

Effects of exercise on muscle transverse relaxation determined by MR imaging and in vivo relaxometry

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Saab, George, R. Terry Thompson, and Greg D. Marsh. Effects of exercise on muscle transverse relaxation determined by MR imaging and in vivo relaxometry. *J. Appl. Physiol.* 88: 226–233, 2000.—The purpose of this study was to determine the effects of intense exercise on the proton transverse (T_2) relaxation of human skeletal muscle. The flexor digitorum profundus muscles of 12 male subjects were studied by using magnetic resonance imaging (MRI; 6 echoes, 18-ms echo time) and in vivo magnetic resonance relaxometry (1,000 echoes, 1.2-ms echo time), before and after an intense handgrip exercise. MRI of resting muscle produced a single T_2 value of 32 ms that increased by 19% ($P < 0.05$) with exercise. In vivo relaxometry showed at least three T_2 components (>5 ms) for all subjects with mean values of 21, 40, and 137 ms and respective magnitudes of 34, 49, and 14% of the total magnetic resonance signal. These component magnitudes changed with exercise by $-44%$ ($P < 0.05$), $+52%$ ($P < 0.05$), and $+23%$ ($P < 0.05$), respectively. These results demonstrate that intense exercise has a profound effect on the multicomponent T_2 relaxation of muscle. Changes in the magnitudes of all the T_2 components synergistically increase MRI T_2 , but changes in the two shortest T_2 components predominate.

proton transverse relaxation; skeletal muscle; handgrip; magnetic resonance

EXERCISE-INDUCED CHANGES IN the transverse (T_2) relaxation of skeletal muscle have been extensively studied for over three decades, but the underlying mechanism is not well understood. This phenomenon was originally described in 1965, when Bratton and co-workers (5) reported an increase in the T_2 of isolated frog skeletal muscle after isometric contractions with electrical stimulation. The authors suggested that the apparent T_2 of muscle increased because protein-bound water molecules, in rapid exchange with “free” water molecules, were released during muscle contraction (5). In the next few years, the T_2 of isolated animal muscle was determined with greater accuracy by using relaxometry protocols (7, 30). The T_2 relaxation of ex vivo muscle was found to be multiexponential, characterized by three distinct T_2 components of <5 , 20–40, and >80 ms, with the intermediate component comprising the majority of the signal (4, 20). Because more than a

single component was measured, Hazlewood et al. (20) interpreted these results as evidence against two fractions of cellular water in rapid exchange (5). They ascribed the three T_2 components to protein-bound and intra- and extracellular water compartments, respectively, which were thought to exchange water molecules slowly but could also include fast-exchanging subcompartments (20). According to this interpretation, exercise-induced T_2 changes reflect a shift of water among cellular compartments. Fung and Puon (18), however, described muscle T_2 as not being multiexponential but instead reflecting a complicated function of hydrogen ion exchange between water and the functional groups of protein filaments. This was further argued to support the notion that an exercise-related intracellular pH drop accounted for the exercise-induced T_2 changes.

The early relaxometry experiments involved excised muscle tissue because in vivo T_2 data obtained with those protocols (7, 30) would have been subject to signal contamination from extraneous tissues. The advent of magnetic resonance imaging (MRI) provided a means for in vivo T_2 measurements, but with less accuracy than the relaxometry techniques (19). Nevertheless, MRI was used to demonstrate a single T_2 value between 30 and 40 ms for human skeletal muscle. With exercise, T_2 increases by 10–30% (12), a phenomenon similar to the original observation of Bratton et al. (5). Consequently, active muscles appear brighter on T_2 -weighted MRI images, which have been useful for muscle recruitment studies (11, 22, 44). Conventional imaging protocols do not show multicomponent T_2 relaxation for in vivo muscle (1, 11, 12, 22, 34, 38), so investigators can only speculate whether the MRI T_2 increases actually involved changes in the long (>80 -ms) (12, 38) or short (20- to 40-ms) (1, 11, 22) T_2 components.

We have recently reported multicomponent T_2 relaxation for in vivo skeletal muscle at rest (36), with some T_2 components not previously observed ex vivo (4, 20). These data were obtained with a novel in vivo relaxometry technique that has measurement parameters [echoes (n) and signal-to-noise ratio (SNR)] two orders of magnitude greater than standard imaging methods (19). With this technique, at least three T_2 components (>5 ms) with values of 21, 39, and 114 ms were resolved from the flexor digitorum profundus (FDP) (36). The objective of the present study was to discover which, if any, T_2 components of in vivo muscle change with

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intense exercise and to determine their relationship to the increase in the MRI T₂ with exercise.

METHODS

General design. This study involved two experiments, Exp 1 and Exp 2. Exp 1 was conducted to determine the changes in muscle T₂ relaxation after intense exercise. Both MRI and in vivo relaxometry were used to measure the T₂ of the FDP before and after a single bout of a maximal-intensity handgrip exercise to fatigue. To analyze the relaxometry data of Exp 1, three common techniques were used: nonnegative least squares using all even echoes [NNLS ($n = 1,000$)] (43), NNLS on even echoes that approximate logarithmic sampling [NNLS ($n = 195$)] (3), and the Marquardt-Levenberg nonlinear minimization using all echoes [MQ ($n = 1,000$)] (29).

Exp 2 was conducted on a different day to determine the rates of change in the magnitudes of each T₂ component throughout rest, exercise, and recovery. Relaxometry data from the FDP were acquired every 30 s over a 32-min period. During this time there were 5 min of rest followed by a handgrip exercise identical to that in Exp 1 (~2 min) and 20 min of recovery. The relaxometry data of Exp 2 were analyzed with NNLS ($n = 1,000$) as it did not require the formulation of initial guesses.

Subjects. Twelve healthy and active men with a mean age of 26 ± 2 yr volunteered to participate in Exp 1 and gave informed consent to the procedures. Five of the subjects (mean age 25 ± 2 yr) also participated in Exp 2. The experiments outlined in this paper were approved by the University of Western Ontario Review Board for Research Involving Human Subjects.

Experimental protocol for Exp 1. Proton nuclear magnetic resonance (NMR) data were acquired by using a 30-cm bore, 1.89-Tesla magnet (SMIS, Oxford Research Systems) interfaced to a computer console, and a custom-built gradient/shim system (Magnex). Within the bore of the magnet, a 14-cm-diameter, high-pass, 16-rung-quadrate birdcage coil functioned as both a transmitter and receiver. Placed inside the birdcage coil was an armrest, on which the subject's right forearm was secured with Velcro straps to reduce involuntary motion. The magnetic resonance (MR) sequences used in this study have been described in greater detail elsewhere (36).

The armrest was positioned to ensure that the FDP was at the isocenter of the magnetic field, and this was verified with "scout" MR images. The scout images had a localization scheme (identical to that of the relaxometry technique used next) combined with a single spin-echo imaging sequence [1-cm slice thickness; field of view (FOV) = 15 cm; 128×128 matrix; repetition time (TR) = 200 ms; echo time (TE) = 20 ms; total scan time = 26 s]. The scout images allowed for a quick determination of the suppression quality, which is the ratio between the mean signal intensity within the localized volume to the extraneous outer volume (39). In addition, the scout images were used to determine whether the localized volume for the T₂ measurements was located within a region of the FDP, excluding visible fat and blood vessels. If this was not the case, repositioning of the arm was necessary. Once the arm positioning was deemed acceptable, the magnet was shimmed and the 90 and 180° radio-frequency pulse settings were determined.

Resting muscle T₂ data were then acquired with the in vivo relaxometry technique, Projection-Presaturation-Carr-Purcell-Meiboom-Gill (PP-CPMG) (36). This technique eliminates the MR signal from tissue extraneous to a cylindrical volume (2-cm diameter, 5-cm length) within the FDP to acquire T₂ data with 2,000 echoes, TE = 600 μ s, TR = 15 s, and 6 averages. A single T₂ measurement, therefore, was

attained in 90 s. Only the even echoes were used for subsequent analysis, so the data in its final form were characterized by $n = 1,000$ and an effective TE of 1.2 ms.

Next, a conventional MRI T₂ measurement of resting muscle was obtained in the form of an axial T₂-weighted forearm image. The MRI technique (1-cm slice thickness; FOV = 128 mm; 128×128 matrix, $n = 6$, TE = 18 ms, TR = 1 s, and 1 average) took 128 s to completion.

The subject was then instructed to keep the forearm still and perform a maximal-intensity handgrip exercise, with contractions at 1 Hz, until fatigue (typically 1 min). When the subject could no longer perform the exercise, the relaxometry and T₂-weighted imaging sequences were repeated. Because the relaxometry sequence was executed immediately after exercise and the imaging sequence was executed 90 s later, the two techniques measured T₂ values at different times in the recovery from exercise.

The total experiment time per subject, including arm positioning, MRI, and T₂ relaxometry, for Exp 1 was ~25 min.

Experimental protocol for Exp 2. This experiment was conducted on a separate day from Exp 1 and had an identical procedure for positioning the subject's arm in the magnet. Once the scout MRI confirmed that the FDP muscle was in the isocenter of the magnetic field, the PP-CPMG sequence was executed. For this experiment, the parameters of the PP-CPMG sequence were modified so as to collect a T₂ relaxation curve every 30 s (2,000 echoes, TE = 600 μ s, TR = 30 s, and 1 average) from the localized volume in the FDP (2-cm diameter, 5-cm length). As in Exp 1, only even echoes were kept for subsequent analysis, so the data were also characterized by $n = 1,000$ and an effective TE of 1.2 ms. The PP-CPMG sequence was executed for a 32-min period, which involved 5 min of rest followed by a handgrip exercise identical to that in Exp 1 (~2 min) and 20 min of recovery.

The total experiment time per subject, including arm positioning and T₂ relaxometry, for Exp 2 was ~45 min.

Data analysis. The T₂-weighted MR images acquired in Exp 1 were analyzed to determine the forearm cross-sectional area (CSA) and T₂ value. The forearm CSA was calculated with Image Display software (SMIS) and excluded bone and subcutaneous fat. T₂ values were determined from a region (0.5 \times 0.5 \times 1 cm) within the FDP. T₂ values were calculated by plotting the average signal intensity of this region at each of the six echoes as a function of TE (18 ms, 32 ms, . . . , 108 ms) and fitting the data to a monoexponential curve.

The SNR of the raw relaxometry data from both Exp 1 and Exp 2 were calculated as the ratio of the first data point (first even echo amplitude) to the SD of the baseline (the last 50 data points).

The relaxometry data from Exp 1 were analyzed with three standard multiexponential algorithms: NNLS ($n = 1,000$), NNLS ($n = 195$), and MQ ($n = 1,000$). First, the NNLS algorithm was employed because it is robust in the presence of noise and has the advantage of not requiring a priori knowledge. The fitting parameters used for the NNLS analysis were 500 linearly spaced values, or data bins, between 0 and 1,000 ms, with a bin width of 2 ms. The value of the NNLS regularizer term (43) was determined from the results of previously reported phantom studies (36). Next, the NNLS analysis was repeated with the same fitting parameters and regularizer term using only selected data points from the T₂ results. The data sets were reduced from 1,000 to 195 points by including every echo up to 50 ms, every second echo up to 200 ms, every fourth echo up to 400 ms, every eighth echo up to 600 ms, and every sixteenth echo up to 1.2 s. Selecting data points in this manner approximates logarithmic sampling and thereby gives equal weighting to the relaxation times of

each component (2). Finally, the NNLS results were used to formulate the model function for the next method of analysis, the MQ algorithm. The data were modeled to three, four, and five components, with the best fit determined by that which minimized the χ^2 statistic without increasing the Cramér-Rao SD.

All three multiexponential algorithms resolved several T_2 components from the relaxometry data of Exp 1 that were labeled A, B, C, D, and E in order of increasing time constant. Component A, with a T_2 value of <5 ms, was disregarded because components in this range cannot be resolved with the same accuracy as those with longer values (36). The magnitude of each component (M_B , M_C , M_D , and M_E) was expressed as a percentage of the total component magnitude at rest ($\%MT_r$). For the NNLS analysis, the total component magnitude was the integral of the resulting T_2 -NNLS spectrum (43). For the MQ analysis, the total component magnitude was determined by the magnetization at $t = 0$.

The relaxometry data of Exp 2 consisted of 64 T_2 measurements for each subject, whereas Exp 1 only had two T_2 measurements per subject: rest and postexercise. The data of Exp 2 were analyzed with the NNLS ($n = 1,000$), and the results were expressed as described above, with one exception. In this experiment there was more than a single T_2 measurement at rest, so MT_r was the average of the total magnitudes from each T_2 measurement in the initial 5-min resting period. The results were plotted as component magnitude ($\%MT_r$) vs. time (min), and time constants of the changes over time were determined by exponential fits of the data.

The statistical testing involved analysis of variance for repeated measures, and the results were considered significant at $P < 0.05$. The values of M_B and M_C in Exp 2 were compared by using Pearson's correlation. All data are presented as means \pm SE.

RESULTS

Axial forearm images acquired in Exp 1 are shown in Fig. 1, A (rest) and B (postexercise). The MR images were used to calculate an average CSA of $4,300 \pm 230$ mm² that increased by 4.5% after exercise to $4,500 \pm 220$ mm². The FDP muscle exhibited increased signal intensity after the handgrip exercise in all 12 subjects. The images yielded a single MRI T_2 value for the FDP of 31.9 ± 1.2 ms at rest, which increased to 37.9 ± 1.1 ms after exercise.

In Exp 1, scout images were used to determine that the suppression quality was $95.8 \pm 0.8\%$. From the relaxometry data, the SNR was shown to be $\sim 4,000$. The relaxometry data [analyzed with the NNLS ($n = 1,000$), NNLS ($n = 195$), and MQ ($n = 1,000$)] revealed at least three T_2 components (>5 ms) for each subject. The component magnitudes are displayed in Table 1, and the corresponding T_2 values are shown in Table 2. After exercise, there were significant changes in the magnitudes of all the T_2 components, and these results are illustrated in Fig. 2.

In Exp 2, despite a lower SNR of $\sim 1,500$, the same T_2 components observed in Exp 1 were usually resolved for all five subjects. Data from the few T_2 measurements where the components could not be resolved were discarded.

The total component magnitude of all the subjects throughout rest, exercise, and recovery is shown in Fig.

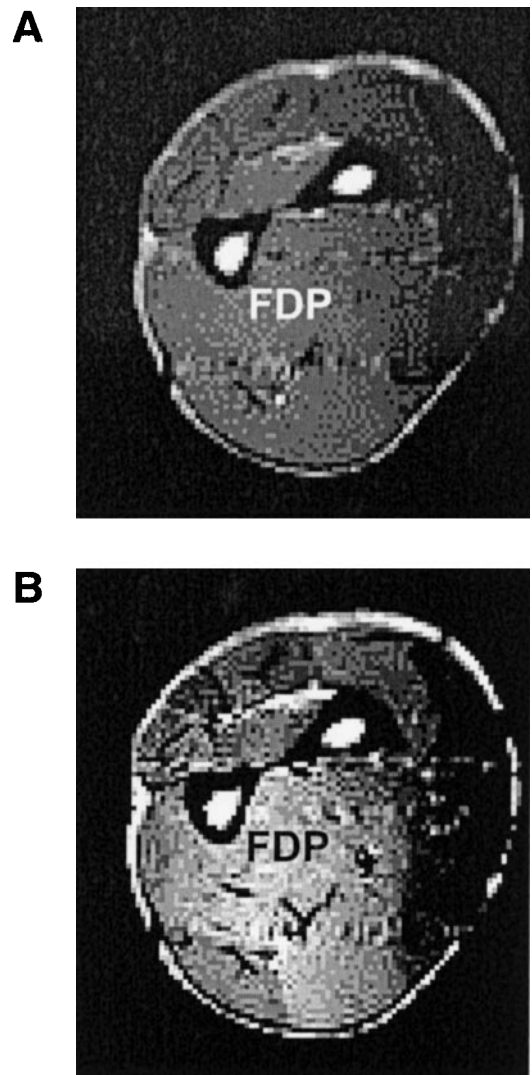


Fig. 1. Experiment 1 (Exp 1) magnetic resonance imaging (MRI) results. Representative proton transverse (T_2)-weighted axial forearm images at rest (A) and after exhaustive maximal-intensity handgrip exercise (B) are shown. Increased signal intensity can be observed in exercised muscles such as flexor digitorum profundus (FDP). In all subjects, postexercise images were used to determine that forearm cross-sectional area increased by 4.5%, and signal enhancement corresponded to a 19% increase in T_2 of FDP.

3. The duration of the exercise period was 48 ± 12 s and is indicated as the area between the two dotted lines (also in Fig. 4 and Fig. 6). The total component magnitude reached a maximal value after the exercise had ceased. After exercise, return to baseline values appeared to be biexponential, with a fast (time constant, $\tau = 1$ min) and a slow ($\tau = 74$ min) decay.

Figure 4 represents M_B and M_C throughout rest, exercise, and recovery. The changes with exercise were fit to monoexponential curves that were both characterized by a τ value of 1 min. During the recovery period, the rate of change in M_B and M_C could be described with single exponential fits with a τ value of 9 min. Figure 5 shows the strong negative correlation observed between the magnitudes of these two T_2 components ($r = -0.878$, $P < 0.01$). Finally, Fig. 6 illustrates the changes

Table 1. Component magnitude (%total magnitude at rest)

T ₂ Component	NNLS (n=1,000)		NNLS (n=195)		MQ (n=1,000)	
	Rest	Postexercise	Rest	Postexercise	Rest	Postexercise
B	33.6 ± 4.5	16.5 ± 1.7*	41.3 ± 4.1	22.8 ± 2.4*	36.5 ± 5.0	19.0 ± 2.5*
C	49.0 ± 3.1	71.1 ± 2.6*	43.5 ± 3.4	68.2 ± 3.5*	51.8 ± 4.0	72.3 ± 39.6*
B + C	82.6 ± 2.0	87.6 ± 1.8*	84.8 ± 1.8	91.0 ± 1.9*	88.3 ± 1.6	91.3 ± 2.7*
D	13.5 ± 1.6	18.1 ± 1.5*	12.0 ± 1.2	15.8 ± 1.6*	10.6 ± 1.4	16.9 ± 2.1*
E	3.4 ± 0.7 (9)	2.7 ± 1.2 (6)	3.0 ± 0.9 (8)	2.6 ± 1.1 (6)	1.1 ± 0.6 (3)	0.6 ± 0.4 (2)
Total	100	108.6 ± 2.0*	100	109.5 ± 2.2*	100	108.7 ± 2.0*

Values are means ± SE. *Experiment 1* (Exp 1) relaxometry results for magnitudes of resolved proton transverse (T₂) components as determined by 3 methods: nonnegative least squares (NNLS; n=1,000), NNLS (n=195), and Marquardt-Levenberg nonlinear minimization (MQ; n=1,000) are shown. *Component A*, with a T₂ value of <5 ms, was disregarded because it could not be determined with the same accuracy as those with longer T₂ values. Results are expressed as %total magnitude at rest (M_{T,r}). *Components B, C, and D* were resolved for all subjects. No. of subjects for whom *component E* could be resolved is displayed in parentheses. *Significant changes after exercise (P<0.05).

in M_D throughout the experiment. The recovery was fit to an exponential with τ = 13 min.

DISCUSSION

This study used a method for in vivo T₂ relaxometry (36) that permits a comprehensive examination of proton T₂ relaxation in human skeletal muscle. The relaxometry method was critical to this study because many data points with a high SNR are required to observe multicomponent T₂ decay [which cannot be achieved with conventional MRI methods (19, 34)]. With an appropriate analysis technique (29, 43), the relaxation value and magnitude of each T₂ component can be extracted from the data. Conceptually, a component magnitude (M_j) is the apparent population, or concentration, of the proton spin group that gives rise to the T₂ component *j*. At the slow exchange limit (where no exchange occurs between spin groups), the component magnitude reflects the actual population of the proton spin group. Moreover, the sum of all the component magnitudes likely reflects the hydration state of the tissue because the vast majority of protons producing the signal are found in water molecules (16).

The most prominent finding of Exp 1 was that intense forearm exercise appreciably alters the multicomponent T₂ relaxation of skeletal muscle. Immediately after the handgrip exercise, large changes were observed in M_B (34–44% decrease), M_C (52–63% increase), and M_D (45–66% increase). The decrease in M_B (which has the shortest T₂ value) and the increase in M_C and M_D (which are characterized by longer relaxation times) would synergistically increase the appar-

ent MRI T₂. Indeed, the MRI data showed a 19% increase in the T₂ value of the FDP. The MRI T₂ increase and the 4.5% increase in forearm muscle CSA also demonstrated that the handgrip exercise was appropriate for recruiting the FDP muscle in all subjects.

Although the absolute percent changes in M_B, M_C, and M_D were similar (Fig. 2), the resting values of M_B and M_C were considerably larger than that of M_D. This means that the actual changes in M_B and M_C (and therefore their contributions to the increase in MRI T₂) were the most substantial. This finding is important because in previous years investigators have questioned whether MRI T₂ increases involve changes in long (>80-ms) (12, 38) or short (20- to 40-ms) (1, 11, 22) T₂ components. Before our recent in vivo muscle study (36), there was only evidence of one “short” T₂ component in the range of 20–40 ms (4, 20), so the effects of exercise on two components in that range had not been previously considered.

Quantitative interpretation of NMR relaxation data from complex systems such as in vivo muscle is a nontrivial problem that requires a precise determination of the distribution of relaxation times in the sample. For this reason, the data of Exp 1 were analyzed with three different methods: NNLS with n = 1,000 (43), NNLS with n = 195 (3), and MQ with n = 1,000 (29). The similarity in the T₂ values, component magnitudes, and changes with exercise derived from all three methods indicates the relaxometry results are independent of the chosen algorithms.

Exp 2 was conducted to obtain more data pertaining to the T₂ components of in vivo muscle, specifically the

Table 2. T₂ values

T ₂ Component	NNLS (n=1,000)		NNLS (n=195)		MQ (n=1,000)	
	Rest	Postexercise	Rest	Postexercise	Rest	Postexercise
B	21.1 ± 1.1	18.2 ± 1.1*	23.2 ± 1.3	20.5 ± 1.3	20.6 ± 1.1	16.4 ± 1.4*
C	39.8 ± 1.6	42.7 ± 1.3	42.7 ± 2.0	45.2 ± 1.3	47.3 ± 4.4	44.6 ± 1.5
D	136.5 ± 9.4	132.7 ± 11.7	141.0 ± 9.9	143.5 ± 10.5	183.1 ± 16.1	169.9 ± 11.8
E	301.0 ± 20.5 (9)	379.3 ± 85.2 (6)	345.0 ± 43.6 (8)	397.7 ± 99.7 (6)	258.9 ± 5.8 (3)	297.7 ± 23.5 (2)

Values are means ± SE in ms. n, No. of echoes. Exp 1 relaxometry results for T₂ values corresponding to the components described in Table 1 are shown. *Component A*, with a T₂ value of <5 ms, was disregarded because it could not be determined with the same accuracy as those with longer T₂ values. T₂ components *B, C, and D* were resolved for all subjects. The number of subjects for whom *component E* could be resolved is displayed in parentheses. *Significant changes after exercise (P<0.05).

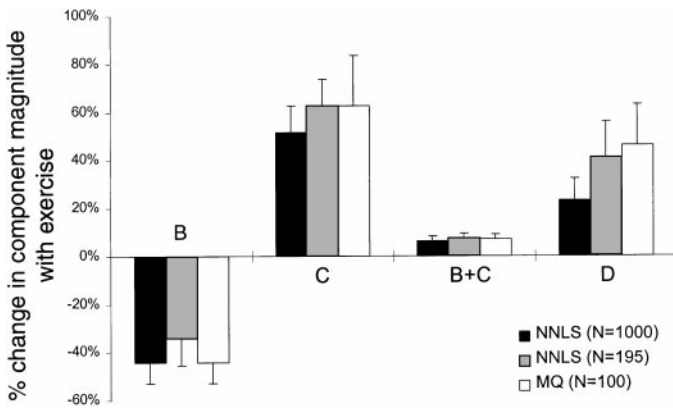


Fig. 2. Exp 1 relaxometry results. %Change in magnitudes of each T₂ component when analyzed by using 3 methods: nonnegative least squares (NNLS) using all even echoes with $n = 1,000$ (solid bars), NNLS on even echoes that approximate logarithmic sampling with $n = 195$ (shaded bars), and Marquardt-Levenberg (MQ) nonlinear minimization using all echoes with $n = 1,000$ (open bars) are shown. B, C, and D: T₂ components. All of these components showed significant changes with exercise ($P < 0.05$).

rates of change in their magnitudes throughout rest, exercise, and recovery. The improved time resolution that made this experiment possible came at the expense of data averaging, so the SNR of this data was consequently lower. Nevertheless, all the components observed in Exp 1 were also found in Exp 2. M_B and M_C were found to have identical rates of change during and after exercise and were inversely correlated ($r = -0.878$, $P < 0.01$), which suggests that they both were involved in a similar physiological process. Another interesting finding of Exp 2 was that the maximal value of the total component magnitude occurred after the exercise had ceased.

The 8–9% increase in total component magnitude (which is influenced by the hydration state of the tissue) was likely due to an uptake of fluid from the vasculature. This is because fluid transfer does not

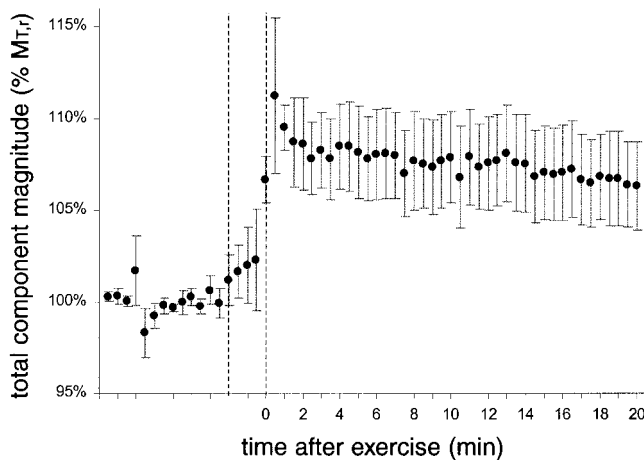


Fig. 3. Experiment 2 (Exp 2) relaxometry results. Total component magnitude throughout rest, exercise, and recovery, expressed as %mean of first 13 data points (M_{T,r}), is shown. Exercise period is indicated as time between vertical dotted lines, and error bars are SE. After exercise, recovery appeared to be biexponential, with τ values of 1 and 74 min.

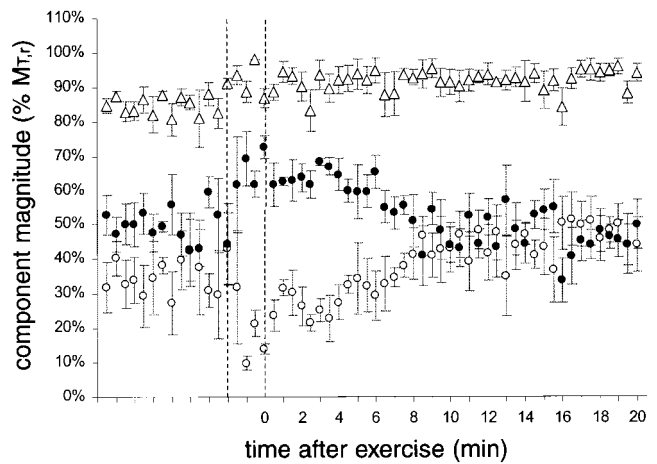


Fig. 4. Exp 2 relaxometry results. Magnitudes of T₂ components B (○), C (●), and sum of B+C (△), expressed as %M_{T,r} throughout rest, exercise, and recovery, are shown. Exercise period and error bars are defined as in Fig. 3. T₂ components B and C had similar rates of change during exercise and recovery.

occur from inactive to active muscles (40), and fluid gain by metabolic water production is negligible in short-term exercise (27). The finding in Exp 2 that the tissue hydration reached a maximal value after exercise is suggestive of exercise hyperemia, when local blood vessels were no longer compressed by contracting muscle fibers. Moreover, the recovery of the total component magnitude in the minutes after exercise was biexponential, in agreement with previous observations of tissue hydration recovery after exercise (14, 27).

The exact reasons certain tissues exhibit multicomponent T₂ relaxation are not known. Perhaps the simplest (and most widely cited) way to interpret the data is in terms of the water compartment model (5, 20, 33), where T₂ components are thought to originate from anatomic compartments that exchange water molecules slowly (relative to their T₂ relaxation times). This model does not require physical barriers such as a semipermeable membrane to distinguish the water compartments; nor does it rule out intermediate ex-

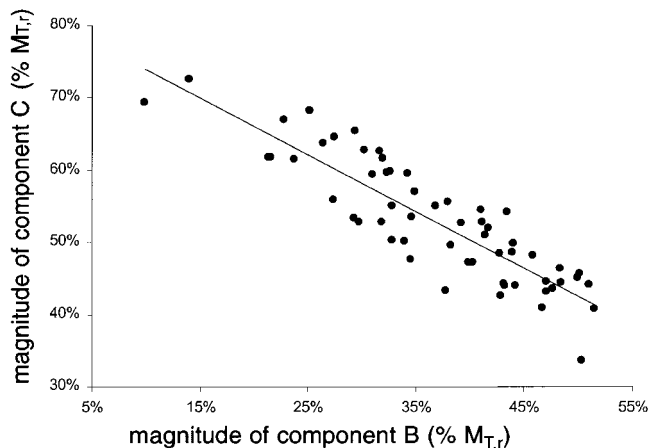


Fig. 5. Exp 2 relaxometry results. Magnitudes of T₂ components B and C show a strong negative correlation between them ($r = -0.878$, $P < 0.01$).

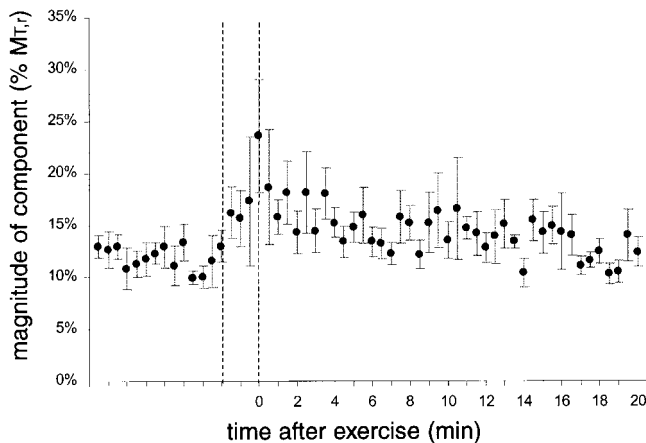


Fig. 6. Exp 2 relaxometry results. Magnitude of T₂ component D, expressed as %MT,r throughout rest, exercise, and recovery, is shown. Exercise period and error bars are defined as in Fig. 3. After exercise, return to resting values was exponential ($\tau = 13$ min).

change (28). The in vivo muscle T₂ relaxation that can be measured for all subjects, therefore, may be described as

$$y(t_i) = M_B e^{-TE_i/T_{2,B}} + M_C e^{-TE_i/T_{2,C}} + M_D e^{-TE_i/T_{2,D}} \quad (1)$$

$$i = 1, 2, 3, \dots, n$$

where $y(t_i)$ is the signal intensity at the i th echo, TE_i is the echo time of the i th echo, n is the number of echoes, and M_j and $T_{2,j}$ are the magnitude and T₂ relaxation value of the j th component, respectively. In a compartment model, components B and C would be considered intracellular because their T₂ values and combined magnitude ($M_B + M_C = 83\text{--}88\%$ MT,r) were similar to those of the single component determined in excised muscle attributed to intracellular water, (4, 15, 20). Also, the magnitude (11–15% MT,r) and T₂ value (>100 ms) of component D closely resemble those of the component attributed to extracellular fluid (4, 15, 20). Critics of the water compartment model have suggested that the ex vivo T₂ component attributed to extracellular fluid was a postmortem artifact (17), but the present observation of a similar component in vivo contradicts that argument. The relative volumes of the both the extra (M_D)- and intracellular fluid ($M_B + M_C$) in resting muscle were in close agreement with values determined with radio tracers (8, 26, 36, 40). Finally, component E, with the longest T₂ value and smallest magnitude, was not included in Eq. 1 because it is relatively small and could not be resolved for all subjects. In a recent study, Noseworthy et al. (31) used a compartment model to analyze MRI T₂ data of muscle. Although the authors could not resolve this long T₂ component, they speculated that it did exist and attributed it to lymphatic fluid (31). Interestingly, the magnitude of component E is in excellent agreement with the plasma space found for in vivo rat muscle tissue (8).

The application of the compartment model to the in vivo relaxometry data may also explain the effect of exercise on MRI T₂. The ~7% increase in intracellular

fluid ($M_B + M_C$) and ~40% increase in extracellular fluid (M_D) with intense exercise are in accord with results from radio tracer studies (26, 36, 40). However, it is generally accepted that gross changes in intra- or extracellular water cannot fully explain the increase in MRI T₂ (1, 10, 11, 44). This has led several investigators to suggest the MRI T₂ change is induced by a complex reorganization, rather than net increase, in intracellular fluid during exercise (1, 10). It is possible that the individual changes in M_B and M_C indicate this reorganization. One speculation is that component B includes water associated with heavily hydrated glycogen molecules and component C was cytoplasmic fluid, so M_B would decrease and M_C would increase as glycogen is utilized during heavy exercise. Other factors must also be operant, as the changes in magnitude of components B and C are similar but not identical (Fig. 4). Indirect evidence to link component B with glycogen can be found in patients with myophosphorylase deficiency. Glycogenolysis is blocked in these individuals, and they do not exhibit the typical increase in MRI T₂ with exercise (13). Moreover, the rapid depletion of glycogen postmortem (6) may explain why components B and C were not resolved in excised muscle preparations (4, 20). A recent study by Price and Gore (35) has shown a correlation between muscle MRI T₂ and a glycolytic intermediate, glucose-6-phosphate. Future experiments need to be conducted to evaluate the relationship between multicomponent T₂ relaxation and glycogen.

Although the water compartment model appears to explain the results of this study well, it is by no means a unique interpretation and has never been directly proven. Perhaps the NMR literature has devoted so much attention to whether the T₂ relaxation curve is dictated by the physical distribution of cellular water that other effectors have been ignored. The liberation of bound water molecules has remained a compelling possibility since the original observation of the change in T₂ with muscle stimulation (5). This effect is more likely to be prevalent in the myoplasm, which contains ~23% protein by weight (16), but may not be limited to the intracellular environment. This might mean that T₂ components represent water molecules of similar translational and rotational freedom regardless of their physical location. In vivo relaxometry, therefore, can potentially be used to study the net liberation of cellular water molecules during exercise, which is thought to play a critical role in muscle contraction (32).

Cellular water molecules that contribute to the T₂ signal may be located in erythrocytes as well as myofibrils. In this study, T₂ data were acquired from a localized volume of skeletal muscle that did not include any visible blood vessels but could not exclude capillaries. The relative quantity of erythrocytes at the capillary level is likely to be quite small, as would be their influence on T₂. Nevertheless, it has been shown that T₂ relaxation of in vitro blood samples is profoundly dependent on erythrocyte volume (41), which changes with exercise (42). This dependence may account, in

part, for the dramatic changes in the T₂ components with exercise.

This discussion has focused on water molecules because the vast majority of protons that give rise to the T₂ signal comprise tissue water (15). However, other cellular moieties such as intracellular lipids may also be important. The in vivo relaxometry technique was effective in eliminating the MR signal from fatty tissue located outside the localized volume of muscle (36), but intracellular triglycerides from within the volume warrant further consideration. Early lipid mobilization during exercises of various intensities has been reported (21), and it is quite possible that would influence T₂ component magnitudes. This would involve both intracellular triglyceride stores and increased delivery of plasma lipids to active muscle. Some investigators, however, argue that glycogenolysis is a more important fuel than lipid mobilization during intense short-duration exercise and that the delivery of plasma lipids is dependent on blood flow, but the T₂ effect is not (2).

There is evidence that muscle T₂ relaxation is affected by the level of oxygenation in the blood (24, 31). Elevated levels of paramagnetic deoxyhemoglobin during exercise cause perturbations in local magnetic fields that dephase spins and thereby could contribute to a decrease in the measured T₂ signal of muscle (24). This phenomenon could contribute to the changes in component B, which was the only component to exhibit a decreased magnitude (and T₂ value) with exercise. Such a reduction in this relatively short component would therefore be expected to prolong the MRI T₂ of muscle.

In summary, this study demonstrates that intense exercise has a profound effect on the multicomponent T₂ relaxation of muscle. The decrease in magnitude of the component with the shortest T₂ value (component B) and an increase in magnitude of those with longer relaxation times tend to synergistically increase MRI T₂. Because of the large initial magnitudes of the two shortest T₂ components (components B and C), their changes with exercise and therefore their contribution to the MRI T₂ effect, would predominate.

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