Magn Reson Med Sci 2019; 18; 184–193
doi:10.2463/mrms.mp.2018-0059
Published Online: November 19, 2018

MAJOR PAPER

T_2 Distribution in the Forearm Muscles and the T_2 Threshold for Defining Activated Muscle

Masayoshi Takamori[1,2], Sumikazu Akiyama[1,3], Kazuya Yoshida[1,3], Hidefumi Wakashin[1], and Yoshiteru Seo[1*]

Purpose: In order to detect exercised muscles by the increase in T_2, we have defined a Gaussian T_2 distribution and reference values (T_{2r} and SD_r) in resting state muscles, and a threshold for detecting exercised muscles.
Methods: The subjects were healthy adult volunteers (14 males and 12 females). Multiple-spin-echo (MSE) MR images were obtained with 10 TE values from 10 to 100 ms using a 0.2T MRI system. T_2 values for 10 forearm muscles were obtained in the resting state and after isometric wrist flexion exercise with 5%, 15%, and 25% of the maximum voluntary contraction (MVC). Z values were obtained by $(T_{2e} - T_{2r})/SD_r$, where T_{2e} was T_2 after exercise. Based on sample size calculations, three thresholds (Z_T = 1.00, 2.56, and 3.07) were applied to agonist and antagonist muscles.
Results: A normal distribution of T_2 was detected in resting muscles at 34 ± 3 ms (mean ± standard deviation [SD]) in 26 subjects using the Kolmogorov–Smirnov test, the Shapiro–Wilk test, and the Jarque–Bera test ($P > 0.05$). No gender differences were shown between the T_2 or SD, and a similar result was obtained in 12 measurements on a single subject ($P < 0.01$). The T_{2r} and SD_r were used for reference values. The threshold Z_T = 1.00 showed the highest sensitivity (0.86) even with 5% MVC, but it showed a lower specificity (0.85) than the other thresholds. Z_T = 3.07 showed the highest specificity (1.0), but it showed a lower sensitivity (0.36) with the 5% MVC, compared with Z_T = 2.56 (0.50). The receiver operating characteristics analysis also supported these results.
Conclusion: We found that the T_2 distribution in muscles was Gaussian, suggesting that a one-sample *t*-test can be applied, and that Z_T = 2.56 could cover low-intensity exercise with high specificity and a low false-positive rate.

Keywords: *normal distribution, receiver operating characteristics analysis, sensitivity, specificity, transverse relaxation time*

Introduction

Increases in the transverse relaxation time of muscles have been used to detect exercised muscle, not only in athletes but also in patients.[1–5] A threshold with a mean + 1.0 standard deviation (SD) in the T_2 of resting muscle has been used for the detection of contracted muscles.[6–8] This criterion is assumed to be a normal (Gaussian) distribution of T_2 in muscles. Several reports have been published on studies investigating the T_2 distribution of ROI that covered the whole muscle.[3,9,10] In one study, Prior et al.[9] examined changes in the T_2 distribution in exercised muscle, and they assumed a Gaussian distribution of T_2 values at rest. However, judging from the pixels in an ROI covering the whole muscle, for example, Fig. 3 in Prior's paper, the T_2 distribution in resting muscle was not symmetrical, but rather, it was skewed to higher T_2 values.[3,9,10] Ploutz-Snyder et al.[3] considered that a high T_2 component (> 35 ms) in resting muscle showed non-muscle tissue, such as fat. This assumption might be true because the distribution of T_2 in exercised muscle becomes more symmetrical, even though there is a tendency for an increase in the SD of the T_2 values.[3,9] In addition, in the muscles of the lower limbs, a large T_2 difference (around 10 ms) has been reported.[9] Therefore, a threshold (mean + 1.0 SD) detected by this method is not general, but rather, it can be applied only for one particular muscle and subject.

In the clinical laboratory, a reference value is often used to define normal or abnormal value.[11] This reference value is obtained by measurements taken using reference subjects. If the distribution of the values is Gaussian, the reference

[1]Department of Regulatory Physiology, University of Dokkyo School of Medicine, 880 Kitakobayashi, Mibu-machi, Shimotsuga-gun, Tochigi 321-0293, Japan
[2]Department of Physical Therapy, Aoi Medical Academy, Saitama, Japan
[3]Department of Rehabilitation, Faculty of Health Sciences, University of Human Arts and Sciences, Saitama, Japan
*Corresponding author, Phone: +81-282-87-2125, Fax: +81-282-86-7835, E-mail: yseo@dokkyomed.ac.jp

Received: May 10, 2018 | Accepted: September 7, 2018

limits are the mean ± 2 SD.[11] As far as we have found in a search of the literature available, there are no reports that mention the reference value of T_2 in skeletal muscle. Till now, similar T_2 values have been assumed for all muscles in the resting state.[3,6] However, some reports have mentioned the presence of T_2 differences (4–10 ms) in the muscles of the lower limbs.[9,12] Therefore, we first measured the T_2 of 10 muscles in the forearm, and differences in the T_2 were examined. If the T_2 values found in the 10 muscles showed a Gaussian distribution and were similar to each other, we determined T_2 and SD to obtain a reference value for resting muscles in healthy adult subjects. Based on the reference value, three thresholds were selected by sample size calculations, and we applied them to detect agonist muscle after wrist flexion exercise. Sensitivity, specificity, and receiver operating characteristic (ROC) curves were examined for the three workload levels usually used for rehabilitation. Finally, we proposed a threshold for defining exercised muscle.

Materials and Methods

Subjects

Healthy adult volunteers (14 males and 12 females) participated in the study from January 2013 to December 2015. The age, height, and weight of the subjects averaged 33.4 ± 9.4 years, 174.8 ± 6.0 cm, and 72.8 ± 13.0 kg for males, and 27.3 ± 5.0 years, 162.4 ± 4.4 cm, and 54.2 ± 5.5 kg for females (mean ± SD), respectively. All of the subjects examined were right-handed, and the exercise performed was done using the left hand. None of the subjects regularly engaged in forearm exercise prior to the study. All of the examinations were conducted in daytime within 1 h from 10:00 to 17:00, except for one experiment that finished at 19:00. T_2 values in the resting state were obtained from 26 subjects, and T_2 values after exercise were obtained from seven subjects. The procedures, purpose, and risks associated with the study were explained to all of the subjects, and written consent was provided prior to the commencement of the study. The study was approved by the Human Research Review Board at the University of Dokkyo School of Medicine (#24003).

MR imaging

The ^{1}H MR images were obtained with a 0.2T compact MRI system (MRTechnology, Inc., Ibaraki, Japan) equipped with an oval ^{1}H solenoidal radiofrequency (RF) coil (120 × 160 mm) and a shell-type arm holder.[4] Since the T_1 and T_2 of muscle are around 500 and 35 ms, respectively,[13–15] the parameters for T_2 multiple-spin-echo MRI were set as follows: a 20 × 20 cm FOV, a data matrix of 128 × 128, a single slice of 15 mm slice thickness, a 2000 ms TR, 10 TE from 10 to 100 ms with a 10 ms step, and one accumulation. The slice position was set at one-third of the length of the ulna from the olecranon. Images were Fourier transformed with a data matrix 256 × 256 after zero filling of data (Fig. 1a). The T_2 values in each pixel were calculated by nonlinear fitting to single exponential decay, and the T_2 images (T_2 map) were reconstructed using iPlus software (MRTechnology, Inc.). MR images were evaluated by three physical therapists (M.T., S.A., and K.Y.) with more than 5 years of experience using 0.2T MRI. These therapists presented a good intraobserver agreement in a previous study.[4] Muscles were assigned using a comparison with an Atlas of the human forearm, using the flexor carpi radialis (FCR) muscle, flexor carpi ulnaris (FCU) muscle, palmaris longus (PL) muscle, flexor digitorum superficialis (FDS) muscle, flexor digitorum profundus (FDP) muscle, extensor carpi ulnaris (ECU) muscle, extensor carpi radialis (ECR) longus/brevis muscle, supinator muscle (SM), pronator teres (PT) muscle, and extensor

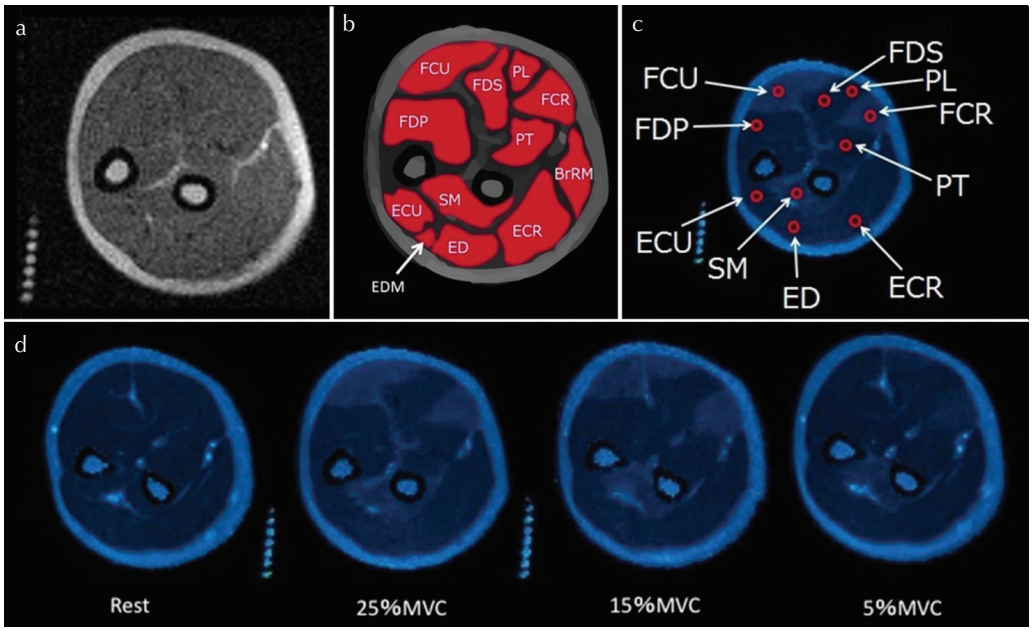

Fig. 1 Transverse MRI of the forearm muscle. (**a**) T_2-weighted MRI with TE = 40 ms. (**b**) Muscle traces. (**c**) ROI positions for 10 muscles. (**d**) T_2 maps of resting muscle and after exercise of 25%, 15%, and 5% MVC. BrRM, brachioradialis muscle; EDM, extensor digiti minimi muscle; ECR, extensor carpi radialis; ECU, extensor carpi ulnaris; ED, extensor digitorum; FCR, flexor carpi radialis; FCU, flexor carpi ulnaris; FDP, flexor digitorum profundus; FDS, flexor digitorum superficialis; MVC, maximum voluntary contraction; PL, palmaris longus; PT, pronator teres; SM, supinator muscle.

digitorum (ED) muscle (Fig. 1b).[16] In each of the T_2 map images, the ROIs with 16 pixels (9.8 mm^2) were set for muscles (Fig. 1c), and we obtained the means and SDs of the T_2 values using iPlus and ImageJ software (version 1.44p, National Institute of Health, Bethesda, MD, USA). In each case, the ROI was set near the center of the muscle, but we excluded visible fat tissue and vessels. Using MR position markers in the shell-type arm holder, the slice position could be reproduced accurately within 4 mm.[4] Rotation errors in the MR images were corrected using the position of the radius, the ulna, and the vessels.

Muscle exercise

During the exercise with palmar flexion of the wrist joint, the left forearm was fixed in the supinated position. Based on our knowledge of kinesiology and electromyography of manual muscle test,[17] the FCR, and FCU are the agonist muscles, PL is the synergist muscle, and ECU, ECR, and ED are the antagonist muscles for palmar flexion of the wrist joint (Table 1a). In order to measure the maximum isometric muscle contraction, the force of the palmar flexion was measured by a muscle dynamometer (μTAS F-1, Anima, Tokyo, Japan) during maximum voluntary isometric contraction for 4–5 s. The average force of three measurements was used for the MVC. Three levels of exercises (5%, 15%, or 25% of MVC) were applied in random order at intervals longer than 1 week. T_2 MRI was measured before the muscle exercise. The forearm was then moved to the anterior side of the magnet, and a string of a weight (5%, 15%, or 25% of MVC) was positioned at the metacarpophalangeal (MP) joint. The wrist joint flexed the palmar side against the weight, and we had the subjects keep that position for 1 s. This isotonic palmar flexion of the wrist joint was repeated at 2 s intervals until the subject was unable to continue the palmar flexion of the wrist. Immediately after the exercise, the arm position was restored to the original position and the T_2 values were measured again.

Statistics

Normality was tested using the Lilliefors corrected Kolmogorov–Smirnov test (KS test) and the Shapiro–Wilk test (SW test) using IBM SPSS Statistics software (V25, IBM, Corporation, Armonk, NY, USA). Skewness, kurtosis and Jarque–Bera values were calculated by Excel 2016 (Microsoft Corporation, Redmond, WA, USA) for Jarque–Bera test (JB test). In a normal distribution, skewness and kurtosis are equal to zero. A *P* value greater than 0.05 indicates normality. Tests for normality were based on (1) the empirical cumulative distribution with the theoretical normal cumulative distribution (KS test), (2) empirical quantiles with the theoretical normal quantiles (SW test), or (3) the sample skewness and sample kurtosis (JB test). Therefore, the normality of T_2 was tested in three different ways. The KS test requires sample size at least 100, and the SW test requires sample size at least 50 to get good power for normality test.[18] The SW test was originally specialized for a small sample size less than 50,[19] and could be used until 2000.[20] The JB test could use sample size from 20 to 300,[21] and is superior for detecting the normal distribution (α error < 0.05 in the sample size from 8 to 150).[22] The SW and KS tests were hard to reach α error less than 0.05, but can be used for practical purpose.[22] Therefore, based on the sample

Table 1 T_2 in 10 forearm muscles
(**a**) T_2 values and distribution in resting muscle

Muscle	Abbreviation	Function	T_2					Test against normal distribution
			Mean (ms)	SD (ms)	*n*	Skewness	Kurtosis	Jarque–Bera (*P* value)
Flexor carpi radialis	FCR	Agonist	32.83	2.96	26	0.253	−1.180	0.409
Flexor carpi ulnaris	FCU	Agonist	33.99	3.08	26	−0.724	−0.254	0.310
Palmaris longus	PL	Synergist	34.06	3.28	26	−0.049	−0.151	0.983
Flexor digitorum superficialis	FDS		33.48	2.42	26	0.065	1.027	0.560
Flexor digitorum profundus	FDP		32.12	2.58	26	0.508	1.050	0.314
Extensor carpi ulnaris	ECU	Antagonist	34.70	3.11	26	0.223	−0.564	0.756
Extensor carpi radialis longus/brevis	ECR	Antagonist	33.51	3.35	26	0.862	−0.178	0.197
Extensor digitorum	ED	Antagonist	35.99	2.71	26	−0.510	−0.474	0.504
Supinator	SM		34.46	2.76	26	−0.290	−1.293	0.337
Pronator teres	PT		32.53	2.83	26	−0.372	−0.679	0.577

P values higher than 0.05 indicate normality of the T_2 distribution, where *n*: number of muscles. Due to small sample number, only the Jarque–Bera test was applied. Post-hoc analysis showed a significant difference in T_2 ($P < 0.01$) for the following combinations: FCR/ED, FDS/ED, FDP/ECU, FDP/ED, FDP/SM, ECR/ED, and ED/PT. Agonist, synergist and antagonist muscles were derived from knowledge of kinesiology and electromyography of palmar flexion of the wrist joint.[17] SD; standard deviation.

Table 1 (**b**) Changes in T_2 due to exercise

Exercise		25% MVC		15% MVC		5% MVC	
		T_2		T_2		T_2	
Muscle	Number of subjects	Mean (ms)	SD (ms)	Mean (ms)	SD (ms)	Mean (ms)	SD (ms)
FCR	7	46.12*	4.77	47.76*	3.36	41.00*	3.97
FCU	7	48.95*	1.52	46.05*	6.85	41.98*	7.27
PL	7	40.07	8.04	50.11*	5.01	40.66	7.88
FDS	7	34.49	3.22	34.34	3.01	35.51	3.90
FDP	7	32.16	1.43	32.02	1.74	30.26	3.39
ECU	7	33.56	1.95	34.26	4.82	32.46	3.37
ECR	7	35.70	2.67	35.03	2.41	32.46	3.19
ED	7	35.81	2.95	37.46	2.51	34.77	2.29
SM	7	38.61	3.30	41.57*	3.05	35.99	5.07
PT	7	32.87	2.51	31.07	2.07	31.49	3.19

*Indicates $P < 0.01$ (one-side), compared with the resting muscle, which was considered as a significant increase in T_2. ECR, extensor carpi radialis; ECU, extensor carpi ulnaris; ED, extensor digitorum; FCR, flexor carpi radialis; FCU, flexor carpi ulnaris; FDP, flexor digitorum profundus; FDS, flexor digitorum superficialis; MVC, maximum voluntary contraction; PL, palmaris longus; PT, pronator teres; SD, standard deviation; SM, supinator muscle.

size used in this study, the results of the JB test were considered as the most important. The number of tests judged to show a normal data distribution was determined by an appropriate fit of the data to a known normal distribution. Significant differences between the T_2 values were tested using the one-sample one-sided *t*-test or one-way analysis of variance (ANOVA) using MS Excel 2016. *P* value less than 0.01 was regarded as significant.

Results

Distribution of T_2 of resting muscle

The distributions of the T_2 values obtained from ten muscles were defined as normal distributions by the JB test ($P > 0.05$) (Table 1a). Statistically, the T_2 values obtained in the 10 muscles showed a significant difference ($P < 0.01$ obtained by one-way ANOVA). The results of the post-hoc analysis are also shown in the legend of Table 1a. However, the T_2 values were distributed within a range of 2 from 34 ms (Table 1a). Accordingly, thereafter the T_2 distribution was analyzed using all of the data from 10 muscles.

Histogram and Quantile–Quantile (Q–Q) plots of T_2 values for 10 muscles obtained in a resting state from 26 subjects are shown in Fig. 2a and 2b. The histogram looks like a Gaussian shape and the Q–Q plot is almost straight along the whole range of T_2. The results of the three statistical tests for the normal distribution are summarized in Table 2. The results showed a normal distribution of T_2 in resting muscle at 34 ± 3 ms (mean ± SD) ($P > 0.05$). A histogram showing the SD of the T_2 is shown in Fig. 3a. The median and mode of the SD were 3.55 and 3.11 ms, respectively. The SD values for the 5th and 95th percentiles were 2.06 and 5.92 ms, respectively. The skewness (5.90) and kurtosis (1.97) values of the SD were too large for a normal distribution ($P < 0.05$). The correlation coefficient between SNR_{T_2} (=T_2/SD) and T_2 was 0.035 (Fig. 3b), and that between %SD (=SD/T_2) and T_2 was 0.077. Neither of these values indicated any statistical significance ($P < 0.05$).

Data from the male ($n = 14$) and female ($n = 12$) subjects were separated, and the resulting histograms and Q–Q T_2 plots are shown in Fig. 2c–2f. Both distributions were defined as normal distributions ($P > 0.05$) as shown in Table 2. There was no significant difference shown between the male and female T_2 values, obtained by one-way ANOVA ($P < 0.01$). In regard to the 12 measurements on a single subject, one of the three statistical tests showed a normal distribution (Table 2), and there was no T_2 difference compared with the T_2 values obtained in the single measurements for 26 subjects ($P < 0.01$ obtained by one-way ANOVA).

T_2 of exercised muscle

A typical set of T_2 maps before and after exercise is shown in Fig. 1d. In these T_2 maps, after exercise with palmar flexion of the wrist joint of 25% and 15% MVC, the T_2 of the FCR, FCU, and SM increased. In the statistical results obtained from seven subjects (Table 1b), the T_2 of the FCR and FCU increased significantly, compared with that of the pre-exercised muscles ($P < 0.01$). The T_2 values for the rest of the muscles did not show any significant increases, except for the T_2 of the PL and SM after exercise at 15% MVC ($P < 0.01$).

Thresholds for detection of exercised muscle

In order to detect exercised muscle, *Z* values were obtained by $(T_{2e} - T_{2r})/SD_r$, where the T_{2e} values were the T_2 values obtained after exercise, and T_{2r} and SD_r are the mean and SD of the resting muscle, respectively. From the results shown above, we used a T_{2r} of 34 ms and an SD_r of 3 ms as the reference value. We choose three thresholds (Z_T = 1.0, 2.56,

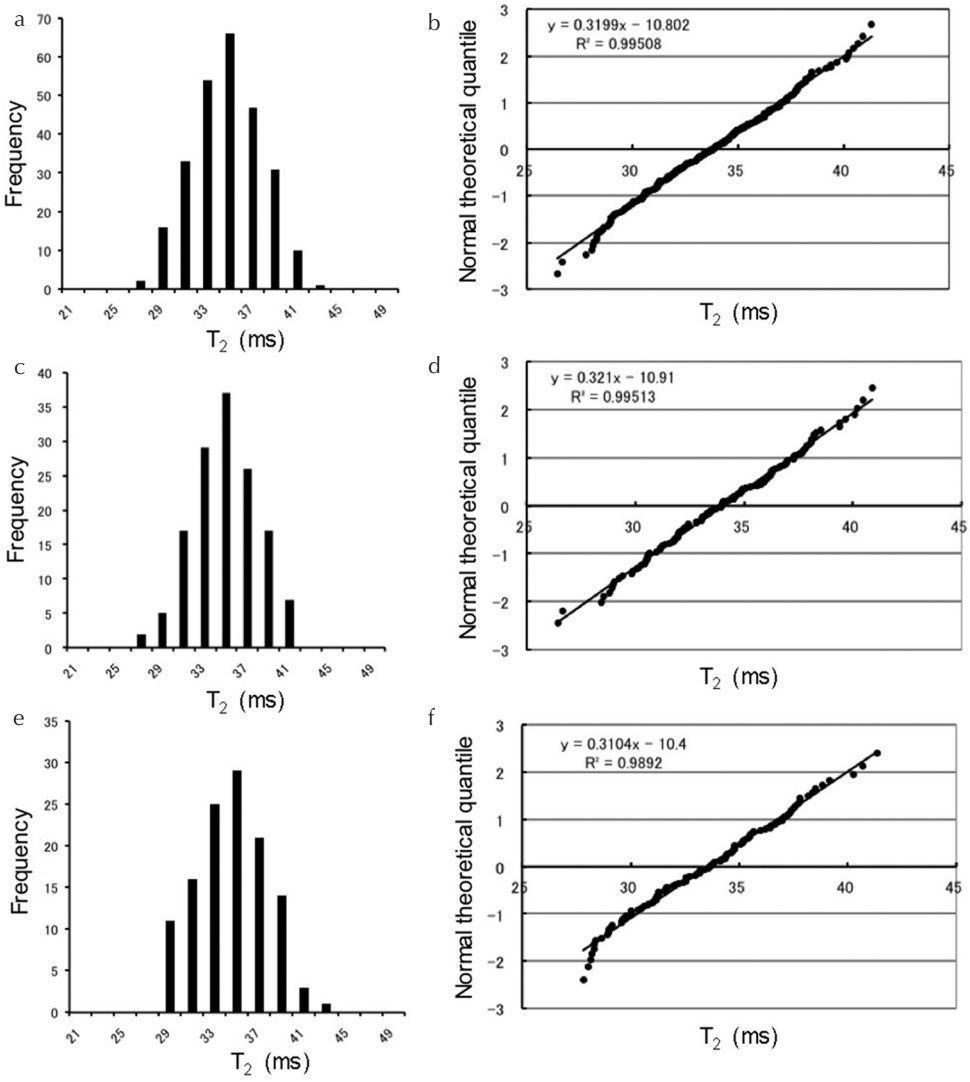

Fig. 2 Histogram and Quantile–Quantile (Q–Q) plot of T_2 values obtained from 10 muscles in the resting state. (**a** and **b**) Results from single measurements obtained from 26 subjects. (**c** and **d**) Results obtained from 14 male subjects. (**e** and **f**) Results obtained from 12 female subjects. R, correlation coefficient.

Table 2 T_2 distribution in resting muscles

Subject	T_2					Test against normal distribution		
	Mean (ms)	SD (ms)	*n*	Skewness	Kurtosis	Kolmogorov–Smirnov (*P* value)	Shapiro–Wilk (*P* value)	Jarque–Bera (*P* value)
Single experiment								
26 Subjects	33.77	3.07	260	0.064	−0.565	>0.200*	0.174*	0.162*
14 Male subjects	33.99	3.03	140	0.012	−0.479	>0.200*	0.606*	0.511*
12 Female subjects	33.51	3.11	120	0.137	−0.606	>0.200*	0.138*	0.275*
Repetitive experiments								
Three experiments with 7 subjects	34.04	2.81	210	0.229	−0.039	>0.200*	0.145*	0.396*
12 Experiments with one subject	33.40	3.03	120	0.381	−0.680	0.002	0.006	0.074*

*Indicates *P* values higher than 0.05, indicating normality for the T_2 distribution, where *n*, number of muscles. SD; standard deviation.

and 3.07) for one-sample *t*-tests for agonist and antagonist muscles. The number of subjects defined as showing exercised muscle are summarized in Table 3a. The sensitivity, specificity, and positive likelihood ratio were calculated from the results of the agonist muscles (FCR, FCU) and the antagonist muscles (ECU, ECR, and ED) (Table 3b). The false-positive rate for resting muscles before exercise is also shown in Table 3c. The Z_T = 1.00 showed the highest sensitivity (0.86) even in 5% MVC, but it showed a lower specificity (0.67–0.86), compared with the other thresholds. This was because the false-positive rate (14%) for resting muscle was much higher than those obtained using Z_T = 2.56 or 3.07 (0%). The Z_T = 3.07 threshold showed the highest specificity (1.0) in all of the MVCs, but it had the lowest sensitivity (0.36) in 5% MVC.

Discussion

Distribution of T_2 of resting muscle

Even though many researchers have used the T_2 method for detecting activated muscles, the distribution of T_2 in resting muscle has not been examined in detail. In this experiment, we used a small ROI (16 pixels: 9.8 mm^2), not an ROI that covered the whole muscle. There were three reasons for this strategy: (1) It is easy to select an ROI that excludes non-muscle tissues, (2) we can apply the same ROI size for 10 muscles in the forearm, and (3) the sample size of 16 is enough to discriminate a 1.0 SD difference in the T_2 values with a one-sided α error of 0.05 and a β error of 0.2.[23] As shown in Fig. 3a, 90% of the SD values were in a range of 2.06–5.92 ms, and furthermore, the variations in the SD did not affect the observed T_2 values. In one case, one of 16 pixels were fat tissue (T_2 = 100 ms) and the rest were muscle (T_2 = 34 ms). In this case, SD values should be increased at around 20 ms. Judging from the maximum observed SD (around 10 ms), the contribution of fat tissues might be half of a pixel, and the increase in the mean T_2 values might be up to 2 ms. Thus, as shown in Fig. 3b, the observed T_2 values did not depend on the SD. In other words, the mean T_2 is not sensitive to the contamination of fat tissue in the ROI, but the SD is much more sensitive. Therefore, it is suggested that when we get a high SD, such as 10 ms, we may need to readjust the position of ROI to minimize the contamination due to fat tissue. Considering these results, we admit that 16 pixels are too small to obtain a stable SD for each muscle. Indeed, any SD value higher than 6 ms forces a decrease *Z* value. As a result, the number of false-negative cases should be increased. Therefore, we did not use the SD values obtained from each subject, but rather, we used the SD obtained from the T_2 in the resting muscles of 26 subjects as the reference value. The distributions of the T_2 shown in the 10 muscles from 26 subjects were normal distributions (Table 1a). There was no difference between the male and female subjects. Results showing no gender dependency have been reported.[12,13] Azzabou et al.[24] detected a significant, but relatively small (0.8–1.3 ms at 3T), difference in water T_2 between the muscles in men and women. Morrow et al.[25] also concluded that muscle lipid increases with weight, but it is not gender dependent. Therefore, we could apply the same T_2 parameters to all subjects. It is true that the T_2 values in the 10 muscles showed a statistical difference (Table 1a). However, the differences in the T_2 values were less than 2 from 34 ms. A small variation in the T_2 values in the FCU, FCR, FDP, FDS have also been reported in the forearm (27.5 ± 0.8 ms at 0.35T).[26] In addition, the T_2 differences are much smaller

Table 3 Results of one-sample one-sided *T*-test
(**a**) Number of subjects where exercised muscle was detected

Exercise			25% MVC			15% MVC			5% MVC		
			Number of subjects			Number of subjects			Number of subjects		
Muscle	Function	Number of subjects (all subjects)	$Z > 3.07$	$Z > 2.56$	$Z > 1.0$	$Z > 3.07$	$Z > 2.56$	$Z > 1.0$	$Z > 3.07$	$Z > 2.56$	$Z > 1.0$
FCR	Agonist	7	6	6	7	7	7	7	3	4	6
FCU	Agonist	7	7	7	7	6	6	6	2	3	6
PL	Synergist	7	3	4	4	6	7	7	2	3	4
FDS		7	0	0	1	0	0	1	1	1	1
FDP		7	0	0	0	0	0	0	0	0	0
ECU	Antagonist	7	0	0	0	0	1	2	0	0	0
ECR	Antagonist	7	0	0	2	0	0	2	0	0	1
ED	Antagonist	7	0	0	2	0	1	3	0	0	2
SM		7	1	2	6	2	5	6	2	3	4
PT		7	0	0	0	0	0	0	0	0	0

ECR, extensor carpi radialis; ECU, extensor carpi ulnaris; ED, extensor digitorum; FCR, flexor carpi radialis; FCU, flexor carpi ulnaris; FDP, flexor digitorum profundus; FDS, flexor digitorum superficialis; MVC, maximum voluntary contraction; PL, palmaris longus; PT, pronator teres; SM, supinator muscle.

Table 3 (**b**) Performance of detection against agonist muscle (FCR, FCU), compared with antagonist muscle (ECU, ECR, and ED)

Exercise	Z_T	Sensitivity	Specificity	Positive likelihood ratio
25% MVC	Z > 3.07	0.93	1.00	∞
	Z > 2.56	0.93	1.00	∞
	Z > 1.0	1.00	0.81	5.25
15% MVC	Z > 3.07	0.93	1.00	∞
	Z > 2.56	0.93	0.90	9.75
	Z > 1.0	0.93	0.67	2.79
5% MVC	Z > 3.07	0.36	1.00	∞
	Z > 2.56	0.50	1.00	∞
	Z > 1.0	0.86	0.86	6.00

ECR, extensor carpi radialis; ECU, extensor carpi ulnaris; ED, extensor digitorum; FCR, flexor carpi radialis; FCU, flexor carpi ulnaris. MVC, maximum voluntary contraction.

Table 3 (**c**) False-positive rate for resting muscle

Z_T	Before exercise	Resting muscle of 26 subjects
Z > 3.07	0	0
Z > 2.56	0	0
Z > 1.0	0.14	0.15

than the increase in the T_2 values due to exercise (7–15 ms, Table 1b). As shown in Table 1b, the T_2 of the antagonist muscle (ECU, ECR, ED) did not present any significant increase ($P > 0.05$). A cumulative curve of the antagonist muscle shows a small shift (0.3) to the right side (Fig. 4a), and an almost straight ROC curve and the small area under the curve (AUC) (0.58) confirm that antagonist muscles were not activated by the exercise. Judging from these results, we decided to use a reference T_2 value of 34 ms, with an SD of 3 ms, for the detection of forearm muscle contraction using the one-sample *t*-test.

Threshold for the detection of exercised muscle

We applied three thresholds for the detection of exercised muscle using the one-sample *t*-test for agonist and antagonist muscles. The $Z_T = 1.0$ threshold was first proposed by Adams et al.[6] This designation was supported by an analysis of the T_2 distribution.[3,9] Thereafter, $Z_T = 1.0$ has been used to detect activated muscle due to exercise.[7,8,27] As mentioned above, previous studies used SD values from the whole resting muscle of each subject, while we have used a single SD value (3 ms) from 10 muscles for calculation of the *Z* value. Therefore, the *Z* values were not exactly the same. However, when the number of pixels in the whole muscle is large enough, the SD values in each muscle were similar to those shown in Table 2 and similar to 3 ms. As shown in the cumulative curve for 5% VMC (Fig. 4a), $Z_T = 1.0$ could detect even weak exercise. However, 16% of the T_2 values in resting muscle is distributed higher than $Z > 1.0$. Indeed, the false-positive rates for resting and antagonist muscles were 14–15% (Table 3c). Thus, the $Z_T = 1.0$ threshold showed the highest sensitivity (0.86) even in 5% MVC, but it had a lower specificity (0.67–0.86), compared with the other thresholds.

To obtain higher specificity, we need to increase the threshold. In brain functional imaging, a higher Z_T is used, such as 2.3 and 3.1.[28,29] These Z_T values correspond to *P* values 0.01 and 0.001 for one-sample one-sided *t*-tests, respectively. These values are preferable to $Z_T = 1.64$ ($P = 0.05$) to avoid false-positives. However, the best threshold for a higher specificity is still an open question.[30] In sample size calculations, we can determine the sample size based on α error (false-positive) and β error (false-negative) calculations for data obtained from a normal distribution. Aside from the statistical significance, in the mathematical expression, a sample size of 1 is obtained at a higher Z_T value.[23] The $Z_T = 2.56$ and 3.07 correspond to an α or β error of 0.10/0.30 and 0.05/0.30, respectively. Since the *Z* values for 15% and 25% MVC overlapped each other, a cumulative curve was plotted by data obtained in both cases (Fig. 4a). As shown in the cumulative curve (Fig. 4a), $Z_T = 2.56$ and 3.07 are suitable for detecting agonist muscle by 15% and 25% MVC. Indeed, the false-positive rates for resting muscle were zero for both $Z_T = 2.56$ and 3.07. Thus, the $Z_T = 3.07$ threshold showed the highest specificity (1.0) for all of the MVCs, but it had the lowest sensitivity (0.36) in 5% MVC. In physical therapy for patients, such as cerebrovascular disease, a manual muscle test (MMT) has been used for muscle training and maintaining muscle strength.[17]

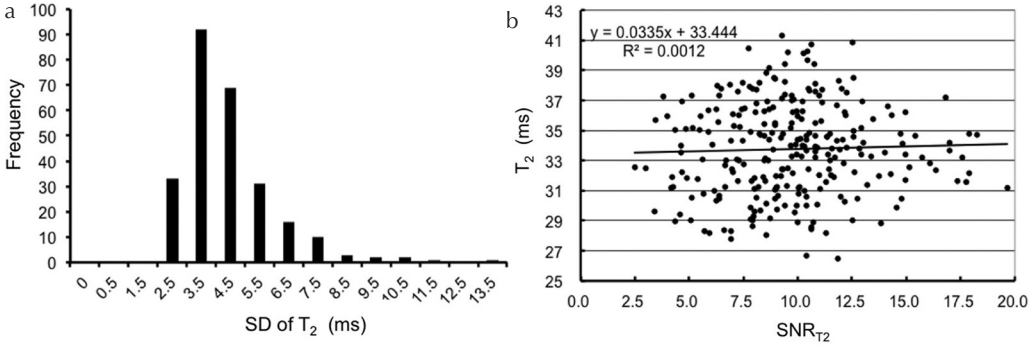

Fig. 3 SD of T_2 values obtained from 10 muscles in the resting state of 26 subjects. (**a**) Histogram of SD. Values of median, skewness and kurtosis were 3.55 ms, 5.90 and 1.97, respectively. (**b**) Correlation of SNR_{T_2} (= T_2/SD) vs T_2. There are no significance of the correlation of coefficient ($R = 0.035$) ($P < 0.05$). SD, standard deviation; SNR; signal-to-noise ratio.

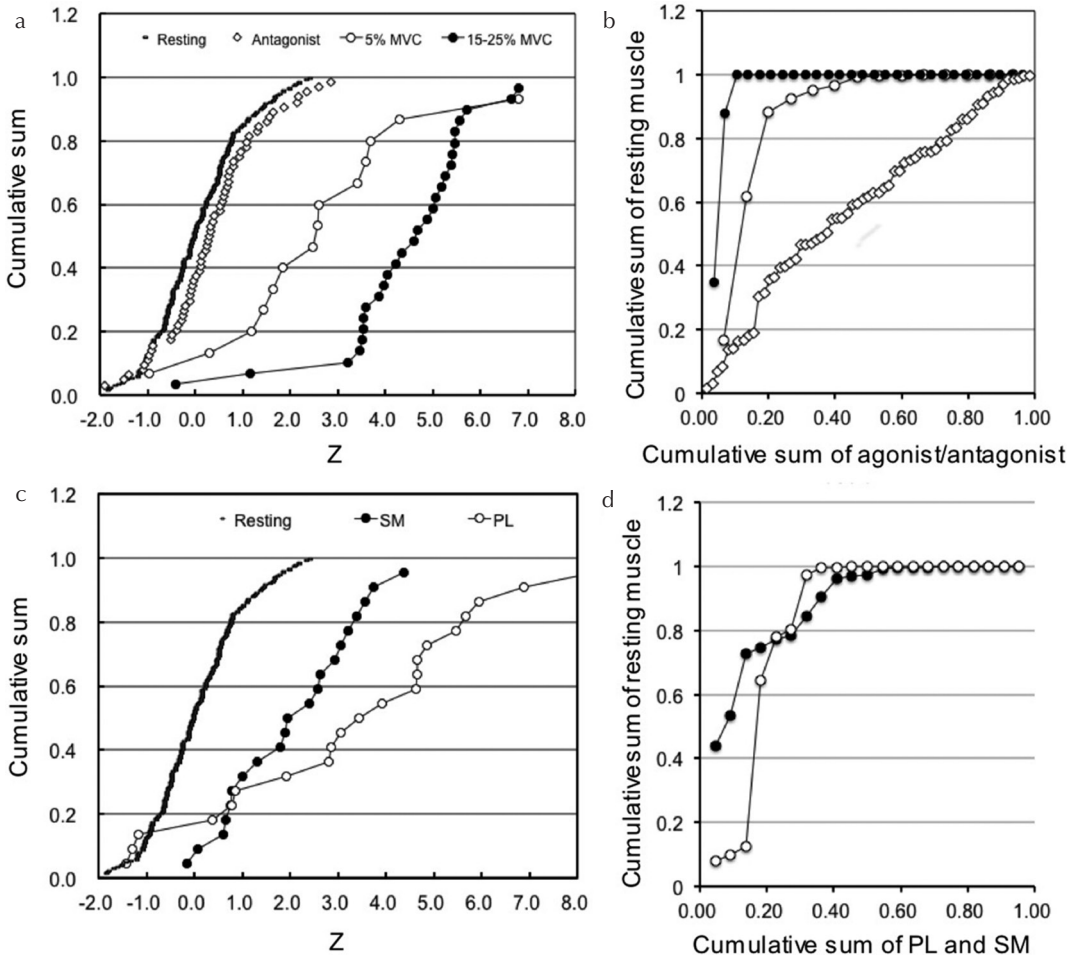

Fig. 4 Receiver operating characteristics (ROC) analysis of T_2. (**a**) Cumulative curves for resting and exercised agonist/antagonist muscle. (**b**) ROC curves for the cumulative sum of resting muscle vs that of exercised agonist muscle and that of antagonist muscle. The area under the curve (AUC) were 0.83, 0.94 and 0.58 for 5% MVC, 15–25% MVC and antagonist muscle, respectively. (**c**) Cumulative curves for resting muscle, SM and PL muscles. (**d**) ROC curves for the cumulative sum of resting muscle vs that of SM muscle and that of PL muscle. The AUC were 0.85 and 0.80 for SM and PL muscles, respectively. MVC, maximum voluntary contraction; SM, supinator muscle; PL, palmaris longus.

It is considered that the strength of the manual resistance of the MMT is important. A 25% MVC is usually used for rehabilitation. Therefore, Z_T values of 2.56 and 3.07 are acceptable due to their high specificity and sensitivity. However, a lower strength of exercise is commonly used for rehabilitation for patients with severe muscle paralysis. Therefore, the sensitivity of Z_T = 2.56 for 5% MVC is probably acceptable. Otherwise, we may go down to Z_T = 1.0 with a 15% false-positive risk. Future studies are necessary to judge the proper threshold for exercise with at a lower strength.

Detection of exercised muscle

The FCR and FCU are the agonist muscles and PL is the synergist muscle for palmar flexion of the wrist joint.[17] These muscles are detected by an increase in the T_2 values (Tables 1b and 3). Increases in the T_2 values of the FCR and FCU were detected in more than 90% of the subjects (Z_T = 1.0). However, an increase in the T_2 value in the PL was detected only in 70% of the subjects (Table 3a), and the histogram showed a bimodal distribution with an additional peak at the lower T_2 (data are not shown) that corresponds to a step-wise increase in the ROC curve (Fig. 4d). Thus, it could be considered that some of the subjects did not use the PL muscle for the palmar flexion of the wrist joints. The slope of the cumulative curve is smaller than that of the 15–25% MVC, and it is similar to that of the 5% MVC (Fig. 4a and 4c). Therefore, the ROC curve and the slope of cumulative curve might be useful to detect the synergist muscle.

We found that the T_2 of the SM increased after exercises of 15% MVC ($P < 0.01$, Table 1b). Since the Z values for the three exercises overlapped each other, a cumulative curve was plotted using the data obtained from all of the subjects (Fig. 4c). The increase in the T_2 value in the SM (70% by Z_T = 1.0) was similar to that of the PL (Table 3a). The slope of the cumulative, the ROC and the AUC were also similar to those of 5% MVC. It is possible that SM muscle could maintain the supinated position of the palm, and assist palmar

flexion of the wrist joint. Therefore, these results suggested that 70% of subjects use the SM as one of synergist muscles for palmar flexion of the wrist joint. Since the increase in the T_2 value is much smaller than that of the agonist muscle, recruiting the SM muscle might be lower than the agonist muscle. It can be considered that the increase of T_2 will be useful to analyze MMT results for patients, since we need not predict specific candidate muscles.

Limitations of this study

One important limitation of the reference T_2 value is the field strength dependency of the T_2. Due to the relaxation mechanisms of ^{1}H nuclei, T_2 decreased when there was an increase in field strength.[31] In addition, the SD of T_2 is not only influenced by the subject variations, but also by the MR pulse sequence and the stability of the MRI scanner. Therefore, the reference value is valid only for 0.2T MRI. However, in the clinical laboratory, new reference values are usually obtained when the analyzers are updated. As shown in this study, once we get the reference value, as long as we keep the same MR protocol, we can apply the reference value on all subjects. Thus, the effort required to obtain this reference value might be acceptable. We admit that we only obtained a reference value for muscle in the forearm of adult subjects. Schwenzer et al.[32] reported no difference between the T_2 calf muscle values obtained from younger (31 years) and older (66 years) subjects. However, Morrow et al.[25] reported a positive age dependency for the T_2 values obtained from lower limb muscle. Therefore, future studies are necessary to obtain reference values for children and elderly subjects, and we also need to examine T_2 variations in the lower limb muscles, to determine whether we can use a single reference value or not.

Conclusion

In conclusion, the T_2 values in the resting forearm muscles showed a normal distribution with the reference value of T_2 (34 ms) and SD (3 ms) at 0.2T. A threshold ($Z_T = 2.56$) for a one-sample one-sided *t*-test is useful to detect activated muscle after 25–15% MVC. Due to its high specificity, we may detect other muscle activity that was not expected, and the ROC analysis might be useful to analyze the increase in the T_2, which could be useful to analyze exercise for patients undergoing physical therapy.

Acknowledgments

The authors would like to thank Prof. Emer. K. Yagi (Kyoto Prefectural University of Medicine) for his advice related to the statistics. We would also like to thank Dr. T. Haishi (MRTechnology, Inc.), as well as Ms. M. Yokoi-Hayakawa and Ms. Y. Imaizumi-Ohashi (Dokkyo Medical University School of Medicine) for their technical assistance. S.A. received support in the form of a grant from the Ministry of Education, Science, and Culture of Japan (Grant-in-Aid for Scientific Research (C) 24500609, 15K01430). Y.S. received support from the Seki Minato Memorial Awards 2005 for Medical Research, Dokkyo Medical University.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Fleckenstein JL, Canby RC, Parkey RW, Peshock RM. Acute effects of exercise on MR imaging of skeletal muscle in normal volunteers. AJR Am J Roentgenol 1988; 151: 231–237.
2. Adams GR, Duvoisin MR, Dudley GA. Magnetic resonance imaging and electromyography as indexes of muscle function. J Appl Physiol (1985) 1992; 73:1578–1583.
3. Ploutz-Snyder LL, Tesch PA, Crittenden DJ, Dudley GA. Effect of unweighting on skeletal muscle use during exercise. J Appl Physiol (1985) 1995; 79:168–175.
4. Takamori M, Akiyama S, Yoshida K, et al. Changes to muscle T_2 after single-finger exercise measured with 0.2T MR imaging. Magn Reson Med Sci 2015; 14:359–366.
5. Segal RL. Use of imaging to assess normal and adaptive muscle function. Phys Ther 2007; 87:704–718.
6. Adams GR, Harris RT, Woodard D, Dudley GA. Mapping of electrical muscle stimulation using MRI. J Appl Physiol (1985) 1993; 74:532–537.
7. Kinugasa R, Kawakami Y, Fukunaga T. Muscle activation and its distribution within human triceps surae muscles. J Appl Physiol (1985) 2005; 99:1149–1156.
8. Gooding TM, Feger MA, Hart JM, Hertel J. Intrinsic foot muscle activation during specific exercises: a T2 time magnetic resonance imaging study. J Athl Train 2016; 51:644–650.
9. Prior BM, Foley JM, Jayaraman RC, Meyer RA. Pixel T2 distribution in functional magnetic resonance images of muscle. J Appl Physiol (1985) 1999; 87:2107–2114.
10. Fouré A, Le Troter A, Guye M, Mattei JP, Bendahan D, Gondin J. Localization and quantification of intramuscular damage using statistical parametric mapping and skeletal muscle parcellation. Sci Rep 2015. doi:10.1038/srep18580
11. Marshall WJ. The interpretation of biochemical data, In: Marshall WJ, Bangert SK, eds. Clinical Biochemistry: Metabolic and Clinical Aspects, 2nd ed., Churchill Philadelphia: Livingstone/Elsevier, 2008; 17–27.
12. Varghese J, Scandling D, Joshi R, et al. Rapid assessment of quantitative T_1, T_2 and T_2* in lower extremity muscles in response to maximal treadmill exercise. NMR Biomed 2015; 28:998–1008.
13. Pettersson H, Slone R. Magnetic resonance relaxation characteristics of muscle, fat and bone marrow of the extremities. Normal values in a low field strength unit. Acta Radiol 1987; 28:363–364.
14. Polak JF, Jolesz FA, Adams DF. NMR of skeletal muscle. Differences in relaxation parameters related to extracellular/intracellular fluid spaces. Invest Radiol 1988; 23:107–112.

15. Kjos BO, Ehman RL, Brant-Zawadzki M. Reproducibility of T1 and T2 relaxation times calculated from routine MR imaging sequences: phantom study. AJR Am J Roentgenol 1985; 144:1157–1163.
16. Ellis H, Logan BM, Dixon AK. Human Sectional Anatomy, 2nd ed., London: CRC Press, 2001; 1-256.
17. Hislop H, Avers D, Brown M. Daniels and Worthingham's Muscle Testing: Techniques of Manual Examination and Performance Testing, 9th ed., St. Louis: Saunders, 2013; 1–528.
18. Razali NM, Yap BW. Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. J Stat Mod Anal 2011; 2:21–33.
19. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika 1965; 52:591–611.
20. Royston JP. An extension of Shapiro and Wilk's W test for normality to large samples. J R Stat Soc Ser C Appl Stat 1982; 31:115–124.
21. Jarque CM, Bera AK. A test for normality of observations and regression residuals. Int Stat Rev 1987; 55:163–172.
22. Öztuna D, Elhan AH, Tüccar E. Investigation of four different normality tests in terms of type 1 error rate and power under different distributions. Turk J Med Sci 2006; 36:171–176.
23. Florey CD. Sample size for beginners. Br Med J 1993; 306:1181–1184.
24. Azzabou N, Hogrel JY, Carlier PG. NMR based biomarkers to study age-related changes in the human quadriceps. Exp Gerontol 2015; 70:54–60.
25. Morrow JM, Sinclair CD, Fischmann A, et al. Reproducibility, and age, body-weight and gender dependency of candidate skeletal muscle MRI outcome measures in healthy volunteers. Eur Radiol 2014; 24:1610–1620.
26. Fleckenstein JL, Haller RG, Lewis SF, et al. Absence of exercise-induced MRI enhancement of skeletal muscle in McArdle's disease. J Appl Physiol (1985) 1991; 71:961–969.
27. Akima H, Kuno S, Takahashi H, Fukunaga T, Katsuta S. The use of magnetic resonance images to investigate the influence of recruitment on the relationship between torque and cross-sectional area in human muscle. Eur J Appl Physiol 2000; 83:475–480.
28. Ashburner J. SPM: a history. Neuroimage 2012; 62:791–800.
29. Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM. FSL. Neuroimage 2012; 62:782–790.
30. Eklund A, Nichols TE, Knutsson H. Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci USA 2016; 113:7900–7905.
31. Bloembergen N. Nuclear Magnetic Relaxation, New York: Benjamin, 1961; 1-178.
32. Schwenzer NF, Martirosian P, Machann J, et al. Aging effects on human calf muscle properties assessed by MRI at 3 Tesla. J Magn Reson Imaging 2009; 29:1346–1354.