RESEARCH

Evaluation of T_2 values and apparent diffusion coefficient of the masseter muscle by clenching

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Objective: The aim of this study was to evaluate the changes in T_2 values and apparent diffusion coefficient (ADC) in the masseter muscle by clenching in healthy volunteers.

Methods: 37 volunteers were enrolled in the study. We measured bite force using pressure-sensitive paper and a T_2 map. The ADC map was obtained at rest, during clenching, immediately after and 5 min after clenching. The spin-echo sequence was used to calculate T_2 , and single-shot spin-echo echo planar imaging was used to calculate the ADC. The motion-probing gradients (MPGs) were applied separately along the posterior-to-anterior (PA), right-to-left (RL) and superior-to-inferior (SI) directions, with b values of 0, 300 and 600 s mm⁻² in each direction. ADC-PA, ADC-RL, and ADC-SI values were obtained, and we calculated the ADC-iso for the mean diffusivity.

Results: There were no significant differences between the stronger and weaker sides of bite force before, during or 5 min after clenching for T_2 and ADC. The bite force had little effect on these parameters; thus, we used the average of the two sides for the following analyses. Time course analysis of ADC-iso, ADC-PA, ADC-RL and ADC-SI demonstrated a marked increase after clenching and a rapid decrease immediately after clenching, although they did not completely return to the initial values; however, the change in ADC-RL was significantly greater than those in ADC-PA or ADC-SI ($P < 0.001$ each). The changes in T_2 were similar to those of ADC, although not as marked.

Conclusions: ADC (especially ADC-RL) was altered by contraction of the masseter muscle. *Dentomaxillofacial Radiology* (2011) **40**, 35–41. doi: 10.1259/dmfr/15607259

Keywords: T_2 values; apparent diffusion coefficient; masseter muscle; clenching

Introduction

Several studies of the extremities have demonstrated the location and relative intensity of muscle recruitment during various tasks using T_2 weighted imaging or maps.^{1–5} Many studies have demonstrated the close relationship between muscle fibre activation and the increase in T_2 time.¹ Recently, diffusion-weighted imaging (DWI) of human skeletal muscle of the extremities has been used for many purposes including evaluating skeletal muscle damage⁶ and the changes that accompany contraction.^{7–10}

Many studies have examined morphological changes in the masticatory muscles by clenching or jaw opening;

however, to our knowledge, there are no previous reports regarding the physiological changes (T_2 time or apparent diffusion coefficient (ADC)) caused by clenching.

The present study evaluated changes in T_2 values and ADC in the masseter muscle caused by clenching in healthy volunteers. The study has possible clinical applications in the evaluation of masticatory muscle fatigue and pain associated with temporomandibular disorder (TMD).

Materials and methods

Participants

We examined 37 healthy volunteers (20 men, 17 women; average age 32.4 ± 7.3 years). This study was approved by the Committee on Clinical Studies of Fukuoka Dental College (number; 133). We explained the purpose

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Received 7 September 2009; revised 15 November 2009; accepted 22 November 2009

of the research, the content of the experiments, risks and possible adverse effects to the volunteers by verbal instruction and documentation, and informed consent was obtained from all the volunteers. The study selection criteria were no symptoms of temporomandibular joint or jaw muscle disorders, no significant gap between the upper and lower jaw midline and no significant anomaly of occlusion.

Measurement of bite force

All measurements were made using pressure-sensitive paper (Dental Prescale 50H, type R; Fuji Photo Film Co., Tokyo, Japan) and an analyzing apparatus (Dental Occlusion Pressuregraph FPD-705; GC Co., Tokyo, Japan). When the sheet was bitten, microcapsules containing a colour-forming material were broken and reacted with a colour-developing material. The bite force (N) and occlusal contact area (mm²) were calculated from the degree of colouring.

The pressure-sensitive paper was scanned using the analyzing apparatus and the data were transferred to a personal computer (Macintosh G4; Apple Computer Japan, Inc., Tokyo, Japan) for analysis. During measurement the participants were seated with their heads in an unsupported natural position; they were then asked to clench as hard as possible with their maxillary and mandibular teeth and to maintain the same level of contraction for 5 s. The bite forces of the right and left sides were calculated.

MRI examinations and parameter calculation

All the examinations were performed on a 1.5 T scanner (Intera Achieva; Philips Medical Systems, Eindhoven, the Netherlands) that used a sensitivity-encoding SENSE-NV-16 coil. Transverse images were taken to obtain a T_2 map and an ADC with a field of view (FOV) of 240 mm at rest, during, immediately after and 5 min after clenching.

Single-shot spin-echo echo planar imaging (SS SE-EPI) sequences were used to calculate the ADC with a repetition time (TR) of 3.5 s, echo time (TE) of 60 ms; spectral attenuated with inversion recovery (SPAIR) for fat suppression; reduction factor of sensitivity encoding (SENSE) = 2; matrix = 104×103 ; number of excitation (NEX) = 6, bandwidth = $2.26 \text{ Hz pixel}^{-1}$; and total imaging time = 4 min 15 s. The motion-probing gradients (MPGs) were applied separately along the posterior-to-anterior (PA), right-to-left (RL) and superior-to-inferior (SI) directions, with b values of 0, 300 and 600 s mm^{-2} in each direction. The ADC was calculated separately along each direction of the MPG for evaluation of anisotropic diffusion; thus, we obtained the ADC-PA, ADC-RL and ADC-SI values. We also calculated the ADC-iso for mean diffusivity.

The signal intensity (SI) for three b factors were fitted to the equation:

$$SI = M \exp(-bD)$$

where SI is the measured signal intensity, M is the steady-state magnetization, b is the strength of MPG and D is the ADC. Images with a slice thickness of 6.0 mm were taken to obtain the ADC.

To calculate T_2 , we used a spin-echo (SE) sequence with a matrix = 104×103 and a total imaging time of 3 min 28 s. TR was 2000 ms. TE increased from 10 to 80 ms in 10 ms intervals. Two-parameter curve-fitting was performed to obtain a T_2 map:

$$SI = M \exp(-TE/T_2)$$

where SI denotes the signal intensity and M corresponds to the signal amplitude at $TE = 0$ ms. TE increased from 10 to 80 ms in 10 ms intervals.

The region of interest (ROI) was placed in each slice image of the masseter muscle, and we examined the changes in the ADC and T_2 value on the console of the MRI unit.

Protocol of the MRI study

We obtained DWI images using SS SE-EPI sequences before, during, immediately after and 5 min after clenching. We obtained the T_2 maps using the fast spin-echo (FSE) sequence before, during, immediately after and 5 min after clenching. The intervals of these sequences were all 5 min. The total imaging time for DWI was 4 min 15 s and that for T_2 measurements was 3 min 28 s. As previous studies have indicated that the haemodynamic changes with clenching disappeared within about 10 min, we evaluated ADC and T_2 every 5 min.^{11,12}

The duration of the clenching was 5 min (Figure 1). During clenching the volunteers were instructed to clench slightly and maintain a constant bite force.

Statistical analyses

The T_2 values and ADCs of the sides with the stronger and weaker bite force were compared using the paired t -test. There was no significant difference between the two sides except immediately after clenching; thus, we used the average of the two sides for the rest of the analyses (Table 1).

Next, we evaluated whether clenching had an effect on T_2 and ADC (ADC-iso, ADC-PA, ADC-RL and ADC-SI) of the masseter muscle. Time course analyses were performed among the four measurements. Repeated ANOVA was used to evaluate whether the time course of the experiment affected these indices, followed by *post hoc* comparisons for any pairs of measurements.

Finally, to evaluate the anisotropy of the ADC of the masseter muscle, we compared the ADC in each direction (ADC-PA, ADC-RL and ADC-SI) for all four measurements. We also calculated and compared the percentage change in ADC (ADC-PA, ADC-RL and ADC-SI) induced by clenching. Repeated measures ANOVA was used to evaluate whether the direction of MPG had an effect on the indices, followed by *post hoc*

Figure 1 The study protocol. We measured the apparent diffusion coefficient (ADC) and T_2 values before, during, immediately after and 5 min after clenching at 5 min intervals. The duration of the clenching was 5 min

comparisons for all pairs of three ADCs along each direction of MPG. All data were analysed using Stat View J (version 5; SAS Institute, Cary, NC) and $P < 0.05$ was taken to indicate significance.

Results

Comparison of T_2 and ADC between sides with stronger and weaker bite force

The mean bite force of the right side was 459.0 ± 231.2 N, and the left side was 469.7 ± 237.7 N. The mean stronger bite force was 474.0 ± 241.0 N and the weaker force was 454.6 ± 230.5 N.

For the ADC-iso there were no significant differences between the stronger and weaker side before, during or 5 min after clenching ($P = 0.90$, 0.63 and 0.85 , respectively; Table 1). ADC-iso differed significantly between the two sides ($P < 0.05$) only immediately after clenching. Similarly for ADC-PA and ADC-RL, a significant difference between the two sides was observed only immediately after clenching. ADC-SI showed no significant difference between the two sides (Table 1). Immediately after clenching the muscles, with the

stronger bite force had higher ADC-iso, ADC-PA, and ADC-RL than those with the weaker force.

For T_2 values, there were no significant differences between the stronger and weaker sides before, during or 5 min after clenching ($P = 0.09$, 0.31 and 0.20 , respectively). Only the T_2 value immediately after clenching was significantly different between the two sides ($P < 0.05$). The results suggested the relative distribution of bite force had little effect on either the T_2 value or ADC. Thus, we calculated the average T_2 value and ADC for the rest of the analyses.

Changes in ADC and T_2 values after clenching

Time course analysis of ADC-iso demonstrated a marked increase with clenching. A rapid decrease in ADC-iso was demonstrated immediately after clenching, although it did not completely return to the base level before clenching (Figures 2 and 3). Similarly, ADC-PA, ADC-RL and ADC-SI demonstrated a marked increase with clenching, followed by a decrease after clenching (Figure 4).

T_2 values also showed an increase with clenching and returned approximately to the initial value after clenching (Figure 3b). However, the percentage change

Table 1 Indices of the side with stronger bite force and those with weaker bite force

		Stronger	Weaker	* P -values
ADC-iso ($\times 10^{-6} \text{ mm}^2 \text{ s}^{-1}$)	Before	1492.2 ± 90.4	1490.8 ± 99.8	0.90
	During	1806.6 ± 303.4	1824.7 ± 379.1	0.63
	Immediately after	1603.4 ± 157.7	1569.6 ± 143.9	*
	5 min after	1559.0 ± 126.6	1561.7 ± 147.6	0.85
ADC-PA ($\times 10^{-6} \text{ mm}^2 \text{ s}^{-1}$)	Before	1571.5 ± 134.7	1547.0 ± 120.0	0.09
	During	1876.8 ± 343.1	1863.6 ± 401.7	0.75
	Immediately after	1685.5 ± 170.6	1632.1 ± 149.5	*
	5 min after	1631.0 ± 154.2	1620.2 ± 136.9	0.53
ADC-RL ($\times 10^{-6} \text{ mm}^2 \text{ s}^{-1}$)	Before	1316.8 ± 119.9	1311.0 ± 135.4	0.09
	During	1707.9 ± 331.8	1742.2 ± 425.0	0.75
	Immediately after	1413.7 ± 173.5	1374.5 ± 169.5	*
	5 min after	1371.2 ± 132.6	1377.0 ± 197.9	0.53
ADC-SI ($\times 10^{-6} \text{ mm}^2 \text{ s}^{-1}$)	Before	1570.0 ± 125.1	1604.0 ± 135.2	0.16
	During	1801.8 ± 266.0	1866.5 ± 362.7	0.11
	Immediately after	1693.9 ± 214.8	1691.9 ± 183.7	0.94
	5 min after	1649.2 ± 199.8	1681.8 ± 184.6	0.21
T_2 values (ms)	Before	38.0 ± 2.1	38.6 ± 2.7	0.09
	During	39.5 ± 2.8	40.0 ± 3.2	0.31
	Immediately after	38.7 ± 2.7	39.6 ± 3.2	*
	5 min after	38.4 ± 2.2	39.0 ± 2.9	0.20

ADC, apparent diffusion coefficient; PA, posterior-to-anterior; RL, right-to-left; SI, superior-to-inferior;

*Paired t -test; $P < 0.05$

Figure 2 Representative images of apparent diffusion coefficient (ADC) and T_2 values. ADC maps (a) before, (b) during, (c) immediately after and (d) 5 min after clenching. T_2 maps (e) before, (f) during, (g) immediately after and (h) 5 min after clenching

in T_2 value with clenching ($3.65\% \pm 2.78\%$) was smaller than that for ADC ($P < 0.0001$).

Comparison of ADC-PA, ADC-RL and ADC-SI

The *post hoc* test demonstrated that ADC-RL was the smallest of the three ADCs throughout the time course (at rest, during clenching, immediately after clenching and 5 min after clenching; Figure 5). The percentage changes in ADC-PA, ADC-RL and ADC-SI were $19.8\% \pm 19.7\%$, $31.5\% \pm 25.1\%$ and $15.5\% \pm 16.7\%$, respectively. The change in ADC-RL was significantly greater than those in ADC-PA or ADC-SI (both $P < 0.001$).

Discussion

Surface electromyography (EMG) is currently part of the physiological assessment of the masticatory muscles to evaluate muscle activity during clenching and the pathology of dysfunction. However, the actual electrical source detected by surface EMG is vague and the signal is faint when derived from deeply located muscles.^{13–17} MRI has advantages over surface EMG in that it provides anatomical and physiological information.

Several studies of the extremities have demonstrated the location and relative intensity of muscle recruitment during various tasks using T_2 weighted imaging or maps.^{1–5} Many studies have demonstrated the close relationship between muscle fibre activation and increase in T_2 times.¹

In the present study, T_2 values increased during clenching. Although the difference was significant, the average percentage change in T_2 was only $3.65\% \pm 2.78\%$. The change in T_2 value of the masseter muscle during clenching was smaller than that of the lower extremities during dynamic ankle dorsiflexion and greater than that of the rotator cuff muscle during pitching.^{2,3} Many researchers have demonstrated a strong relationship between T_2 values and muscle activity measured by electromyography.¹ Thus, we felt that the main reason for the small change in T_2 values was the low level of muscle activity due to instructing the volunteers to clench lightly, but to not apply maximum voluntary contraction, during MRI.

The most widely cited explanation for the increase in T_2 during exercise or constriction is increased extra-cellular and/or vascular fluid volume. The intracellular accumulation of inorganic phosphate and lactate is another reason for the increase in T_2 , because they draw

Figure 3 Time course of changes in (a) apparent diffusion coefficient (ADC)-iso and (b) T_2 for all volunteers. (a) ADC increased during clenching and decreased immediately after clenching; however, it did not return fully to the initial value. (b) T_2 also increased during clenching and decreased after clenching; however, the changes in T_2 were not as marked as those for ADC-iso.
*Significant difference ($P < 0.05$; *post hoc* test)

water into the muscle fibres as a result of osmotic pressure gradients.^{1,4} However, when contracting muscles, as in the present study, there is a rise in intramuscular pressure, which causes mechanical compression of blood vessels. This compression reduces the blood flow, which competes against the local myogenic and metabolic factors that induce vasodilation to maintain adequate muscle perfusion.^{11,12} The shortening of T_2 is a result of decreased oxygen saturation and an increased proportion of deoxyhaemoglobin.^{18,19}

The limitation of blood flow (perfusion) has a negative effect on the increase in T_2 . Previous studies demonstrated that the increase in T_2 after exercise occurred in the presence of vascular occlusion.^{20,21} The increase in extra-cellular fluid was one of the most important factors involved.

Our results demonstrated increases in ADC-iso, ADC-PA, ADC-RL and ADC-SI during clenching.

The ADCs increased during and decreased rapidly after clenching, although they did not return fully to the pre-clenching level. The percentage change in ADC during clenching was larger than the change in T_2 values.

Several studies of the extremities have demonstrated that the increase in ADC results from morphological changes in the muscles,^{7,8} increased extra-cellular fluid⁹ and heat generated by constriction.

Many researchers have reported that because skeletal muscle consists of highly ordered elongated muscle fibres, the diffusion of the skeletal muscle is anisotropic.^{22,23} Our results regarding the masseter muscle also demonstrated anisotropic diffusion. The masseter muscle runs from the zygomatic arch to the masseteric tuberosity. Thus, it runs in an anterosuperior to posteroinferior direction. Many studies have verified that water diffusion is greatest in parallel with the long axis of the muscle fibres by analyzing the diffusion

Figure 4 Time course of changes in (a) apparent diffusion coefficient (ADC)-posterior-to-anterior (PA), (b) ADC-right-to-left (RL) and (c) ADC-superior-to-inferior (SI). The graphs show the mean and standard deviation. The three ADCs increased during clenching and decreased immediately after clenching, the changes were similar to those for ADC-iso.

*Significant difference ($P < 0.05$; *post hoc* test)

Figure 5 Comparison between apparent diffusion coefficient (ADC)-posterior-to-anterior, ADC-right-to-left (RL) and ADC-superior-to-inferior (SI) (a) before, (b) during, (c) immediately after and (d) 5 min after clenching. ADC-RL was the smallest of the three ADCs throughout the time course.

*Significant difference ($P < 0.05$; *post hoc* test)

tensor imaging of skeletal or cardinal muscles. In our study ADC-SI was the largest among the three ADCs and ADC-RL was the smallest, suggesting that the direction of the muscle fibre had a strong effect on the anisotropy of diffusion.

We considered that the rapid increase during clenching and rapid decrease after clenching were related to morphological changes in the masseter muscles. Hatakenaka *et al*⁸ have suggested that an increased size in myofibrils results in an increase in tertiary eigenvalues (λ_3) with passive muscle contraction of the tibialis anterior muscles. We found that during clenching the change in size of the masseter muscle in the RL direction was larger than those in the PA or SI direction; thus, the percentage change in ADC-RL was larger than that in ADC-PA or ADC-SI.

The ADC did not return fully to the pre-clenching level after clenching, which suggests that the increase in extra-cellular fluid may also be associated with an increase in ADCs.

No method is currently available to measure bite force during MRI examination; however, Karibe *et al*²⁴ reported that the occlusal force distribution on the dental arch has its own pattern and that the clenching

strength had no effect on this pattern. Thus, we postulate that the bite force distribution recorded with pressure-sensitive sheets was approximately retained during the MRI examination. In our study, there was no significant difference in T_2 values or ADCs between the stronger and weaker side before, during or 5 min after clenching; therefore, our results suggest that the relative distribution of the bite forces had little effect on the T_2 values or ADCs except immediately after clenching.^{1,2} This result was similar to that of a previous EMG study. Van der Bilt *et al*¹³ demonstrated that the masseter muscles showed no difference in activity between the ipsilateral and contralateral side during unilateral clenching. The masseter muscle acted to close the jaw by symmetrical craniocaudal movement.

Similar muscle activity and symmetrical morphological changes resulted in similar changes in T_2 values or ADCs in the masseter muscles on both sides. The difference was significant only immediately after clenching. The morphological structure of the muscle fibres returned to the basal state before clenching. These observations suggest that factors other than morphological changes in the masseter muscles were related to the ADCs. Therefore, we suggest that the volume of

extra-cellular fluid⁵ (*i.e.* blood flow) was responsible for the significant difference between the sides.

In conclusion, ADC (especially ADC-RL) was sensitive to changes by clenching. We suggest that T_2 values and ADCs may be used to evaluate muscle

fatigue and injuries that accompany bruxism and TMDs. For this purpose, further studies including MRI, bite force measurement and evaluation of muscle activity by EMG in patients with bruxism or TMD are needed.

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