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FULL PAPER

In vivo morphological and functional evaluation of the lateral pterygoid muscle: a diffusion tensor imaging study

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Objective: To explore the feasibility of morphological and functional evaluation of the lateral pterygoid muscle (LPM) by diffusion tensor imaging (DTI) in vivo.

Methods: 30 healthy volunteers underwent DTI with the jaw in the rest position, opening and clenching. Diffusion parameters of the superior head of the LPM (SHLP) and the inferior head of the LPM (IHLP) at different jaw positions were calculated.

Results: When the jaw was in the rest position, λ_3 of the SHLP was significantly lower than that of the IHLP; fractional anisotropy (FA) value of the SHLP was significant higher than that of the IHLP. There was no significant difference in λ_1 , λ_2 and apparent diffusion coefficient (ADC) value. During jaw opening, there was significant increase of all three eigenvalues and ADC value, and significant decrease of FA value both at the SHLP and IHLP. Clenching caused a significant increase in the ADC and all three

INTRODUCTION

The lateral pterygoid muscle (LPM), consisting of a superior head and inferior head, is one of the most important masticatory muscles due to its morphological and functional relationship with jaw movement. LPM dysfunction, $1-4$ $1-4$ including muscle hyperfunction, muscle hypofunction and low muscular co-ordination between the two heads of the muscle, is closely associated with temporomandibular joint disorders (TMJDs), thus it is important to evaluate in vivo the morphology and function of the LPM in clinic.

In previous studies about the LPM, the evaluation of its function during movement of the jaw has been limited to electromyography (EMG), in which single motor units were recorded from the superior head of the LPM (SHLP) and the inferior head of the LPM (IHLP) with the placement of stainless steel wire electrodes within the muscles while the electrodes were carried by a spinal needle directed

eigenvalues, and caused a significant decrease of FA at the SHLP. However, at the IHLP, the variations of all diffusion parameters by clenching in the intercuspal position showed no significance when compared with those at rest. Conclusion: The morphological and functional changes of LPM fibres caused by jaw movements could be sensitively detected by DTI which may serve as a new and noninvasive method for simultaneously investigating the functional and morphological features of the LPM during jaw movement.

Advances in knowledge: A new application of DTI is proposed for the morphological and functional evaluation of the LPMs. The results show that the significant change of three eigenvalues indicates the activity of the LPM in a specific jaw movement, a finding that shows the potential value of DTI serving as a new and non-invasive method for investigation of the LPM.

below the zygomatic arch and retracted to leave the wires within the muscle. EMG study had been proved as a useful and accurate way to evaluate the function of the LPM in jaw movement.^{[5](#page-7-0)–[10](#page-7-0)} However, EMG could neither demonstrate the morphological characters of the muscles nor show the morphological change induced by muscle activity. In addition, EMG studies do not achieve a consensus result about the function of the IHLP in clenching. Murray et $al⁵$ $al⁵$ $al⁵$ showed that no electromyographic activities were recorded at the IHLP during clenching. On the contrary, Huo et $al¹¹$ $al¹¹$ $al¹¹$ found that the IHLP was active during the intercuspal clenching, and the action potential of the IHLP was slightly lower than that of the SHLP.

MRI, with high spatial resolution, has been used in muscular anatomical imaging. In addition, functional MRI technique, such as diffusion tensor imaging (DTI), has been successfully used to non-invasively investigate the function of muscular

tissues. Based on the restriction of water diffusion by cell membranes and other subcellular structures and via measuring the intensity of water diffusion in three orthogonal directions, including the primary (λ_1) , secondary (λ_2) and tertiary (λ_3) eigenvalues, DTI allows the mapping of the diffusion characteristic of water molecules of biological tissues in vivo. From the previous studies, DTI has been successfully used to non-invasively investigate the diffusive characteristics and microstructure changes of muscular tissues, such as the diffusive properties of the extraocular muscle, 12 the effect of blood flow obstruction and reperfusion, 13 skeleton muscle injury^{[14](#page-7-0),[15](#page-7-0)} and changes after exercise.^{16,17} The results demonstrated that there was a geometric correlation between the eigenvalues and muscle fibre architecture.^{[18](#page-7-0),[19](#page-8-0)} Therefore, DTI has a huge potential to be a non-invasive imaging tool for both functional and morphological evaluation of the LPM, which may subsequently be beneficial for patients with TMJD.

To the best of our knowledge, there is so far no study evaluating the influence of occlusal changes on the LPM using DTI. Therefore, we designed this prospective study to explore the feasibility of DTI in the functional evaluation of the LPM in vivo in the healthy volunteers.

METHODS AND MATERIALS

Subjects

This study was approved by the institutional review board of Tongji Medical College, Huazhong University of Science and Technology, and a written consent form was obtained from each participant.

30 healthy volunteers (22 males and 8 females; mean age, $24 \pm$ 2.3 years; age range, 19–26 years) were enrolled. All volunteers were students at Tongji Medical College, Huazhong University of Science and Technology; all of whom gave informed consent for participation in the study before the examination. The inclusion criteria included: no missing tooth, no TMJDs, no jaw muscle dysfunction, no chew-side preference and with normal occlusion.

MRI

MRI of bilateral LPM was performed at a 3.0-T MR unit by using an eight-channel head coil for each subject. The imaging parameters of DTI were as follows: repetition time/echo time, 6000/ 65.6 ms; matrix, 128×128 ; thickness/gap, 2.0/0 mm; field of view, 22 cm; number of excitations, 2; and motion-probing gradients were applied for 25 different directions with b-values of 0 and 600 s mm^{-2} . The total imaging time was 318 s. To specify the regions of interest (ROIs) for the selected muscle, a high-resolution three-dimensional (3D) brain volume imaging (3D-BRAVO, GE Medical Systems, Milwaukee, WI) was obtained with the following parameters: repetition time/echo time, 6.8/2.5 ms; matrix, 256×256 ; thickness/gap, 0.9/0 mm; number of excitations, 1; and field of view, 22 mm. The total imaging time was 138 s.

In this study, postural position was imposed as a supine position rather than upright position to adapt to the criteria of MR examination. The supine position was defined as the position in which the angle between the floor and Frankfort horizontal plane was 90 $^{\circ}$. According to Yotsuya et al, 20 20 20 the anteroposterior postural

Figure 1. The method for setting the region of interest at the superior head of the lateral pterygoid muscle (SHLP) (a) and the inferior head of the lateral pterygoid muscle (IHLP) (c). We traced the SHLP and IHLP on the b0 images using axial three-dimensional brain volume imaging (3D-BRAVO, GE Medical Systems, Milwaukee, WI) (a, c) images as anatomical images; the diffusion tensor tractography images (axial) were fused with 3D BRAVO images (axial) to provide detailed anatomy of SHLP (c) and IHLP (d).

change did not affect function of the LPM, therefore, it is possible to use supine position as postural position in this study.

Initially, the subject underwent both DTI and 3D BRAVO sequence after lying down on the examining table and keeping the jaw in the postural position for 15 min. Then, they underwent the second DTI and 3D BRAVO sequences with their mouth opened by holding a 20-ml syringe barrel covered with a sheet of gauze between the upper and lower incisors. Finally, the syringe barrel was removed; the subject had a rest for 15 min and was then instructed to imitate masticatory movement for 3 min; then, the third DTI was performed when the subject was instructed to bite slightly in the centric occlusion. The 3D BRAVO sequence was not performed in the centric occlusion so as to reduce the examination time. Owing to intolerability of some volunteers to the discomfort caused by the opening aid device, only 23 volunteers underwent the 3D BRAVO sequences with their mouth opened. The 15 min of jaw rest before the entire MR examination and the 15 min of jaw rest between the second and third DTI acquisitions was determined by the previous study performed by Chikui et al. 21

Image analysis

The diffusion-related parameters including the apparent diffusion coefficient (ADC), fractional anisotropy (FA) and three eigenvalues (λ_1 , λ_2 , λ_3) were calculated using the Diffusion Tensor Visualize Tool implemented in a dedicated off-line workstation (AW4.5; GE Medical Systems). The ROI was manually placed by tracing the contours of bilateral SHLP and IHLP on B0 images. In addition, the BRAVO images (anatomical images) were superimposed on the DTI images in order to confirm the level of ROIs. Fascia, blood vessels and fat were avoided from the ROIs to keep the measurements consistent and accurate.

The cross-sectional areas (CSAs) of the SHLP and IHLP when the jaw was in the postural position and open state were calculated at the centre of the muscles; meanwhile, the maximum length of the SHLP and IHLP was also measured.

All the measurements were repeated three times by two radiologists, and the mean value was present as the final result for each parameter.

Statistical analyses

Statistical analysis was performed by using SPSS® v. 19.0 for Windows® (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL). The paired t -test was applied to evaluate the symmetry between the right and left sides of the LPM as well as the difference between the SHLP and IHLP when the jaw was in the posture position. The paired t -test was also used to evaluate whether occlusal changes had an effect on the DTI-derived parameters, the CSA and the length of the SHLP and IHLP. The statistical significance was set to $p < 0.05$ for all analyses.

RESULTS

DTI permits to directly observe the geometrical morphology of muscles via fibre tractography and to quantitatively assess the changes caused by occlusal variation [\(Figures 1](#page-1-0)–[4](#page-4-0)).

Figure 2. The oblique sagittal fused images of the superior head of the lateral pterygoid muscle (LPM) (c) and the inferior head of the LPM (f) using diffusion tensor tractography images and three-dimensional brain volume imaging (3D BRAVO, GE Medical Systems, Milwaukee, WI) images (b, e). The white lines in axial 3D BRAVO (a, superior head of LPM; d, inferior head of LPM) supplied as a reference image of the oblique sogittal images (b, c, e, f).

All the DTI-derived parameters (ADC, FA, λ_1 , λ_2 and λ_3), CSA and fibre length had no significant difference between the right and left sides of the SHLP and IHLP, respectively [\(Table 1](#page-4-0)). Therefore, the mean values from the bilateral SHLP and IHLP were used for analyses [\(Figure 5\)](#page-5-0).

When the jaw was in the postural position, λ_3 of the SHLP was significantly lower than that of the IHLP ($p = 0.002$), and the FA value of the SHLP was significantly higher than that of the IHLP ($p = 0.03$). There were no significant differences in λ_1 , λ_2 and the ADC value [\(Table 2](#page-5-0)).

During jaw opening, all three eigenvalues and ADC values of the SHLP and IHLP were significantly higher than those at rest. The FA values of the SHLP and IHLP were significantly lower than those at rest ([Table 3\)](#page-6-0). Morphologically, the CSA of both heads were significantly larger than those at rest, whereas the muscle length got significantly shorter ([Table 4,](#page-6-0) Figures 3 and [4](#page-4-0)).

Clenching induced a significant increase in ADC and all three eigenvalues, and a significant decrease in FA value at the SHLP. However, at the IHLP, the variations of all the DTI parameters by clenching in the intercuspal position showed no significant difference as compared with those at rest ([Table 3\)](#page-6-0).

DISCUSSION

In the current study, we investigated the feasibility of DTI in evaluation of the function of the LPM in vivo. Our results

demonstrate that the changes of geometrical morphology and diffusive properties caused by occlusal variation of the SHLP and IHLP could be sensitively detected by DTI. The variations of the diffusion parameters imply the presence of a functional difference between the SHLP and IHLP in specific jaw movements.

Unlike EMG, MRI could spontaneously achieve morphological visualization and functional evaluation of the LPM. 3D BRAVO with isotropic imaging and high spatial resolution enables reformations in arbitrary planes, optimizing visualization of the muscle and is helpful in quantitative assessment of the CSA and length in different status. Diffusion tensor tractography could directly depict the geometrical morphology of the SHLP and IHLP in different status via fibre tractography, which has a high concordance with the 3D BRAVO reformation images.

In the current study, we successfully calculate all the DTIderived parameters (ADC, FA, λ_1 , λ_2 and λ_3) of the bilateral SHLP and IHLP, respectively. We demonstrated the symmetry between the left and the right sides of the two heads of the LPM and the difference of diffusive parameters between the SHLP and IHLP. Previous DTI studies $18,19$ $18,19$ have demonstrated that there was a geometric correlation between the eigenvalues and muscle fibre architecture. λ_1 , defined as the diffusivity parallel to the long axis of the muscle fibre, has been verified.^{[19](#page-8-0),[22](#page-8-0)} λ_2 was assumed to represent the diffusive transport along the sheets of individual muscle fibres within the endomysium. The SHLP and IHLP are two heads of the LPM, consisting of the same type of

Figure 3. The three-dimensional (3D) representations of axial views of diffusion tensor fibres and volume rendering (VR) images of the superior head of the lateral pterygoid muscle at different occlusal status (a–c) corresponding to diffusion tensor tractography images of rest, open and clench; (d–f) corresponding to VR images of rest, open and clench. During jaw opening and clenching, both diffusion tensor imaging and 3D brain volume imaging (3D BRAVO, GE Medical Systems, Milwaukee, WI) detect a shortening of fibre length and an increase of cross-sectional area in comparison to the postural position, reflecting muscle contraction.

Figure 4. The three-dimensional (3D) representations of axial views of diffusion tensor fibres and volume rendering (VR) images of the inferior head of lateral pterygoid muscle (IHLP) at different occlusal status (a–c) corresponding to diffusion tensor tractography images of rest, open and intercuspal position (ICP) clench; (d–f) corresponding to VR images of rest, open and ICP clench. During jaw opening, both diffusion-tensor imaging and 3D BRAVO detect a shortening of fibre length and an increase of cross-sectional area in comparison to the postural position, reflecting muscle contraction. Non-significant changes of the IHLP are observed during jaw clenching.

muscle fibre and making the water diffusion parallel to the long axis (λ_1) and within the endomysium (λ_2) non-significant when the jaw is at the postural position in this study. λ_3 might be related to the physiological cross-sectional area (PCSA) through the radii of the muscle fibre. 23 Theoretically, the PCSA is the sum of the CSAs of all the muscle fibres within the muscle. Our results demonstrated that the λ_3 of the IHLP was statistically higher than that of the SHLP in the postural position, which may suggest that the IHLP has a bigger PCSA than the SHLP.

This is consistent with the results from studies using cadavers, 24 in which the IHLP (2.82 cm^2) has a much bigger PCSA than the SHLP (0.95 cm^2) . The PCSA is the only architectural parameter that is directly proportional to the maximum tetanic tension generated by the muscles, $23,25$ $23,25$ $23,25$ making it an important architectural parameter in defining the function role of the muscles. In this study, we demonstrated that a higher λ_3 was associated with a bigger PCSA, since the PCSA is directly proportional to the maximum tetanic tension; our result may indicate that the

Table 1. Symmetry between the right and left sides of the superior head of the lateral pterygoid muscle (SHLP) and the inferior head of the lateral pterygoid muscle (IHLP)

 $\lambda_1-\lambda_3$, three eigenvalues; ADC, apparent diffusion coefficient; CSA, cross-sectional areas; FA, fractional anisotropy.

BSHLP ADC FA 135 RES \overline{ab} RES $\frac{1}{0.000}$ \overline{one} \overline{OP} REST OPEN CLENCH \mathbf{B} SH₁₄ **BSKP** λ 2 λ 3 σ^{73} α RES OPEN cusivo REST \overline{open} \overline{c}

Figure 5. Changes in diffusion parameters [three eigenvalues $(\lambda_1-\lambda_3)$, apparent diffusion coefficient (ADC) and fractional anisotropy (FA)] when the superior head of the lateral pterygoid muscle (LPM) and the inferior head of the LPM are at different occlusal status. Data are mean values (\times 10⁻³ mm² s⁻¹) of all the DTI-derived parameters.

 λ_3 may be related to the ability to produce the maximum muscle force, making it a possible index in assessing the function of muscles. The ADC and FA were calculated from the three eigenvalues as follows:

$$
ADC = (\lambda_1 + \lambda_2 + \lambda_3)/3
$$

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

According to these equations, the changes of ADC and FA values in this study were brought about by the three eigenvalues.

During the jaw-opening process, morphological changes were observed both in reformatted images of 3D BRAVO and the

diffusion tensor tractography images. We observed a significantly increasing CSA and decreasing muscle length both in the SHLP and IHLP. This may demonstrate a reciprocal activity of both heads of the LPM in the generation and control of forces to the temporomandibular joint (TMJ) required in the jaw-opening process. This result is consistent with the result from EMG studies, $8,10$ which showed that both the SHLP and IHLP contracted to generate forces in fine controlling of the protrusive, opening and contralateral jaw movement tasks. Besides the changes of the CSA and the muscle length, a significant increase in the three eigenvalues of both the SHLP and IHLP was observed. These increased diffusion parameters at the SHLP and IHLP reflected the anatomical changes of muscle fibres caused by the contraction of the SHLP and IHLP. Physiologically, muscle contraction (a shortening of muscular fibres) will result in a decrease of muscle fibre length, an increase of muscle fibre diameter and a diastolic of the endomysium. These changes will significantly

Table 2. Differences of diffusion tensor imaging-derived parameters of the superior head of the lateral pterygoid muscle (SHLP) and the inferior head of the lateral pterygoid muscle (IHLP) at rest

| Muscles | ADC. | FA | | Λ_2 | Λ 3 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| SHLP | 1.49 ± 0.05 | 0.42 ± 0.07 | 2.19 ± 0.15 | 1.41 ± 0.07 | 0.90 ± 0.13 |
| IHLP | 1.51 ± 0.06 | 0.40 ± 0.05 | 2.19 ± 0.14 | 1.40 ± 0.07 | 0.94 ± 0.08 |
| <i>p</i> -value | 0.102 | 0.030 | .000 | 0.237 | 0.002 |

 λ_1 - λ_3 , three eigenvalues; ADC, apparent diffusion coefficient; FA, fractional anisotropy.

Table 3. Comparisons of diffusion tensor imaging-derived parameters of the superior head of the lateral pterygoid muscle (SHLP) and the inferior head of the lateral pterygoid muscle (IHLP) during occlusal changes

 $\lambda_1-\lambda_3$, three eigenvalues; ADC, apparent diffusion coefficient; FA, fractional anisotropy; ICP, intercuspal position.

 $\partial \rho$ < 0.01 for the comparison between rest and opening.

 b_D < 0.01 for the comparison between rest and ICP clench.

affect the water molecular diffusion parallel of the long axis of the muscle fibres and diffusion along the sheets of individual muscle fibres within the endomysium, as well as the diffusion within the cross-section of individual muscle fibres, which may cause an increase of λ_2 and λ_3 and a decrease of λ_1 , respectively. The changes of λ_2 and λ_3 were also proved by Schwenzer et al, 26 26 26 who reported that increased fibre radius due to muscular shortening allows for facilitated diffusion of water in the radial directions, resulting in an increase of λ_2 and λ_3 . According to our prediction, the muscle activity should cause a decrease of λ_1 ; however, in the current study, we noticed a slight increase of λ_1 . We assume this contradicting change of λ_1 may be a comprehensive impact of two factors. The presence of some subcellular structure, such as the sarcoplasmic reticulum, may serve as a strong barrier to radial diffusion instead of to axial diffusion. Therefore, the changes in λ_1 due to muscle shortening are minor regarding diffusion hindrances[.26](#page-8-0) Meanwhile, the exercise of the muscular tissues was accompanied by heat production 27 and the increasing of local blood perfusion.[28](#page-8-0) Both will promote the water diffusion in vivo. The slight increase of λ_1 probably suggests that the promotion of water diffusion caused by heat production and the increase of local blood perfusion may exceed the hindrance caused by muscle shortening. Thus, we assume that the changes of the diffusion parameters (especially λ_2 and λ_3) reflect the morphological and functional changes of the LPM, and they could be used as an index for assessment.

According to Karibe et al ,^{[29](#page-8-0)} the occlusal force distribution on a dental arch had its own pattern and that the clenching strength had no effect on that pattern. Chikui et $al²¹$ showed that the variation of bite force had no influence on the diffusion parameters of the masseter muscle. In this study, there is no method for conducting the chewing and for monitoring the bite force during MRI examination; therefore, this study focused on the effect of the distribution of the bite force by static clenching in the intercuspal position on the diffusion parameters.

During the clenching process, morphological changes and significant variations of the three eigenvalues were observed in the SHLP but not in the IHLP. This finding indicates that SHLP contraction is closely associated with the jaw-clenching movement, whereas IHLP may not participate or just plays a less prominent role in this movement. Our results reveal the difference in morphology and diffusion parameters; this may reflect the functional difference of the SHLP and IHLP in the jaw-clenching movement. There was an argument about whether SHLP and IHLP played an important role in clenching.^{[5,11](#page-7-0)[,30](#page-8-0)} From the prospective of morphology and function, our findings are consistent with the EMG study of Murray et al, 5 who believed that intercuspal clenching may activate the SHLP and other muscles, such as the medial pterygoid muscle, instead of the IHLP. Therefore, DTI has a great applied potential in the functional evaluation of muscles.

Table 4. Comparisons of cross-sectional areas and muscle length of the superior head of the lateral pterygoid muscle (SHLP) and the inferior head of the lateral pterygoid muscle (IHLP) during occlusal changes

| Muscles | | Cross-sectional areas | Muscle length | | |
|-------------|--------------------|----------------------------|------------------|--------------------------|--|
| | Rest | Open | Rest | Open | |
| SHLP | 77.40 ± 16.57 | 110.93 ± 21.41^a | 35.82 ± 2.49 | 24.77 ± 2.50^a | |
| IHLP | 230.72 ± 34.10 | $286.28 \pm 43.82^{\circ}$ | 35.41 ± 2.30 | $28.35 \pm 2.44^{\circ}$ | |

 $\partial \rho$ < 0.01 for the comparison between rest and opening.

Recently, many investigations $31-34$ $31-34$ $31-34$ about the relationship between LPM attachment and internal derangement of the TMJ have been reported already. However, these studies focused on attachment of the LPM to the disc-condyle complex and found no consensus regarding the correlation between LPM attachment and TMJ pathology. In our study, DTI successfully demonstrated changes of the LPM during different jaw movement tasks. The morphological and functional evaluation method of the SHLP, as a whole muscle bundle, can offer a new idea for further research of TMJD's aetiopathogenesis.

This study has several limitations. First, due to the intolerability to maintain clenching, only a few volunteers underwent 3D BRAVO sequence during jaw clenching, thus the CSA and muscle length in clenching in the intercuspal position could not be used in statistication. Second, the sample size is relatively small, with a narrow range of subjects' age. Finally, the water diffusion in vivo is a comprehensive process influenced by many factors. Our result showed that the diffusion parameters are sensitive to changes by jaw movement, and further studies are required to specify the relationship between the changes of diffusion parameters and the physiology of muscle fibres, studies about DTI in detecting the function of LPM when it is under pathological conditions are also required.

CONCLUSION

DTI successfully demonstrated the morphological and functional changes of the LPM during different jaw movement tasks, it also proved synchronous action of the SHLP and IHLP in the jaw opening as well as the action of SHLP rather than IHLP in the jaw clenching. DTI may serve as a new and non-invasive method for simultaneously investigating the functional and morphological features of the LPM during jaw movement. Further studies could potentially use DTI to evaluate the LPM dysfunction in patients with TMJD.

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