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# Functional anatomy of the equine temporomandibular joint: Collagen fiber texture of the articular surfaces



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#### ABSTRACT

In the last decade, the equine masticatory apparatus has received much attention. Numerous studies have emphasized the importance of the temporomandibular joint (TMJ) in the functional process of mastication. However, ultrastructural and histological data providing a basis for biomechanical and histopathological considerations are not available. The aim of the present study was to analyze the architecture of the collagen fiber apparatus in the articular surfaces of the equine TMJ to reveal typical morphological features indicating biomechanical adaptions. Therefore, the collagen fiber alignment was visualized using the split-line technique in 16 adult warmblood horses without any history of TMJ disorders.

Within the central two-thirds of the articular surfaces of the articular tubercle, the articular disc and the mandibular head, split-lines ran in a correspondent rostrocaudal direction. In the lateral and medial aspects of these articular surfaces, the split-line pattern varied, displaying curved arrangements in the articular disc and punctual split-lines in the bony components. Mediolateral orientated split-lines were found in the rostral and caudal border of the articular disc and in the mandibular fossa. The complex movements during the equine chewing cycle are likely assigned to different areas of the TMJ. The split-line pattern of the equine TMJ is indicative of a relative movement of the joint components in a preferential rostrocaudal direction which is consigned to the central aspects of the TMJ. The lateral and medial aspects of the articular surfaces provide split-line patterns that indicate movements particularly around a dorsoventral axis.

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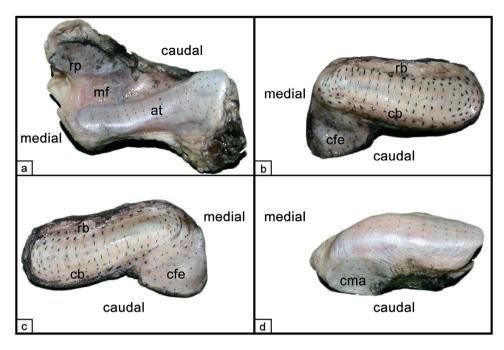
#### Introduction

The equine temporomandibular joint (TMJ) lacks in-depth objective clinical studies and studies investigating its role in pathological conditions (Carmalt, 2014; Witte, 2015).

To allow a precise diagnosis of pathological changes within the structures of the equine TMJ, the gross anatomical features of this complex joint have been described in detail by anatomical dissections (Rodríguez et al., 2006). Concomitantly, several studies have been performed to evaluate the suitability and potential relevance of advanced imaging techniques, i.e. radiography (Ebling et al., 2009), computed tomography (Rodríguez et al., 2008; Carmalt et al., 2016), ultrasonography (Rodríguez et al., 2007), magnetic resonance imaging (Rodríguez et al., 2010) and TMJ-arthroscopy (May et al., 2001; Weller et al., 2002). Besides these diagnostic and gross anatomical investigations, functional examinations of the equine masticatory movements have been performed. The initial tests utilized a molograph (Leue, 1941), while more recent research has used

\* Corresponding author. E-mail address: Carsten.Staszyk@vetmed.uni-giessen.de (C. Staszyk). video analysis (Collinson, 1994). Similar to other herbivorous mammals (Hiiemae, 1978) the equine chewing cycle has been described as consisting of an opening stroke, a closing stroke and a power stroke (Collinson, 1994; Baker and Easley, 1999; Baker, 2002). The power stroke in equids is described generally as unimodal and mediolateral movements of the mandible (Fortelius, 1985; Kaiser, 2002; Williams et al., 2007). Using optical tracking systems, the detailed 3-D kinematics of the TMI have been described in each phase of the strokes considering a lateroventral movement of the working side during the opening stroke and a marked mediodorsal movement of the working side during the power stroke (Collinson, 1994; Bonin et al., 2006; Staszyk et al., 2006). Both the direction and the range of rotational and translational movements have been quantified (Bonin et al., 2006). This technique also enabled examinations focused on the influence of different feeds (Bonin et al., 2007), dental corrections (Simhofer et al., 2011) and the effect of acute unilateral TMJ inflammation (Smyth et al., 2015b) on the kinematics of the TMJ. Additionally, masticatory forces have been estimated by placing force sensors on the second and third premolars (Staszyk et al., 2006).

Although a case report has demonstrated histopathological changes in the equine TMJ (Smyth and Carmalt, 2015a), the normal microscopical anatomy of the equine TMJ remains widely



**Fig. 1.** Articular surfaces obtained from a right temporomandibular joint of a horse demonstrating the split-lines. (a) Temporal components of the TMJ. Rp, retroarticular process; mf, mandibular fossa; at, articular tubercle. (b) Articular disc, dorsal side. rb, rostral border; cb, caudal border; cfe, caudomedial fibrous expansion. (c) Articular disc, ventral side. rb, rostral border; cb, caudal border; cfe, caudomedial fibrous expansion. (d) Mandibular head. cma, caudomedial aspect.

undescribed (Ramzan, 2006). However, ultrastructural features may be important for the understanding of TMJ pathologies, regenerative capabilities and biomechanical considerations.

Therefore, the aim of the present study was to visualize and analyze the architecture of the collagen fiber apparatus in the articular surfaces of the healthy equine TMJ to reveal typical morphological features indicating biomechanical adaptions.

## Material and methods

Sixteen adult warmblood horses without any history of TMJ disorders or dental diseases were included. The horses were euthanized for reasons not related to this study and the left and right TMJ were dissected. The articular surface bearing components of the temporal bone (articular tubercle, mandibular fossa and retroarticular process), the condylar process of the mandible (mandibular head, caudomedial aspect of the mandibular head) and the articular disc were removed. The dissected TMJs were examined for signs of degenerative joint disease (chondral lesions, ulcerations, hyperplasia, pannus, osteophytes) by gross inspection. Only joints free of all signs of degenerative joint disease were further evaluated.

The collagen fiber alignment in the articular surfaces was visualized using the split-line technique (Below et al., 2002). This involves the following procedure: a dissecting needle was dipped in commercial grade India ink (Pelikan, Scribtol) and inserted into the joint surfaces of the mandibular head, the dorsal and ventral side of the articular disc, the articular tubercle, the mandibular fossa and the retroarticular process of the temporal bone. The depth of penetration on the ventral and dorsal side of the articular disc was about 3 mm. In the mandibular and temporal components of the TMJ, a dissecting needle was inserted into the joint cartilage until the level of subchondral bone was reached. This procedure was repeated in a grid pattern at intervals of 5 mm until the articular surfaces were completely pricked. Each penetration was performed perpendicularly to the articular surface and resulted in a colored line, enabling visualization of the main orientation of the collagen network. This pattern of lines - termed 'split-lines' - arose on each articular surface. Articular surfaces were photographed (Nikon SB-29S, Macro Speedlight) and the split-line patterns were recorded on a schematic map. Subsequently, the split-lines were evaluated according to two parameters: length and orienta-

The length was assigned to one of three categories: punctiforme (<2 mm), medium (from 2 mm to <4 mm) or large ( $\ge$ 4 mm). The orientation was assigned to one of four categories: rostrocaudal, mediolateral, oblique or diffuse (no clear orientation visible).

Additionally, the orientation, presence and length of the split-lines and the general split-line patterns of the articular surfaces of the temporal bone, the mandibular head and the articular disc (dorsal and ventral surface) were compared.

Statistical analyses were performed using the data analysis software GraphPad Prism (v. 6.07; GraphPad Software) and BiAS (v. 9.08; Ackermann, 2010). The split-

line patterns of the central aspects of the articular surfaces were divided into 'rostrocaudal' and 'non-rostrocaudal'. On the medial and lateral aspects of the articular surfaces, the split-line patterns were classified into 'curved' and 'non curved' in case of the articular disc, and into 'punctiforme' and 'non punctiforme' in case of the articular tubercle and mandibular head. To test the relationship between the aspects of the articular surfaces examined and the split-line pattern, the Pearson Chi-Square-test for contingency tables was applied. Initially, the global comparison of all split-line patterns was performed. In case of global statistically significant differences, pair-wise comparisons were performed with the central aspects, controlling the type I error rate using the Bonferroni-Holm-procedure. P values of less than or equal to 0.05 were assumed to express statistical significance.

### Results

The most constant alignment of split-lines was present in the central two-thirds of the articular surfaces of the articular tubercle, the articular disc and the mandibular head. In these areas, medium sized (articular tubercle and mandibular head) or large sized (articular disc) split-lines were arranged most frequently in rostrocaudal direction (P < 0.001; Figs. 2–4). Variations in length and orientation were obtained in the peripheral (lateral and medial) areas of the articular tubercle, the articular disc and the mandibular head and in the mandibular fossa, the retroarticular process and at the caudomedial aspect of the mandibular head (Tables 1 and 2).

#### Articular tubercle

The orientation of split-lines in the central and medial aspects of articular tubercle was consistent for all specimens. In the centre, medium-sized split-lines were most frequently oriented in a rostrocaudal direction (P < 0.001). Towards the medial margin, punctiforme split-lines were identified most commonly (P < 0.001). In contrast, the lateral part of the articular surface demonstrated three different arrangements of split-lines. In 14 of 32 specimens, a combination of oblique orientated and punctiforme split-lines was noted (Fig. 3; Table 1). Punctiforme split-lines became visible in 13 of 32 specimens (P < 0.001; Fig. 3; Table 1). Finally, a diffuse design was shown in five of 32 specimens (Fig. 2; Table 1).

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