

Alterations of the Proton- T_2 Time in Relaxed Skeletal Muscle Induced by Passive Extremity Flexions

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Purpose: To demonstrate reciprocal changes of the apparent proton- T_2 time in the biceps and triceps due to passive contraction and extension of the muscle fibers.

Materials and Methods: The contraction state of the upper arm muscles of six healthy volunteers was passively changed by alternating the forearm position between the straight-arm position and an elbow flexion of 90°. The relaxation of the muscle during passive contraction and extension was measured with the use of muscle electromyography (EMG) experiments. Spin-echo (SE) MRI with increasing echo times (TEs) of 12–90 msec was used to acquire the averaged signal decay of the segmented biceps and triceps. The apparent T_2 was deduced using monoexponential least-square fitting.

Results: The median T_2 alterations in biceps and triceps among all volunteers were found to be 1.2 and –1.3 msec in the straight and bent forearm positions, respectively. The confidence intervals (0.5 to 1.7 msec in biceps, and –2.6 to –1.1 msec in triceps) clearly indicate that proton- T_2 in MR images is significantly ($P < 0.05$) prolonged with muscle contraction.

Conclusion: The observed increase of the proton- T_2 time was correlated with a passive contraction of skeletal muscle fibers. This passive effect can be attributed to changes in the intracellular water mobility corresponding to the well-known “active” T_2 increase that occurs after stimulation of muscle.

Key Words: skeletal muscle MRI; relaxometry; T_2 ; muscle contraction; water mobility; diffusion

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THE MEASUREMENT of the proton transverse relaxation time (T_2) in skeletal muscle is particularly useful for investigating muscle activity by NMR. It is known that the proton- T_2 time increases during exercise of the skeletal muscles (1). This effect has become an important factor in T_2 -sensitive MRI, in which signal intensity can be correlated to muscle recruitment during different motor tasks (2–4). Most of the literature data show that T_2 examinations on the timescale of conventional imaging protocols (10–200 msec) yield a monoexponential T_2 relaxation with values between 26 and 33 msec for resting in vivo skeletal muscle (2–9). This apparent muscle T_2 was found to increase up to 30% depending on the intensity and dynamics of the exercise involved (6–11). Although the physiological cause of the effect is not entirely understood, it is being exploited as a correlative means of measuring muscle activity in sports and rehabilitation medicine (12–15).

Intense research during the last few years has illuminated the relationship between the biomolecular environment of water protons and their T_2 relaxation in muscles. From ^1H -spectroscopy it is known that the T_2 relaxation of muscle tissue displays a multiexponential decay that consists of two (16), three (17), or more (18) distinct T_2 components. Some investigators also observed a biexponential muscle T_2 by MRI that consisted of one short T_2 ($T_2\text{-S}$) corresponding to the apparent T_2 , and one additional minor (<10%) and long (>100 msec) relaxing T_2 component ($T_2\text{-L}$) (5,8,19–21). This multi-component T_2 decay is widely ascribed to anatomical compartmentation of water molecules, i.e., the existence of extra- and intracellular water in the extra- and intravascular spaces (16–18,20,22). Since the blood-volume fraction is low (2–3%) in skeletal muscles (23), the compartmentation model suggests that the vast majority of the ^1H -NMR signal is related to the extravascular spaces. Correspondingly, the activity-induced increase of the apparent T_2 is also observable in the absence of blood flow (24,25). On the other hand, the $T_2\text{-L}$ component varies with changes in the blood oxygenation (20,26). Since blood volume and oxygenation state are related to muscle work, the $T_2\text{-L}$ component is sensitive to muscle activation (26,27) in a manner similar to that of a blood oxygen level-dependent (BOLD)-

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type signal contrast, which can be measured by muscle functional MRI (fMRI) (28–30). Since this intravascular effect can account for only a minor portion of the T_2 increase during muscle work, most of the information about physiological processes in activated muscles is carried by the T_2 -S component. The high sensitivity of this relaxation component to muscle activation is presumably due to complex reorganization processes of the intracellular water molecules rather than to shifts between extra- and intracellular fluids, which have been found to have only a limited influence on T_2 -S (5,8,21,27). The mechanism behind such reorientation is still speculative. One study showed that metabolism plays an important role in exercise-induced muscle modifications of MR images (31), which could further be assigned to intracellular water reorientation (27). Other investigators have proposed that this mechanism involves the release of protein-bounded water molecules during muscle contraction (1) or water diffusion governed by variable cell boundaries (32).

In vivo T_2 studies on skeletal muscle have illuminated the complex relationship between transverse proton relaxation and the metabolism, perfusion, and morphology (e.g., sarcomere length and filament overlap) of muscle fibers. In this study we sought to change the morphology of muscle cells without disturbing the equilibrium of the metabolites, and to measure the effect of that change (if any) on the apparent T_2 time in MRI. To avoid putting any work load on the examined muscles, their contraction state was passively changed by changing the position of the attached extremity. Axial spin-echo (SE) MRI images of the biceps and triceps of six volunteers were acquired, yielding T_2 maps of the flexor and extensor muscles in one image (i.e., the relaxation times of contracted and extended muscles were available simultaneously). By just changing the forearm position, the elbow was flexed and extended so that the contraction of the biceps and triceps was inversely affected. If the resting condition is properly maintained, such a simple experimental protocol allows T_2 variations to be investigated separately from metabolic processes.

MATERIALS AND METHODS

Figure 1 demonstrates the protocol of alternating positions between contraction and extension of the biceps and triceps in the right extremity of six volunteers in the prone position. The participants were asked to maintain a resting position with two distinct flexion states of the elbow. The angle between the forearm and upper arm was passively alternated between 90° and 180° (denoted in the following as positions 1 and 2, respectively, as shown in Fig. 1) by an operator. The extremities were supported during the entire experiment by the table of the MR scanner. There was a delay of at least three minutes between each position change and T_2 measurement.

To further ensure that the muscle was not activated during the position change of the forearm, electromyography (EMG) measurements were applied. Surface electrodes were placed over the midbelly of the long and short heads of the biceps and the long head of the

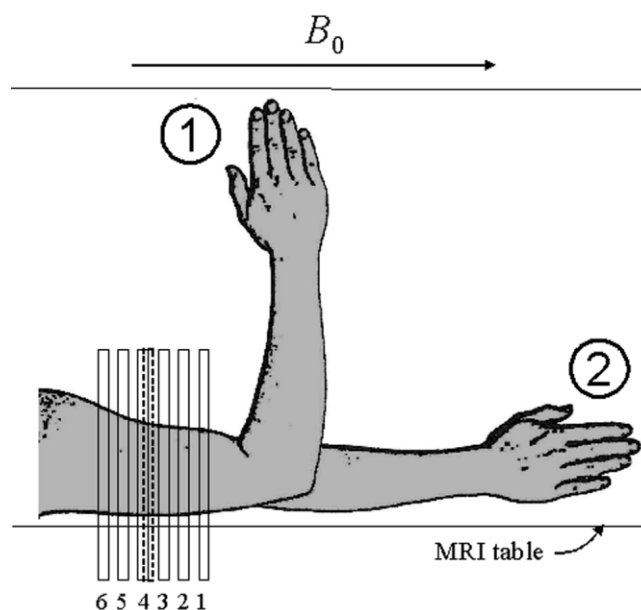


Figure 1. Sketch of the arm position during T_2 examinations of the biceps and triceps. The open boxes numbered 1–6 indicate the locations of the axial slices in the TSE experiments. The dashed box corresponds to the approximated slice position during the SE-EPI experiment.

triceps and fed into an EMG device (Keypoint EMG, Medtronic, Denmark) to record possible changes of the action potential induced by the extremity flexion (4).

The MRI protocol was based on two experiments, as described below:

1. For highly resolved sampling of the T_2 signal decay, an SE echo-planar imaging (EPI) sequence was used with variable echo lengths. A total of 29 echo times (TEs) were logarithmically incremented from 12 to 90 msec. The approximate slice position of the axial images is given in Fig. 1 (dashed box). The forearm position was passively altered 20 times between positions 1 and 2. A statistical analysis based on a Wilcoxon signed-rank test was used to calculate the confidence intervals and the significance of the T_2 changes (ΔT_2).
2. To cover the muscle volume by several image slices, and thus be able to estimate the position dependency of ΔT_2 , a turbo SE (TSE) experiment was applied that incorporated six distinct TEs of 11, 22, 34, 45, 56, and 79 msec. Six axial slices were acquired, which were numbered in ascending order from the distal to the proximal end of the arm (see Fig. 1). The experiments were repeated 10 times with interchanging arm positions between positions 1 and 2.

Experiments were performed on a 1.5 T scanner (Siemens Magnetom Sonata, Erlangen, Germany) using a standard extremity coil. The acquisition parameters for both protocols were: TR = 2 sec, matrix = 128×128 , 256×256 mm (SE-EPI); FOV = 150×150 mm (TSE), slice thickness = 10 mm.

The spatially resolved T_2 values were determined from decaying signal amplitudes of the SE-EPI and TSE images by a pixelwise linear regression on a logarithmical

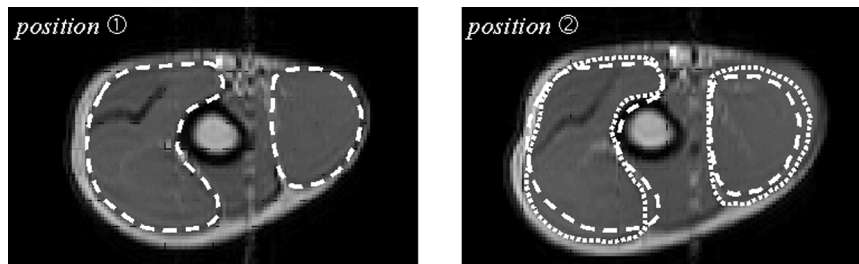


Figure 2. Anatomical TSE images (TE = 11 msec) of the upper arm of a volunteer with segmentation lines of biceps and triceps in the bent-arm (position 1: dashed lines) and straight-arm (position 2: dotted lines) positions. On the right-hand side the segmentation lines of position 1 are superimposed to demonstrate the increase of the effectively visualized CSA in both triceps and biceps with elbow flexion.

scale using a noise-correction as described by Hardy et al (9). The resulting T₂ maps were manually segmented with regard to the boundaries of the biceps and triceps based on short-TE magnitude images, so that fat and blood flow artifacts would be excluded from the regions of interest (ROIs; see Fig. 2). Furthermore, the T₂ values of the biceps and triceps were spatially averaged over the selected regions. Their variances were calculated by means of the T₂ distribution inside the segmented areas. The interindividual average of the results was based on the medians to avoid biases due to a non-normal distribution of the data.

RESULTS

The protocol of passively changing the muscle contraction was supported by EMG data acquired by surface electrodes on the biceps and triceps of two volunteers. It was found for both subjects that the EMG signal was approximately constant on a level of 100 μ V during passive forearm motion. In contrast, an active contraction of the muscle caused a significant EMG increase of slightly more than one order of magnitude. However, this decayed to the initial signal level within 10–20 seconds.

The observed T₂ signal decay of water protons in resting skeletal muscles is well represented by a monoexponential decay that results in a linear slope on a logarithmic scale (see Fig. 3). A higher number of independent fit parameters (e.g., using a biexponential model) did not improve the quality of the fit, as revealed by chi-squared tests. Therefore, one apparent T₂ was determined for each muscle with values ranging from 24 to 30 msec in all SE-EPI studies. The Δ T₂ medians of all volunteers, as determined by SE-EPI experiments, were 1.2 msec and –1.3 msec for biceps and triceps,

respectively. The confidence intervals (0.5 to 1.7 msec in biceps, and –2.6 to –1.1 msec in triceps) suggest that both T₂ changes are significantly ($P < 0.05$) different from zero. The direction of the T₂ alteration corresponds to the change of the forearm position from position 2 to position 1. The T₂ response to the position change is illustrated in Fig. 4. Figure 5 shows Δ T₂ plotted against the change in the cross-sectional areas (Δ CSA) of the biceps and triceps. Δ T₂ and Δ CSA are designated here as the changes in the quantities corresponding to position 1 minus those corresponding to position 2. It is visible in Fig. 5 that the muscle area increases between 4% and 30% in both the biceps and triceps of all volunteers. Thus, no correlation between Δ T₂ and Δ CSA was found.

The T₂ values measured by TSE experiments were found to be prolonged by approximately 5 msec compared to the SE-EPI experiments. This difference can be attributed to the characteristics of the CPMG-type TSE sequence, in which echo spacing can affect the T₂ dephasing of the magnetization (in contrast to the Hahn-echo type of SE-EPI sequence used) (9,33). Since our TSE experiments were intended to reveal relative effects as well as the slice-position dependency of Δ T₂, all TSE data were further analyzed as difference data.

The difference data of each subject and image slice position (Fig. 6a) correspond to the T₂ change revealed by SE-EPI. The interindividual average (1.1 ± 0.6 msec in biceps, and -1.2 ± 0.4 msec in triceps; Fig. 6b) indicates a similar range of T₂ changes as revealed by SE-EPI. Only a minor position dependency of Δ T₂ is deducible from Fig. 6, which is determined by a slight increase of T₂ in the vicinity of both muscle ends.

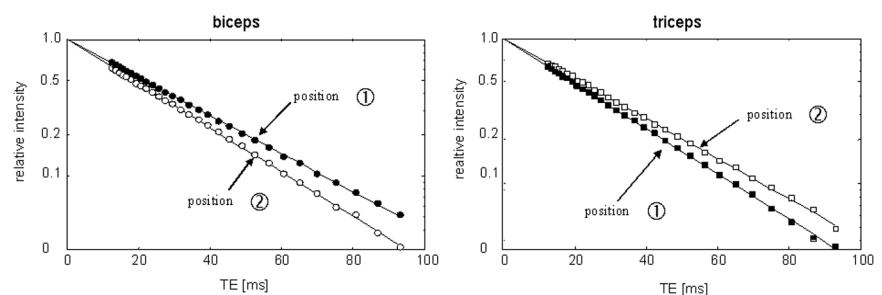


Figure 3. Experimental (SE-EPI) and simulated ¹H-T₂ dephasing in biceps and triceps of volunteer 6 with arm positions 1 and 2 corresponding to experiments 13 and 14, respectively, in Fig. 4.

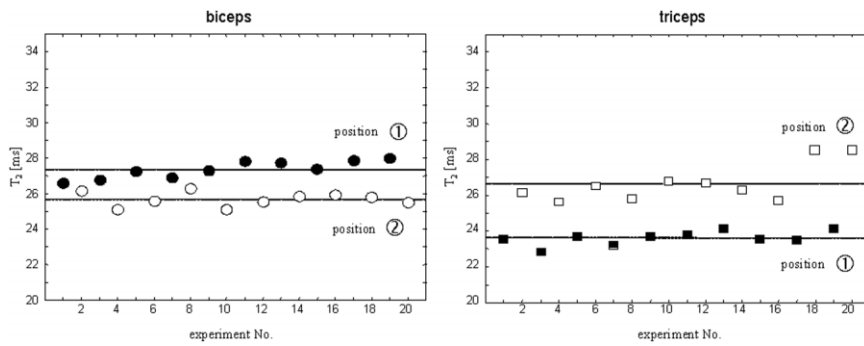


Figure 4. T_2 alterations determined by SE-EPI experiments in the course of the repeatedly performed elbow flexions of volunteer 6 for biceps and triceps. The solid lines indicate the mean of the data.

DISCUSSION

Our results from human biceps and triceps suggest that the apparent T_2 time in skeletal muscle significantly increases about 1–2 msec with passive muscle contraction. This principal behavior is demonstrated in Figs. 3 and 4, which show that the signal decays more slowly in biceps with a flexed elbow than it does in an extended straight-arm position. The inverse relaxation behavior is observed in the triceps. Here, the muscle acts as an extensor and thus it is contracted in position 1 and extended in position 2, vice versa to the contraction of the biceps. The alternation of the arm position consistently reflected the T_2 increase and decrease with muscle contraction and extension, as shown in Fig. 4.

Although the T_2 effect was found to be significant over all subjects, its magnitude exhibited larger individual variations in ΔT_2 from -0.4 to 1.7 msec (biceps) and -2.6 to -1.1 msec (triceps). These variations are not related to interindividual variations in the amount of contractile tissue measured by the anatomical cross section in the MR image. The change in the CSA (ΔCSA) with biceps contraction and triceps extension is shown in Fig. 5. The tendency of ΔCSA to increase in both the biceps and triceps is visible in Fig. 2. While this ΔCSA

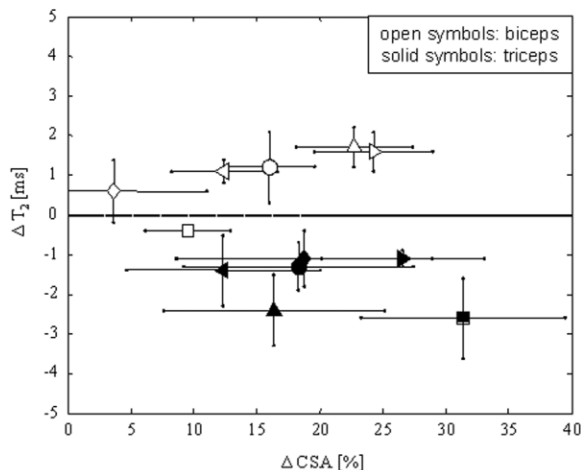


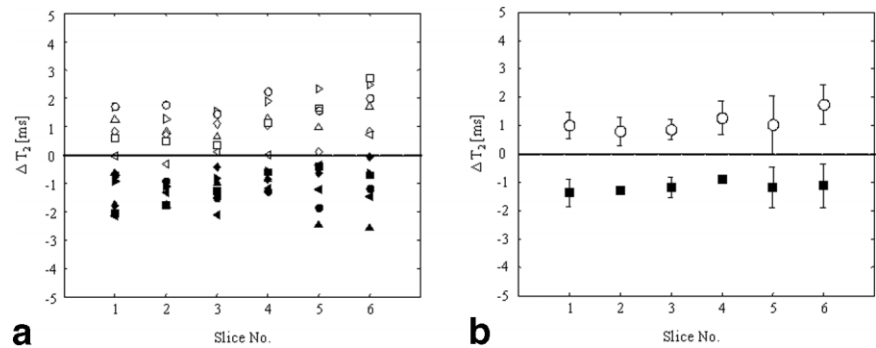
Figure 5. ΔT_2 of the biceps and triceps of six subjects (1: \circ ; 2: \square ; 3: \triangleleft ; 4: \triangleright ; 5: \diamond ; 6: \triangle) vs. the changes in the anatomical CSA (ΔCSA) of the muscles, which are due to elbow flexion. ΔCSA and ΔT_2 are not correlated to each other with respect to both muscles; however, when biceps and triceps are viewed separately a reverse tendency of the data is noticeable.

increase is expected in the biceps, the increase in the triceps area with muscle extension indicates that the image slice is probably not aligned with the anatomical cross section of the triceps. A changing projection of the anatomical cross section onto the image slice could then yield an increased CSA image even if the muscle diameter is effectively shrinking (34). The variation of the ΔCSA (from 4% to 25% (biceps), and 12% to 32% (triceps)) does not show any correlation to the vertically plotted ΔT_2 variation with respect to both muscles. Instead, the mean positive ΔT_2 of the biceps in contrast to the negative ΔT_2 of the triceps clearly indicates that the size of the muscle cross section is not the crucial factor in the observed effect. Therefore, the reverse tendency of the ΔT_2 in the biceps and triceps to increase or decrease, respectively, was not further pursued in this study.

If the fiber orientation changes relative to the magnet field, dipolar effects (35) might occur, which however should equally change T_2 in both biceps and triceps. Regarding the limited experimental accuracy of arm positions, dipolar effects are most likely not important, since T_2 clearly increases with contraction of the biceps while it simultaneously decreases in the triceps. Since the direction of the fibers relative to B_0 varies within the muscle, a possible directional dependence of the measured effect would be visible in Fig. 6. However, the T_2 changes in biceps and triceps are perspicuously separated for all of the image slice positions.

In the literature, T_2 components in the range of the apparent MRI T_2 are assigned to intracellular fluids (18). Accordingly, the monoexponential signal decay of our data was not influenced by a slow-relaxing extracellular T_2 component (>100 msec), as revealed by chi-square tests of multicomponent models. Thus, the measured apparent T_2 provides distinct information about water mobility inside muscle cells. In this crowded environment, water molecules are differently associated with dilute metabolites, enzymes, and structure proteins. Since the examined muscles were not involved in any work, a metabolically induced change of T_2 can be excluded. To ensure the validity of this crucial point, muscle EMG measurements were performed, and the results showed that no significant change in the measured action potential in the biceps and triceps occurred when the position of the relaxed arm was passively changed. In contrast, a single, active contraction of the biceps elevated the potential of the biceps significantly. Short-term changes in T_2 following a single con-

Figure 6. a: Mean ΔT_2 values calculated from 10 TSE experiments with interchanging arm position (from position 2 to position 1) of the biceps (open symbols) and triceps (solid symbols) of six volunteers (1: \circ ; 2: \square ; 3: \triangleleft ; 4: \triangleright ; 5: \diamond ; 6: \triangle). The positions of the axial image slices are given on the x-coordinate with numbers corresponding to Fig. 1. b: Mean of the volunteer data in part a.



traction have been reported to occur in the regime of the slow-relaxing T₂-L component, and to decay within 20 seconds after stimulus (29,30). Such an effect was not measured in the described experiments, since the delay between position change and start of data acquisition exceeded this scale of seconds, and no activation occurred due to the passive operation used.

Therefore, we attribute the observed T₂ alterations to changes in the microanatomy of the muscle that occur during contraction. In this context, relevant anatomical changes have been attributed to a variation in sarcomere length and filament overlap resulting in a variation of the muscle fiber length (36). The effect of such microscopic changes on water proton relaxation is still speculative. The release of water molecules has remained a compelling possibility since the original observation of the change in T₂ with muscle stimulation (1). This model relies on the existence of at least two water fractions (one slow- and one fast-relaxing component). With muscle contraction the amount of fast-relaxing water decreases and the apparent T₂ time increases. According to Saab et al (27), these two fractions may reflect two components of T₂ of approximately 22 and 43 msec, respectively. These two components are not resolvable by MRI T₂ measurements because of the limited number of echoes and the relatively small signal-to-noise (SNR) (37). However, the transition of water molecules from a more bounded state to a less associated state may explain the current findings. Since the fiber length was the only apparent parameter that changed with alternation of the forearm position, one can speculate that a shortening of the sarcomere length is correlated with a release of associated water due to a reduction of hydrophilic surfaces and hydrogen bridges. The vice versa loss of T₂ signal intensity with muscle extension would then indicate a reinforcement of water bounds to polar groups of proteins that become accessible with stretched filaments. Apart from the degree of association of intracellular water, a simple change in the size and shape of the cells may contribute to the observed T₂ increase with muscle contraction. As shown by Brownstein and Tarr (32), the presence of diffusion alone yields an increase in T₂ with increasing cell diameters. This elegant physical description is based on coarse approximations of cell geometry and intracellular water diffusivity; nevertheless, the quantity of the effect is in agreement with spectroscopic data from rat muscles. Thus, our experiments cannot exclude the possibility that a change in the

muscle cell diameter has some influence on the observed T₂ alteration. Morvan et al (38) observed an increase in the apparent diffusion coefficient (ADC) in muscle after exercise, which was ascribed to temperature effects. If diffusion is important for the ΔT_2 effect observed in this study, our data suggest the existence of an additional temperature-independent effect that influences the ADC in skeletal muscle. However, ADC studies incorporating our protocol of passive contraction are required to examine such an effect in skeletal muscle.

In stimulated muscles, metabolism becomes an important parameter that governs intracellular water reorientation (27,31). Consequently, the entire T₂ increase during muscle activity is the sum of multiple effects on intracellular water mobility. The demonstrated effect may significantly contribute to the signal increase in muscle-activation images. Therefore, it is important to consider changes in the contraction state of muscles in MRI studies of muscle function.

In conclusion, this study demonstrates that passive contraction of the skeletal muscles affects the apparent T₂ relaxation time in MR images. The increase in T₂ (by about 1–2 msec) found with shortening of the biceps and triceps is attributable to an increase in intracellular water mobility. Such a release of water molecules during the contraction of muscle fibers may be caused by liberation of protein-bound water or enhanced water diffusivity.

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