TECHNICAL NOTE

Effect of Muscular Contraction on Magnetization Transfer Detected at 1.5T

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The effect of rigor (formation of actomyosin complexes) on magnetization transfer was observed with a 1.5T clinical magnetic resonance (MR) imaging system. The magnetization transfer ratio of chemically skinned (calcium-sensitive) muscle fiber preparations increased much more in a rigor state than in a relaxed state, while that of calcium-insensitive fiber preparations and solutions showed no difference. These results suggest that the formation of actomyosin complexes increased the magnetization transfer ratio. A clinical MR system is not only effective for medical imaging, but also has the potential to demonstrate physiological characteristics.

Keywords: MRI, magnetization transfer, muscle contraction, actomyosin

Introduction

Magnetic resonance (MR) imaging is widely used in musculoskeletal imaging. Relaxation time has also been used for muscle fiber characterization and for the detection of aging effects.1-5 Magnetization transfer contrast (MTC), which is often used in the fields of neuro-imaging, 6-8 is a sensitive parameter for physiological characteristics because it relates the exchange of magnetization between the free and restricted hydrogen protons in the tissue.6,8

The muscle contraction-relaxation cycle—the actin-myosin interaction—in the vertebrate-striated muscle is regulated by calcium ions through regulatory proteins: troponin and tropomyosin. The molecular mechanisms of muscle contraction are one of the most challenging areas of physiology. However, few studies have been reported that investigate the molecular mechanisms of muscle contraction with MR imaging.

Several studies regarding conformational changes due to actin-myosin interaction have been reported, and their results suggest that the actomyosin complex has hydrophobic interactions at its center and ionic and polar residues around the center.9 We considered that the formation of actomyosin complexes probably affected the ratio of free to restricted hydrogen protons around the complex and that this effect could be detected through clinical MR imaging with MTC.

To test this hypothesis, the effect of rigor (formation of actomyosin complexes) on magnetization transfer was observed by comparing the magnetization transfer ratio (MTR) of the fiber preparations in a relaxed with those in a rigor state. We used chemically skinned muscle fiber preparations. This preparation offers two advantages: calcium ions in a solution are able to bind troponin C directly on a thin filament; and muscle states can be controlled between relaxation and contraction, or rigor, with a change in ingredients in a solution.10,11 We also used trans-1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid (CDTA)-treated chemically skinned fiber preparations as a control, as they are calcium insensitive following the removal of troponin C components from the thin filaments.10

Materials and Methods

Fiber preparation

Female Japanese albino rabbits were anesthetized with an intravenous injection of sodium pentobarbital and sacrificed. The animal experiment was approved by our hospital’s Animal Use and Care Committee.

Bundles of psoas muscles were dissected and soaked in a solution containing 100 mM KCl, 20 mM 4-morpholinepropanesulfonic acid (MOPS) (pH 7.0), 5 mM MgCl2, 2 mM ethylenbis (oxo-nitrilo) tetraacetic acid (EGTA), 2 mM ATP, 1 mM dithiothreitol and 1% Triton-X100 for one day at 4°C. These bundles were then transferred to a

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solution of the same composition but containing 50% (by volume) glycerol instead of 1% Triton-X100, left for one day at 4°C, and stored at −20°C. After about three weeks of incubation, the samples were used in the experiments.

**Fiber treatment**
Small bundles of muscle fibers (5–8 mm in thickness, 15–20 mm in length, and about 0.4 g in weight) were dissected from glycerinated muscle bundles and tied to plastic tubes. For the preparation of calcium-sensitive fibers, bundles were washed in a 100-ml solution (washing solution) containing 100 mM KCl, 20 mM MOPS (pH 7.0), 5 mM MgCl₂, 2 mM EGTA, and 1 mM dithiothreitol for 45 min at 4°C. This procedure was repeated three times, after which MR imaging was performed. In contrast, for calcium-insensitive fiber preparation, fiber bundles were washed in a 100 ml volume of washing solution for 45 min at 4°C, a procedure that was repeated twice. Bundles were incubated subsequently in a solution containing 5 mM CDTA and 20 mM tricine (pH 8.4) for one hour at 25°C to remove troponin C on the thin filament and render the fiber calcium insensitive. The bundles were then washed in a washing solution for 45 min at 4°C. MR imaging was then performed. The schema of the experimental procedures is shown in Fig. 1.

**MR imaging**
MR imaging was performed at 25°C on a 1.5T whole-body MR system (Signa, General Electric Medical Systems, Milwaukee, WI) with a transmitting/receiving head coil, a field of view of 12 cm, a matrix of 256×128, a slice thickness of 5 mm, and an excitation number of one.

Following fiber treatment, each fiber bundle tied to a tube was placed in a 15-ml test tube containing 10 ml of solution comprising 100 mM KCl, 20 mM MOPS (pH 7.0), 5 mM MgCl₂, 2 mM EGTA, 1 mM dithiothreitol and 2 mM ATP at 25°C for 30 min (a relaxing condition). A test tube plate with three to six test tubes was subsequently placed at the center of a head coil. One spin echo image was obtained with a TR of 5000 ms and a TE of 20 ms, and one spin echo image with MT pulse was subsequently performed. The pulse shape, frequency offset, duration, and mean amplitude of the MT pulse were sinc function with no side lobes, 400 Hz, 16 ms, and 4.48 μT, respectively. MR images with and without MT pulse were obtained under the same scanning conditions, water resonance frequency, receiving attenuation parameters, and transmit attenuation parameters. In order to induce muscle contraction, an adequate volume of CaCl₂ was added to reach a 2 mM concentration (an activating condition). After 30 min, when almost all Mg-ATP in the solution was catalyzed and fibers attained rigor, MR images with the same sequences described above—spin echo sequences with and without MT pulse—were performed. The schema of the experimental procedures is shown in Fig. 1.

**Image analysis**
The region of interest (ROI) was set at each of the muscle bundles (4–8 mm² including 9–18 pixels) and at each of the solutions (8 mm²), and the signal intensity was measured. The signal intensities of the ROI on an image without an MT pulse (Mo) and that with an MT pulse (Ms) were obtained. The value of MTR was calculated as (Mo-Ms)/Mo.
Figure 2 shows a representative MR image.

Statistics
Using a pared t-test, we compared the MTR values of the fibers and the solutions in a relaxing condition and an activating condition.

Results
The MTR values of the calcium-sensitive fiber bundles increased significantly (P < 0.01) under the activating condition (rigor state) compared with those under the relaxing condition (relaxed state). In the calcium-insensitive fiber bundles and in the solutions, no significant difference in MTR values was observed between the activating and relaxing conditions (Table).

Discussion
Among the several methods of off-resonance saturation imaging, the method used most often with a high-field clinical MR system is the off-resonance pulsed method, mainly because of its specific absorption rate. The off-resonance pulsed method was also used in the present experiment. The exchange of magnetization between the free and restricted hydrogen protons is a primary mechanism of MT.6,8 The restricted hydrogen protons are those bound to macromolecular proteins or lipids.6,8 In the lower range of frequency offset (<300 Hz), however, direct saturation effects such as the spin-tip effect can occur irrespective of macromolecules.6

In this experiment, the MTR values of the calcium-sensitive fibers showed a larger increase in the rigor state than in the relaxed state. No significant change in the MTR values was observed in the calcium-insensitive fibers or in the solutions. These results suggest that the increase in the MTR values of the calcium-sensitive fibers was induced by the formation of actomyosin complexes, not by the direct saturation effect mentioned above.6 We presume that this can be explained by the following mechanism: the actin-myosin binding site appears
to be centered on hydrophobic interactions involving helices at the actin (Ala₁₄₄, Ile³⁴₁, Ile³⁴₅, Leu³⁴₉ and Phe³₅₂) and myosin surfaces (Pro⁵₂⁹, Ile³₅₅, Met⁵₄₁, Phe⁴₄₂ and Pro⁵₄₃), and around this site are complementary ionic and polar residues (Tyr⁶²₆-Gln⁶₄₇ in myosin and Asp¹, Glu¹, Asp³, Glu³ in actin for the first group; Lys⁵₆₇-His⁵₇₈ in myosin and Glu⁴⁹, Glu¹₀₀ in actin for the second group; and Pro⁴⁰₄-Lys⁴₁₅ in myosin and Pro³₃₂-Glu³₃₄ in actin for the third group). The conformation of the actin-myosin complex probably causes an interaction between ionic or polar residues present at the surface of the complex and the hydrogen protons around the complex and is likely to increase the MTR values. Another possible mechanism is the following: if the formation of actomyosin complexes induces the decrease in the extent of the solution present in muscle fibers through, for example, the mechanism by which the diameter of each fiber increases, the MTR value of each muscle bundle increases because that of the solution is low. Further examinations are required to clarify these mechanisms in detail.

The MTR values of the skeletal muscle in vivo are much larger than those shown in this experiment. This is attributable to the concentration of proteins in the chemically skinned fiber bundle, which is much lower than that of muscle in vivo. Other differences—such as imaging parameters (spin echo/gradient echo, TR/TE), MT pulse shape, frequency offset, duration and amplitude—also affect the results.

In a glycerinated fiber, cellular membranes and sarcoplasmic reticula are destroyed. Thus, the degree of muscle contraction is controllable by changing the concentration of calcium ions in the solution. These chemically skinned fibers are widely used in physiological studies. The effects and mechanisms of CDTA treatment have been reported elsewhere. In brief, it removes troponin C components from the thin filaments and renders the fibers calcium insensitive.

In summary, the effect of rigor (formation of actomyosin complexes) on MT is detectable with a clinical MR system. MR imaging is not only effective for clinical imaging, but it also has the potential to demonstrate physiological characteristics.

References