

## CASE REPORT

# Microstructural investigation of masticatory muscles: a pre- and post-treatment diffusion tensor imaging study in a bruxism case

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We evaluated, by means of a non-invasive procedure based on MRI, the masticatory muscular microstructure in a 55-year-old-female patient affected by bruxism. The patient underwent MR examination before and after 1 month of splint therapy, when she mentioned the complete disappearance of all symptoms. By means of diffusion tensor imaging we observed changes at microstructural level of masticatory muscular complex. We conclude that diffusion tensor imaging may be a useful instrument both to perform panoramic reconstruction of the masticatory muscle complex and to investigate microstructural modifications related to the pain relief in bruxism.

*Dentomaxillofacial Radiology* (2018) **47**, 20170275. doi: [10.1259/dmfr.20170275](https://doi.org/10.1259/dmfr.20170275)

**Cite this article as:** Mormina E, Granata F, Gaeta M, Longo M, Calamuneri A, Arrigo A, et al. Microstructural investigation of masticatory muscles: a pre- and post-treatment diffusion tensor imaging study in a bruxism case. *Dentomaxillofac Radiol* 2018; **47**: 20170275.

**Keywords:** tractography; bruxism; diffusion tensor imaging; masticatory muscles; clenching

## Introduction

Bruxism has been defined as a repetitive jaw muscle activity characterized by clenching or grinding of teeth as well as by bracing or thrusting of the mandible.<sup>1–3</sup> It is often misdiagnosed, and can cause dental wear, jaw muscle pain and fatigue, temporal or chronic headaches with neck irradiation.<sup>4</sup>

Several therapies, both medical and mechanical, have been proposed in order to treat bruxism, with different results.<sup>3</sup> In order to objectively evaluate the effect of mechanical therapy on masticatory muscle on patients affected by bruxism, surface electromyography is commonly used.<sup>5</sup> Unfortunately this technique is not able to provide information about each single component of

masticatory muscle complex, and is usually adopted only for masseter muscle analysis.<sup>5</sup>

Herein we evaluated the masticatory muscular microstructure in a patient affected by bruxism, pre- and post-splint therapy. To this end, we adopted a MRI non-invasive procedure, called diffusion tensor imaging (DTI) tractography. By means of DTI tractography we assessed differences in muscular microstructure.

## Methods and materials

### *Clinical evaluation*

For this study we chose a 55-year-old-female referring chronic headaches with cervical irradiation, which has been lasting for 6 years. She reported previous medical treatments with analgesics and physiotherapy for several years. None of them were able to solve her chronic pain.

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Received 13 July 2017; revised 08 January 2018; accepted 13 February 2018

Moreover, she referred a sense of pain and constriction mostly present in the early morning subsequent to a regular night sleep. Based on clinical history and dental wear on oral cavity inspection, the suspect of bruxism was raised. The patient refused to undergo polysomnography examination and surface electromyography. A full cover smooth splint on the superior arch was then built and dressed by the patient during the regular night sleep for 30 days. After 1 week of therapy, the patient reported a complete recovery from all symptoms. Written informed consent for the case to be published (incl. images, case history and data) was obtained from the patient for publication.

#### *MR acquisition parameters*

The subject underwent head and masticatory MRI with a 1.5T MR scanner (Ingenia, Philips Healthcare, Best, Netherlands) before undergoing splint therapy. After 1 month treatment the patient referred the complete disappearance of any symptom; subsequently, MRI scans were acquired again. The second MRI examination was performed to assess if DTI was able to detect any microstructural change which may be linked to pain relief. MRI protocol consisted in a 3D- $T_1$  weighted fast field echo sequence, with repetition time (TR) of 25 ms, echo time (TE) of 4.6 ms, reconstruction matrix  $256 \times 256$  and voxel size  $1 \times 1 \times 1$  mm, covering from the vertex to an axial plane through the body of C5. In addition, high resolution  $T_1$  weighted turbo spin echo (TSE) images were acquired in true sagittal and coronal planes of the pterygoid muscles, covering from 1 cm above the nasion to C5 level on axial planes, with TR of 500 ms, TE 9 ms, reconstruction matrix  $512 \times 512$ , slice thickness 2.5 mm and no interslice gap.

Spatial measures of length, width and height were performed on  $T_1$  weighted TSE images.

DTI sequence was acquired in the axial plane, with the same coverage of high resolution  $T_1$  weighted TSE. Following parameters were adopted: TR of 4320 ms, TE of 90 ms, reconstruction matrix  $128 \times 128$ , voxel size  $2 \times 2 \times 2$  mm; 32 unique diffusion encoding gradient directions ( $b = 1000 \text{ s mm}^{-2}$ ) and one volume with no diffusion encoding ( $b = 0 \text{ s mm}^{-2}$ ) were gathered.

MRI protocol lasted less than 20 min. During acquisitions, the subject laid in a comfortable supine position with a postural position of jaw, without any masticatory or talking or clenching movement. The subject was asleep for the whole MR examination time. A visual check for movement artifact on images was performed and the examination was evaluated suitable for the study, since no movement artifacts were present.

As recently described by St-Jean *et al*<sup>6</sup> in order to improve image quality and analysis reliability, a denoising procedure was applied on all DTI data before the evaluation.

#### *MR fiber tracking parameters*

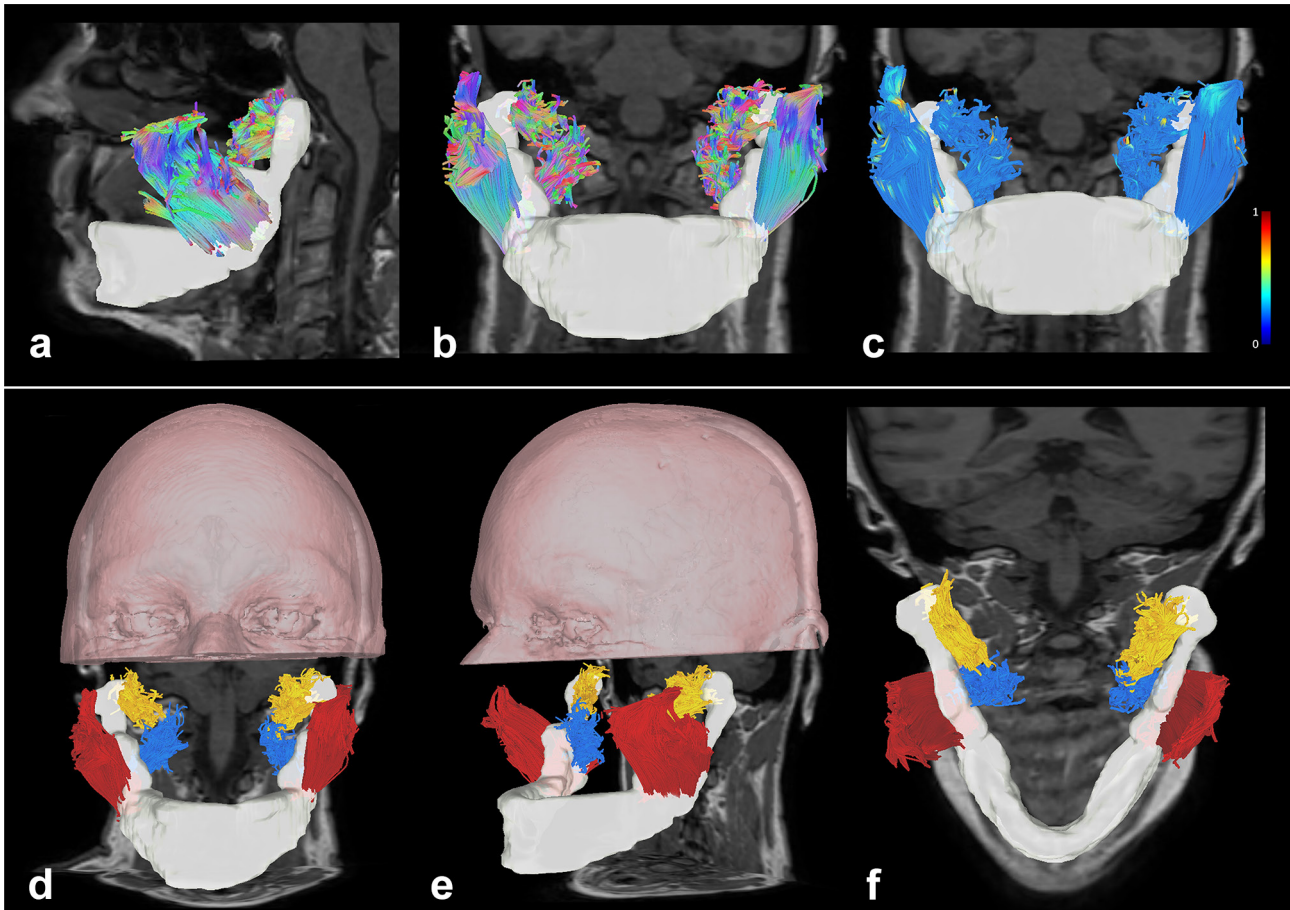
Linear co-registration of MRI images was performed using Statistical Parameter Mapping tool (Wellcome Trust

Centre for Neuroimaging, University College of London - SPM12 - [www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)), running on the scientific computing software called Matlab [MathWorks (R)], release R2013a (<https://www.mathworks.com/products/matlab.html>). To this end, fractional anisotropy (FA) maps were extracted from DTI datasets;<sup>7</sup> subsequently,  $T_1$  weighted images and FA maps were co-registered using tools described above. DTI tractography consists in following the most likely water diffusion pathway based on Diffusion Tensors estimated. Diffusion tensor estimation, FA map extraction and deterministic tractography were performed using DSI Studio (<http://dsi-studio.labsolver.org/>).<sup>7</sup> The following parameters were adopted: fractional anisotropy threshold as termination index 0.19, angular threshold  $80^\circ$ , step size 0.98 mm with no smoothing, minimum fiber length 6 mm. A single seed approach was used in order to start fiber tracking in both directions. Manual regions of interest (ROIs) were drawn on DTI images to select the area where the muscle is larger, in order to increase the number of reconstructed muscle fibers. ROIs were moreover checked on coregistered  $T_1$  weighted images. Three separate ROIs were selected: medial pterygoid muscle (MPM), lateral pterygoid muscle (LPM) and masseter muscle (MM). We decided to exclude temporal muscles from DTI evaluation since it was only partially included in the field of view of the MR images: the field of view indeed included only its insertion on medial and anterior aspect of coronoid process of mandible.

Manual segmentation, slice by slice, of mandible was also performed on  $T_1$  weighted MRI images in order to have a better three-dimensional (3D) rendering visualization, as well as to ease the evaluation of spatial relationship of masticatory muscles and their morphology. Mandibular volume was set as a region of avoidance (ROA) to avoid any unreliable reconstructions going through this region.

#### *MR fiber tracking 3D rendering*

Muscle tract colors were set following the color-coded tractography convention of Pajevic and Pierpaoli for white matter fibers, with the blue color representing fibers oriented craniocaudally, the red color representing fibers oriented latero-laterally and the green color representing fibers oriented anteroposteriorly.<sup>8</sup> A whole set of intermediate color was automatically selected from these primary colors for intermediate fiber muscle orientation (Figure 1a,b). To better discriminate muscles, an arbitrary color coding was given to all fibers belonging to a single muscle (Figure 1d-f). For each muscle group, we extracted and averaged the following diffusion tensor parameters: FA, mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). FA maps were furthermore adopted for visualization purposes to highlight fiber muscle diffusivity characteristics (Figure 1c). This procedure gives us the opportunity to investigate locally FA variability. Eventually, the percentage of variation of DTI parameters was calculated to assess the amount of alterations after 1 month of therapy.



**Figure 1** Tractographic reconstructions of masticatory muscular complex. (a, b) Sagittal and frontal view of the masticatory muscular complex with a directional color coding representation, overlaid on a  $T_1$  weighted image (the mandible is reconstructed as a white volume). (c) Frontal view of the masticatory muscular complex with a local index of FA color coding representation overlaid on a  $T_1$  weighted image (lateral scale represents FA values from 0 to 1). (d–f) Volume rendering of the upper portion of the head of the patient with tractographic reconstructions of masticatory muscular complex (axial and coronal  $T_1$  weighted images are overlaid). Arbitrary colors were given to fibers: masseter muscles are represented in red, lateral pterygoid muscles are represented in yellow and medial pterygoid muscles are represented in blue.

## Results

DTI tractography was able to reconstruct each component of the masticatory complex on both sides and in both pre- and post-treatment MRI examinations. The volume rendering representation of masticatory muscles did not show shape differences. No differences in length, width or height were observed on LPM, MPM and MM muscles.

Visual inspection of FA local variation in fiber muscles did not show any intramuscular abnormal peak, whereas a slight FA increase was detected in correspondence of muscular insertion.

The patient, after 1 month of splint therapy, referred the complete disappearance of any symptom. Comparison of DTI parameters pre- and post-mechanical treatment showed FA decrement and MD, AD and RD increment in each analyzed muscle. In particular, there was a mean FA decrement of 14.05% in MMs (left -20.26%; right -7.84%), 15.15% in LPMs (left -17.60%;

right -12.71%) and 1.17% in MPMs (left -0.53%; right -1.81%). Average MD increased of 14.5% in MMs (left +24.80%; right +4.20%), 18.67% in LPMs (left +22.52%; right +14.83%), and 4.88% in MPMs (left +7.89%; right +1.86%). DTI parameter variations are reported in detail in [Table 1](#).

## Discussion

Bruxism has been estimated in 5–8% of the adult population with any documented sex differences.<sup>4</sup> Patients with an anxious habit seem to have a higher risk to develop bruxism, although the pathogenesis is still unclear.<sup>9</sup> The main treatments available are sleep hygiene measures combined with relaxation techniques, pharmacological therapy, contingent electrical stimulation and splint therapy.<sup>3,10,11</sup> Splint therapy is a mechanical treatment which covers the whole dental arch and focuses on avoidance of dental wear and muscle spasticity, which cause typical headache affecting patients suffering of bruxism.<sup>10</sup>

**Table 1** Pre- and post-splint therapy diffusion tensor parameter values

		<i>Masseter muscle</i>		<i>Lateral pterygoid muscle</i>		<i>Medial pterygoid muscle</i>	
		<i>Left</i>	<i>Right</i>	<i>Left</i>	<i>Right</i>	<i>Left</i>	<i>Right</i>
FA	Pre	0.385 ± 0.17	0.395 ± 0.17	0.392 ± 0.12	0.362 ± 0.10	0.380 ± 0.11	0.386 ± 0.80
	Post	0.307 ± 0.11	0.364 ± 0.14	0.323 ± 0.10	0.316 ± 0.09	0.378 ± 0.12	0.379 ± 0.12
	Variation	↓ <b>20.26</b>	↓ <b>7.84</b>	↓ <b>17.60</b>	↓ <b>12.71</b>	↓ <b>0.53</b>	↓ <b>1.81</b>
MD	Pre	0.915 ± 0.41	0.927 ± 0.42	0.937 ± 0.29	0.998 ± 0.25	0.824 ± 0.19	0.913 ± 0.20
	Post	1.142 ± 0.30	0.966 ± 0.31	1.148 ± 0.32	1.146 ± 0.29	0.889 ± 0.25	0.930 ± 0.25
	Variation	↑ <b>24.80</b>	↑ <b>4.20</b>	↑ <b>22.52</b>	↑ <b>14.83</b>	↑ <b>7.89</b>	↑ <b>1.86</b>
AD	Pre	1.193 ± 0.45	1.215 ± 0.47	1.252 ± 0.33	1.330 ± 0.30	1.096 ± 0.23	1.206 ± 0.22
	Post	1.449 ± 0.34	1.266 ± 0.34	1.467 ± 0.36	1.464 ± 0.32	1.185 ± 0.28	1.215 ± 0.28
	Variation	↑ <b>21.45</b>	↑ <b>4.20</b>	↑ <b>17.17</b>	↑ <b>10.07</b>	↑ <b>8.12</b>	↑ <b>0.75</b>
RD	Pre	0.776 ± 0.39	0.783 ± 0.39	0.780 ± 0.18	0.831 ± 0.19	0.688 ± 0.28	0.767 ± 0.24
	Post	0.989 ± 0.29	0.816 ± 0.30	0.989 ± 0.24	0.988 ± 0.24	0.740 ± 0.30	0.787 ± 0.28
	Variation	↑ <b>27.45</b>	↑ <b>4.21</b>	↑ <b>26.80</b>	↑ <b>18.89</b>	↑ <b>7.56</b>	↑ <b>2.61</b>

AD, axial diffusivity; FA, fractional anisotropy; LPM, lateral pterygoid muscle; MD, mean diffusivity; MM, Masseter muscle; MPM, medial pterygoid muscle; Pre, pre splint therapy; Post, post splint therapy; RD, radial diffusivity; SD, standard deviation. FA, MD, AD and RD values, ± SD are evaluated as mean of all tracts for each muscle. Percentage variation (variation) is represented in bold as increment (↑) and decrement (↓) values of diffusion tensor parameters between pre and post splint therapy.

In this study, we investigated masticatory muscles microstructure changes in bruxism by means of DTI parameters and DTI tractography, evaluated before and after 1 month of mechanical treatment. The aim of the study was to evaluate whether it was possible to detect any microstructural alteration after mechanical therapy and how this latter may influence masticatory muscle complex. Our results showed variations in diffusion parameters of MPM, LPM and MM muscles, when comparing pre- and post-treatment MRI datasets.

Notably, the complete regression of subject's symptoms was accompanied by a corresponding variation of diffusion metrics. On the contrary, muscles morphology, namely width, height and length, did not show any change.

DTI tractography is a technique which permits the non-invasive evaluation of microstructural as well as macroscopic properties of masticatory muscles.<sup>12–20</sup> The use of isotropic high resolution  $T_1$  images allowed image reformatting in arbitrary planes, making the selection of ROIs and the evaluation of masticatory muscle shapes easier to perform if compared to the use of DWI b0 images only.

To the best of our knowledge, this is the first study which have evaluated the mechanical treatment outcome on masticatory muscles by means of diffusion tensor parameters.

From a physiological point of view, in muscular contraction reduction in muscular fiber length and increase of fiber muscular diameter usually occur. During contraction, fibers diameter increment may cause a bigger amount of fibers to insist over the same MR voxel; this in turn may affect diffusion tensor estimation resulting in FA increment. FA is a measure describing the amount of water molecules diffusion asymmetric displacement.<sup>21</sup> High FA values correspond to a cigar

shape of the diffusion tensor ellipsoid indicating a prominent diffusion directionality, whereas low FA values indicate the lack of a precise water molecules diffusion directionality.

In our study, mechanical therapy was able to decrease muscle contraction of masticatory complex, which was demonstrated by chronic pain and headaches disappearing. The longitudinal diffusion tensor evaluation showed FA decrease in each muscle evaluated, and similarly MD, AD and RD increment (Table 1). These changes in diffusion tensor parameters are probably related to the decrement of masticatory muscle chronic contraction, which may have influenced changes in muscular microstructure.

Several studies showed how muscular contraction and relaxation may result in heterogeneous change of diffusion parameters.<sup>13–20</sup> Schwenzer et al reported how the passive shortening of leg muscles may cause a FA decrement with a consensual MD increment and how stretching muscles have an inverse effect on diffusion parameters.<sup>19</sup> On the other hand, Liu et al<sup>12</sup> detected FA increment and MD decrement, when comparing active clenching and rest position of LPM inside an MR scanner. In our study, we performed MR scans in neutral postural position with no active clenching. Moreover, gender differences have been observed.<sup>13–20</sup> It is important to remark that our analyses are based on a single patient affected by bruxism. For this reason case-control studies involving longitudinal evaluations are needed in order to confirm and validate our results.

## Conclusion

DTI tractography, a non-invasive technique based on MRI is able to easily reconstruct the masticatory muscle

complex in order to assess its morphology as well as its microstructural properties. By means of diffusion tensor parameters were able to detect post-treatment changes in fiber microstructure in a patient affected by chronic bruxism.

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## Consent

Written informed consent for the case to be published (including images, case history and data) was obtained from the patient for publication.