**MAJOR PAPER**

T2 Distribution in the Forearm Muscles and the T2 Threshold for Defining Activated Muscle

Masayoshi Takamori1,2, Sumikazu Akiyama1,3, Kazuya Yoshida1,3, Hidefumi Wakashin4, and Yoshiteru Seo1*

**Purpose:** In order to detect exercised muscles by the increase in T2, we have defined a Gaussian T2 distribution and reference values (T2r and SD) 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. T2 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 (T2 - T2r)/SD, where T2r was T2 after exercise. Based on sample size calculations, three thresholds (Zr = 1.00, 2.56, and 3.07) were applied to agonist and antagonist muscles.

**Results:** A normal distribution of T2 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 T2 or SD, and a similar result was obtained in 12 measurements on a single subject (P < 0.01). The T2r and SD were used for reference values. The threshold Zr = 1.00 showed the highest sensitivity (0.86) even with 5% MVC, but it showed a lower specificity (0.85) than the other thresholds. Zr = 3.07 showed the highest specificity (1.0), but it showed a lower sensitivity (0.36) with the 5% MVC, compared with Zr = 2.56 (0.50). The receiver operating characteristics analysis also supported these results.

**Conclusion:** We found that the T2 distribution in muscles was Gaussian, suggesting that a one-sample t-test can be applied, and that Zr = 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 T2 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 T2 in muscles. Several reports have been published on studies investigating the T2 distribution of ROI that covered the whole muscle.9

In one study, Prior et al.9 examined changes in the T2 distribution in exercised muscle, and they assumed a Gaussian distribution of T2 values at rest. However, judging from the pixels in an ROI covering the whole muscle, for example, Fig. 3 in Prior’s paper, the T2 distribution in resting muscle was not symmetrical, but rather, it was skewed to higher T2 values.3,9 Ploutz-Snyder et al.3 considered that a high T2 component (> 35 ms) in resting muscle showed non-muscle tissue, such as fat. This assumption might be true because the distribution of T2 in exercised muscle becomes more symmetrical, even though there is a tendency for an increase in the SD of the T2 values.3,9 In addition, in the muscles of the lower limbs, a large T2 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

1Department of Regulatory Physiology, University of Dokkyo School of Medicine, 880 Kitakobayashi, Mibu-machi, Shimotsuga-gun, Tochigi 321-0293, Japan
2Department of Physical Therapy, Aoi Medical Academy, Saitama, Japan
3Department of Rehabilitation, Faculty of Health Sciences, University of Human Arts and Sciences, Saitama, Japan
4Corresponding author, Phone: +81-282-87-2125, Fax: +81-282-86-7835, E-mail: yseo@dokkyomed.ac.jp

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limits are the mean ± 2 SD. As far as we have found in a search of the literature available, there are no reports that mention the reference value of T2 in skeletal muscle. Till now, similar T2 values have been assumed for all muscles in the resting state. However, some reports have mentioned the presence of T2 differences (4–10 ms) in the muscles of the lower limbs. Therefore, we first measured the T2 of 10 muscles in the forearm, and differences in the T2 were examined. If the T2 values found in the 10 muscles showed a Gaussian distribution and were similar to each other, we determined T2 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. T2 values in the resting state were obtained from 26 subjects, and T2 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 1H MR images were obtained with a 0.2T compact MRI system (MRTechnology, Inc., Ibaraki, Japan) equipped with an oval 1H solenoidal radiofrequency (RF) coil (120 × 160 mm) and a shell-type arm holder. Since the T1 and T2 of muscle are around 500 and 35 ms, respectively, the parameters for T2 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 T2 values in each pixel were calculated by nonlinear fitting to single exponential decay, and the T2 images (T2 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. These therapists presented a good intraobserver agreement in a previous study. 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) muscle, extensor carpi radialis (ECR) longus/brevis muscle, supinator muscle (SM), pronator teres (PT) muscle, and extensor...
digitorum (ED) muscle (Fig. 1b).\(^6\) 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,\(^7\) 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 MVC.\(^8\) 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 Lillieforrs corrected Kolmogorov–Smirnov test (KS test) and the Shapiro–Wilk test (SW test) using IBM SPSS Statistics software (V25, IBM, Corporation, Armonik, 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 (\(\alpha\) error < 0.05 in the sample size from 8 to 150).\(^22\) The SW and KS tests were hard to reach \(\alpha\) 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       | FDS          |           | 33.48    | 2.42     | 26       | 0.065 | 1.027 | 0.560 |
| superficialis           |              |           |          |          |          |        |        |        |
| Flexor digitorum       | FDP          |           | 32.12    | 2.58     | 26       | 0.508 | 1.050 | 0.314 |
| profundus               |              |           |          |          |          |        |        |        |
| Extensor carpi ulnaris | ECU          | Antagonist| 34.70    | 3.11     | 26       | 0.223 | −0.564 | 0.756 |
| Extensor carpi radialis| ECR          | Antagonist| 33.51    | 3.35     | 26       | 0.862 | −0.178 | 0.197 |
| longus/brevis           |              |           |          |          |          |        |        |        |
| Extensor digitorum      | ED           | Antagonist| 35.99    | 2.71     | 26       | −0.510 | −0.474 | 0.504 |
| Supinatores             | 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. SD: standard deviation. 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.\(^7\)
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 $34 \pm 3$ ms (mean $\pm$ 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 ($P < 0.05$).

Table 1b Changes in $T_2$ due to exercise

| Exercise | Number of subjects | $T_2$ (Mean ms) | $T_2$ (SD ms) | $T_2$ (Mean ms) | $T_2$ (SD ms) | $T_2$ (Mean ms) | $T_2$ (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.

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 significant difference shown between the pre-exercised muscles and SM increased. In the statistical results obtained from seven subjects (Table 1b), 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, FCU, and SM increased significantly, compared with that of the pre-exercised muscles ($P < 0.01$).

$T_2$ 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 $(T_2 - T_{2e})/SD_e$, where the $T_{2e}$ values were the $T_2$ values obtained after exercise, and $T_{2e}$ and $SD_e$ are the mean and SD of the resting muscle, respectively. From the results shown above, we used a $T_2$ of 34 ms and an SD of 3 ms as the reference value. We choose three thresholds ($Z_1 = 1.0, 2.56,$
Table 2 $T_2$ distribution in resting muscles

| Subject                        | $T_2$ 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 0.67) 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 $\alpha$ error of 0.05 and a $\beta$ error of 0.2. As shown in Fig. 3a, 90% of the SD values were in a range of 0.35–0.86 ms, 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.

**Table 3** Results of one-sample one-sided t-test.

| Exercise | 25% MVC | 15% MVC | 5% MVC |
|----------|---------|---------|--------|
| 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 | 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 | 1 | 1 |
| FDP | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECU | Antagonist | 7 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECR | Antagonist | 7 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ED | Antagonist | 7 | 0 | 0 | 2 | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SM | 7 | 1 | 2 | 6 | 2 | 5 | 6 | 2 | 3 | 4 | 3 | 4 | 3 | 4 |
| PT | 7 | 0 | 0 | 0 | 0 | 0 | 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 | $\infty$ |
|          | $Z > 2.56$ | 0.93 | 1.00 | $\infty$ |
|          | $Z > 1.0$  | 1.00 | 0.81 | 5.25 |
| 15% MVC  | $Z > 3.07$ | 0.93 | 1.00 | $\infty$ |
|          | $Z > 2.56$ | 0.93 | 0.90 | 9.75 |
|          | $Z > 1.0$  | 0.93 | 0.67 | 2.79 |
| 5% MVC   | $Z > 3.07$ | 0.36 | 1.00 | $\infty$ |
|          | $Z > 2.56$ | 0.50 | 1.00 | $\infty$ |

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

The threshold in activated muscles

Before exercise

| $Z_T$ | Sensitivity | Specificity | Positive likelihood ratio |
|-------|-------------|-------------|--------------------------|
| 0.67  | 0.93 | 1.00 | $\infty$ |
| 0.93  | 0.93 | 1.00 | $\infty$ |
| 2.56  | 0.93 | 0.90 | 9.75 |
| 1.00  | 0.93 | 0.67 | 2.79 |
| 7     | 0.36 | 1.00 | $\infty$ |
| 8     | 0.50 | 1.00 | $\infty$ |
| 27    | 0.86 | 0.86 | 6.00 |

$T_2$ Threshold in Activated Muscles

Before exercise

| $Z_T$ | Sensitivity | Specificity | Positive likelihood ratio |
|-------|-------------|-------------|--------------------------|
| 0.67  | 0.93 | 1.00 | $\infty$ |
| 0.93  | 0.93 | 1.00 | $\infty$ |
| 2.56  | 0.93 | 0.90 | 9.75 |
| 1.00  | 0.93 | 0.67 | 2.79 |
| 7     | 0.36 | 1.00 | $\infty$ |
| 8     | 0.50 | 1.00 | $\infty$ |
| 27    | 0.86 | 0.86 | 6.00 |

$T_2$ Threshold in Activated Muscles

Resting muscle of 26 subjects

| $Z_T$ | Sensitivity | Specificity | Positive likelihood ratio |
|-------|-------------|-------------|--------------------------|
| 0.67  | 0.93 | 1.00 | $\infty$ |
| 0.93  | 0.93 | 1.00 | $\infty$ |
| 2.56  | 0.93 | 0.90 | 9.75 |
| 1.00  | 0.93 | 0.67 | 2.79 |
| 7     | 0.36 | 1.00 | $\infty$ |
| 8     | 0.50 | 1.00 | $\infty$ |
| 27    | 0.86 | 0.86 | 6.00 |

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

| $Z_T$ | Before exercise | Resting muscle of 26 subjects |
|-------|-----------------|------------------------------|
|       | 0.14            | 0.15                         |

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

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. This designation was supported by an analysis of the $T_2$ distribution. Thereafter, $Z_T = 1.0$ has been used to detect activated muscle due to exercise. 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. 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. In sample size calculations, we can determine the sample size based on $\alpha$ error (false-positive) and $\beta$ 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. The $Z_T = 2.56$ and 3.07 correspond to an $\alpha$ or $\beta$ 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.

![Fig. 3 Standard deviation (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 ($R = 0.0355$) ($P < 0.05$). SNR; signal-to-noise ratio.]
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 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. 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

![Fig. 4](image-url)
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.\(^{31,32}\) 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 (\( Z_2 = 2.56 \)) 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.

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**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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