Diffusion imaging of mouse skeletal and cardiac muscle

Gustav J. Strijkers, PhD

Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, PO Box 513, 5600 MB, Eindhoven, the Netherlands
g.j.strijkers@tue.nl

In the last decade diffusion imaging of skeletal and heart muscle has developed into a powerful technique to characterize muscle architecture as well as to provide insight in local histopathological status of muscle cells. The function of muscle is to provide movement and generate force. This can be used for locomotion, limb or eye movement, and also to enable the function of internal organs, such as heart contraction or peristaltic of stomach and intestines. To facilitate these diverse sets of activities mammals have three different types of muscles, viz. skeletal muscle, cardiac muscle and smooth muscle, which have different functions and structure. Most skeletal muscles are connected to bones, and as such they are of vital importance in posture and locomotion being the “motors” of the skeleton. Contraction of the striated muscle fibers in the heart propels the blood through the circulatory system. Smooth muscle is a non-striated muscle type, mainly present in internal organs, and serves many functions related to contraction of hollow organs such as blood vessels, the gastrointestinal tract and the bladder.

Because muscle tissue is such an abundant tissue, pathologies affecting muscle structure and function usually have a direct impact on the quality of life. Neuromuscular diseases comprise a large number of clinically and diverse disorders affecting skeletal muscle and their nerves. Examples of common neuromuscular disorders are dystrophies (e.g. Duchenne and Becker Muscular Dystrophy), motor neuron diseases (e.g. Amyotrophic Lateral Sclerosis), and metabolic diseases (e.g. Type-2 diabetes). Even more so, malfunction of the heart, for example as a consequence of myocardial infarction, hypertrophy or heart failure is a life-threatening condition. Likewise there are many other pathologies affecting the heart that lead to a severely diminished quality of life. Skeletal muscle weakness is a debilitating condition and in extreme cases skeletal muscle dysfunction is even life threatening, for example when respiratory muscles are affected. Non-invasive imaging techniques to evaluate muscle structure, function and metabolism are of tremendous importance for diagnosis and characterization of various muscle pathologies, as well as for the development and evaluation of treatment strategies.

Diffusion imaging is a specialized MRI technique that is particularly suited to study muscle structure. Diffusion imaging provides quantitative information on muscle geometry, which is the main mechanical determinant of muscle performance, and on the local histopathological status of muscles to monitor disease progression or the effect of physical exercise or a pharmacological intervention. Diffusion MRI, like all other versions of MRI, is a non invasive technology that provides a full three dimensional overview of the tissue of interest. Most of the information gained by diffusion MRI is unique, in the sense that no alternative procedures exist that deliver similar insights.

Skeletal muscle

In most vertebrates, muscles consist of contractile fibers, which are connected to bones via non-contractile collagenous fibers, lying in tendons and tendon plates. Muscle geometry is a main determinant of muscle ability to generate force and to actuate movement. Often, muscles are modeled rather like a parallelogram. The most important
geometrical parameters are: cross sectional area, muscle fiber length, muscle volume, pennation angle, moment arm and distance from insertion on the bone to the joint. The total cross section of a muscle perpendicular to the fibers at optimal muscle length is called the physiological cross sectional area (PCSA), which is proportional to the muscle strength. Determination of PCSA is most relevant for mechanical evaluation of muscles in individual subjects, e.g. in muscle modeling and evaluation of interventions such as training studies. However, PCSA is also the parameter that is the most difficult to determine experimentally, and is therefore normally indirectly determined from the muscle volume and fiber length. Fiber length and pennation angle may be determined by ultrasound and anatomical reconstruction methods. However, both of these techniques have severe limitations, as ultrasound does not account for spatial variation in fiber length and orientation within a muscle. The disadvantage of traditional anatomical reconstruction techniques is that they are restricted to ex vivo preparations, which precludes their use in longitudinal studies. DTI provides an alternative, noninvasive method used to repetitively measure the skeletal muscle fiber structure allowing for the reconstruction of whole muscles yielding the desired architectural parameters, including the PCSA.

Because of the success of diffusion imaging in the brain in obtaining early readouts of tissue damage, e.g. after stroke, there is increasing interest in using diffusion imaging in skeletal muscle to provide non invasive measures of the histopathological status of muscle tissue. Commonly, contrast in muscle MRI is obtained via traditional contrast mechanisms, such as T1- and T2-weighted MRI, complemented with fat suppression techniques. Because of its sensitivity to the muscle cell microanatomy, diffusion imaging would possibly contribute to provide additional and more specific readouts of tissue status in terms of relevant histological characteristics like inflammation, necrosis, regeneration, fatty infiltration, and fibrosis.

Diffusion imaging measurements of small rodent skeletal muscle can conveniently be performed with a standard two-dimensional multi slice diffusion weighted spin-echo sequence, which guarantees a high signal-to-noise ratio and artifact free diffusion weighted images. Three dimensional diffusion weighted spin echo imaging can be used when high resolution is needed, at the expense of very long acquisition time. Heemskerk et al. have introduced a three dimensional diffusion weighted fast spin echo sequence that allowed for in vivo high resolution DTI of mouse hind limb within approximately two hours at a resolution of 234x234x234 μm³ (1). Frequently a standard multi slice diffusion weighted spin-echo sequence is used for in vivo diffusion imaging of rodent skeletal muscle.

Non-spatially resolved diffusion NMR in skeletal muscle dates back to the seventies, when first papers reported on restricted diffusion and diffusion anisotropy in skeletal muscle (2-6). Diffusion measurements in skeletal muscle regained interest in the nineties, when MR imaging hardware was developed up to a standard that allowed reliable diffusion-weighted and diffusion tensor imaging.

Diffusion tensor imaging and fiber tracking to determine muscle architectural parameters were first applied to study small animals. Van Doorn and coworkers reported on DTI to determine muscle fiber orientation in cat semimembranosus muscle (7). In a seminal paper by van Donkelaar et al. DTI was applied to fixed rat lower leg (8). Fiber directions based on the principal eigenvector of the diffusion tensor showed a high degree of correspondence with high-resolution T2-weighted images. By careful comparison with histology, it was shown that the fiber directions could be determined with sufficient accuracy to serve as input for a biomechanical finite-element simulation of muscle contraction. Subsequently Damon and coworkers demonstrated the ability of fiber tracking to measure muscle pennation angles quantitatively (9). In this study of rat gastrocnemius muscle, DTI was performed in vivo after which the animals were
sacrificed to allow for a visual comparison of fiber tracts and pennation angles with photographs of the muscle anatomy. Heemskerk et al. applied a three-dimensional diffusion-weighted fast spin echo technique, which permitted high-resolution diffusion tensor in vivo imaging of mouse skeletal muscle within reasonable measurement time (1). The technique allowed for a fiber reconstruction of the complete lower hind limb muscle architecture. Additionally, physiological cross-sectional area (PCSA), fiber length, and pennation angle for the tibialis anterior (TA) were determined. In a follow-up study the technique was used to measure mouse muscle fiber architecture for different ankle angles, which demonstrated that DTI and fiber reconstruction offer valuable tools in biomechanical research of muscle anatomy and function (10).

Recently, Heemskerk et al. applied a multi-spectral approach using a combination of quantitative T2 imaging and DTI to study mouse muscle degeneration and regeneration after ischemic injury inflicted by femoral artery ligation (11). Histological analysis revealed that this approach allowed for differentiation between areas with large, swollen cells and areas with high levels of necrosis and fibrosis. This study supports the power of multi-parameter imaging for determining muscle tissue status.

Cardiac muscle

For the left ventricle, it is generally accepted that the myocardial fibers display a gradual change of direction from the endocardium to the epicardium. Seen from the apex, the subepicardial fibers follow a left-handed helical path, while the subendocardial fibers follow the path of a right-handed helix. In the midwall region the fibers are oriented mainly circumferential (12). The local fiber orientation with respect to myocardial wall is commonly characterized by the helix angle and transverse angle.

DTI has been used extensively to study the myocardial architecture of mainly the left ventricle. The primary, secondary and tertiary eigenvectors of the tensor have been proven to correspond to myofiber, sheet plane and sheet normal directions, respectively (13-16). MR diffusion imaging is therefore extremely suited to study the myocardial architecture non-destructively in whole-heart specimens and has been used to both corroborate as well as oppose several of the conflicting models described above.

Studies aiming to understand structure and function relations of the healthy heart form the basis for understanding cardiac adaptation and remodeling as a consequence of pathological conditions, such as acute and chronic myocardial infarction, hypertrophy, various forms of heart failure as well as cardiac dyssynchrony. The profound changes in cardiac function and geometry induced by such pathologies can be studied using a combination of functional cardiac MRI and diffusion MR methods. Classical histological characterization is extremely time consuming and restricted to small sections. MR diffusion imaging, on the other hand, is in essence non-destructive, and therefore provides a powerful tool to study adaptation and remodeling in pathological areas as well as in adjacent and remote myocardium as a whole.

In animal models, diffusion imaging is restricted to ex vivo imaging of fixed excised hearts. Since here motion artifacts are not an issue, any two- or three-dimensional diffusion-weighted imaging technique can be used, such as 2D diffusion-weighted spin-echo (13), 3D diffusion-weighted spin-echo (17) and diffusion-weighted EPI (18). Provided the hearts are suitably fixed and immobilized, measurements may take 12 hrs or longer in order to optimize signal-to-noise and resolution. For these reasons, most myocardial diffusion MRI studies have been carried out on excised post-mortem specimens.

In a number of studies the detailed left ventricular architecture was quantified in ex vivo specimens of healthy animals, including rabbit (19), goat (20), mouse (17,21), rat (22),...
swine (18,23), dog (24), sheep (25) and human (26). There is general consensus that across species the fiber helix angles vary continuously from about +60° to -60° from endocardium to epicardium. From apex to base, the subepicardial fibers follow a left-handed helical path and the subendocardial fibers a right-handed helix, while in the midwall region the fibers are oriented mainly circumferential. The midwall transverse angle ranges from approximately -10° at the base to about +10° near the base. Changes in myocardial architecture as a consequence of myocardial infarction were studied in various species, including rat (27), sheep (28), swine (29,30) and humans (31). From these studies it is clear that changes in myocardial architecture are difficult to quantify, because the heart undergoes rather large morphological changes that prevent a straightforward analysis in terms of helix and transverse angles. Furthermore changes are likely dependent on infarct size, time and possibly on species.

A number of studies have focused on DTI as a means to characterize tissue microstructure and myocardial fiber architecture as a consequence of myocardial infarction. After myocardial infarction, cardiomyocytes are replaced by scar tissue that lacks contractile function. A remodeling process is initiated during which the non-contracting tissue expands and becomes thinner, leading to left-ventricular dilatation. Compensatory mechanisms result in hypertrophy of remote areas. Hsu et al. studied changes in diffusion parameters after acute regional ischemia in isolated perfused rabbit hearts (32). The ADC of the ischemic region displayed a gradual decrease over a period of 160 min, while the ADC in remote areas remained constant. Increased ADC accompanied with decreased FA with respect to remote regions was observed in the infarct regions in fixed rat hearts four weeks after surgery (27) and in fixed swine hearts 13 weeks after induction of the myocardial infarction (30). These changes can be attributed to necrosis, loss of cellularity and edema. In contrast, reduced ADC values accompanied with increased FA were observed in the infarct regions of fixed mice hearts 7 and 14 days after myocardial infarction (33). The high FA in infarct regions was accompanied by a large degree of fiber disarray. As compared to remote regions, the ADC normalized at 28 days after infarction, while the FA remained higher. These observations were linked to the formation of structured collagen fibers in this infarct model.


