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Diffusion Tensor MRI Assessment of Skeletal Muscle Architecture

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Abstract

Diffusion-tensor magnetic resonance imaging (DTI) offers great potential for understanding structure-function relationships in skeletal muscle. The basis for these studies is that water diffuses more readily along the long axes of muscle fibers than along their transverse axes. This diffusion anisotropy can be characterized using a tensor, with the orientation of the principal eigenvalue corresponding to the long axis of the muscle fiber. These local, voxel-based directions can be combined by a fiber tracking algorithm to reconstruct the whole-muscle architecture. The fiber tracking data can be used to characterize important muscle architectural parameters, such as pennation angle, fiber length, and physiological cross-sectional area. The second and third eigenvalues convey information about muscle structural properties along the fibers' transverse axes. A comprehensive description of the sources of transverse diffusion restriction in muscle and how their relative importance may vary with the image acquisition conditions does not yet exist, but may ultimately make DTI a useful tool in studies of skeletal muscle microstructure as well. Ultimately, DTI-based longitudinal studies of changes in muscle architecture may provide insight into the relationships between structure and function in muscle, the time frames of muscle wasting, and in studying adaptations that maintain muscle functionality.

Keywords

DTI; anisotropy; fiber tracking; pennation angle; skeletal muscle

Introduction

The architectural arrangement of a skeletal muscle's fibers with respect to the line of action of the muscle and the microscopic architectural properties of these fibers are important determinants of the muscle's functional properties, including force, power, and shortening velocity [1, 2]. Biomechanical models relating muscle structure to muscle function require a highly spatially resolved, mathematical, and three-dimensional description of this architecture. The historical approaches to this problem have included histological reconstructions of cadaver muscle [3] and *in vivo*, real-time, brightness-mode ultrasound [4]. More recently, a magnetic resonance imaging (MRI) technique has proven useful for these studies as well. That technique relies on the correspondence between the cell geometry and the anisotropic nature of water diffusion in muscle.

The basis for these studies lies in the seminal paper by Cleveland *et al.* on excised skeletal muscle [5]. During the time since, diffusion tensor MRI (DTI) has been firmly established as an important technique for studying *in vivo* tissue structure. Previous reviews of diffusion imaging and spectroscopy of muscle have focused on the clinical aspects of diffusion MRI [6] or have provided general overviews of diffusion imaging and spectroscopy, including metabolite diffusion [7]. Original work has examined the changes in the diffusion coefficient and in diffusion-like processes in exercising skeletal muscle [8, 9]. In this review we focus on the particular use of DTI for reconstructing whole-muscle architecture [10–14] and for evaluating muscle microstructure [15–19].

The review is divided into five sections. First, we provide a brief description of muscle structure and function, focusing on the properties that are most relevant to shortening and force generation and that are observable using DTI. Next, we describe the theoretical basis of DTI, including its measurement and the basis for DTI-based fiber tracking. The third and fourth sections review, respectively, the small amount of literature on DTI-based muscle fiber tracking and DTI-based studies of muscle microstructure, in health and disease. Finally, we provide general conclusions and identify key areas for future research and application.

Skeletal muscle structure and function

This section provides a brief overview of the histological, biochemical, and morphological characteristics of muscles that allow them to produce their principal mechanical actions, shortening and force production. Examination of these properties over size scales ranging from micrometers to centimeters shows a high degree of structural and functional organization. This organization is also highly structurally anisotropic, which is necessary for generating mechanical events that have directions and magnitudes appropriate to the functional requirements of the task.

Skeletal muscles are composed of hundreds to thousands of muscle fibers, which have an elongated, generally cylindrical shape and are multinucleated. The fibers can be a few millimeters to several centimeters long and in mammals have diameters between 20 and 70 μm . The fibers are arranged within the whole muscle so as to be stacked on top of one

another, originating at a stiffer structure (generally proximal within the body) and inserting into a more compliant, and generally distal, structure.

Each fiber consists of densely packed contractile protein filaments called myofibrils. The myofibrils are arranged parallel to the longitudinal axis of the fiber and contain contractile proteins (actin and myosin), regulatory proteins (such as troponin and tropomyosin), and structural proteins (such as titin and nebulin). The contractile and regulatory proteins are involved in regulated, active force generation, while the structural proteins (as well as other structures within the muscle-tendon complex) contribute to passive, elastic forces. The proteins are arranged regularly along the myofibril, giving the skeletal muscle its characteristic striated pattern. Muscle fibers also contain the sarcoplasmic reticulum (SR), which forms interconnecting tubules that surround the myofibrils. Like the myofibrils, the SR also has a predominant orientation parallel to the muscle fiber's longitudinal axis. The SR contains the cell's calcium store and plays a crucial role in the activation and relaxation kinetics of muscle. Activation occurs as a result of a transient elevation in the intracellular calcium concentration, causing the actin and myosin filaments to bind to and slide with respect to each other, producing the length change and force associated with muscle contraction. Release of the actomyosin complex and the restoration of the intracellular ionic environment require the energy released by ATP hydrolysis. The ATP concentration is maintained by up-regulated rates of the flux through the creatine kinase, glycolytic and oxidative phosphorylation reaction.

Muscle fibers can differ in their mechanical, histological and biomechanical properties. Slow twitch/oxidative (SO) fibers have lower specific tensions (*i.e.*, force normalized to cross-sectional area) and twitch speeds. They also have histological and biochemical properties (including smaller fiber diameter, higher myoglobin content, and many mitochondria) that result in higher oxidative capacities and levels of fatigue resistance. Fast-twitch/glycolytic (FG) fibers have fast twitch speeds, low oxidative capacities, and lower levels of fatigue resistance (characteristics are large fiber diameter, low myoglobin content, and few mitochondria). Fast-twitch/oxidative/glycolytic (FOG) fibers have fast twitch speeds, and intermediate levels of fatigue resistance (larger fiber diameters but with intermediate-to-high oxidative capacities). In general, SO fibers are more present in postural muscles, whereas FG fibers are primarily used for short bursts of muscle contraction.

The influences of these histological and biochemical properties on force production and shortening velocity are further modified by a muscle's gross morphological properties. For example, the main determinant of a muscle's force production capacity is its volume [2]. However, for a given muscle volume different architectural designs exist that also modulate muscle force and shortening velocity [20, 21]. Muscle architecture is defined as the arrangement of muscle fibers relative to the axis of force generation [1, 2], and can be described as fusiform, unipennate or multipennate (Fig. (1)). Fusiform muscles, such as the hamstrings, have long muscle fibers arranged parallel to the muscle's long axis, allowing the muscle to undergo large length changes during a given contraction period. Consequently, these muscles are optimally designed for high shortening velocities. Pennate muscles, in which shorter fibers insert into an aponeurosis at an oblique angle (the pennation angle), have a larger number of sarcomeres in parallel and are therefore well designed for high

force, low length excursion contractions. The soleus muscle, a postural muscle, is one example. More complex geometries (such as the bipennate gastrocnemius and multipennate trapezius muscle) also exist. In addition to fiber length, pennation angle, and muscle volume, muscle architecture can also be characterized with fascicle curvature and the anatomical and physiological cross-sectional areas (ACSA and PCSA, respectively). The ACSA is simply the largest cross-section across the whole muscle, while the PCSA is the sum of the cross-sectional area of all fibers. By accounting for pennation, the PCSA better predicts a muscle's maximal force production than the ACSA [22, 23].

This relationship between structure and function of muscles is central to understanding the physiological basis of force production and movement, especially as muscle architecture can be altered by bed rest [24], training [25], or as the result of genetic defects (*e.g.* Duchenne's muscular dystrophy). Structure-function relationships are commonly investigated by muscle architecture-based biomechanical models [2, 26]. These models are often simplified to one or two dimensions, representing muscles as a series of line segments. However, these models do not account for variations in muscle architecture that occur along the muscle length [10, 27, 28]. More complex three-dimensional (3D) models offer the potential to improve the predictive accuracy of biomechanical models [29, 30], but require knowledge of the whole muscle architecture.

Muscle architecture can be determined by several methods, ranging from those that are highly invasive (3D reconstruction of histological images of cadaver muscles) to those that are completely non-invasive (such as brightness- (B-) mode ultrasound (US) and magnetic resonance imaging (MRI)). Histological analysis of cadaver muscles was for many years the only available option of studying muscle architecture, and has yielded a large database of pennation angle estimates that have been widely used in biomechanical models [3]. However, this process is very time consuming and is prone to errors arising from the fixation process (such as anisotropic shrinkage [31]) and the likelihood that the subjects are unrepresentative with regard to age and health status. B-mode US uses the returned echoes from the connective tissues to visualize fascicles in real time, even during dynamic contractions. These studies have revealed that pennation angles increase and fascicles shorten during contraction [4, 32]. When combined with muscle volume data, the pennation angle measurements from US allow estimation of the PCSA. However, US is limited because it is a projection, rather than tomographic, imaging technique; this makes it difficult to characterize a whole muscle's architecture. Also, virtually all applications of US to date have used two-dimensional methods, making it difficult to capture the 3D aspects of muscle architecture. Recently, it has been shown that DTI also has the ability to determine the 3D structure of muscle *in vivo*, including measurements of the pennation angle, fiber length, and PCSA [10, 11, 13, 14].

Theoretical basis for diffusion tensor MRI

Brownian motion causes molecules to randomly displace in time depending on the temperature, the viscosity of the fluid, and the size of the molecule. For water, the free diffusion coefficient is $3.3 \times 10^{-3} \text{ mm}^2 \cdot \text{s}^{-1}$ at 37°C . *In vivo*, the diffusion of water in tissue is influenced by physical barriers (*e.g.*, cellular membranes and cellular constituents).

Therefore, an effective diffusivity is measured that is usually called the apparent diffusion coefficient (ADC) [33, 34] which is lower than the free diffusion coefficient. For tissues with fiber-like structure, such as skeletal muscle, cardiac muscle, and central nervous system, the diffusion is more hindered perpendicular to the fibers than along the fibers [34–36]. Therefore, in these tissues the diffusion is anisotropic, with typical values of $2.2 \times 10^{-3} \text{ mm}^2 \cdot \text{s}^{-1}$ parallel to human muscle fibers and $1.3 \times 10^{-3} \text{ mm}^2 \cdot \text{s}^{-1}$ perpendicular to the fibers.

Water diffusion can be studied by diffusion-sensitized MRI, using the pulsed-field gradient (PFG) technique proposed by Stejskal and Tanner [37]. For the PFG experiment, two identical gradient pulses are applied on either side of the 180° refocusing radio frequency (RF) pulse in a spin-echo pulse sequence. During the first gradient the spins will dephase according to their position in the gradient field. The second gradient will reestablish phase coherence. In the absence of diffusion, the net phase effect is zero for all spins, resulting in a signal intensity determined by the transverse relaxation time constant (T_2) of the water. Spins that have moved will experience a different magnetic field during rephasing than during dephasing, resulting in phase dispersion and therefore signal loss.

In case of rectangular gradient pulses, the dependence of the signal intensity on the experimental settings and ADC is given by

$$\ln \left(\frac{S(b)}{S_0} \right) = -\gamma^2 G^2 \delta^2 (\Delta - \delta/3) ADC = -b \cdot ADC, \quad [1]$$

in which $S(b)$ and S_0 are the MR signal intensities with and without diffusion sensitization, respectively, γ is the gyromagnetic ratio of the nucleus, G is the gradient amplitude, δ is the duration of the gradient pulses, Δ is the delay between the leading edges of the two gradient pulses, and the b-factor is used to indicate the strength of the diffusion weighting (often given in units of $\text{s} \cdot \text{mm}^{-2}$) [38, 39].

The diffusion in elongated tissue is anisotropic and the extent of signal loss strongly depends on the direction of the diffusion gradients [5]. Skeletal muscle clearly shows a stronger signal attenuation as a function of b-value when the diffusion gradient is applied approximately parallel to the muscle fiber direction. In media with structural anisotropy, the ADC cannot be considered a scalar quantity, but rather must be described using a tensor. The tensor model expresses molecular mobility along each direction and the correlations among the diffusional displacements in these directions. This diffusion tensor $\mathbf{D}$ is given by

$$\mathbf{D} = \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix} \quad [2]$$

The diagonal elements of $\mathbf{D}$ – D_{xx} , D_{yy} and D_{zz} – represent diffusion along the x, y and z axes of the laboratory's frame of reference, while the off-diagonal elements of $\mathbf{D}$ – D_{xy} , D_{xz} and D_{yz} – represent the correlation between the diffusion in orthogonal directions. In the case of isotropic diffusion, there are no correlations between the different directions, the off-

diagonal elements are zero, and D_{xx} , D_{yy} , and D_{zz} are equal. After diagonalizing the tensor, the off-diagonal terms are removed and the tensor is reduced to its diagonal terms, which are co-aligned with the principal direction of diffusivity.

The complete diffusion tensor conveys information regarding the orientation dependence of diffusion. From the diffusion tensor, three eigenvalues (in descending order, λ_1 , λ_2 and λ_3) with corresponding eigenvectors (ε_1 , ε_2 and ε_3) can be calculated. In order to simplify the characterization of the diffusion tensor data, several scalar rotationally and scaling- invariant indices are used [33, 34]. The apparent diffusion coefficient

$$ADC = D_{av} = \frac{Tr(D)}{3} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \quad [3]$$

is a commonly used index to quantify the mean diffusivity of biological tissue. The anisotropy of the diffusion can be described by the fractional anisotropy (FA)

$$FA = \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_1 - \lambda_3)^2}}{\sqrt{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}, \quad [4]$$

which measures the fraction of the diffusion tensor that can be ascribed to anisotropic diffusion [33]. This index is zero for isotropic diffusion ($\lambda_1 = \lambda_2 = \lambda_3$) and approaches one for a highly anisotropic, cylindrically symmetric medium ($\lambda_1 \gg \lambda_2 = \lambda_3$).

The orientation of ε_1 has been investigated in terms of its relationship to the muscle fiber direction [10, 40–43]. All of these studies showed that the direction of the principal eigenvalue corresponded with the local fiber orientation. By comparing DTI findings with histology, it has been shown that, within experimental error, the direction of the highest diffusivity indeed corresponds to the local muscle fiber orientation [41]. This correspondence enables a powerful application of DTI, which is the ability to reconstruct fiber trajectories [44], a technique that has become known as DTI tractography. The task of tractography is to assign mathematical associations between adjacent voxels based on eigenvalue and eigenvector information. Several fiber tracking algorithms have been developed to visualize the main diffusion pathways in the 3D diffusion tensor volume using deterministic or probabilistic approaches [44–51]. In deterministic approaches, a fiber is generally reconstructed by starting from a seed point defined with a planar region of interest (ROI) and then following the path of the principal eigenvectors until it reaches one of several predefined stop criteria. These criteria are usually a point with low FA or with large curvature; the latter is often defined as the inner product between two adjacent eigenvectors. Probabilistic approaches compute all possible connections between voxels, thereby determining the likelihood of these pathways and accounting for diverging fibers. This approach is especially useful for brain, where fibers branch, “kiss” or cross.

Although DTI is very appealing for studies of skeletal muscle microstructure and whole-muscle architecture, there are several practical challenges. The transverse relaxation time constant (T_2) of muscle is relatively low (approximately 40 ms at 3 Tesla), which results in a

low signal-to-noise ratio (SNR) and limits to the range of diffusion weighting and diffusion times that can be applied. For human applications, the length of a typical limb muscle is larger than the RF coil length and the sufficiently homogeneous B_0 region of the magnet. In addition, there are imaging artifacts due to spatial distortions of echo-planar imaging (EPI) sequences, chemical shift, and limb motion. Furthermore, using a planar ROI to start reconstructing the fibers will not contain all fibers of the pennate muscle. However, studies have been performed that overcome these problems [12, 52, 53].

DTI-based studies of skeletal muscle architecture

The initial skeletal muscle DTI-based fiber tracking studies were performed in small animals. The small animal model reduces motion artifact by fixing the limb and through anesthesia; therefore, a conventional image acquisition scheme can be used. At the cost of increased total scan duration, the conventional image acquisition method also allows for improved SNR, an absence of chemical shift artifact, and no need for distortion corrections. The small animal model also allows, as was performed in the initial DTI-based muscle fiber tracking study [10], a comparison of pennation angle measurements using DTI and direct anatomical inspection. In this study of the rat gastrocnemius muscle, seed points were defined in a planar ROI at a density of one point per voxel. Similar fiber tracts were grouped into bundles based on their anatomical similarity, and for each bundle the diffusion properties and the pennation angle along the length of the fiber were calculated. The pennation angle was calculated as the angle formed by the tangent lines to each point along the fiber bundle and to the central axis of the aponeurosis. This method is analogous to the method of US-based pennation measurements, but is objective, expands the method to three dimensions, and allows the assessment of pennation heterogeneity within a fiber. A high correlation ($r=0.89$) was found between the pennation angle measured with DTI (mean $\theta = 30 \pm 8^\circ$) and that obtained by direct anatomical inspection (mean $\theta = 31 \pm 7^\circ$). This study demonstrated that DTI-based muscle fiber tracking is not only feasible but is also a valid tool for pennation angle measurements in of small-animal skeletal muscle.

Heemskerk et al. [11] subsequently applied DTI-based fiber tracking to mouse hind limb muscles. They made a number of anatomical measurements of interest, including the PCSA, fiber length, and pennation angle for the tibialis anterior (TA) muscle. Images were obtained using a 3D diffusion-weighted fast spin echo sequence with an isotropic resolution of $234 \mu\text{m}^3$. In addition, a T_2 -weighted scan was made while contracting the dorsiflexors, resulting in a T_2 enhancement in the activated muscles. It was shown that essentially all reconstructed fiber trajectories remained within the activated muscle, indicating the ability to discriminate between adjacent muscles. A follow-up study determined the changes in the architectural parameters upon foot extension [14]. A 30 degree increase in ankle angle resulted in a 7% increase in fiber length and a decrease in pennation angle and PCSA of 10 degrees and 4%, respectively. The calculated moment arm was 1.64 mm. This study showed that subtle changes in muscle architecture can be determined with DTI-based fiber tracking.

As alluded to above, translation of these techniques to human studies introduces additional challenges, including coil length, imaging time, and motion artifacts. However, Steidle and Schick have recently described several technical advances that aid *in vivo* human DTI

studies considerably, including eddy-current nulling and stimulated echo acquisitions [53], and Sinha *et al.* [12] recently demonstrated the feasibility of DTI-based fiber tracking on human muscles. In the Sinha study, the mid-calf region was examined with an extremity coil and an EPI sequence. The quality of the DTI data is described in detail showing images of all the diffusion directions and the calculated eigenvalue and eigenvector maps. Fibers were traced started from small manually selected ROIs within 5 different muscles. Fig. (2) shows two panels from Fig. (7) of their paper, which together demonstrate the ability of DTI-based fiber tracking to detect known muscle architectural patterns. The human TA muscle is bipennate (*i.e.*, an architecture such as that depicted in (Fig. (1)). As illustrated in the left panel of Fig. (2), Sinha *et al.* specified a small planar ROI straddling the TA's central aponeurosis. The fibers emerging from this ROI are shown in the right panel, and are consistent with the bipennate architecture of this muscle. These authors also demonstrated the possibility of tracking fibers from aponeurosis of origin to aponeurosis of insertion.

More recently, a technique was presented for quantitatively analyzing DTI-based human muscle architectural reconstructions [13]. A total of four EPI-based acquisitions, two requiring coil repositioning, were made in order to compensate for the limited RF coil length and to reduce artifacts related to static magnetic field inhomogeneities. From T₁-weighted images, the aponeurosis was manually defined and a mesh was created as the seed surface for fiber tracking (Fig. (3)). The mean pennation angle was calculated for each fiber tract, enabling a 3D representation of the pennation angle distribution. The calculated pennation angles agreed well with a previous 3D US study, but did so using an objective measurement technique that inherently provides a mathematical description of the fiber locations. As noted by the authors, the fiber tracts can also serve as an architectural scaffold onto which other functional information, such as mechanical strain or perfusion, can be overlaid and understood with respect to the local fiber architecture.

DTI-based fiber tracking of skeletal muscle therefore has exciting possibilities for understanding muscle structure-function relationships, but some challenges remain. One relates to the typical use of criteria such as low FA or high curvature to terminate the fiber tracking procedure. The reasons for using low FA as a stop criterion are that 1) the estimate of the principal eigenvector can be inaccurate and so terminating fiber tracts in regions of low FA prevents the generation of erroneous fiber tracts and 2) non-muscle tissues might have intrinsically low FA values, and so terminating fibers in voxels with low FA will prevent tracking fibers outside the muscle boundaries. Terminating the fiber tracts when there is high curvature between adjacent points also prevents the generation of fiber tracts that cross muscle boundaries. These criteria are appropriate when there is high SNR and spatial resolution, but may be problematic under conditions of low SNR, spatial distortions, and partial volume artifacts. Low SNR and spatial distortions result in imperfect specification of the first eigenvector, potentially leading to high apparent curvature and premature termination of the fiber tracking procedure. Other fibers appear to stop because of the intrinsic muscle composition, such as fat infiltration. This may affect the underlying tissue architecture by causing the fibers to adopt a highly curved trajectory. However, this high curvature would potentially lead to premature fiber tract termination. In addition, the fat suppression that is typically used to avoid chemical shift artifacts would result in low

SNR for voxels containing both muscle and fat. This would lead to inaccurate estimates of FA and ε_1 and therefore premature fiber termination. These issues are expected to be particularly problematic in the muscles of persons with a high level of intramuscular fat or in clinical conditions such as muscular dystrophy or inflammatory muscle diseases, in which muscle tissue is replaced with fat and connective tissue. There is a need to develop fiber tracking algorithms that can accurately determine fiber trajectories under these conditions.

Also, it should be noted that it is the muscle architecture during contraction, not at rest, that is important in determining the mechanical properties of the contraction. While there is therefore a great interest and advantage to performing DTI studies of contracting muscle, there are several additional anticipated challenges. First, the time to perform a single DTI sequence is about 2–4 minutes and is therefore too long to perform under many muscle contraction conditions. In addition, a contracting muscle will introduce movement of the muscle, introducing image registration errors and problems with the measurement of the diffusion coefficient. The problem is not intractable, however, and the intelligent and creative design of image acquisition and repeatable muscle contraction protocols could enable reconstruction of fiber architecture in the active state.

DTI-based studies of skeletal muscle microstructure, in health and disease

The eigenvectors and eigenvalues of the diffusion tensor provide information on the local tissue structure, and as discussed above, λ_1 and ε_1 correspond respectively to the magnitude and direction of greatest diffusion – parallel to the fibers' long axes [10, 40–43]. The large reduction of λ_2 and λ_3 relative to λ_1 suggests that there is specific structural information than can be obtained from these values as well. Because ε_1 corresponds to the long axes of the fibers and the three eigenvectors are mutually orthogonal, ε_2 and ε_3 must lie perpendicularly to the long axis of the fiber. In the heart, there is a higher level of fiber structuring beyond the orientation of the fibers' long axes such that the fibers are arranged in layered sheets. Tseng *et al.* found that ε_2 and ε_3 correspond to the directions parallel to myocardial sheets and normal to these sheets, respectively [54]; a subsequent study showed that there are patterns of strain development that correspond to these diffusion directions [55]. However, we know of no studies demonstrating an analogous laminar structure in skeletal muscle. This creates some uncertainty as to the structural origin of λ_2 and λ_3 in skeletal muscle.

The general possibilities for sources of diffusion hindrance lying transverse to the fibers' longitudinal axes include the myofibrils, SR, and cell membranes. Significantly, these structures' dimensions vary by several orders of magnitude. Thus it is likely that the relative importance of the myofibrils, SR, and cell membrane in hindering transverse diffusion will vary with the details of the diffusion acquisition. In particular, the δ term from Eq. 1 is expected to be quite important. The reason for this is that it is the mean diffusion distance during the time δ that will determine the size (and therefore the identity) of the structures that hinder diffusion. An additional factor is the multiexponential character of T_2 , in which intracellular water has a mean T_2 of ~35 ms and interstitial water has a mean T_2 of ~125 ms [56]. Therefore as echo time increases, the difference in the relaxation properties of the intracellular and interstitial spaces progressively increases the weighting of the diffusion signal attenuation on the structural characteristics of the interstitium. To complicate the

interpretation even more, the diffusion decay (signal attenuation versus b-value) is also bi-exponential [57]. However, the volume fractions of the two diffusion compartments did not correlate with the volume fractions of simultaneously measured T_2 compartments, indicating that factors other than (or in addition to) intra versus extracellular water compartmentation play a role in the diffusion decay. It might be possible to identify these factors by exploring diffusion decay as function of diffusion time using oscillating gradient [58] or stimulated echo [53] sequences for the short and long limits of Δ , respectively.

A fundamental issue to resolve is whether the λ_2 and λ_3 values in skeletal muscle truly represent different aspects of tissue structure (*i.e.*, $\lambda_1 > \lambda_2 > \lambda_3$) or if λ_2 and λ_3 are equal ($\lambda_1 > \lambda_2 = \lambda_3$) but with apparent differences created by noise (the “sorting bias”). Despite several attempts [15–19] there has as yet been no convincing study reporting the morphological origin of λ_2 and λ_3 in skeletal muscle. Galban *et al.* found systematic variations in λ_3 between muscle groups of the human calf and, using the tissue structure model employed by Tseng *et al* in the heart, attributed these variations to differences in cell diameter [15]. These authors subsequently reported that inter-gender differences in eigenvalues exist, with greater relative differences in λ_3 than in λ_1 [16]. Both of these findings imply a model in which $\lambda_1 > \lambda_2 > \lambda_3$. Conversely, Sinha and colleagues reported that it is λ_1 that varies most between human calf muscles; also, they concluded that $\lambda_2 \approx \lambda_3$ [12]. Resolving these different observations and conclusions concerning the nature of the diffusion tensor in human skeletal muscle is difficult, for several reasons. First, the noise effects on the tensor will be great at the SNR levels typically observed in muscle DTI studies. This creates the usual problem of sorting bias and introduces a rotationally variant noise characteristic to the data, because one of the six sampled diffusion directions (along the Z axis) is almost exactly coincident with the predominant muscle fiber direction. Also, the TE values used in these studies (69 ms for the Sinha *et al* study, 95 ms for the Galban *et al* studies) mean that signals from the intracellular space are contributing very little (for TE=69 ms) or almost not at all (for TE=95 ms) to the measured diffusion tensor. The differing spatial dimensions and amounts of non-membrane, diffusion-hindering structures in the intracellular and interstitial spaces add additional confounding factors that preclude a simple interpretation of diffusion data from acquisitions of differing echo times.

Several studies investigating the dynamic response of the diffusion indices to muscle injury or muscle disease not only support the possibility that the differences in λ_2 and λ_3 and their corresponding eigenvectors are real but also demonstrate that information about muscle quality can be obtained from DTI that is not available from other MRI measures. The first case studies on muscles with inflammatory myopathies indicated that diffusion-weighted provides additional information to T_1 and T_2 that may be of value for studying the pathophysiology of muscle disease [59]. Also, muscle injury (muscle tear or intramuscular hematoma injury) results in a higher ADC, lower FA, and a disorganized fiber structure [60]. A detailed analysis of the relationship of DTI indices to muscle damage was performed by Heemskerk *et al.* [18], who evaluated the response of the DTI indices to acute ischemia/reperfusion injury. They found that the diffusion coefficients decreased during ischemia and increased upon reperfusion. Superimposition of stimulated muscle contractions upon an already ischemic muscle indicated that the extent and durations of the changes in

eigenvalues depended on the severity of ischemic stress. Moreover, there was a differential response of the eigenvalues such that changes in λ_3 showed the largest relative increase during reperfusion. Twenty-four hours following reperfusion damage, it was found that λ_1 correlated with no histological damage indices, λ_2 correlated with interstitial fluid accumulation and the percentages of damaged and round cells, and λ_3 also correlated with these histological measures and with the general damage score and the amount of infiltrates. This finding supports the hypothesis that λ_2 and λ_3 convey different information about muscle structure. In another study, they evaluated DTI for monitoring tissue regeneration following prolonged ischemia after ligation of the femoral artery [19]. Regional differences existed in the response between DTI and T_2 , demonstrating that DTI can be used as an *in vivo* marker of ischemia-induced muscle damage and the combination of DTI and T_2 might enable discrimination between muscle disorders. Finally, Saotome et al. [17] evaluated diffusional anisotropy and microscopic structure of muscle atrophy induced by denervation of the sciatic nerve in rats. They found that atrophy caused an increase in FA and a decrease in λ_2 after 4 weeks and in λ_3 after 8 weeks. They also measured fiber diameters during this same period. Although the measured fiber diameters did not correlate perfectly with the changes in λ_2 and λ_3 , this study does also provide additional support for the idea that at least some of the transverse hindrance to diffusion is provided by the cell membrane, and differential time courses of the changes in λ_2 and λ_3 suggest that these eigenvalues have distinct biological bases.

Conclusions

This review has considered the microstructural and gross morphological characteristics of muscles that impact upon functional properties of skeletal muscles, such as shortening velocity and tension development. The sensitivity of DTI to muscle architecture suggests that these data may provide a potentially powerful tool for examining how these structural and functional properties are interrelated. Specifically, the first eigenvector of the tensor reflects the predominant fiber orientation within a voxel, and may be used to reconstruct the local fiber trajectory. Also, the second and third eigenvectors lie transverse to the longitudinal fiber axis, and may reflect structural characteristics of muscle fibers such as cell diameter. The microscopic and macroscopic structural properties of muscles are potentially influenced by factors such as disease, disuse, and exercise training, and its non-invasive, non-destructive nature may make DTI a useful tool for these studies as well. There are several important issues to be resolved for DTI to be fully useful, however. For fiber tracking studies, algorithms need to be developed that can accurately determine the fiber trajectory around intramuscular fat infiltrations and acquisition protocols need to be developed for contracting muscles. For DTI studies of muscle fiber microstructure, the identification of the structures that hinder diffusion transverse to the fibers' long axes, including a quantitative analysis of how these structures' relative importance changes with the diffusion acquisition parameters, needs to be performed.

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References

1. Burkholder TJ, Fingado B, Baron S, Lieber RL. Relationship between muscle fiber types and sizes and muscle architectural properties in the mouse hindlimb. *J Morphol.* 1994 Aug; 221(2):177–90. [PubMed: 7932768]
2. Lieber RL, Friden J. Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve.* 2000 Nov; 23(11):1647–66. [PubMed: 11054744]
3. Wickiewicz TL, Roy RR, Powell PL, Edgerton VR. Muscle architecture of the human lower limb. *Clin Orthop Relat Res.* 1983 Oct;(179):275–83. [PubMed: 6617027]
4. Rutherford OM, Jones DA. Measurement of fibre pennation using ultrasound in the human quadriceps in vivo. *Eur J Appl Physiol Occup Physiol.* 1992; 65(5):433–7. [PubMed: 1425649]
5. Cleveland GG, Chang DC, Hazlewood CF, Rorschach HE. Nuclear magnetic resonance measurement of skeletal muscle: anisotropy of the diffusion coefficient of the intracellular water. *Biophys J.* 1976 Sep; 16(9):1043–53. [PubMed: 963204]
6. Baur A, Reiser MF. Diffusion-weighted imaging of the musculoskeletal system in humans. *Skeletal Radiol.* 2000 Oct; 29(10):555–62. [PubMed: 11127677]
7. Strijkers GJ, Drost MR, Heemskerk AM, Kruiskamp MJ, Nicolaay K. Diffusion MRI and MRS of skeletal muscle. *Israel Journal of Chemistry.* 2003; 43:71–80.
8. Morvan D. In vivo measurement of diffusion and pseudo-diffusion in skeletal muscle at rest and after exercise. *Magn Reson Imaging.* 1995; 13(2):193–9. [PubMed: 7739360]
9. Morvan D, Leroy-Willig A. Simultaneous measurements of diffusion and transverse relaxation in exercising skeletal muscle. *Magn Reson Imaging.* 1995; 13(7):943–8. [PubMed: 8583872]
10. Damon BM, Ding Z, Anderson AW, Freyer AS, Gore JC. Validation of diffusion tensor MRI-based muscle fiber tracking. *Magn Reson Med.* 2002 Jul; 48(1):97–104. [PubMed: 12111936]
11. Heemskerk AM, Strijkers GJ, Vilanova A, Drost MR, Nicolay K. Determination of mouse skeletal muscle architecture using three-dimensional diffusion tensor imaging. *Magn Reson Med.* 2005 Jun; 53(6):1333–40. [PubMed: 15906281]
12. Sinha S, Sinha U, Edgerton VR. In vivo diffusion tensor imaging of the human calf muscle. *J Magn Reson Imaging.* 2006 Jul; 24(1):182–90. [PubMed: 16729262]
13. Lansdown DA, Ding Z, Wadington M, Hornberger JL, Damon BM. Quantitative Diffusion Tensor MRI-Based Fiber Tracking of Human Skeletal Muscle. *J Appl Physiol.* 2007 Apr 19.
14. Heemskerk, AM.; Strijkers, GJ.; Drost, MR.; Nicolay, K. Diffusion tensor MRI for the in vivo determination of skeletal muscle architecture in two ankle angles. *International Society for Magnetic Resonance in Medicine 14th Scientific Meeting and Exhibition; 2006; Seattle, WA.* 2006.
15. Galban CJ, Maderwald S, Uffmann K, de Greiff A, Ladd ME. Diffusive sensitivity to muscle architecture: a magnetic resonance diffusion tensor imaging study of the human calf. *Eur J Appl Physiol.* 2004 Dec; 93(3):253–62. [PubMed: 15322853]
16. Galban CJ, Maderwald S, Uffmann K, Ladd ME. A diffusion tensor imaging analysis of gender differences in water diffusivity within human skeletal muscle. *NMR Biomed.* 2005 Dec; 18(8):489–98. [PubMed: 16075414]
17. Saotome T, Sekino M, Eto F, Ueno S. Evaluation of diffusional anisotropy and microscopic structure in skeletal muscles using magnetic resonance. *Magn Reson Imaging.* 2006 Jan; 24(1):19–25. [PubMed: 16410174]
18. Heemskerk AM, Drost MR, van Bochove GS, van Oosterhout MF, Nicolay K, Strijkers GJ. DTI-based assessment of ischemia-reperfusion in mouse skeletal muscle. *Magn Reson Med.* 2006 Aug; 56(2):272–81. [PubMed: 16826605]
19. Heemskerk AM, Strijkers GJ, Drost MR, van Bochove GS, Nicolay K. Skeletal muscle degeneration and regeneration after femoral artery ligation in mice: monitoring with diffusion MR imaging. *Radiology.* 2007 May; 243(2):413–21. [PubMed: 17384238]

20. Wickiewicz TL, Roy RR, Powell PL, Perrine JJ, Edgerton VR. Muscle architecture and force-velocity relationships in humans. *J Appl Physiol*. 1984 Aug; 57(2):435–43. [PubMed: 6469814]
21. Lieber RL, Blevins FT. Skeletal muscle architecture of the rabbit hindlimb: functional implications of muscle design. *J Morphol*. 1989 Jan; 199(1):93–101. [PubMed: 2921772]
22. Powell PL, Roy RR, Kanim P, Bello MA, Edgerton VR. Predictability of skeletal muscle tension from architectural determinations in guinea pig hindlimbs. *J Appl Physiol*. 1984 Dec 1; 57(6):1715–21. [PubMed: 6511546]
23. Fukunaga T, Roy RR, Shellock FG, Hodgson JA, Day MK, Lee PL, et al. Physiological cross-sectional area of human leg muscles based on magnetic resonance imaging. *J Orthop Res*. 1992 Nov; 10(6):928–34. [PubMed: 1403308]
24. Appell HJ. Muscular atrophy following immobilisation. A review. *Sports Med*. 1990 Jul; 10(1):42–58. [PubMed: 2197699]
25. Frisch H. Growth hormone and body composition in athletes. *J Endocrinol Invest*. 1999; 22(5 Suppl):106–9. [PubMed: 10442579]
26. Oomens CW, Maenhout M, van Oijen CH, Drost MR, Baaijens FP. Finite element modelling of contracting skeletal muscle. *Philos Trans R Soc Lond B Biol Sci*. 2003 Sep 29; 358(1437):1453–60. [PubMed: 14561336]
27. Agur AM, Ng-Thow-Hing V, Ball KA, Fiume E, McKee NH. Documentation and three-dimensional modelling of human soleus muscle architecture. *Clin Anat*. 2003 Jul; 16(4):285–93. [PubMed: 12794910]
28. Hiblar, T.; Bolson, E.; Hubka, M.; Sheehan, F.; Kushmerick, M. Three dimensional ultrasound analysis of fascicle orientation in human tibialis anterior muscle enables analysis of macroscopic torque at the cellular level. In: Sugi, H., editor. *Molecular and Cellular Aspects of Muscle Contraction*. New York, NY: Springer; 2003. p. 635–45.
29. Blemker SS, Asakawa DS, Gold GE, Delp SL. Image-based musculoskeletal modeling: applications, advances, and future opportunities. *J Magn Reson Imaging*. 2007 Feb; 25(2):441–51. [PubMed: 17260405]
30. Blemker SS, Pinsky PM, Delp SL. A 3D model of muscle reveals the causes of nonuniform strains in the biceps brachii. *J Biomech*. 2005 Apr; 38(4):657–65. [PubMed: 15713285]
31. Quester R, Schroder R. The shrinkage of the human brain stem during formalin fixation and embedding in paraffin. *J Neurosci Methods*. 1997 Jul 18; 75(1):81–9. [PubMed: 9262148]
32. Fukunaga T, Ichinose Y, Ito M, Kawakami Y, Fukushima S. Determination of fascicle length and pennation in a contracting human muscle in vivo. *J Appl Physiol*. 1997 Jan; 82(1):354–8. [PubMed: 9029238]
33. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B*. 1996 Jun; 111(3):209–19. [PubMed: 8661285]
34. Pierpaoli C, Basser PJ. Toward a quantitative assessment of diffusion anisotropy. *Magn Reson Med*. 1996 Dec; 36(6):893–906. [PubMed: 8946355]
35. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed*. 2002 Nov-Dec; 15(7–8):435–55. [PubMed: 12489094]
36. Garrido L, Wedeen VJ, Kwong KK, Spencer UM, Kantor HL. Anisotropy of water diffusion in the myocardium of the rat. *Circ Res*. 1994 May; 74(5):789–93. [PubMed: 8156627]
37. Stejskal E, Tanner J. Spin diffusion measurements: spin echoes in the presence of a time-dependent field gradient. *J Chem Phys*. 1965; 42:288–92.
38. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. *Biophys J*. 1994 Jan; 66(1):259–67. [PubMed: 8130344]
39. Le Bihan D. Molecular diffusion nuclear magnetic resonance imaging. *Magn Reson Q*. 1991 Jan; 7(1):1–30. [PubMed: 2043461]
40. Napadow VJ, Chen Q, Mai V, So PT, Gilbert RJ. Quantitative analysis of three-dimensional-resolved fiber architecture in heterogeneous skeletal muscle tissue using nmr and optical imaging methods. *Biophys J*. 2001 Jun; 80(6):2968–75. [PubMed: 11371469]

41. Van Donkelaar CC, Kretzers LJ, Bovendeerd PH, Lataster LM, Nicolay K, Janssen JD, et al. Diffusion tensor imaging in biomechanical studies of skeletal muscle function. *J Anat.* 1999 Jan; 194(Pt 1):79–88. [PubMed: 10227669]
42. Hsu EW, Muzikant AL, Matulevicius SA, Penland RC, Henriquez CS. Magnetic resonance myocardial fiber-orientation mapping with direct histological correlation. *The American journal of physiology.* 1998 May; 274(5 Pt 2):H1627–34. [PubMed: 9612373]
43. van Doorn A, Bovendeerd PH, Nicolay K, Drost MR, Janssen JD. Determination of muscle fibre orientation using Diffusion-Weighted MRI. *European journal of morphology.* 1996; 34(1):5–10. [PubMed: 8743092]
44. Basser PJ, Pajevic S, Pierpaoli C, Duda J, Aldroubi A. In vivo fiber tractography using DT-MRI data. *Magn Reson Med.* 2000 Oct; 44(4):625–32. [PubMed: 11025519]
45. Conturo TE, Lori NF, Cull TS, Akbudak E, Snyder AZ, Shimony JS, et al. Tracking neuronal fiber pathways in the living human brain. *Proceedings of the National Academy of Sciences of the United States of America.* 1999 Aug 31; 96(18):10422–7. [PubMed: 10468624]
46. Mori S, Kaufmann WE, Pearlson GD, Crain BJ, Stieltjes B, Solaiyappan M, et al. In vivo visualization of human neural pathways by magnetic resonance imaging. *Ann Neurol.* 2000 Mar; 47(3):412–4. [PubMed: 10716271]
47. Stieltjes B, Kaufmann WE, van Zijl PC, Fredericksen K, Pearlson GD, Solaiyappan M, et al. Diffusion tensor imaging and axonal tracking in the human brainstem. *NeuroImage.* 2001 Sep; 14(3):723–35. [PubMed: 11506544]
48. Mori S, van Zijl PC. Fiber tracking: principles and strategies - a technical review. *NMR Biomed.* 2002 Nov-Dec; 15(7–8):468–80. [PubMed: 12489096]
49. Parker GJ, Wheeler-Kingshott CA, Barker GJ. Estimating distributed anatomical connectivity using fast marching methods and diffusion tensor imaging. *IEEE transactions on medical imaging.* 2002 May; 21(5):505–12. [PubMed: 12071621]
50. Ciccarelli O, Parker GJ, Toosy AT, Wheeler-Kingshott CA, Barker GJ, Boulby PA, et al. From diffusion tractography to quantitative white matter tract measures: a reproducibility study. *NeuroImage.* 2003 Feb; 18(2):348–59. [PubMed: 12595188]
51. Parker GJ, Haroon HA, Wheeler-Kingshott CA. A framework for a streamline-based probabilistic index of connectivity (PICO) using a structural interpretation of MRI diffusion measurements. *J Magn Reson Imaging.* 2003 Aug; 18(2):242–54. [PubMed: 12884338]
52. Lansdown DA, Ding Z, Wadington M, Hornberger JL, Damon BM. Quantitative diffusion tensor MRI-based fiber tracking of human skeletal muscle. *J Appl Physiol.* 2007 in review.
53. Steidle G, Schick F. Echoplanar diffusion tensor imaging of the lower leg musculature using eddy current nulled stimulated echo preparation. *Magn Reson Med.* 2006 Mar; 55(3):541–8. [PubMed: 16450364]
54. Tseng WY, Wedeen VJ, Reese TG, Smith RN, Halpern EF. Diffusion tensor MRI of myocardial fibers and sheets: correspondence with visible cut-face texture. *J Magn Reson Imaging.* 2003 Jan; 17(1):31–42. [PubMed: 12500272]
55. Dou J, Tseng WY, Reese TG, Wedeen VJ. Combined diffusion and strain MRI reveals structure and function of human myocardial laminar sheets in vivo. *Magn Reson Med.* 2003 Jul; 50(1):107–13. [PubMed: 12815685]
56. Cole WC, LeBlanc AD, Jhingran SG. The origin of biexponential T2 relaxation in muscle water. *Magn Reson Med.* 1993 Jan; 29(1):19–24. [PubMed: 8419738]
57. Ababneh Z, Beloeil H, Berde CB, Gambarota G, Maier SE, Mulkern RV. Biexponential parameterization of diffusion and T2 relaxation decay curves in a rat muscle edema model: decay curve components and water compartments. *Magn Reson Med.* 2005 Sep; 54(3):524–31. [PubMed: 16086363]
58. Schachter M, Does MD, Anderson AW, Gore JC. Measurements of restricted diffusion using an oscillating gradient spin-echo sequence. *J Magn Reson.* 2000 Dec; 147(2):232–7. [PubMed: 11097814]
59. Qi, J.; Olsen, N.; Price, R.; Park, J. Diffusion-weighted imaging provides a detailed evaluation of polymyositis: Inflammation, fat infiltration and replacement of muscle. *International Society of Magnetic Resonance in Medicine 13th Annual Meeting and Exhibition; 2005; Miami, FL.* 2005.

60. Zraiskaya T, Kumbhare D, Noseworthy MD. Diffusion tensor imaging in evaluation of human skeletal muscle injury. *J Magn Reson Imaging*. 2006 Aug; 24(2):402–8. [PubMed: 16823776]

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Figure 1.

Illustration of fusiform (left) and bipennate (right) architectures. In fusiform muscles, the fibers are generally oriented parallel to the muscle's longitudinal axis. The fibers of the bipennate muscle insert into a central aponeurosis. In the muscle at right is also illustrated the measurement of a planar pennation angle, θ , as the angle between the local tangent to the muscle fibers and the local tangent to the aponeurosis. The fusiform architecture causes the muscle at left to be optimally designed for high shortening velocities, while pennation causes the muscle at right to be optimally designed for force production. This figure has not previously been published.

Figure 2.

Demonstration of utility of DTI-based muscle fiber tracking for detecting the bipennate architecture of the human TA muscle. The panel at left illustrates the placement of a planar ROI straddling the TA's central aponeurosis; the panel at right illustrates the fiber tracts emerging from this ROI. [Reproduced in part from Figure 7 of *J Magn Reson Imaging*, 24(1), 2006, 182–190 (Ref. [12]), with permission from the author and Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.].

Figure 3.

Example muscle fiber tracking result from the human TA muscle. Two axial slices through the leg are shown. The central blue structure is a 3-D mesh representation of the TA's central aponeurosis. The gold and green lines represent fiber tracts, and also illustrate the bipennate muscle structure. Color variations in the fiber tracts exist only for contrast. This figure has not previously been published.