**MAJOR PAPER**

**Diffusion Fractional Anisotropy-based Transformation in Skeletal Muscle Caused by Pressure**

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**Purpose:** We used diffusion tensor imaging (DTI) to evaluate anisotropic changes in skeletal muscle cells under external pressure.

**Materials and Methods:** In 6 healthy volunteers, we compared DTI of the tibialis anterior (TA) and soleus (SOL) muscles under pressure. We performed imaging using a 1.5-tesla magnetic resonance (MR) scanner and diffusion-weighted stimulated-echo echo-planar pulse sequences optimized for skeletal muscle. We calculated diffusion tensor eigenvalues ($\lambda$), apparent diffusion coefficients, and fractional anisotropy (FA) values in a series of axially acquired DTI and compared them between the pressurized and nonpressurized lower limbs. We also measured a cross-sectional area of skeletal muscle.

**Results:** We observed clear differences in FA and $\lambda_3$ between pressurized muscles and the nonpressurized muscles we used as controls. The mean control FAs were $0.36 \pm 0.02$ (TA) and $0.30 \pm 0.02$ (SOL). The mean control $\lambda_3$s were $0.74 \pm 0.02$ s/mm$^2$ (TA) and $0.85 \pm 0.03$ s/mm$^2$ (SOL). FA values in the pressurized (200 mmHg) limbs increased to $0.39 \pm 0.02$ (TA) and $0.35 \pm 0.04$ (SOL) compared with those values in the nonpressurized controls. $\lambda_3$ values in the pressurized (200 mmHg) limbs decreased to $0.68 \pm 0.05$ s/mm$^2$ (TA) and $0.77 \pm 0.06$ s/mm$^2$ (SOL) compared with those in controls. Moreover, the mean value of cross-sectional area of skeletal muscle (control) was $907.3 \pm 140.1$ mm$^2$ (TA) and $1522 \pm 201.0$ mm$^2$ (SOL). The cross-sectional area in the pressurized (200 mmHg) limbs decreased to $590.3 \pm 68.1$ mm$^2$ (TA) and $1131 \pm 112.6$ mm$^2$ (SOL) compared with those in controls. One-way analysis of variance (ANOVA) and post hoc Tukey-Kramer tests showed significant differences.

**Conclusion:** Anisotropy changed markedly on pressurizing the lower limb based on the correlation of the cross-sectional area and $\lambda_3$ suggested marked changed in anisotropy following application of pressure to the lower limb. If compression of the cross-sectional area is assumed to represent compression of the cell, change in $\lambda_3$ reflected the change in the size of muscle cells.

**Keywords:** diffusion tensor, fractional anisotropy, pressurization, skeletal muscle, stimulated echo

**Introduction**

In 1965, Stejskal and Tanner advocated the use of diffusion-weighted imaging (DWI) for measuring water diffusion; DWI enables such measurement in vivo. Acquisition of DWI data from more than 6 axes, termed diffusion tensor imaging (DTI), allows assessment of anisotropy. DTI has been widely used to evaluate the anisotropic structures of nerves and skeletal muscle. However, difficult signal acquisition for the very short T$_2$ relaxation time of skeletal muscle do not permit sufficient impression of the gradient magnetic field and sufficient reflection of the diffusion phenomenon.
In 1970, Tanner reported the efficacy of diffusion-weighted stimulated-echo (DW-STE) method in assessing tissues with short T2 values. The signal intensity of the DW-STE sequence, which employs three 90° pulses (Fig. 1), is expressed as:

\[
S_{\text{ISTimulated echo}} = S_{I_0} \cdot \frac{1}{2} \left( 1 - \exp \left( \frac{-TR}{T_1} \right) \right) \times \exp \left( \frac{-TE}{T_2} - \frac{TM}{T_1} - bD \right).
\]

This method uses the motion-probing gradient (MPG) after the first and third radiofrequency (RF) pulses. Magnetization is governed by the longitudinal relaxation between the second and third RF pulses (mixing time [TM]). Therefore, the T1 value as well as the T2 value greatly influences the change in signal intensity. Moreover, a strong signal can be acquired when the ratio of echo time (TE) and TM becomes a definite value. However, this ratio differs according to the imaging object and is approximated using \( \delta: \Delta = T_2:T_1 \) for the respective T1 and T2 values. The STE method is useful to break weak signals with short T2 value. It allows an increase in the b-value by augmenting TM rather than TE, and relaxation-dependent signal losses are clearly lower during the TM than TE interval.

Studies of skeletal muscles that have evaluated anisotropy report different fractional anisotropy (FA) values for various reasons. We aimed to clarify the relationship between FA and muscle morphology by applying pressure to skeletal muscles in the lower limbs. Moreover, we applied the STE method to a clinical machine to permit sufficient reflection of the diffusion phenomenon.

Materials and Methods

Subjects

We obtained study approval from the Tokyo Metropolitan University Ethical Committee and informed consent from 6 male volunteers (aged 21.1 ± 1.6 years [mean ± standard deviation]; height, 170.7 ± 6.2 cm; weight, 60.7 ± 5.3 kg; body mass index, 20.9 ± 1.0 kg/m²). Subjects were nonathletes with motor function in the lower limbs and no history of myogenic disease.

Pressure application

We used a cuff (Tanaka Industry Inc., Tokyo, Japan) to apply pressure, which we monitored using a mercury gauge (Tanaka Industry Inc.). Outside the MR imaging room, we rolled the manchet onto the lower limb, turned the manchet valve so that the pressure did not change, and removed the meter. The pressure was set to 3 stages —0, 100, and 200 mmHg —values at which pain is generally not experienced.

MR imaging

We carried out MR imaging using a 1.5-tesla MR scanner (Signa Horizon Lx Ver. 9.0; GE Healthcare, Tokyo, Japan) and knee coil (GE Healthcare) and achieved best performance with magnetic field strength of 22 mT/m and maximum slew rate of 77 mT/m/m. Each volunteer was positioned supine with feet first and the imaging apparatus centered at the largest diameter of the lower limb.

DTI was performed using an originally developed single-shot DW-STE pulse sequence with parameters: repetition time (TR)/TE/TM, 4000/44.1/208.2 ms; \( \Delta/\delta \), 225.9/11.4 ms; b-value, 1000 s/mm²; number of excitations (NEX), 4; field of view (FOV), 240 × 240 mm²; matrix size, 128 × 128; number of slices, 6; slice thickness, 8 mm without a gap; and MPG moment, 6 axes (xy, xz, yz, −xy, −xz, −yz). Total DW-STE scanning time was 224 s.

We scanned T1-spoiled gradient recalled acquisition in the steady state (SPGR) to measure the ana-
tomical form using parameters: TR/TE, 20/5 ms; flip angle, 40°; NEX, one; FOV, 240 × 240 mm²; matrix size, 128 × 128; and slice thickness, 8 mm without a gap.

Analyses

We evaluated DWI data using freeware provided by the University of Tokyo (dTV2; http://www.volume-one.org/) and calculated apparent diffusivity coefficient (ADC) using b-values of 0 and 1000 s/mm². Moreover, we estimated the diffusion tensor by fitting the ADC to a 3-dimensional (3D) ellipsoid model based on tensor analysis results. The lengths of the longest ($\lambda_1$), medium ($\lambda_2$), and shortest ($\lambda_3$) axes, called eigenvalues, were calculated, and FA was then calculated as:

$$ FA = \sqrt{\frac{3}{2}} \left( \frac{\lambda_1 - \lambda}{\lambda_1 + \lambda_2 + \lambda_3} \right)^2 $$

We targeted 2 skeletal muscles in the lower limb—the tibialis anterior (TA) and soleus (SOL) muscles—and selected a region of interest (ROI) for each muscle (Fig. 2) from the T₁ SPGR image and calculated diffusion values (ADC, FA, $\lambda_1$, $\lambda_2$, and $\lambda_3$). We also calculated cross-sectional areas from this ROI. As a statistical analysis, we detected a between-group difference on one-way ANOVA and post hoc Tukey-Kramer tests at a significance level of 5%.

Results

We applied pressure to the lower limbs and performed MR imaging. Figure 3 shows ADC calculated from the results of the DTI measurements. ADC changed little in proportion to pressure. Significant difference in ADC between the 2 muscles was not admitted, though ADC by pressure was given official approval (TA: $P=0.27$, SOL: $P=0.10$). Figure 4 shows FA, the result of the rise in pressure, and Fig. 5 shows eigenvalue, which provides a measure of the directionality of diffusion. Significant difference in FA between the 2 muscles was admitted though FA by pressure was given official approval (TA: $P<0.001$, SOL: $P<0.01$). There was a marked change in $\lambda_3$, but the difference in $\lambda_1$ was limited and not significant (TA: $P=0.93$, SOL: $P=0.47$). In contrast, changes in $\lambda_2$ (TA: $P<0.05$, SOL: $P<0.01$) and $\lambda_3$ (TA: $P<0.01$, SOL: $P<0.005$) were significant. Eigenvalues, which divide FA according to direction, tended to decrease when pressure increased. Thus, the influence of pressure was in the order of $\lambda_3 > \lambda_2 > \lambda_1$. Figure 6 shows the results in the cross-sectional area, in which the value decreased in proportion to pressure. A significant difference was obtained for the cross-sectional area following application of pressure (TA: $P<0.001$, SOL: $P<0.005$).

![Fig. 2. Anatomical imaging of the lower limb to select the region of interest (ROI). Solid line, tibialis anterior muscle (TA); dotted line, soleus muscle (SOL).]

![Fig. 3. Bar graph shows the averages and standard deviation (SD) of apparent diffusion coefficients (ADC) (mm²/s) represented by the pressure (mmHg) applied to the tibialis anterior (TA) and soleus (SOL) muscles. n.s., not significant; *$P<0.05$; **$P<0.01$.]
Fig. 4. Bar graph shows the averages and standard deviation (SD) of fractional anisotropy (FA) (arbitrary unit) represented by the pressure (mmHg) applied to the tibialis anterior (TA) and soleus (SOL) muscles. n.s., not significant; *P < 0.05; **P < 0.01.

Fig. 5. Bar graph shows the averages and standard deviation (SD) of each eigenvalue (mm²/s) represented by the pressure (mmHg) applied to the tibialis anterior (TA) and soleus (SOL) muscles. n.s., not significant; *P < 0.05; **P < 0.01.

Discussion

The T₂ value of skeletal muscle is very short and cannot provide an appreciable b-value. In this study, we used the STE method to evaluate the diffusion anisotropy of skeletal muscle after application of pressure. Some studies have used this method to show disease-related changes in skeletal muscle.7,17,18 However, the changes appeared to occur externally, and I understand it to refer to the other studies.

We applied pressure to the lower limb and measured diffusion value. The value changed in the pressurized limb compared with the control. Heemskerk reported the relationship between ADC and properties of muscle cells. ADC changes with disease and cell edema.19 However, the extent of change in ADC was not great to our study (Fig. 3). The change in ADC caused by pressure resulted in muscle ischemia and change in muscle properties.

Eigenvalue provides ADC directionality and offers a greater understanding of the details of muscle transformation. During on and off pressure application produced small variations in λ₁ in some muscles (Fig. 5). We attribute these results to insufficient diffusion time along the long axis of the muscle fiber20,21; failure to set diffusion time to permit adequate diffusion of water molecules prohibits visualization of the entire structure. We could set a long mixing time using the STE method, so this method might be useful in evaluating structural limitation.

Because λ₂ and λ₃ were assessed in the same cross section, we expected to obtain the same value by water diffusion, but results differed (Fig. 5). These results suggest that λ₁ reflects muscle cell size and λ₂ reflects layers or sheets of muscle cells, an explanation analogous to Tseng’s description of fiber orientation.22,23 The geometry of the muscle fibers lacked a well defined symmetry. Therefore, λ₂ is sensitively reflected in the cross-sectional orientation of the fiber.24 λ₃ changed most sensitively, so this value is believed to greatly reflect the radius of a muscle cell. Galbán and Karampions used a different approach to compare diffusion value and a cross-sectional area.25,26 Their studies were presented to demonstrate the linear relationship between the muscle physiological cross-sectional area (PCSA)25 and λ₃, which was due to a similar dependence on the muscle cross-sectional area. Figure 6 shows the change in a muscle cross-sectional area, a great change when pressure was applied. Therefore, both λ₃ values are needed to evaluate the extent of muscle transformation caused by pressure.

Other determinants, such as temperature change in the muscle, may affect diffusion parameters, but we did not monitor muscle temperature. Nevertheless, we maintained room temperature at 26°C. We believe temperature had little effect on results because the time from pressure application until MR imaging was very short, and pressure in the lower limb was changed passively. It is also impossible to
explain the decrease in \( \lambda_3 \) and invariability in \( \lambda_1 \) changes based on muscle temperature. Changes in blood flow or perfusion may also have affected the results. We conclude it for the following reason. Use of a low b-value (<200 [s/mm\(^2\)]) may affect blood flow and perfusion,\(^{12,13} \) but our use of a b-value of 1000 [s/mm\(^2\)] counters this explanation.

The size of the skeletal muscle cell was believed to be greatly reflected by \( \lambda_3 \). Skeletal muscle cells are currently classified by staining but may be classified in the future by MR imaging using the \( \lambda_3 \) value and q-space imaging.\(^{27-29} \)

**Conclusion**

We evaluated human skeletal muscle to uncover the relationship between anisotropy, diffusion coefficient, and application of external pressure to the muscle. Our results suggest that the \( \lambda_3 \) of skeletal muscle reflects the size of a muscle cell.

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**References**

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