ARTICLE

Functional Magnetic Resonance Imaging of Muscle

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Muscle functional magnetic resonance imaging (MRI) is used to compare the relative involvement of different muscles recruited during exercise. The method relies on the activity-induced increase in the nuclear magnetic resonance transverse relaxation time (T_2) of muscle water, which is caused by osmotically driven shifts of fluid into the myofibrillar space. In addition to imaging of whole muscle recruitment, muscle MRI may reveal changes in motor unit organization during disease. **Keywords:** skeletal muscle recruitment, nuclear magnetic resonance, muscle metabolism

INTRODUCTION

The availability and sophistication of magnetic resonance imaging (MRI) scanners have increased enormously during the last decade. MRI is the now the method of choice for clinical imaging of most soft tissue pathologies, including sports-related injuries of muscles and joints. Furthermore, because MRI is completely noninvasive and does not depend on ionizing radiation, exercise scientists increasingly use it for basic and applied morphometric studies of human subjects. For example, MRI can easily measure the effects of exercise training on muscle and fat volume and the inflammation associated with delayed-onset muscle soreness. However, the potential of MRI for exercise science extends beyond the mere visualization of anatomy. Using specialized techniques, MRI can noninvasively measure vessel blood flow and tissue perfusion (8) and map the velocities of muscle and tendon motions (3). Most remarkably, MRI enables real-time or near real-time "functional imaging" of both muscle and brain.

Functional MRI refers to imaging not only the anatomy of a tissue but also the extent to which the tissue is involved in performing some task. Functional MRI of the brain was first reported in 1992 and has already been applied in hundreds of studies, including basic studies of motor control. The purpose of this review is to introduce the less well-known and less well-understood technique of muscle functional MRI.

The basic phenomenon of muscle functional MRI is illustrated by Figure 1. Figure 1A is an axial image across the upper arm of a healthy human subject at rest. Figure 1B is an image acquired from the same location 3 min after the subject performed 30 repetitions of a "military press" (i.e., extension of a barbell from behind the neck to over the head). Note that the medial and lateral heads of the triceps muscle are "bright" compared to the other arm muscles after the exercise. The obvious implication of these images is that the bright portions of the triceps muscle were recruited during the exercise, and therefore that the image acquired after the exercise can be interpreted as a "recruitment" image. This activity-induced increase in intensity is detectable after as few as two contractions and rises to a work-rate dependent plateau within a few min (7). Recovery of the phenomenon after exercise takes 20 min or more (4,11), so it is quite possible to acquire functional images after performing a task outside the scanner room.

This phenomenon was first reported by Fleckenstein et al. (5) in 1988. Soon thereafter, Fisher et al. (4) suggested that it could be exploited as a noninvasive, quantitative measure of muscle recruitment. Despite continuing controversy surrounding the idea, muscle MRI has already been used to examine changes in recruitment during various exercises (e.g., 15) and after exercise training (9). Furthermore, at least one study recently implied that spatial variations in motor unit recruitment within a muscle can be measured by MRI (14). In this article, we will explore the validity of these applications by considering three questions: 1) what is the cause of the phenomenon, 2) how is it related to muscle recruitment, and 3) can it yield any information about motor unit distributions?

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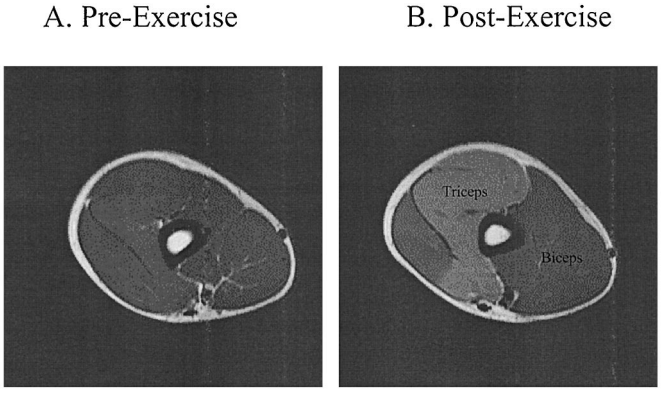

Figure 1 Axial MR images (TE = 50 ms, TR = 1.5 s, 16 cm field-of-view, 1 cm thick slice) across upper left arm of a healthy subject before (A) and after (B) performing elbow extension exercise.

BASIS OF MUSCLE FUNCTIONAL MRI

Why does muscle look bright in MR images after exercise? This is impossible to explain without a brief digression into nuclear magnetic resonance (NMR) physics. The NMR signal from which MR images are constructed arises from the magnetic behavior of the hydrogen nuclei in tissue water and fat molecules when the tissue is placed in a strong magnetic field. Inside a strong magnet, the hydrogen nuclei can be excited by the input of energy, specifically, by a "pulse" of energy at the resonant radiofrequency. This excitation causes a fraction of the nuclei to oscillate *together* (phase-coherent oscillation) in the magnetic field in an orientation that generates a detectable magnetic signal that can be recorded electronically. As long as the nuclei continue to oscillate in synchrony, the signal can be observed and can be manipulated to form an image. However, immediately after excitation, nuclei in different magnetic environments begin to oscillate differently, the phase-coherence begins to break down, and the observed signal decays away. Decay of the NMR signal because of loss of phase coherence is referred to as "transverse" or "T_2 relaxation. " T_2 is the time constant that characterizes the exponential decay of the signal after the initial excitation. In simple water solutions, T_2 relaxation is a single exponential process [i.e., $SI(TE) = SI(0) * \exp(-TE/T_2)$, where SI (0) is the signal intensity immediately after the exciting pulse, and SI(TE) is the signal intensity at time TE after the initial pulse, or the "echo-time" in a typical imaging sequence].

Thus, the signal intensity of tissue in an MR image depends on the imaging echo time, TE, and on two properties of the tissue: the concentration of hydrogen nuclei in the tissue, which determines SI (0), and the T_2 of those hydrogen nuclei in the magnetic environment of the tissue. For example, the humerus is black in Figure 1 because bone contains relatively little hydrogen compared with fat or muscle. The humerus bone marrow and subcutaneous fat are brighter than muscle in Figure 1 because the relaxation of fat hydrogen is slow (T_2=50 ms) compared with the relaxation of muscle water hydrogen (T_2= 28 ms). (Note: This description ignores another relaxation process, T_1 relaxation, which also profoundly effects image intensity, but it is not crucial for understanding muscle functional MRI.)

It is now well established that the increased MR signal of muscle during activity is caused by an increase in the T_2 of muscle water and *not directly* by an increase in water concentration. This was demonstrated by acquiring images at many different TE values and extrapolating to the intensity at time zero (10). Exercise results in slower decay of the signal, but has no effect on the extrapolated initial intensity, or water concentration. After intense exercise, T_2 of muscle water can increase by up to 12 ms (from about 28 to 40 ms). This slower relaxation results in the "brightness" of recently active muscle compared with resting muscle in images acquired at long TE, as illustrated by Figure 1.

The main causes of local magnetic field variations (and, hence, T_2 relaxation) in concentrated protein solutions such as muscle are magnetic interactions between neighboring molecules. In pure water, molecular motions are so fast that these magnetic interactions are averaged, such that all molecules experience nearly the same effective magnetic environment. However, in regions where the motion of water is restricted (for example, by hydrogen bonding around proteins and other macromolecules), these interactions are not averaged, and phase dispersion and signal decay results. Once phase coherence is lost by random molecular interactions, it cannot be restored (except by starting over again with a new exciting pulse). Unfortunately, there are not yet any comprehensive theories that quantitatively predict the T_2 relaxation of water in a protein matrix such as the cytoplasm of muscle. The experimental observation is simply that the relaxation of water in protein solutions depends strongly on protein concentration. It is this observation that leads to the conventional view that a major determinant of T_2 in muscle and other tissues is water content.

An additional layer of complexity is added by the fact that muscle is not a uniform protein matrix; rather, it contains intracellular and extracellular membranes that impede the diffusion of water. If the exchange of water between membrane-bound compartments is not fast compared with the intrinsic T_2 of water in the compartments, the T_2 relaxation will exhibit multiexponential behavior. Thus, the T_2 decay of muscle is often reported to include a slow-relaxing, long T_2 component corresponding to the extracellular fluid. At one time, it was thought that the increase in muscle T_2 observed after exercise could be explained by an increase in the volume of the slower-relaxing extracellular fluid. However, this hypothesis is excluded by the fact that expansion of the extracellular fluid without exercise does not mimic the effect of exercise on T_2 (10). Thus, the T_2 increase is clearly linked to activity of the muscle cells.

Considering the above, what physiological change explains the increase in muscle T_2 during activity? The simplest explanation is that the accumulation of osmolites (phosphate, lactate, sodium) in the cytoplasm during activity results in the influx of fluid, "diluting" the effect of the myofibrillar proteins on water relaxation. There are many correlative observations consistent with this hypothesis. For example, there is a correlation between total (but not extracellular) muscle volume and T_2 both during and after exercise (e.g., 4). In rodent muscles stimulated at constant, moderate rates, the T_2 change is greatest in muscles with the lowest aerobic capacity and highest ATPase rate, which

produce the greatest osmotic load during stimulation (13). Treatments or conditions (e.g., McArdle's syndrome) that result in decreased metabolite production tend to diminish the T_2 change. Compared with isometric contractions, eccentric contractions result in a smaller T_2 increase, and dynamic contractions result in a greater increase, as would be expected from the relatively smaller and greater energetic demands of these contraction modes (11). However, despite these observations, the phenomenon cannot be explained solely by an increase in total muscle water, because T_2 increases substantially even during ischemic contractions, so influx of extramuscular fluid is not required. We are left with the somewhat vague conclusion that the T_2 increase results from osmotically driven fluid shifts which alter the mobility or average magnetic environment of myofibrillar water.

DOES MUSCLE MRI MEASURE MUSCLE RECRUITMENT?

It should be clear from the above that MRI does not measure muscle electrical activity directly; rather, it portrays a complex function of muscle cell metabolism and fluid uptake. Because both metabolic capacity and perfusion can vary between muscles of different subjects, or even between different muscles of the same subject, what is the rationale for using MRI to measure recruitment? Empirical studies consistently demonstrate a correlation between whole muscle contractile rate, work intensity, or integrated root-mean-square electromyography (EMG) versus the observed T_2 increase in human muscles. Many such studies have been reported (see ref. 12 and the citations therein), although the details of the exercise vary greatly between studies.

The most common practical application of muscle functional MRI is to compare the relative recruitment of different muscles or muscle groups during various motor tasks. EMG is not well suited for simultaneous measurements from many muscles, some of which may lie deep under the skin. For example, Conley et al. (2) used MRI to investigate the involvement of various neck muscles during head motions. Similarly, Richardson et al. (15) used MRI to demonstrate differences in the recruitment of thigh muscles in bicycling versus knee extension exercise. Of course, these comparisons implicitly assume that the relationship between T_2 and recruitment is not markedly different in the muscles under study. Furthermore, because the images are typically acquired several min after the exercise, these comparisons also assume that the recovery of T_2 after exercise is equally slow in all the muscles. However, inasmuch as the conclusions of these MRI studies are consistent with previous electrophysiological and anatomical studies of the same muscles, it seems that this sort of semiquantitative, within-subject comparison is justified. By extension, it should be straightforward to use functional MRI to detect aberrant patterns of muscle recruitment (e.g., in stroke patients or after peripheral nerve injury). Similarly, it might be possible use MRI to investigate whether atypical patterns of muscle use contribute to repetitive motion or other occupational injuries. Surprisingly, few such medical applications have been reported so far.

The use of MRI to compare the intensity of recruitment between muscles of different subjects is less established. Considering the underlying metabolic basis of the phenomenon, it is likely that the increase in muscle T_2 depends on work rate relative to muscle peak aerobic power rather than on absolute work rate. Thus, the correlation between T_2 and contraction rate is stronger within a single subject than across subjects (7). For example, suppose two subjects with the same size biceps muscles performed an identical biceps curl exercise, and the T_2 increase was found to be greater in the biceps of one subject than in the other? From that measurement alone, it would not be appropriate to conclude that the fractional recruitment of biceps muscle cells was greater in one subject than the other, because the subjects might also differ in metabolic capacity. Similarly, it would difficult to conclude from MRI measurements that recruitment intensity had decreased in a subject after an exercise-training program, because the training might also alter the metabolic profile and vascular dynamics in the muscle. In such cases, the MRI results by themselves would imply only that the metabolic stress was relatively less in the muscle of one of the subjects, or after the exercise training.

Thus, just as brain functional MRI can complement but not replace electrical measurements of neuron activity, muscle functional MRI can complement but not replace quantitative EMG as a direct measure of muscle activity. In both cases, the advantage of MRI lies not in its specificity or quantitative accuracy but in its much greater spatial resolution. Compared with surface EMG, the most novel feature of muscle MRI is the potential to map spatial variations in activity *within* a muscle, and thereby perhaps gain insight into the spatial distribution of the muscle fibers recruited during a task.

CAN MRI MAP MOTOR UNIT ACTIVITY?

The first application of MRI to map spatial variations of activity *within* a muscle was by Adams et al. (1), who measured activation in human leg muscles after electrical stimulation. More recently, Hillegass and Dudley (6) monitored the extent of muscle activation during electrical stimulation of thigh muscles in patients with spinal cord injury. In both studies, the spatial dependence of activation on placement of the stimulating electrodes was easily visualized, and peak force during stimulation was nicely correlated with the cross-sectional area of muscle showing elevated T_2. Of course, during direct electrical stimulation, muscle activation depends on the macroscopic flow of current through the muscle, not on motor unit recruitment. Can MRI also detect spatial variations within human muscles during voluntary activity, when activation does depend on motor neuron recruitment? The answer depends on the spatial distribution of the recruited motor units and on the density of innervated fibers within the motor unit territories.

The planar spatial resolution of standard clinical MR images (around 0.6×0.6 mm, or 0.4 mm^2 per pixel) is crude compared with the size of single muscle fibers (~0.004–0.005 mm^2), so each pixel in an image includes around 100 muscle fibers. In human limb muscles, a "typical" motor

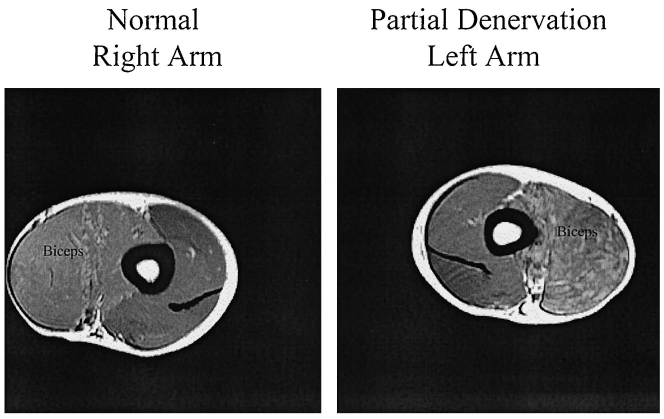

Figure 2 Images (TE = 30 ms, TR = 1s, 16 cm field-of-view, 1 cm slice) acquired after moderate biceps curl exercise in the left and right arms of a 26-yr-old subject with obstetric brachial plexus palsy (i.e., partial denervation at birth) in the left arm. Note the blotchy nature of the signal increase in the affected left compared with the normal right arm after exercise.

neuron innervates around 400 fibers, or about 5% of the cells within a circular unit territory roughly 7 mm in diameter perpendicular to the long axis of the fibers. Thus, the territory of a single motor unit is spread over several dozen pixels in a typical image. The remaining 95% of the fibers in that territory are innervated by 25 or so other motor neurons with overlapping territories. Considering this sparse fiber density and overlapping arrangement of unit territories, it is unlikely that MRI could distinguish the activity of single motor units in healthy muscle. In fact, analysis of the pixel T_2 variance and noise in MR images after exercise indicates that "active" versus "inactive" muscle areas cannot be resolved on a pixel-by-pixel level by simple threshold methods in healthy muscles [i.e., using images acquired on a standard clinical scanner (12)].

If single motor unit activity cannot be resolved, then subregions of activity within a healthy muscle will be observed by MRI only if the location of motor units in the muscle is correlated with recruitment order or varies with the exact nature of the motor task. In hind limb muscles of many animals, there is a correlation between recruitment order and location of motor units; consequently, those muscles exhibit a clear predominance of slower, more aerobic fibers in the region most frequently recruited. However, there is little electrophysiological or histological evidence for such spatial ordering of recruitment in healthy human muscles. Although no study has systematically examined this issue, there is not yet any MRI evidence that subregions within anatomically defined human muscles are recruited preferentially at different exercise intensities. On visual inspection, the T_2 increase appears to be grossly uniform within the recruited muscles of healthy subjects.

Despite the inability to resolve single unit activity in healthy muscles, there is hope that MRI can detect pathological changes in motor unit distribution. A characteristic of many neuromuscular diseases (e.g., amyotrophic lateral sclerosis, postpolio syndrome, diabetic neuropathy) and of advancing age is the gradual loss of motor neurons. However, muscle fibers that were innervated by the lost neurons very frequently do not die, but are instead reinnervated by branches from other motor neurons with overlapping unit territories. The result of this denervation/reinnervation cycle is muscles with fewer motor units but with higher fiber densities and less overlap between territories. These changes are presently diagnosed from fiber-type clumping in muscle biopsy samples or from unusually large EMG motor unit potentials. Recent computer simulations suggest that the same pathological changes might be detected from an increase in T_2 variance, or "clumpiness" of the T_2 response after a mild exercise (12). Figure 2 shows a case in which compensated motor unit loss clearly altered the homogeneity of the T_2 response to mild exercise in the affected limb. If this is a general phenomenon, functional MRI may well enable noninvasive diagnosis of motor unit loss in human muscles.

In sum, muscle functional MRI is well-suited for examining normal and abnormal patterns of muscle recruitment within individuals during exercise and may prove useful for diagnosing and monitoring the progression of motor neuron disease.

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