TECHNICAL NOTE

Correlation between Changes in the Transverse Relaxation Time and Electromyographic Measurements of the Superficial Masseter and Temporal Muscles

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We analyzed the correlations between the T_2 shift and integrated electromyographic (iEMG) values in the masseter and temporal muscles. Six healthy adults engaged in a clenching task over two durations at various bite forces. We evaluated the mean T_2 shift per voxel and assessed their correlations with iEMG using a linear mixed model. The regression coefficients were different for each muscle type, similar for the left and right sides, and decreased upon doubling duration.

Keywords: *electromyography, magnetic resonance imaging, masticatory muscle, transverse relaxation time*

Introduction

Skeletal muscles play an essential role in performing most physical functions, making the multifaceted evaluation of muscle activity a clinical necessity. Muscle activity is typically evaluated with electromyography (EMG); however, muscle functional MRI (mfMRI) is better able to evaluate broad muscle activity than standard EMG.[1] In mfMRI, the transverse relaxation time (T_2) of the skeletal muscles shifts with exercise, facilitating the quantitative and noninvasive evaluation of muscle activity in an arbitrary range.[2] Although the exercise-related T_2 shift is attributed to several biological factors, including intracellular and intercellular water volumes and acidification, the exact mechanism is still unclear.[3] The exercise-induced T_2 shift in skeletal muscle is also caused by blood oxygenation (the blood oxygenation level-dependent [BOLD] effect); however, the contribution of the BOLD effect to large increases in T_2 for several minutes after intensive exercise is smaller than that of the osmotic pressure changes.[4] Despite limited information regarding its mechanism, the T_2 shift showed a sigmoidal relationship with both the repetition number (duration) and intensity of exercise,[5] and was correlated with them within a specific load range.[6]

Some studies have investigated the relationship between the T_2 shift and EMG values in exercising skeletal muscles. A majority of these studies utilized multiple subjects and assessed the data using correlation and linear regression across subjects due to the difficulty in obtaining multiple mfMRI measurements from a single subject.[6] Such an approach cannot consider different characteristics within subjects, thereby increasing the likelihood of erroneous results. In contrast, upon analyzing a spinal muscle during exercise, Dickx et al.[7] reported that a linear regression coefficient adjusted for the effects of individual subjects will differ for each muscle when using a linear mixed model to assess the T_2 shift relative to EMG values. The linear mixed model can analyze the characteristics of the data by considering the characteristics of subjects. Despite the usefulness of the method, Dickx et al.[7] considered only two slice images of MRI (slice thickness was not reported), although they alone may not reflect the T_2 shift for the overall muscle. Considering that the activity distribution in skeletal muscles is not homogeneous, the different linear regression coefficients among muscles they reported might be caused by muscle activity outside the imaged slices. Moreover, it is unclear whether the different linear regression coefficient exists between the left and right sides of the same muscle, since Dickx et al.[7] did not compare the regression coefficients between the left and right sides. If the regression coefficient changes depending on the characteristics of each muscle, the regression coefficients may be similar between the left and right sides of the same muscle.

In mfMRI, since T_2 change in the muscle from before to after the exercise is used as an index of muscle activity, the measured value is affected by the exercise duration. However, total muscle activity can be assessed over the limited range of

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exercise duration by mfMRI due to the finite nature of T_2 extension, while the total muscle activity can be appropriately evaluated over the total range of exercise duration by using integrated EMG (iEMG), in which EMG signals are integrated over time. Although the relationship between T_2 shift and iEMG may be different based on the isometric exercise duration, it has not been sufficiently verified. Since the task in the previously mentioned study by Dickx et al.[7] did not comprise isometric contraction and the duration was constant, the impact of the duration of isometric contraction on the correlation between T_2 shift and EMG measurements is unknown.

The objective of the present study was to analyze the correlations between the T_2 shift and iEMG in the both superficial masseter and temporal muscles, which can be measured by surface EMG, by examining clenching activities of varying intensity and duration in multiple subjects. To this end, we used a linear mixed model that accounts for variations between subjects.

Materials and Methods

Subjects

Six healthy subjects were enrolled (three men, three women; mean age: 30.7 ± 5 years). We thoroughly explained the objective, methods, and safety of this study to each subject before obtaining informed consent. The exclusion criteria of the study were current or previous functional disorders of the stomatognathic system, pain in teeth or periodontal tissues, diseases related to nerve/muscle/metabolism, and MRI contraindication. Although the presence or absence of crown prosthesis was unquestioned, it was confirmed that there were no missing teeth other than the wisdom teeth and there was bilateral molar occlusal support. This study was approved by the Ethics Committee of the Tohoku University Graduate School of Dentistry.

Bite force gauge

Since the increased T_2 of the exercising skeletal muscles begins recovery immediately after the completion of the task, MRI must be performed promptly following the completion of the task.[8] Thus, we applied a compact pressure sensor made of optical fiber (FOP-M-BA; Fiso Technologies Inc., Quebec, QC, Canada) capable of working in a strong magnetic field. In a previous study, using the changes in water pressure in a water bag matching the dental configuration as an index, we developed an MRI-compatible device that could evaluate the bite force in real time (Fig. 1).[9] However, owing to the instability of the occlusion state with a water bag, we prepared a hollow mouthpiece for each subject using 1 and 0.5-mm thermoplastic resin sheets (Yamahachi Dental MFG. Co., Aichi, Japan) and self-curing resin (Unifast III; GC Corporation, Tokyo, Japan), securing the occlusion by replacing the water bag with the mouthpiece (Fig. 1). We adjusted the mouthpiece once it was inside the mouth of the subject to attain uniform occlusion. Immediately before the experiment, we used a force gauge (ZP-1000N; IMADA Co. Ltd., Aichi, Japan) and a resin dentition model to simultaneously measure the internal water pressure while applying the external force–simulating bite force, thereby confirming the relationship between the water pressure and the bite force. In addition, we connected a conditioner (EVO-SD-2; Fiso Technologies Inc.) in the control room to the pressure sensor using a fiber optic cable and used the designated software on a laptop connected to the conditioner, to observe the bite force of subjects in the MRI gantry in real time.

MRI protocol

For all MRI, we used a MAGNETOM Verio 3T (Siemens Healthcare, Erlangen, Germany). All subjects were placed in the supine position, wearing the bite force gauge, with the head fixed to the head coil. We used the spin-echo method (TR, 2300 ms; TE, 20/60 ms; flip angle = 90°; 22 slices; slice thickness, 3 mm; matrix = 256×256; FOV = 250 mm; pixel size, 1×1 mm^2; scan time, 7 min 25 s) and imaged the entire head and neck. Because the T_2 of the muscle decreases with time during MRI, the amount of T_2 change varies between slices with different imaging times. To minimize the influence of interslice T_2 differences on the T_2 differences between muscles, the coronal acquisition plane was selected and care was taken to include all the masticatory muscles in the same slice as much as possible.
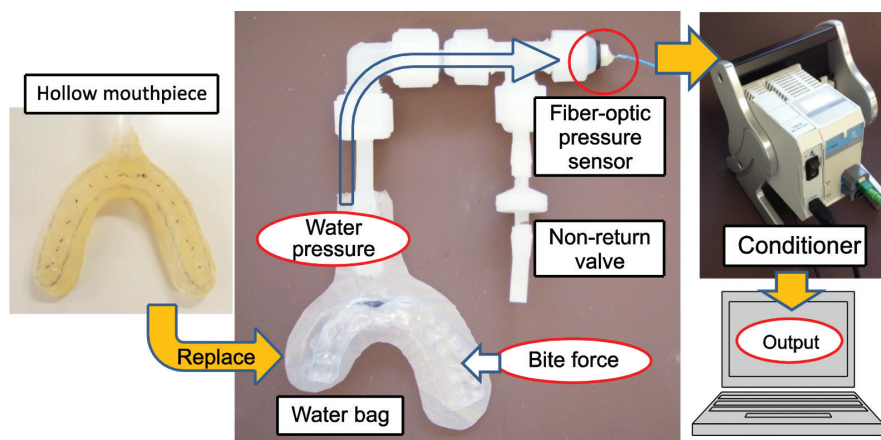

Fig. 1 An outline of the metal-free bite force gauge. We used a hollow mouthpiece molded to the dental configuration and fiber optic pressure sensor and adjusted it in subjects' mouths so that occlusal contact remains uniform.

Experimental design

We placed the mouthpiece of the bite force gauge in the maxilla and asked subjects to perform continuous clenching for 30 and 60 s. The exercise intensities were 10%, 20%, 30%, and 40% of the maximum bite force (maximum voluntary clenching [MVC]), calculated from the water pressure evaluated with the same bite force gauge at maximum clenching. The average value of the maximum bite force of all subjects was 463.3 ± 229.5 N. Because the median endurance time for clenching of teeth at 40% MVC has been reported to be 1.4 min,[10] sustained clenching of teeth for 60 s at 40% MVC was considered achievable for all subjects.

MR images were acquired from subjects while resting before and immediately after all exercise tasks. For each subject, we obtained eight volumes of MR images, each during rest and shortly after the exercise (30 s clenching at 10%, 20%, 30%, and 40% MVC and 60 s clenching at 10%, 20%, 30%, and 40% MVC). Subjects performed tasks in the supine position in the MRI gantry as described previously.[10] Measurements were displayed on the computer and projected onto the screen in the imaging room such that the reflection of the screen was visible to subjects through a mirror attached to the head coil; this provided feedback to subjects for maintaining constant water pressure. We randomized the order of the tasks as much as possible, particularly if a subject performed more than one task in a single day. Subjects were allowed an approximately 40 min rest between each exercise task if multiple tasks were performed on the same day, to allow T_2 to return to its pre-exercise value. There was no subject whose MR images were obtained under all task conditions in 1 day.

Image analysis

We converted MR images to an analysis format using MRIcron (McCausland Center for Brain Imaging, Columbia, SC, USA) and then reconstructed the T_2 images using ImageJ (National Institutes of Health, Bethesda, MD, USA). To calculate T_2, we used the following equation for each voxel signal intensity in an MR image with TE 20 and 60 ms:

$$T_2 = \frac{(t_b - t_a)}{\ln\left(\dfrac{i_a}{i_b}\right)}$$

where t_a and t_b are spin-echo time and i_a and i_b are signal intensities.

We used Avizo6.1 (Visualization Science Group, Burlington, MA, USA) to analyze the volumes of interest (VOIs). We set the VOI using auto-partition with mean and standard deviation to remove potential technician bias as described below:

(1) We selected three arbitrary slices from images with TE of 20 ms, traced the outline of the masseter muscle where signals were stable, and applied this trace to images with TE of 20 and 60 ms to calculate the internal mean signal intensity and standard deviation; this was defined as the standard for the masticatory muscle (Fig. 2a).

(2) We traced the slightly larger outline of superficial masseter and temporal muscles, which is the analytical target, to include the entire muscles in all slices from images with TE of 20 ms; we applied this trace to images with TE of 20 and 60 ms. We selected only the range of mean signal intensity ±2 standard deviation (SD) calculated from Fig. 2a and calculated the mean signal intensity and standard deviation in the range (Fig. 2b).

(3) From images with TE of 20 and 60 ms to which a large trace was applied again, we selected the range with mean signal intensity ±2 SD calculated from Fig. 2b for each superficial masseter and temporal muscles (Fig. 2c).

(4) Comparing selected ranges for TE of 20 and 60 ms, we reselected only the area that was common to both (Fig. 3a).

(5) We applied the range selected in (4) to T_2 images (Fig. 3b).

(6) We manually removed muscles that were clearly not the target and sites where membrane and adipose tissues were included because of the use of a large outline trace and used the remaining area as the VOI (Fig. 3c).

In this study, all VOIs were drawn by a dentist (T.F) with 4 years of experience and verified and corrected by another dentist (S.Y) who was engaged in MRI studies for 12 years. We applied the VOI to T_2 images during rest and after exercise and calculated the mean T_2 for each VOI (mean T_2 for the rest and exercise for each exercise task). In addition, we calculated eight mean T_2 values during rest for the same subject for each VOI. In the case of multiple MR images during the rest period on the same day, we used the minimum mean T_2 during rest on the same day as the representative mean T_2 during rest for that day. Then, by subtracting the representative mean T_2 during rest from mean T_2 after tasks for each VOI, we obtained mean ΔT_2.

Electromyography

We recorded electromyograms by asking subjects to perform the exercise tasks (see Experimental design) as they did during mfMRI acquisition, again in the supine position. After eliminating as much sebaceous matter from the skin surface as possible, we attached surface electrodes over the belly or the lower part of the masseter and the anterior belly of the temporal muscles (Duotrode; Myotronics, Inc., Kent, WA, USA). We used a bio-amplifier (BIOTOP 6R12; NEC San-ei, Tokyo, Japan) and an AD converter (NR-500; Keyence Corp, Osaka, Japan) to assess the signals and simultaneously digitally recorded four channels. The sampling frequency was 5 kHz. MATLAB 2016b (MathWorks Inc., Natick, MA, USA)
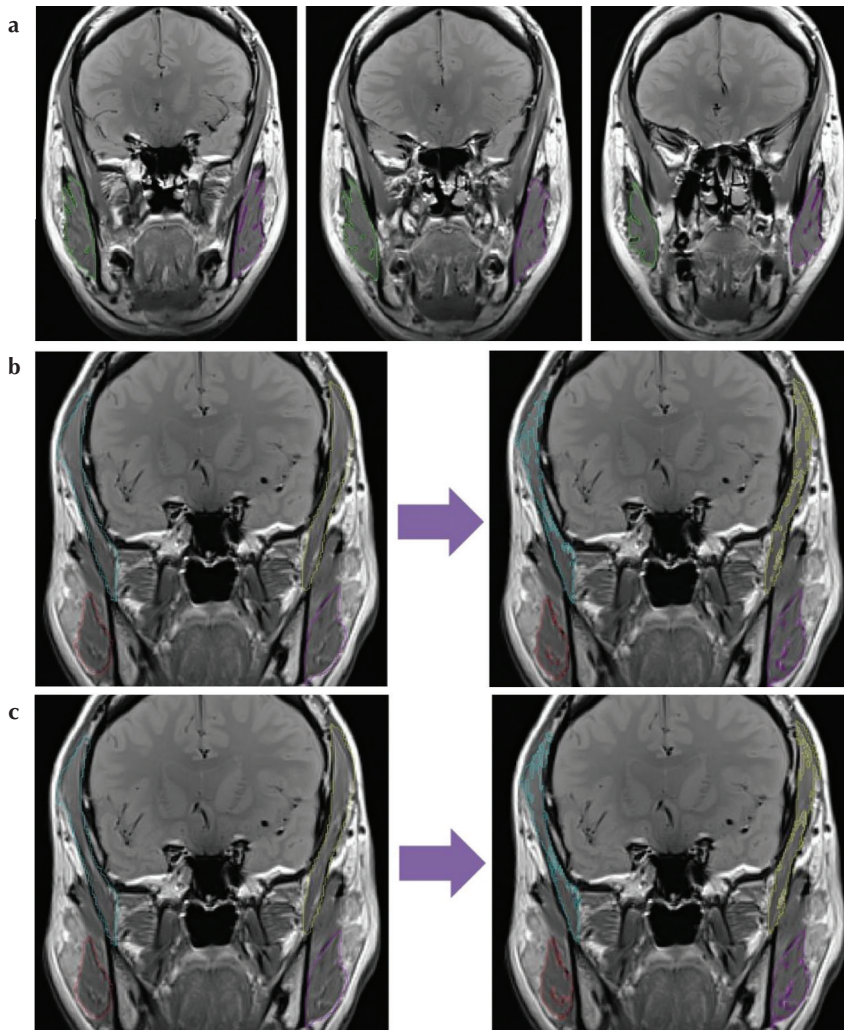
Fig. 2 The method of setting volumes of interest (VOIs) (**a**–**c**). (**a**) Tracing the contour of the masseter muscle in a three-slice image with an TE of 20 ms. (**b**) From the large muscle contour trace, we selected the range of the mean signal intensity ±2 standard deviation (SD) from "**a**". (**c**) From the large trace, we selected the range of the mean signal intensity ±2 SD from "**b**" again (All displayed images have a TE of 20 ms.).

was used to calculate iEMG for the 30 and 60 s clenching exercises and to assess the electromyogram waveform. Although EMG values are standardized in most analyses, the raw measurements were used herein for correlation analysis with the T$_2$ shift.

Statistical analysis

We used the Shapiro–Wilk test to test for normality. Some data were not normally distributed, but the overall shape of the histogram was close to a normal distribution. In addition, the intra-class correlation coefficient (ICC) for the mean ΔT_2 and iEMG distribution between muscles and subjects for evaluation determined that all ICCs were ≥ 0.1. We used the linear mixed model for statistical analysis with the restricted maximum likelihood method to estimate parameters for the model. Furthermore, we used Akaike's information criterion (AIC), which considers not only the likelihood but also the number of parameters, to verify and compare the model. In this study, all statistical analyses were performed with SPSS 22.0 (IBM Japan, Tokyo, Japan) with a significance level of $P = 0.05$.

First, we assessed the correlation between the T$_2$ shift and EMG values for 30 and 60 s clenching using the linear mixed model. The association between iEMG and T$_2$ shift was evaluated individually for each muscle; to account for the different characteristics of subjects, we used a model that considers subjects as a random factor and muscle, iEMG, and their interaction as fixed factors. The mean ΔT_2 was a dependent variable used to assess the difference in the regression coefficient. In the absence of significant interaction, we re-analyzed the data with a model that did not include interaction as a fixed factor for each combination of muscles with similar regression coefficients in the plot and from there calculated common regression coefficients.

Next, the influence of clenching duration on the association between exercise intensity and ΔT_2 and the association between exercise intensity and iEMG were confirmed. We analyzed all the 30 and 60 s clenching data with subject and muscle as random factors, clenching duration (30 and 60 s), exercise intensity (10%, 20%, 30%, and 40% MVC), and interaction of the clenching duration and exercise intensity as fixed factors; notably, the mean ΔT_2 and iEMG were
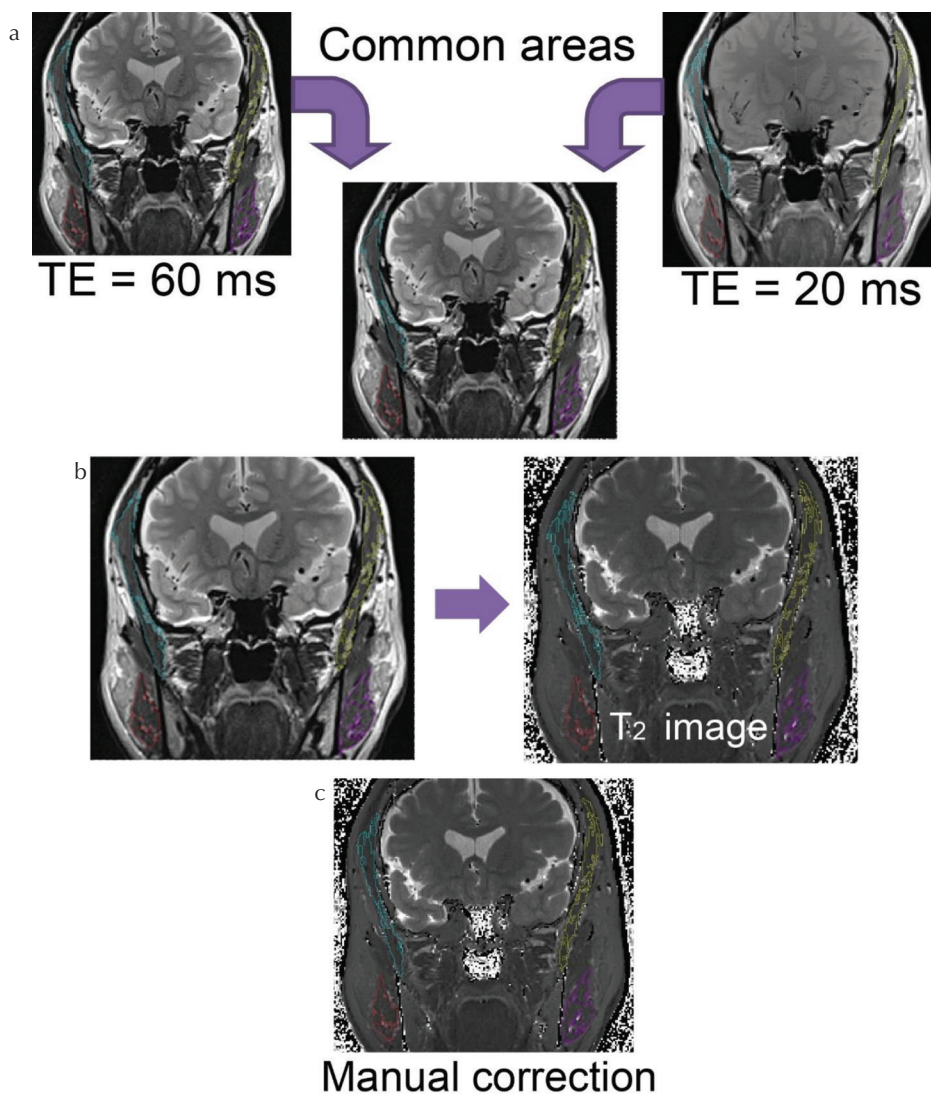
Fig. 3 The volume of interest (VOI) method (**a**–**c**). (**a**) Reselect the part that was common to echo time (TE) of 20 and 60 ms. (**b**) The range selected in "**a**" is applied to the T_2 image. (**c**) We manually excluded the parts that were not the target muscles and parts that were assumed to contain fascia and adipose tissues. We used the remaining area as the VOI.

dependent variables. We verified regression coefficients that considered variations of subjects and muscles.

Results

Correlation between ΔT_2 and iEMG
Table 1 summarizes the results of the test of fixed factors (muscle, iEMG, and interaction) with the linear mixed model that used mean ΔT_2 as dependent variables and subjects as a random factor. Figure 4a and 4b demonstrates the scatter plots for the mean ΔT_2 versus iEMG from which the estimated intercept and regression coefficients were plotted. With all models, a significant change was observed with iEMG ($P < 0.001$), but the interaction was not significant. The scatter plots also demonstrate that the mean ΔT_2 was similar in terms of intercept and regression coefficient between left and right superficial masseter muscles and between left and right temporal muscles. Hence, we prepared a new model with the mean ΔT_2 as the dependent variable and without interaction as a fixed factor for the superficial masseter and temporal muscles separately and re-estimated

Table 1 Test of fixed factors with the linear mixed model

Duration time (s)	Dependent variable	Fixed factor	*P*
30	Mean ΔT_2	iEMG	<0.001
		Muscle * iEMG	0.386
60	Mean ΔT_2	iEMG	<0.001
		Muscle * iEMG	0.267

*Interaction between the two factors. Fixed factors: iEMG, muscles, and interactions. Variable factor: subjects. iEMG, integrated electromyography.

the parameters. The results of the test of fixed factors (Table 2) and scatter plots (Fig. 4c and 4d) demonstrated a significant variation with iEMG ($P < 0.001$), but no significant differences between the left and right side muscles. Regarding the fit of the model that used the mean ΔT_2 as the dependent variable, the model that was separately constructed for the superficial masseter and temporal muscles and did not include interaction as the fixed factor was a better fit (i.e., had a lower AIC).

Table 2 Test of fixed factors with the linear mixed model (separate models for the superficial masseter and temporal muscles)

Duration time (s)	Muscle	Dependent variable	Fixed factor	P
30	Superficial Masseter	Mean ΔT_2	Muscle	0.693
			iEMG	<0.001
	Temporal	Mean ΔT_2	Muscle	0.510
			iEMG	<0.001
60	Superficial Masseter	Mean ΔT_2	Muscle	0.616
			iEMG	<0.001
	Temporal	Mean ΔT_2	Muscle	0.119
			iEMG	<0.001

Fixed factors: iEMG and muscle. Variable factor: subjects. iEMG, integrated electromyography.

Comparison between 30 and 60 s clenching duration

Table 3 summarizes the results of examination of fixed factors, i.e., duration time, exercise intensity, and interactions, of the linear mixed model with subject and muscle as random factors. Figure 5 illustrates the scatter plot for the mean ΔT_2 and iEMG versus exercise intensity in 30 and 60 s clenching exercises. In the model that used iEMG as the dependent variable, the interaction was significant ($P = 0.009$); however, in the model that used the mean ΔT_2 as the dependent variable, the interaction was not significant ($P = 0.630$).

Discussion

This study was the first comprehensive analysis of the correlations between mean ΔT_2 and iEMG in the superficial masseter and temporal muscles, and the impact of exercise duration on such correlations. The isometric exercise was
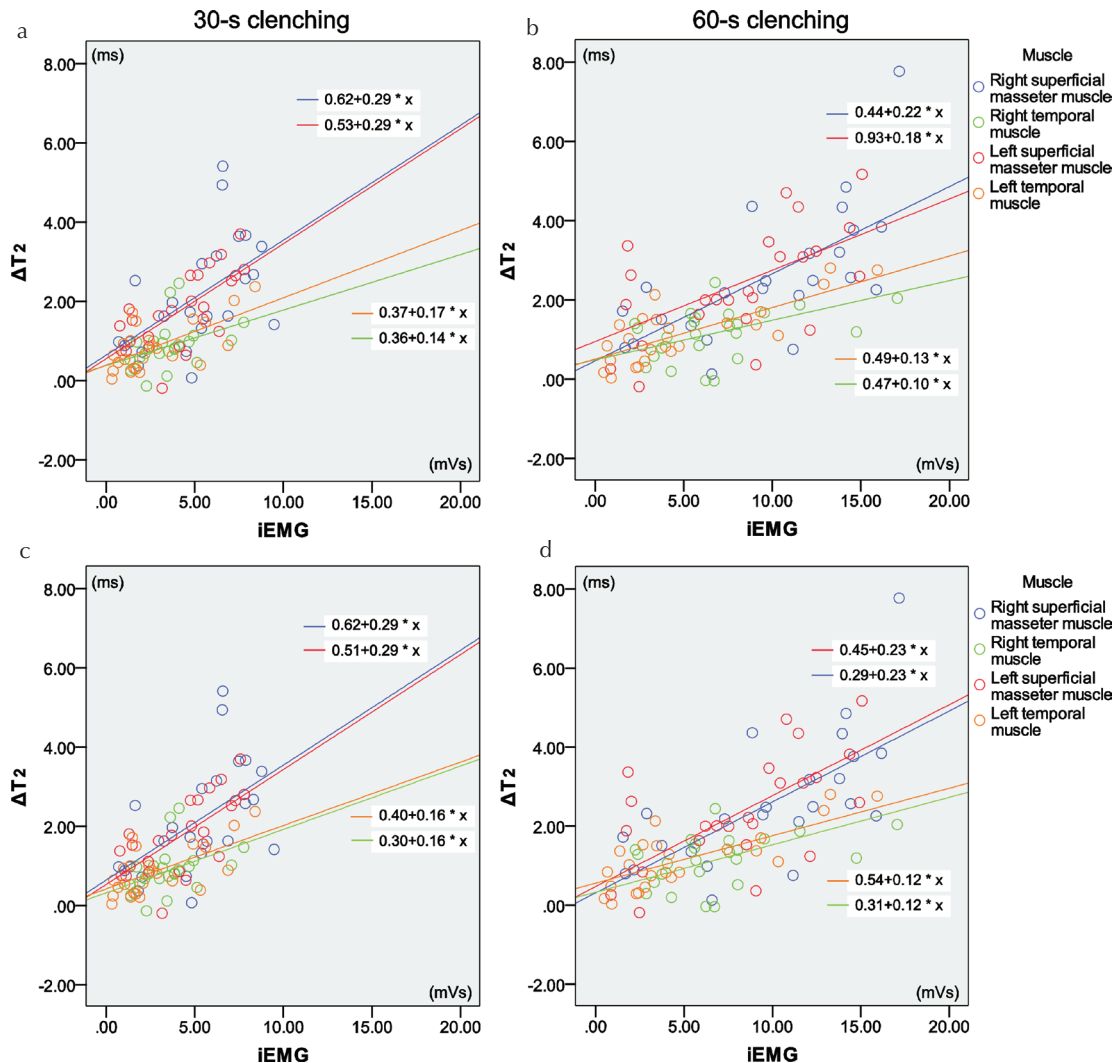

Fig. 4 Scatter plots of ΔT_2 against iEMG and four muscles with intercepts and regression coefficients estimated with the linear mixed model. Plates (**a**) and (**b**), with interaction (all four muscles are included in one model). Plates (**c**) and (**d**), without interaction (common regression coefficient: different models for the masseter and temporal muscles). iEMG, integrated electromyography.
*The color version is available online.

utilized with four levels of exercise intensities and two exercise durations as the task, based on a linear mixed model.

Relationship between T_2 shift and EMG value

The results of present study suggest that the regression coefficients between T_2 shift and iEMG differ by kind of muscle but are similar between the left and right sides of the same kind of muscle. Unlike previous work analyzing only part of the muscle, we analyzed the mean T_2 shift calculated from the entire muscle. Nevertheless, the present results about different regression coefficients between the different kind of muscles were consistent with that previous work.[8] These results indicate that a difference in regression coefficients is not caused by the deviation in localized intramuscular activities. A recent study that used positron emission tomography (PET)/MRI demonstrated that a T_2 increase in the exercising skeletal muscles exhibited a strong correlation with glucose metabolism (simultaneously evaluated with 2-deoxy-2-fluoro-D-glucose [FDG]-PET); since this surpasses the variation among muscle types or individual variation by subjects in both whole muscle and per-pixel analyses, the T_2 shift might be used as a substitute marker for glucose uptake.[11] The regression coefficients between iEMG and mean ΔT_2 are different for each kind of muscle but are similar on the left and right sides, as confirmed in this study, and demonstrated that the relationship between the localized electrical activity and the actual energy metabolism in the whole muscle might vary for each muscle.

30 and 60 s clenching

In the cases of regression coefficients relative to exercise intensity, where impact of subjects and muscle was adjusted, only iEMG was significantly different in 30 and 60 s clenching. Comparing the regression coefficients in 30 and 60 s clenching, that for iEMG nearly doubled, whereas that for mean ΔT_2 mostly remained the same. However, the doubled mean ΔT_2 intercept indicated that the rate of increase from 30 to 60 s clenching was lower in the higher load range. Because the regression coefficient between the mean ΔT_2 and exercise intensity hardly changed between 30 and 60 s clenching, the increment in mean ΔT_2 with respect to the same iEMG increase was smaller in 60 s clenching than in 30 s clenching for the superficial masseter and temporal muscles. This result suggests that because the increase in T_2 extension due to the extension of the exercise duration is lesser compared with the time integral value iEMG, the linear regression coefficient between the T_2 extension and the iEMG changes with the exercise duration. Despite there being few details available about the cause of this phenomenon, it is possible that the increase in T_2 shift associated with exercise in skeletal muscle may decrease over time, even during exercise, given that the increase in the T_2 of skeletal muscles associated with exercise returned to its pre-exercise value in about 20 min.[9] Additionally, some studies have indicated that the association between the T_2 shift and exercise might represent a sigmoid curve change,[12] which, in turn, could be attributed to the decrease in the rate of increase

Table 3 Examination of fixed factors with the linear mixed model

Dependent variable	Fixed factor	P
Mean ΔT_2	Exercise intensity	<0.001
	Duration time * exercise intensity	0.630
iEMG	Exercise intensity	<0.001
	Duration time * exercise intensity	0.009

*Interaction between the two factors. Fixed factors: duration time, exercise intensity, and interaction. Variable factors: subjects and muscles. iEMG, integrated electromyography.
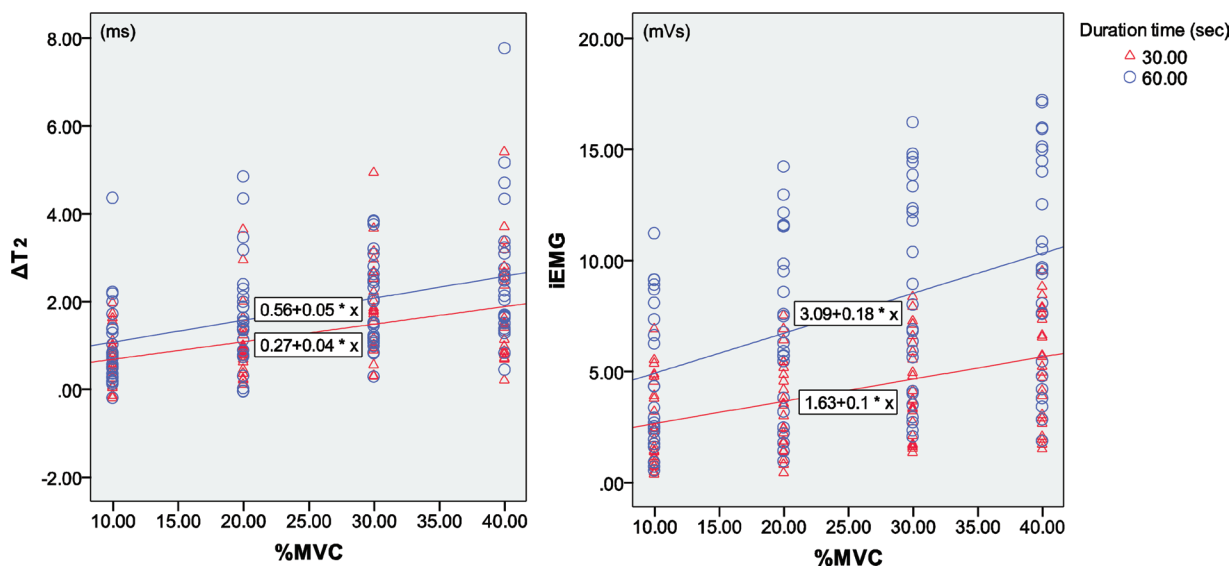

Fig. 5 Scatter plots of ΔT_2 and iEMG against exercise intensity and 30 and 60 s clenching with intercepts and regression coefficients estimated with the linear mixed model. iEMG, integrated electromyography; MVC, maximum voluntary clenching.

in the higher load range. In contrast, the increase in mean ΔT_2 relative to exercise intensity in 60 s clenching was significant, and no clear decrease was observed in the mean ΔT_2 increase rate relative to the increase in exercise intensity. Unlike EMG values, variations in the T_2 of the skeletal muscles relative to exercise intensity and exercise duration might be different. However, since we only measured 30 and 60 s clenching in this study, a detailed analysis was challenging. Hence, in future studies, it will be necessary to use constant intensity with a variety of clenching durations and investigate the relationship between exercise duration and T_2 shift.

Limitations

This study has some limitations. First, the sample size of six subjects might not be adequate. Hence, further extensive studies with a larger study population, more data, and comprehensive analyses are warranted to generalize the results. Second, there was no subject whose all MR images were obtained on a single day. Therefore, there is a possibility that the MR images acquired on other days might affect the T_2 shift. Third, MRI and EMG were not performed at the same time. Despite using the same exercise task, the fact that the actual muscle activity was the same cannot be guaranteed.

Conclusion

The linear regression coefficients between muscle T_2 shift induced by isometric exercise of the superficial masseter and temporal muscles and iEMG according to the same task are different for each type of muscle but are similar on the left and right sides of the same type of muscle. Furthermore, although the exercise duration was extended from 30 to 60 s, T_2 shift did not increase enough in the higher load range, and it showed that T_2 shift is not suitable for the evaluation of the amount of muscle activity accompanying the increase in exercise duration. Hence, mfMRI can be used as a muscle activity evaluation method based on a different index than EMG, as long as the motor tasks are isometric exercises of appropriate intensity (10–40% MVC) and same duration (around 30 s).

Funding

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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