within day (WD) analysis was performed with measurements 1 and 3 and indicates the influence of acquisition noise, positioning, and image analysis procedures (image registration and manual selection of muscle border and aponeurosis). The between days (BD) analysis was performed with measurements 1 and 4 and indicates the influence of acquisition noise, positioning, image analysis procedures and day to day variation.

Subjects

Nine healthy subjects (five male) participated in this study. Their average age ($\pm$ SD) was 29 ± 7 years, with height 172 ± 12 cm and mass 69 ± 23 kg. The subjects lay supine with the right foot strapped into a custom-built exerciser. Subjects were asked not to perform strenuous exercise during the preceding 24 h before participation and not to drink alcohol and caffeine the preceding 6 h. In addition to the measurements described in this paper, data were also acquired with the foot rotated as published previously (35). All procedures were approved by the Vanderbilt University Institutional Review Board, and written consent of the risk, benefits, and procedures was obtained from each subject prior to participation.

MRI

Data were obtained with a Philips 3 T scanner (Philips Medical Systems, Best, The Netherlands) using two double flexible surface coils (14×17 cm each) covering the length of the tibialis anterior (TA) muscle.

The acquisition was performed as described previously (35). For anatomical reference a proton density (PD) weighted scan was obtained using field of view (FOV) = 196×196 mm², matrix size = 256×128 with 512×512 reconstruction, slice thickness = 6 mm, 55 slices, repetition time (TR) = 4152 ms, echo time (TE) = 11 ms, number of excitations (N_{EX}) = 1. DTI images were acquired in 5 continuous stacks of 11 slices each with the center position aligned with the anatomical images. The center of each stack was located at the magnet isocenter and a sensitivity encoding (SENSE) reference scan was obtained for each stack. Scans were acquired using an EPI sequence with FOV = 192×192 mm², matrix size = 96×64 with 128×128 reconstruction, slice thickness = 6 mm, TR = 3300 ms, TE = 48 ms, N_{EX} = 4, and SENSE factor = 1.2. Fat suppression was performed using spectrally selective adiabatic inversion recovery (SPAIR), with a pulse 160 ms before excitation and a spectral width of 200 Hz shimmed over the acquired image volume. The fat-shift direction was set so that residual fat signals were shifted by 20 pixels to outside of the fiber tracking region. Diffusion weighted (Dw) images were acquired with $b = 500$ s/mm² and in ten directions (36). The total scan time for all stacks was 15 minutes. After the DTI a T_2 weighted scan was acquired for registration purposes with the same geometric parameters as the PD scan and TR = 7557 ms, TE = 30 ms, N_{EX} = 1, and SPAIR based fat saturation.

Image registration

Image registration was performed as described previously (35) and in short consisted of registration in four steps: 1) within each stack, Dw images to the $b = 0$ images using affine registration; 2) DTI stacks to each other using rigid registration; 3) T₂w to PD using rigid registration; 4) DTI to registered T₂w using rigid registration. All transformation matrices were concatenated to transform the DTI images, preventing multiple interpolation steps. The diffusion gradient directions were corrected for the combined image transformations and the tensor calculations were performed.

Fiber tracking

Fiber tracking was performed as described previously (5,35). The borders of the TA muscle were traced from the anatomical PD images. The positions of both the superficial and deep aspects of the central aponeurosis were manually digitized. For each side, the average positions were determined using linear filtering. 3D mesh reconstructions were defined with 200 rows $\times$ 100 columns density and the points of intersection were used as seed points. Fiber tracking was performed by following the direction of greatest diffusion from the seed points. Fibers were terminated at the muscle borders, if fractional anisotropy (FA) < 0.15 or FA > 0.75 , or if successive points had a curvature of $> 45^\circ$. These settings have shown to provide suitable stop criteria for the TA muscle (35).

After the fiber tracking, a quantitative assessment of the fiber tracts was performed to exclude erroneous fiber tracking results (35). This method is based on expected patterns of muscle fiber geometry and rejects short fibers, fibers that cross over to the other compartment, prematurely stopping fibers, and fibers that differ more than 4.5 mm in length from their neighbors.

Architectural parameters

Each fiber tract contains a seed point and a series of points corresponding to each fiber tracing step. Position vectors were formed between the seed point and each of its first five points on the tract. For each point, the angle between the position vector and the plane tangent to the seed point was calculated (5). The θ was calculated as the mean of these 5 points and L_{ft} was calculated as the sum of the distance between consecutive fitted points along fiber tract.

Data analysis

The mean values of the DTI indices were calculated at the maximal cross-sectional area of the muscle. The defined muscle border was eroded by 2 pixels to account for partial volume effects and used as the ROI.

To enable statistical analysis of the data, mean values were calculated for 18 evenly spaced segments along the aponeurosis (6 rows height and 3 columns width), as depicted in Figure 1. This also corrects for small variations in digitizing the aponeurosis between multiple measurements. In addition, reporting the repeatability in multiple segments of the aponeurosis is beneficial for future studies investigating spatial heterogeneity in muscle architecture. The height of the rows for each aponeurosis was set as follows. The z-distance

between the most distally and most proximally traced fibers of the deep compartment was determined; this compartment extends further distally than the superficial compartment. The z-distance was divided into six equal segments. The width of the columns was calculated by determining the maximal width of the reconstructed aponeurosis for each mesh row and dividing it by 3. The center of the middle row always aligned with the middle of the aponeurosis. From this center point the segments were calculated by the column width or until the edge of the mesh (see Fig. 1). Therefore, segments do not contain the same number of seed points and distally there are fewer segments. For each segment containing more than 5 fiber tracts, the median value for θ or L_{ft} was calculated.

Statistics

Data outliers were removed where the difference between two measurements was greater than two times the SD of the differences among the nine subjects. This was performed for each segment and each compartment, for both θ and L_{ft} . This resulted in the removal of 20–28 data points from a total of 540 points (15 segments $\times$ 9 subjects $\times$ 4 measurements).

The following analyses were made for θ and L_{ft} for the following conditions: between acquisitions (BA; identical position), within day (WD; after repositioning), and between days (BD). Bland-Altman plots were made by plotting the difference between pairs of measurements against their mean value; these would reveal a possible relationship between the measurement error and the true value (37). The repeatability coefficient (RC) was calculated according to Bland and Altman (38). The RC represents the maximum difference between measurements that is likely due to measurement error and therefore a low RC is desired. For the same conditions and all four measurements, the inter-class correlation coefficient (ICC) was calculated; an ICC of 1 indicates perfect agreement. Finally, one-way analysis of variance (ANOVA) with a Bonferroni *post-hoc* test was performed to test for significant differences between RCs or ICCs among the different conditions. The coefficient of variation (CV) was determined for each subject for all four measurements. Statistical significance was set to $p < 0.05$.

RESULTS

The ICC's between all four measurements of the selected muscle volume (0.994), muscle length (0.966) and maximal cross sectional area (0.961) were high, indicating reliable selection of the muscle border. The average DTI indices did not differ significantly for any of the measurements (Table 1). The ICC values and RC were significantly different between the three conditions using a one-way ANOVA (ICC: $p = 0.014$, RC: $p = 0.009$). However, using the Bonferroni test, no significant differences were present.

Sample fiber tracking data are shown in Figure 2. The distribution of θ along the aponeurosis is depicted in Figure 3 for the two different scan days. A high similarity in both values and typical patterns were present between the two days, including the pattern in the posterior proximal part of the aponeurosis. Figure 4 shows the mean θ and L_{ft} values of all subjects for the 18 segments. Figure 4 also shows the mean values per aponeurosis row (foot–head direction) and column (postero lateral–anteromedial direction).

The Bland-Altman plots revealed that there were no systematic errors and that the data were distributed around zero (Fig. 5). The limits of agreement differed per segment, with higher values for the more proximal segments. The RCs were between 4.6° and 10.2° and between 17 mm and 50 mm for θ and L_{ft} , respectively (Fig. 6), with lower values for the more proximal segments (data not shown). The RC's were significantly different with condition ($p < 0.007$), with the BA condition being lowest. In addition, the deep compartment had slightly lower RC values (θ : $p = 0.009$, L_{ft} : $p = 0.011$).

Tables 2 and 3 show the ICCs for θ and L_{ft} , respectively. The mean of all segments gave high ICC values (>0.6) for all conditions. The ICCs for the separate segments were highest for the middle segments. The ICCs were significantly different with condition ($p < 0.031$), with the BA condition being highest. The Bonferroni test revealed that in all cases the ICC for the BA condition was significantly higher than the BD ($p < 0.024$) conditions. In addition, there was a difference between BA and WD for the superficial θ ($p = 0.002$) and the deep L_{ft} ($p = 0.019$). No significant differences for WD versus BD were found. Two way ANOVAs showed that the ICC's were similar between compartments for θ , while the L_{ft} ICC's for the deep compartment showed higher values ($p = 0.008$).

DISCUSSION

This paper is the first to describe the repeatability of DTI indices in skeletal muscle, a tissue in which DTI is very difficult to implement, and the first systematic repeatability study of DTI fiber tracking results in any tissue. In summary, four different measurements were performed, allowing us to analyze the repeatability between acquisitions, within a single imaging session and between days. As expected, the BA condition showed the highest repeatability for the DTI indices and the architectural parameters (θ and L_{ft}), followed by the WD and BD conditions. In the following discussion we will describe the possible sources of variation and their effects on the BA, WD and BD conditions. The RC and ICC values will be discussed in the light of expected muscular variabilities and compared to ultrasound (US). After that the repeatability within the different segments and variation along the whole aponeurosis is discussed. We will show that DTI and DTI-based fiber tracking, as we have implemented it here, is repeatable and able to quantify practically significant differences in muscle architecture and water diffusivity.

Variability in the DTI indices can be caused by acquisition-related noise and by true differences in muscle water diffusion or architecture. The three conditions (BA, WD and BD) all contain different acquisition-related noise contributions from thermal sources (i.e. incoherent noise related to the subject or coils), system (such as coil positioning, B_0 drift, gradients, or shims), and physiological (motion, the cardiac and respiratory cycles, subject positioning, and temperature). With regard to thermal noise contributions, these are not expected to vary systematically between the BA, WD, and BD conditions. Also, it is noteworthy that the $b = 0$ SNR levels [measured according to Dietrich *et al.* (39)] were 109 and 147 in the superficial and deep compartments, respectively. These values were greater than the SNR level required for 1% accuracy in the eigenvalues and FA in 27-voxel regions of interest (16). The ROIs used in the present study contained ~ 42 voxels and so are expected to be even less noise sensitive than a 27 voxel ROI. The SNR levels for $b = 500$

with the diffusion gradients parallel to the fiber direction, so obtaining the largest signal attenuation, were 70 and 56 in the superficial and deep compartments, respectively. These SNR levels are well above the noise floor which occurs at an SNR lower than 20 (16). In addition, these SNR levels also imply an angular uncertainty in the principal eigenvector of less than $\pm 5^\circ$ in a single voxel (16). Compared to the BA condition, system and physiological noise contribution can reasonably be expected to increase with the WD and BD conditions. In particular, there might be architectural variations related to small differences in joint angle upon repositioning that would affect these conditions but not the BA condition. Next to these acquisition-related factors, there is the potential influence of the data processing factors of manual selection of the muscle borders and aponeurosis. Because we used common muscle mask and aponeurosis definitions for the BA analysis, those factors did not influence the BA data but would influence the WD and BW data to similar extents. Therefore, with all factors combined it is expected that the BA data would have the smallest amount of variation and lowest repeatability coefficient, followed by the WD and BD conditions. This is indeed present in the Bland-Altman plots, the RC calculations, and the ICCs: the BA showed lowest RC's and higher ICCs, whereas the WD and BD values slightly differ (Tables 2 and 3 and Figs. 5 and 6). Although it is not surprising that the BA condition showed the best results among the conditions, it is important to know the extent of the variations before performing application studies.

Therefore, the RC and ICC values must be interpreted within the context of their normal and pathological variabilities. Variations in DTI indices, thought to be due to micro-structural changes, can range from 5–10% in healthy muscle (22–24) and 10–20% in damaged or diseased muscle (25–30). In this study, we calculated RCs of less than 12% of the mean for all the DTI indices, showing that the expected difference due to measurement error is comparable to or less than the expected changes due to alterations in the microstructure. This is an acceptable repeatability level for studies aiming to detect a difference between two typical experimental conditions. The ICCs that we observed for muscle were slightly lower than findings in a brain DTI study (20). The ICCs are highest for BA data ($p < 0.05$) and reduced for WD and BD, and fall above the minimum ICC of 0.4 that suggest fair to good agreement or above 0.75 that is considered excellent agreement (40). The small dynamic range of the DTI indices in this study of resting muscle is a likely cause of the relatively low ICCs. Because the underlying measuring scale influences ICC one should not compare ICC's of different sources (e.g. ADC vs FA or ADC vs θ) (41).

We also assessed the repeatability of the architectural parameters θ and L_{ft} determined by our DTI-based muscle fiber tracking procedures. In order to gain insight into the amount of architectural variation to expect, we reviewed the results of brightness-mode US studies of muscle architecture in vivo. These studies have revealed that both transient and chronic factors can alter muscle architecture. Transient factors such as muscle contraction or joint rotation cause θ to change by $1.5\text{--}5^\circ$ (31,34), while chronic factors such as disease, training or bed rest result in $2\text{--}5^\circ$ change in θ and >4 mm in fiber length (32,33). Therefore, techniques to measure architecture should be able to distinguish θ changes of 1.5° and fiber length changes of >4 mm.

We observed maximum mean RC values for θ of 6.7°, 8.3°, and 10.2° for the BA, WD, and BD conditions, respectively; for L_{ft} , we observed RC values of 24, 29, and 50 mm, respectively. The values tended to be lower in the proximal and middle portions of the muscle, which represent the majority of the muscle's volume. The most likely explanation for this is that these segments are less affected by partial volume artifacts in the muscle with bone, subcutaneous fat tissue, and other muscles. The ICC's were on the order of 0.4–0.9. It is noteworthy that these values are the within-segment values, and so are also affected by relatively small dynamic ranges.

The repeatability depends on the imaging protocol, noise and artifact characteristics, and the fiber tracking algorithm. It is expected that better SNR and/or more diffusion directions will improve the repeatability as long as imaging time does not increase considerably.

Susceptibility induced signal voids could be present in a small portion of the muscle on the anterior side near the proximal tibia; these voids could have affected the fiber trajectories. We did not determine the full extent or origin of the artifacts; however, the quantitative assessment of fiber tracts did remove those incorrect fiber tracts that likely resulted from artifacts and noise-related factors. The contributions of these artifacts to the resulting fiber tract depend on the FA and curvature thresholds, with stringent thresholds resulting in early termination and loose thresholds resulting in incorrect pathways (35,42). Although incorrect tracts are removed, it can be expected that the repeatability will be altered when FA or curvature thresholds other than those in this study are used. This is particularly true for L_{ft} , which is determined by the whole tract and is therefore subject to greater error propagation than θ , which is determined from the first five points along the tract. More advanced fiber tracking algorithms, such as nearest neighbor interpolation or probabilistic methods, might be more robust with regard to local invalid data; therefore improved fiber tracking results and repeatability might result. Further research is needed to assess the contributions of tracking algorithms to the repeatability and if necessary, develop improved protocols.

Few US-based muscle architecture studies have assessed repeatability; indeed, we were only able to find a single study (43). These authors reported a coefficient of variation of 9.8% for θ , corresponding to a within-subject SD (S_w) of 2°, and a fascicle length CV of 5.9% (S_w : 4 mm). By definition, RC is greater than CV: $RC = 2.77 \times S_w$ and $CV = S_w / \text{mean} \times 100\%$. The exact relationship between RC and CV will vary according to the mean ($RC = 0.0277 \times \text{mean} \times CV$), resulting in RC's for the US-study of 7.6° and 10.6 mm for θ and L_{ft} , respectively. Our study shows lower RC values for θ , especially in the area typically used for US: at the maximal CSA of the muscle we observed RC values of 4.5° and 25 mm for θ and L_{ft} , respectively. With regard to the RC of the L_{ft} it should be noted that the determination of L_{ft} occurs in a discrete, not continuous, fashion; one tracking step-size difference in L_{ft} results in an RC of 12.7 mm ($2 \times \text{step size}$). This could most easily be improved with a reduced slice thickness. In addition, the RC is the maximum difference between repeated measurements for a single subject; therefore, an RC higher than the expected changes in architecture can be acceptable as group statistics are generally used. We conclude that DTI-based muscle fiber tracking offers acceptable repeatability for muscle architecture studies.

In addition, the use of DTI has allowed us to reproducibly detect spatial characteristics of the data within muscle. Comparing the two compartments, we found that θ is higher in the superficial than in the deep compartment. There were opposite patterns in the L_{ft} : this variable was greater in the deep compartment than in the superficial compartment and increased in the foot-to-head direction. In the anterior-to-posterior direction, L_{ft} tended to decrease, in the deep compartment; the opposite trend was noted for θ for the superficial compartment. These patterns were highly reproducible. In addition, certain unique structures were present for all datasets. The right corner structure in Figure 3 is present in all four datasets and is likely related to a local inflection in the aponeurosis. All nine subjects had such a minor distribution characteristic present in their datasets. Therefore, in addition to the RC and ICC values, the systematic within-muscle spatial patterns of θ and L_{ft} also highlight the repeatable nature of these data.

CONCLUSIONS

Under these experimental and data acquisition conditions, the repeatability of the data acquisition is very good and repeatability of fiber tracking based architectural measurements is acceptable. We anticipate that similar repeatability characteristics would be observed in other muscles when similar noise characteristics are present. This is very promising for longitudinal studies on muscle and also provides an indication for the repeatability of DTI-based fiber tracking in other tissues, in which comparisons between repeated measurements are difficult to quantify. Therefore, this study provides a framework to which future publications should be evaluated.

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Abbreviations used

BA	between acquisitions
BD	between days
CV	coefficient of variation
DTI	diffusion tensor imaging
FA	fractional anisotropy
ICC	inter class coefficient
L_f	fiber length
L_{ft}	fiber tract length

RC	repeatability coefficient
SNR	signal to noise
TA	tibialis anterior
US	ultrasound
WD	within day
θ	pennation angle

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Figure 1.
Example of the segment selection. The white lines indicate the borders of the segments, while the grey line and arrow indicates the position of the maximal width.

Figure 2.

Example of fiber trajectories after quantitative assessment. The aponeurosis is indicated in blue and the posterior part of the aponeurosis is pointing outward. The fibers of the deep compartment are indicated in shades of yellow, while fibers of the superficial compartment are indicated in shades of green. Color variations within the tracts exist only for contrast.

Figure 3.

Example of pennation angles of the superficial compartment plotted on a 3 dimensional representation of the aponeurosis for 2 different days. Note the overall similarity of pennation angles. Grey values indicate the location where either no fiber was tracked or a tract fiber was determined to be erroneous. The subject depicted did not show the best similarity in pennation angle, but showed a characteristic pattern in the posterior portion of the aponeurosis that was present in all four measurements. The anterior side of the aponeurosis is located on the left.

Figure 4.

Average values of pennation angle (θ) and fiber tract length (L_{ft}). Figures A) and C) represent θ , and B) and D) the L_{ft} , with A) and B) for the superficial compartment and C) and D) for the deep compartment. In the images, the mean values per segment are depicted for all subjects and measurements. The line colors in the graphs represent mean θ or L_{ft} for the different columns or rows for the four different measurements with black: baseline, red: identical position, blue: after repositioning, and green: another day. For the columns, I, II, and III indicate the anterior (0–33%), middle (34–66%) and posterior (67–100%) portion of the aponeurosis, respectively. For the rows, A through F indicate the head and toe direction of the rows. The units for θ are degrees and for L_{ft} are mm.

Figure 5.

Bland-Altman plots for the segment with good overall limits of agreement (A) and bad overall limits of agreement (B). In general the better limits of agreement are at the proximal aspect of the aponeurosis. The average difference (solid lines) and the limits of agreement (average $\pm$ 1.96 SD; dotted lines) are shown. The axes for the deep compartment are the same as for the superficial compartment.

Figure 6.

The repeatability coefficients for the different comparisons. For the pennation angle (θ) all segments are included, whereas for the fiber tract length (L_{ft}) only the 0–66% segments are included, as the most distal segments had very high RC. Black bars: superficial compartment; grey bars: deep compartment. BA: between acquisitions; WD: within day; and BD: between days. Significance is indicated by horizontal bars: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.005$.

Table 1

The inter-class-coefficients and repeatability coefficients for the DTI indices.

	Mean	SD	Range	CV	ICC				RC		
					BA	WD	BD	all scans	BA	WD	BD
ADC	1.64	0.05	1.55–1.75	2.1	0.70 **	0.17	-0.17	0.27 *	0.06	0.12	0.13
λ_1	2.13	0.10	1.98–2.40	2.7	0.89 ***	0.44	0.35	0.48 **	0.12	0.20	0.22
λ_2	1.56	0.08	1.40–1.77	2.9	0.51	0.38	0.30	0.40 ***	0.11	0.18	0.19
λ_3	1.22	0.06	1.04–1.32	2.7	0.53 *	0.48	0.43	0.46 ***	0.13	0.12	0.11
FA	0.28	0.04	0.22–0.39	6.9	0.80 ***	0.58 *	0.57 *	0.57 ***	0.06	0.06	0.07

The indicated mean, SD, CV and range are for all subjects and all scans.

The units for the eigenvalues and ADC are $\times 10^{-3} \text{ mm}^2 \cdot \text{s}^{-1}$, FA is dimensionless. Significant differences of the mean ICC from zero, as indicated by an *F*-test, are shown with

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.005$.

CV indicates the average coefficient of variation (%) of all subjects for the four measurements combined.

BA: between acquisitions; BD: between days; ICC: inter-class coefficient; RC: repeatability coefficient; SD: standard deviation; WD: within day.

The inter-class coefficients of the pennation angles for each of the segments

	Superficial compartment				Deep compartment			
	BA	WD	BD	All Scans	BA	WD	BD	All Scans
Mean segments	0.78 *	0.75 *	0.74 *	0.83 **	0.80 ***	0.90 **	0.62	0.86 ***
Position of segment								
H-F				CV				CV
Anterior	0-16%	0.92 ***	0.78 **	0.89 ***	0.77 ***	0.95 ***	0.65 *	0.57 *
	17-33%	0.94 ***	0.86 ***	0.86 ***	0.84 ***	0.71 *	0.46	0.68 *
	34-50%	0.79 **	0.63 *	0.39	0.58 ***	0.68 *	0.39	0.67 *
	51-66%	0.94 ***	0.83 ***	0.58 *	0.62 ***	0.95 ***	0.72 *	0.91 ***
	67-83%	0.66 *	0.54	0.69 **	0.46 ***	0.69 *	0.68 *	0.70 *
Middle	0-16%	0.80 **	0.41	0.38	0.34 *	0.92 ***	0.64 *	0.19
	17-33%	0.78 ***	0.39	0.82 ***	0.58 ***	0.73 *	0.82 ***	0.36
	34-50%	0.79 **	0.48	0.21	0.58 ***	0.89 ***	0.70 ***	0.28
	51-66%	0.92 ***	0.77 **	0.50	0.58 ***	0.89 ***	0.87 ***	0.68 *
	67-83%	0.84 ***	0.48	0.69 *	0.46 **	0.86 ***	1.01	0.55 *
Posterior	0-16%	0.72 *	0.76 ***	0.61 *	0.63 ***	0.85 ***	0.85 ***	0.65 *
	17-33%	0.83 ***	0.86 ***	0.46	0.68 ***	0.96 ***	0.77 **	0.28
	34-50%	0.70 *	0.31	0.31	0.38 *	0.92 ***	0.64 *	0.12
	51-66%	0.84 ***	0.83 ***	0.00	0.33 *	0.87 ***	0.90 ***	0.54
	67-83%	0.73 *	0.20	-0.01	0.30	0.89 ***	0.70 *	0.60 *

The average ICC is given for the analysis with the mean values of the upper four rows. Significance of an F -test with true value 0 is indicated for the ICC with

 $p < 0.005$.

CV indicates the average coefficient of variation (%) of all subjects for the four measurements combined.

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BA, between acquisitions; BD, between days; WD, within day.

Table 3

The inter-class coefficients of the fiber lengths for each of the segments

	Superficial compartment				Deep compartment				CV
	BA	WD	BD	All Scans	BA	WD	BD	All Scans	
Mean segments	0.87 ***	0.94 ***	0.62	0.86 ***	0.93 ***	0.85 **	0.83 **	0.90 ***	CV
H-F									
Anterior									
0–16%	0.26	0.85	0.38	0.71 ***	0.85 ***	0.49	0.81 *	0.57 ***	14.3
17–33%	0.70 *	0.78 **	0.76 *	0.69 ***	0.83 ***	0.88 ***	0.86 ***	0.85 ***	7.6
34–50%	0.83 ***	0.93 ***	0.83 ***	0.87 ***	0.87 ***	0.80 ***	0.82 ***	0.83 ***	8.4
51–66%	0.90 ***	0.81 ***	0.77 ***	0.81 ***	0.97 ***	0.92 ***	0.83 ***	0.90 ***	5.4
67–83%	0.51	0.78 *	0.37	0.57 ***	0.96 ***	0.35	0.70 *	0.60 ***	8.8
Middle									
0–16%	0.63 *	0.31	0.76 *	0.15	0.87 ***	0.30	0.84 ***	0.22	14.4
17–33%	0.33	0.79 ***	0.40	0.38 ***	0.77 **	0.74 *	0.51	0.59 ***	11.6
34–50%	0.96 ***	0.87 ***	0.51 *	0.62 ***	0.93 ***	0.73 *	0.79 **	0.80 ***	10.5
51–66%	0.89 ***	0.88 ***	0.55 *	0.59 ***	0.98 ***	0.79 *	0.85 **	0.86 ***	10.8
67–83%	0.57	0.88 ***	0.36	0.55 ***	0.93 ***	0.70 *	0.39	0.66 ***	9.0
Posterior									
0–16%	0.91 ***	0.46	0.22	0.23	0.96 ***	0.27	0.46	0.05	22.4
17–33%	0.91 ***	0.86 ***	0.54	0.71 ***	0.96 ***	0.73 *	0.54	0.50 ***	17.5
34–50%	0.94 ***	0.84 ***	0.52	0.68 ***	0.89 ***	0.73 *	0.58	0.68 ***	19.6
51–66%	0.97 ***	0.90 ***	–0.04	0.31 *	0.63 *	0.86 ***	0.74 **	0.74 ***	21.0
67–83%	0.91 ***	–0.39	0.31	–0.15	0.92 **	0.87 ***	0.54	0.71 ***	18.3

The positions of the segments are indicated as shown in Figure 1.

The average ICC is given for the analysis with the mean values of the upper four rows. Significance of an *F*-test with true value 0 is indicated for the ICC with

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.005$.

CV indicates the average coefficient of variation (%) of all subjects for the four measurements combined.

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BA, between acquisitions; BD, between days; WD, within day.