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Regional and disease-related differences in properties of the equine temporomandibular joint disc

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ABSTRACT

Temporomandibular joint (TMJ) disorders affect up to 12% of the human population, and naturally occurring TMJ diseases are increasingly recognized in animals. The TMJ disc plays a major role in TMJ disorders in people, but little is known about its role in TMJ pathology in animals. This study characterizes differences in properties of equine TMJ discs associated with age, disc region, and presence of TMJ osteoarthritis (OA). Discs were dissected from both TMJ's of sixteen horses euthanized for reasons unrelated to this study. Each joint was grossly evaluated and scored as normal, mild OA, or severe OA. Samples from the rostral, caudal, lateral, central, and medial regions of the disc were subject to compressive testing, quantitative biochemistry, and histology. Samples from the lateral, central, and medial region were tested for tensile properties in the rostrocaudal and mediolateral directions. We found that the equine TMJ disc is highly anisotropic, and its glycosaminoglycan (GAG) content and compressive stiffness vary between disc regions. The disc also exhibits increasing GAG content and compressive stiffness with increasing age. While equine TMJ disc properties are generally similar to other herbivores, greater compressive stiffness throughout the disc and greater GAG content in its rostral region suggest that mechanical demands on the TMJ disc differ between horses and other species. Importantly, a region-specific decrease in compressive stiffness was observed associated with joint disease and corresponded to cartilage erosions in the underlying condylar surface.

1. Introduction

Clinical disease of the temporomandibular joint (TMJ) affects up to 12% of the human population (Liu and Steinkeler, 2013), with increased prevalence in women and the elderly (Haskin et al., 1995; Warren and Fried, 2001). Naturally occurring TMJ disorders have also been described in animals, with osteoarthritis (OA) reported to be the most common disorder of the TMJ in dogs and the second most common in cats (Arzi et al., 2013). The TMJ is a synovial joint composed of the mandibular head of the condylar process of the mandible, mandibular fossa of the squamous temporal bone, the retroarticular process of the temporal bone, and an intraarticular, fibrocartilaginous disc. The essential anatomy of the TMJ is similar among humans and domestic animals, although differences in morphology of the bones and TMJ disc exist between species and likely reflect differences in mastication, prehension, diet, and dentition (Herring, 2003). While the TMJ disc is frequently involved in TMJ disorders in people, its role in pathophysiology of TMJ disorders in people or animals is not clear (Murphy et al., 2013b). Although a significant association has been shown between abnormal position of the disc, known as "internal derangement," and TMJ OA in people (Marguelles-Bonnet et al., 1995; Moncada et al., 2014), there is limited information regarding changes in the TMJ disc associated with OA. Further characterization of naturally occurring TMJ OA and the role of the TMJ disc in animals could provide insight into comparable diseases in people (Kol et al., 2015).

Given the differences in morphology and function of the TMJ between people and animals, no single animal species is likely to be sufficient as a model for all TMJ pathophysiology (Almarza et al., 2018). In horses, OA of the TMJ is suspected to be clinically important (Baker, 2002), but is poorly understood (Carmalt, 2014; Carmalt et al., 2017; Ramzan, 2006). A recent retrospective computed tomography study of the TMJ in 1018 horses found mineralization of the TMJ disc in 15% of asymptomatic horses as well as other findings typically associated with OA (Carmalt et al., 2016). Experimentally induced unilateral inflammation of the equine TMJ has shown that horses will compensate by adjusting their masticatory cycle, but are not hesitant to eat and don't show

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pain on palpation (Smyth et al., 2016). The existing literature reflects the difficulty in assessing the presence of equine TMJ disease and interpreting its clinical significance. Nonetheless, the relative prevalence of TMJ OA and the longer lifespan of horses compared to other domestic animals provide an opportunity to examine potential changes in the TMJ disc associated with age and OA.

Knowing the properties of the TMJ disc can help understand the function of the healthy TMJ as well as inform pathophysiology of TMJ disease. Tensile anisotropy and regional variations of properties have been described in healthy TMJ discs of multiple species (Almarza et al., 2006; Arzi et al., 2011; Detamore and Athanasiou, 2003; Kalpakci et al., 2011; Murphy et al., 2013a); however, characterization of the equine TMJ disc has not been performed, and there has been little investigation of pathologic changes in the disc of animals with naturally occurring TMJ disease (Murphy et al., 2013a; Matuska et al., 2016). Normal equine mastication is divided into three phases: opening, closing, and a power phase that involves mediolateral translation (Bonin et al., 2006). We hypothesize that equine TMJ disc properties will vary by region and direction associated with varying stresses experienced by the TMJ during mastication. Additionally, we hypothesize that TMJ OA will be associated with changes in composition and mechanical properties of the TMJ disc. This is the first study characterizing the equine TMJ disc and will improve the understanding of normal TMJ function and potential role of the disc in TMJ disease in horses.

2. Materials and methods

2.1. Specimen procurement

Sixteen equine heads were obtained from client-owned patients that died or were euthanized for reasons unrelated to this study and donated to the UC Davis Veterinary Medical Teaching Hospital with client consent for research use. Skulls were frozen within 12 h of death and kept at −20 °C until the time of dissection. Each joint was dissected and scored as normal, mild OA, or severe OA based on the presence and degree of gross articular cartilage damage and periarticular bone remodeling (Fig. 1). TMJ discs from all joints were dissected from their attachments, washed in phosphate buffered saline (PBS), wrapped in gauze soaked with PBS containing protease inhibitors (10 mM N-ethylmaleimide and 1 mM phenylmethylsulfonyl fluoride, Sigma, St. Louis, MO, USA), and frozen at −20 °C until testing. Both discs from each horse were used for the study. Full-thickness samples were collected from the rostral, caudal, lateral, central, and medial regions of each disc using 3 mm dermal biopsy punches for evaluation of biochemical composition, histologic appearance, and compressive properties (Fig. 2). "Dogbone" shaped samples oriented in rostrocaudal (comparable to anteroposterior) and mediolateral directions were collected from the central, lateral and medial regions of each disc for tensile testing using biopsy punches and scalpel dissection (Fig. 2).

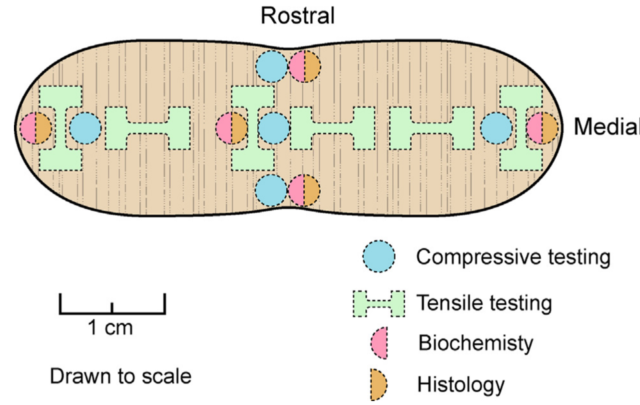

Fig. 2. Schematic of a dorsal view of an equine TMJ disc depicting sample allocation for mechanical, biochemical, and histologic evaluation. Full-thickness samples were obtained using dermal biopsy punches and scalpel dissection. The dorsal and ventral surfaces of compressive samples were trimmed using a scalpel, as necessary, to obtain flattened cylinders for testing.

2.2. Biochemical characterization

Biochemical samples were weighed prior to and following 72 h of lyophilization to assess tissue hydration. The lyophilized tissue was then digested in 2.125 units/mL papain (Sigma) in phosphate buffer (pH 6.5) containing 2 mM N-acetyl cysteine (Sigma) and 2 mM ethylenediaminetetraacetic acid for 18 h at 60 °C. Sulfated glycosaminoglycan (GAG) content was quantified using the 1,9-dimethylmethylene blue binding assay (Blyscan™, Biocolor, Carrickfergus, UK). Total collagen was quantified using a hydroxyproline assay (Cissell et al., 2017) with Sircol™ bovine collagen standards. DNA content was quantified using the Quant-iT™ Picogreen double stranded deoxyribonucleic acid assay (Invitrogen, Carlsbad, CA). Collagen, GAG, and DNA content were normalized to sample dry weight (DW).

2.3. Mechanical characterization

Dogbone samples were prepared with a gauge length: width aspect ratio of approximately 3:1. Orthogonal photographs of each dogbone were taken, and the cross sectional area of the sample gauge length was measured using ImageJ (NIH). Continuous, uniaxial strain-to-failure tests were conducted on a materials testing machine (Instron 5965, Instron, Norwood, MA). Samples were placed between pneumatic grips set to 70 psi and strained at a rate of 1% of the gauge length per second. The tensile Young's Modulus was calculated from the linear portion of each stress-strain curve

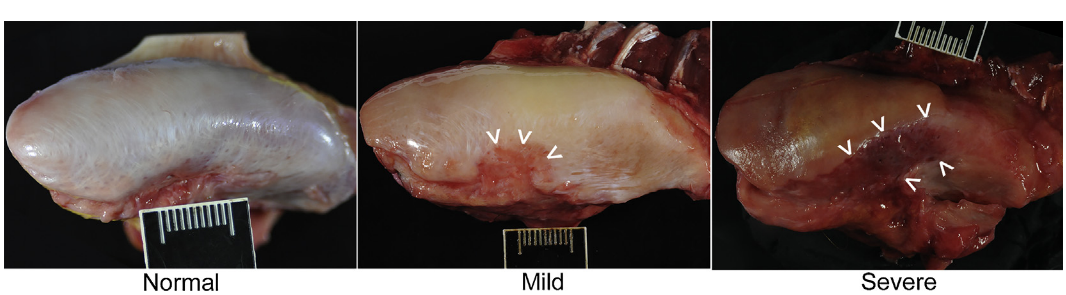

Fig. 1. Dorsal (i.e., superior) view of representative mandibular condyles from normal temporomandibular joints (TMJ) and TMJ with mild or severe osteoarthritis. Arrowheads indicate grossly visible lesions.

and the ultimate tensile strength (UTS) was determined as the maximum stress experienced by the tissue using data analysis software (MATLAB, Mathworks, Natick, MA).

Due to the biconcave shape of the TMJ discs, the dorsal and ventral surfaces of compressive samples were trimmed manually with a scalpel to obtain a flattened cylindrical shape for testing. Samples were immersed in PBS at room temperature and stress-relaxation testing was performed in unconfined compression using an Instron 5965 materials testing machine. Contact between the compression platen and sample was determined by a 0.05N preload. Samples were then preconditioned with 15 cycles of 5% strain at a strain rate of 10% per second. During the test, two serial 10% step-strains were applied, with a 7 min interval after the first step, and a 10 min interval after the second step to allow for stress relaxation. The instantaneous modulus, relaxation modulus, and coefficient of viscosity were calculated by fitting the stress relaxation data to a viscoelastic solution for a standard linear solid using MATLAB (Allen and Athanasiou, 2006).

2.4. Histologic characterization

The scalpel cut surfaces of histology samples were oriented parallel to the sagittal plane and the dorsal surface was marked with dye to maintain sample orientation. Each histology sample was fixed in 10% formalin for a minimum of 24 h, then dehydrated via ascending alcohol concentrations and embedded in paraffin. Histologic sections were prepared from each region of each disc and stained using hematoxylin & eosin (HE), safranin-O/fast green (SOFG), or picrosirius red to evaluate general morphology, GAG, and collagen distribution, respectively. Bright field light microscopy was performed for all samples and stains. Polarized light images were acquired with the polarizer and analyzer at 90° to each other and two orientations of the stage: (1) maximum light transmission and (2) rotated 30° relative to maximum light transmission.

2.5. Statistical analysis

The effects of individual horse, joint (right or left), disc region, and horse age on each response variable were assessed using mixed model ANOVA for a split-split plot design. Each horse was treated as a whole plot, random variable. Joint (right or left) was treated as a fixed, subplot effect nested in individual horse, and the fixed, sub-subplot effect of region was nested in joint and horse to account for potential covariance associated with analysis of two TMJ discs from each horse and multiple regions within each disc. Age was included in the ANOVA model as an independent variable. For analysis of tensile properties, the strain direction (rostrocaudal vs. mediolateral) was entered into the statistical model as an additional fixed effect nested in region, joint, and horse. After initial statistical analysis, residuals were evaluated for constancy of variance, independence, and normality. If necessary, response variables were log-transformed and/or outliers were removed and ANOVA was repeated. Significant differences among levels within a factor were assessed using the Tukey HSD *post hoc* test when indicated.

Potential associations between OA and changes in TMJ disc mechanical properties or biochemical composition were assessed by matched pairs analysis with grouping by disc region within horses with asymmetrical TMJ OA. Matched paired analysis was performed to avoid potential confounding effects of age and individual variation. Potential relationships between biochemical composition and mechanical properties were assessed by multiple linear regression and is further described in the Appendix. Association between horse age and joint disease was examined by logistic regression separately for right and left joints. All statistical analysis was performed using JMP Pro 13. Statistical significance for all tests was defined by $p < 0.05$. All quantitative results are presented as the mean ± standard deviation except where otherwise indicated.

3. Results

Ages of horses included in this study varied from 5 to 25 years old with a median age of 16 years. Horses included fourteen of light riding breeds (three American Paint Horse, three Thoroughbred, two Quarter Horse/Quarter Horse cross, two Standardbred, and one each Appaloosa, Fox Trotter, Lusitano, and Mustang), one warmblood (specific breed not stated), and one light draft breed (Friesian). Of the 32 total joints analyzed, 11 exhibited no gross evidence of osteoarthritis (OA), 12 had mild OA, and 9 had severe OA. Of the 21 joints with OA, cartilage lesions were most commonly located on the caudal (12/21) or medial (6/21) aspect of the mandibular head, retroarticular process (7/21), and mandibular fossa (5/21). Gross pathology was less common in the TMJ discs, with lesions consisting of firm, raised ridges (4/32); small, raised nodules (2/32); fibrillation (2/32); and pannus formation on the disc surface (1/32). Increasing age was associated with greater collagen content ($p = 0.009$), greater GAG ($p < 0.001$), increasing compressive relaxation modulus ($p < 0.001$), decreasing tensile Young's modulus ($p < 0.001$) and decreasing UTS ($p = 0.004$). Joints with OA were also significantly more likely to be associated with older horses ($p \leq 0.002$); the median ages of normal, mild OA, and severe OA joints were 10, 16, and 21 years, respectively. There was no significant effect of joint (right vs. left) on any response variable.

3.1. Biochemical analysis

Quantitative biochemical results are displayed in Fig. 3. Hydration of the discs was significantly lower in the caudal region (65.9 ± 2.8%) than all other regions, which varied minimally between 69.2 and 71.0% (not shown). GAG data were log-transformed to correct variance for final statistical analysis. GAG

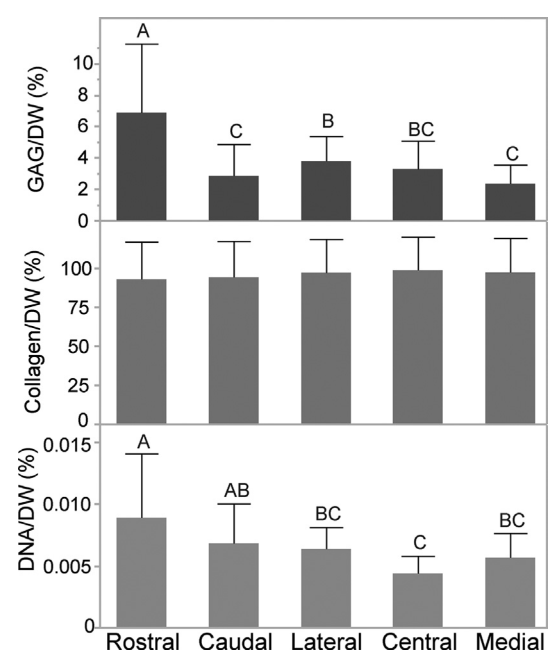

Fig. 3. Mean glycosaminoglycan (GAG), collagen, and DNA content per dry weight (DW) of all equine TMJ discs by region. Groups not connected by the same letter are significantly different by mixed model ANOVA with Tukey's HSD *post hoc* test ($p < 0.05$). Error bars represent one standard deviation.

and DNA content varied significantly between different regions. The rostral region contained the greatest GAG (6.9 ± 4.4%DW) and the medial aspect of the disc contained the least GAG (2.3 ± 1.2%DW). The rostral region of the discs had the highest DNA content ($8.9 \pm 5.2 \times 10^{-3}$ %DW) and the central region of the discs had the lowest DNA content ($4.4 \pm 1.4 \times 10^{-3}$ %DW). The mean collagen content of TMJ discs varied from 90 ± 16% in the rostral region to 98 ± 22% in the central region, but regional differences in collagen content were not significantly different. Associations between biochemical composition and mechanical properties of the equine TMJ disc are described in the Appendix, with significant associations summarized in Table S1.

3.2. Tensile properties

Tensile properties of the equine discs are presented in Fig. 4. Data were log-transformed to correct variance for final statistical analysis. Discs were significantly stiffer and stronger when strained in the rostrocaudal direction than the mediolateral direction. Young's moduli in the rostrocaudal and mediolateral directions were 60 ± 35 MPa and 1.6 ± 2.0 MPa, respectively. UTS in the rostrocaudal and mediolateral directions were 34 ± 15 MPa and 0.92 ± 1.1 MPa, respectively. When strained in the rostrocaudal direction, neither stiffness nor strength significantly differed among regions; however, when strained in the mediolateral direction, the lateral region was significantly stiffer (2.2 ± 2.4 MPa) and stronger (1.3 ± 1.7 MPa) than the central region (0.98 ± 0.62 MPa and 0.61 ± 0.34 MPa, respectively).

3.3. Compressive properties

The compressive properties for the equine disc at 20% strain are shown in Fig. 5. Data were log-transformed to correct variance for final statistical analysis. Compressive stiffness and coefficients of viscosity varied significantly between disc regions ($p < 0.001$). The rostral region of the disc was significantly stiffer than all other regions with 20% relaxation modulus of 0.95 ± 0.48 MPa and instantaneous modulus of 4.6 ± 1.8 MPa. The medial region was the least stiff in compression with 20% relaxation modulus of 0.25 ± 0.15 MPa and instantaneous modulus of 1.4 ± 0.83 MPa. Coefficients of viscosity also significantly varied from 200 ± 120 MPa·s in the rostral region to 61 ± 47 MPa·s in the medial region.

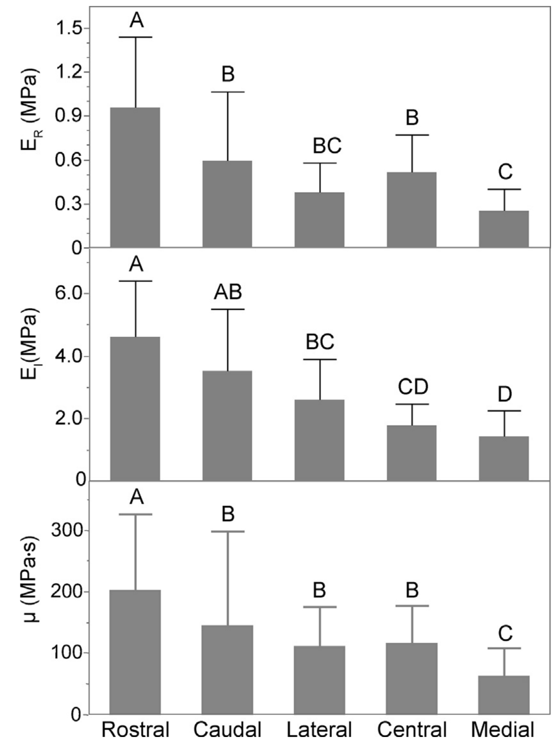

Fig. 5. Mean compressive relaxation modulus (E_R), instantaneous modulus (E_I), and coefficient of viscosity (μ) at 20% strain of all TMJ discs by region. Groups not connected by the same letter are significantly different by mixed model ANOVA with Tukey's HSD *post hoc* test ($p < 0.05$). Error bars represent one standard deviation. The rostral region exhibited the greatest compressive moduli and coefficient of viscosity.

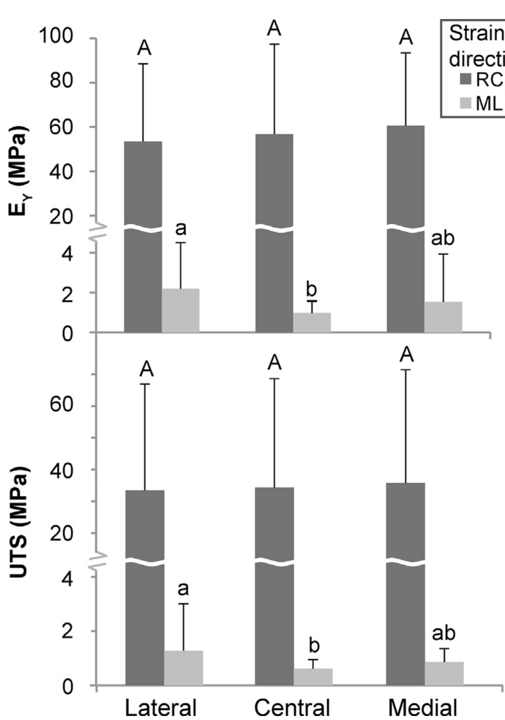

Fig. 4. Mean tensile Young's modulus (E_Y) and ultimate tensile strength (UTS) of all equine TMJ discs by region and direction of strain. E_Y and UTS were significantly greater across all regions when samples were strained in the rostrocaudal (RC) direction than in the mediolateral (ML) direction. Groups not connected by the same case or letter are significantly different by mixed model ANOVA with Tukey's HSD *post hoc* test ($p < 0.05$). Error bars represent one standard deviation.

3.4. Effects of joints disease on disc properties

Eight horses had asymmetrical TMJ disease. Three horses had one grossly normal TMJ with mild OA of the contralateral joint and five horses had mild OA of one TMJ with severe OA of the contralateral joint. For horses with asymmetric TMJ OA, the compressive properties significantly varied between joints in a region-dependent fashion (Fig. 6). On average, the caudal region of discs from more diseased joints had 37% lower relaxation moduli ($p = 0.006$), 40% lower instantaneous moduli ($p = 0.005$), and 49% lower coefficients of viscosity ($p = 0.001$). There was no significant difference between asymmetric joints with respect to composition or tensile properties.

3.5. Histology

Representative histologic images are presented in Fig. 7. Histology of discs from normal TMJ's revealed abundant collagen interspersed with fibroblasts. The central, medial, and lateral regions exhibited highly anisotropic collagen fibers tightly packed in large

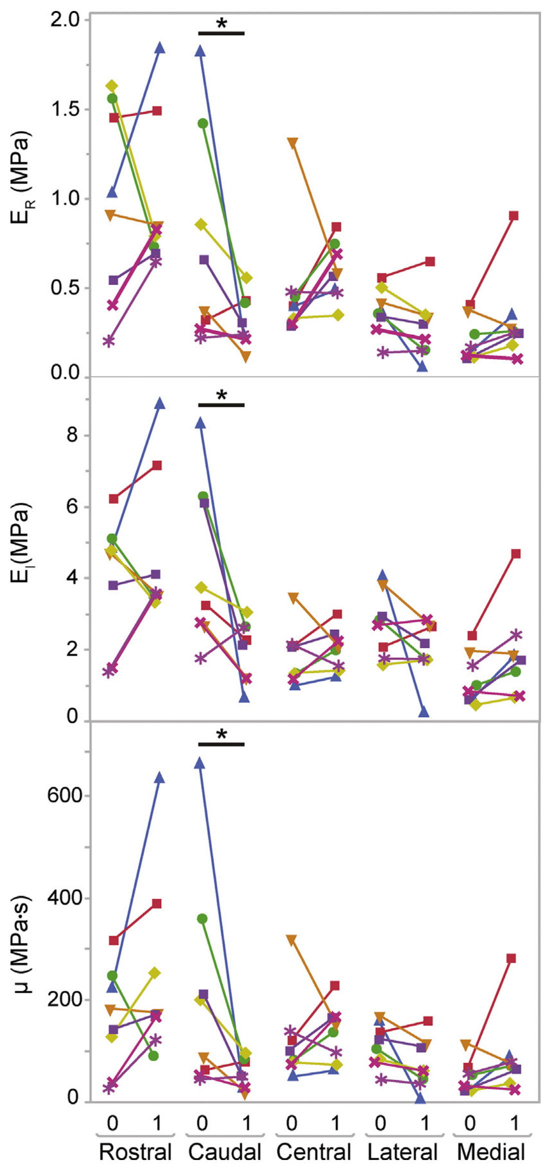

Fig. 6. Scatter plot of compressive relaxation modulus (E_R), instantaneous modulus (E_I), and coefficient of viscosity (μ) at 20% strain for TMJ discs from horses with asymmetrical TMJ osteoarthritis. Within each disc region, points labeled "0" represent samples from normal or mildly osteoarthritic joints and points labeled "1" represent samples from joints with worse osteoarthritis. Matched pairs are connected by solid lines and represent samples from opposite joints within the same horse. Compressive properties were significantly decreased in the caudal region of discs from joints with greater osteoarthritis ($p \leq 0.006$).

bundles oriented in the rostrocaudal direction and negligible GAG staining. The rostral and caudal portions of the disc appeared more complex, with greater GAG and variable collagen fiber orientation. Discs from joints with severe OA exhibited greater GAG content in all regions compared to discs from normal joints. The intensity of collagen staining or degree of anisotropy did not differ between discs from normal joints and joints with OA.

4. Discussion

The significance of TMJ disease in the horse is not well understood. Computed tomography studies of the equine TMJ have shown that changes consistent with OA are common, but the pathologic significance of these findings is not known (Carmalt et al., 2016). In order to better understand the normal physiology of the TMJ as well as the pathophysiology associated with OA of the TMJ, the present study examined the biochemical, mechanical and histologic features of the equine TMJ disc. Broadly, we found that the discs are highly anisotropic, being stiffest and strongest in tension in the rostrocaudal direction. We also found regional variations in disc composition and compressive stiffness and a region-specific decrease in compressive stiffness associated with TMJ OA.

Because the force generated during the equine masticatory cycle is greatest in the mediolateral direction (Cordes et al., 2012), we hypothesized that the disc would show greater tensile properties in the mediolateral direction than species with primarily hinge-like motion or rostrocaudal translation of their joints. Interestingly, we found that the discs were significantly stiffer in the rostrocaudal direction in all regions tested. This anisotropy was preserved among disease groups. These mechanical results are in agreement with our histologic findings and a previously published split line study of the equine TMJ disc (Adams et al., 2016), which demonstrate that its collagen fibers are primarily oriented in the rostrocaudal direction. Multiple studies have found rostrocaudal collagen alignment and corresponding tensile anisotropy across species, regardless of variations in mastication among carnivores, herbivores, or omnivores (Almarza et al., 2006; Arzi et al., 2011; Detamore and Athanasiou, 2003; Kalpakci et al., 2011; Murphy et al., 2013a). The center of the human disc is also anisotropic, albeit to a lesser degree than observed in the equine disc, with ~50% greater tensile stiffness along the anteroposterior axis compared to the mediolateral axis (Wright et al., 2016). Our findings and those of previous studies suggest that the tensile properties of the disc are not necessarily associated with masticatory demands and that there may be other mechanisms by which the stresses are transmitted or dissipated.

Studies in other species have demonstrated that the rostral and caudal bands of the TMJ disc have collagen fibers aligned in the lateromedial direction and possess greater tensile properties in the lateromedial direction (Detamore and Athanasiou, 2003; Kalpakci et al., 2011). A limitation of this study is that tensile testing was not performed in the rostral and caudal regions of the disc; however, histologic evaluation indicates that collagen fiber arrangement is more complex in the rostral and caudal regions and it is likely that these regions exhibit different tensile anisotropy than the central, medial, and lateral regions of the disc. It is also possible that the peripheral, but not the central, regions of the disc experience the greatest tensile forces, as is suggested in a previous split-line study (Adams et al., 2016).

We expected that discs from osteoarthritic joints would exhibit greater compressive stiffness and GAG content. A previous study of experimental TMJ injury found that GAG content increased in response to injury (Embree et al., 2015). We observed increased GAG staining in histologic sections of discs from joints with severe OA, but these joints were found in older horses. Importantly, increasing horse age was significantly associated with greater GAG content and compressive stiffness of the TMJ disc; neither GAG content nor stiffness increased associated with joint disease when comparing discs within horses with asymmetrical disease. In contrast, the caudal region of the disc from joints with greater TMJ OA was found to be less stiff than in the contralateral, healthier joint. It is possible that increasing GAG content and overall compressive stiffness of the TMJ discs of older horses represent adaptive, rather than pathologic changes. Interestingly, the majority of gross pathology in the mandibular condyles was present at their caudal aspects and we observed decreased compressive stiffness in the caudal part of discs from osteoarthritic joints. Disproportionate stress on the caudal part of the joint or failure of adaptive changes may make the caudal part of the TMJ more susceptible to injury or degeneration in horses.

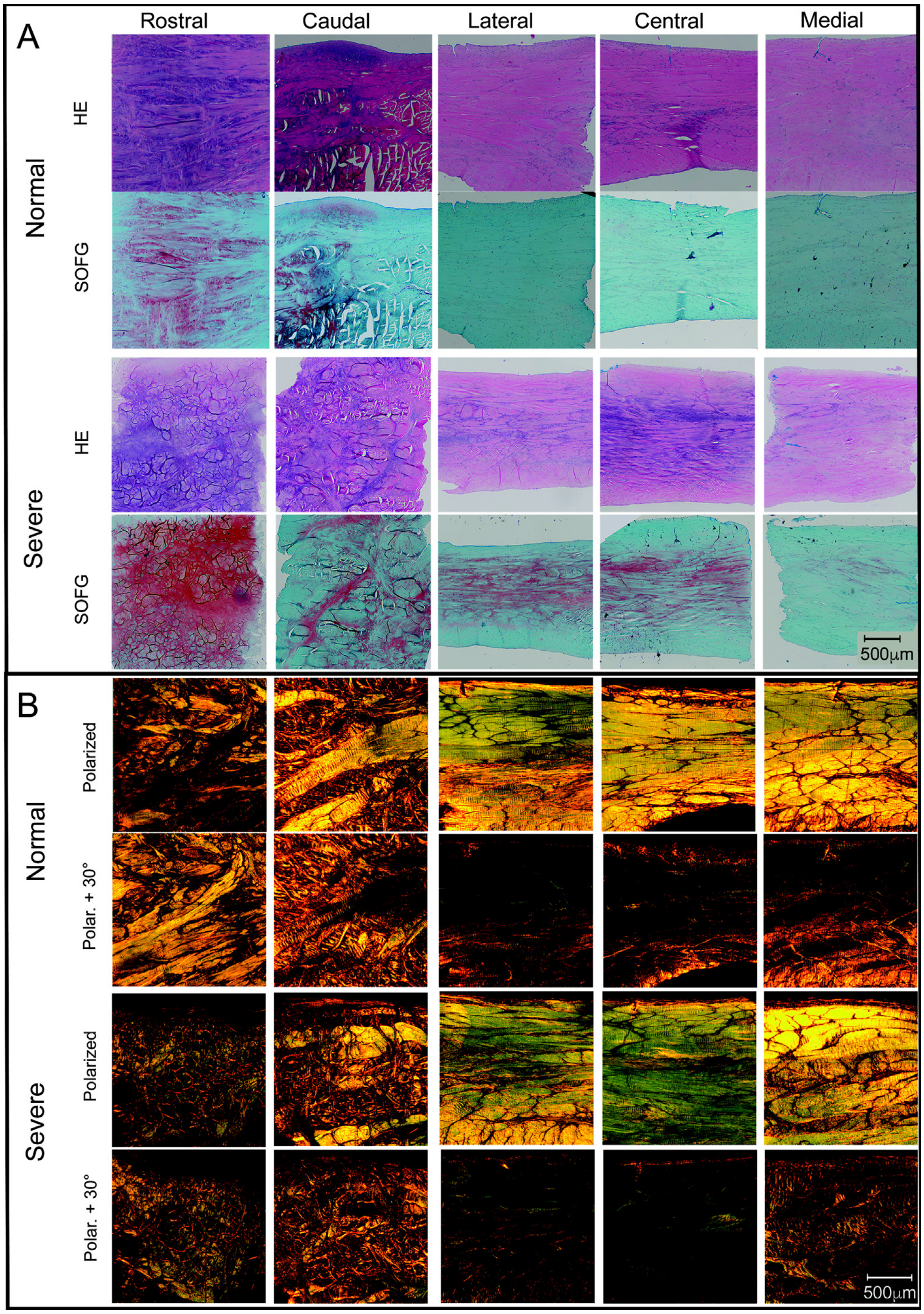

Fig. 7. Representative histologic images of equine TMJ discs from normal joints and joints with severe OA, by region. (A) Bright field microscopy of sections stained with hematoxylin & eosin (HE) or safranin-O/fast green (SOFG). (B) Polarized light microscopy of sections stained with picrosirius red oriented at maximum light transmission (0°) and after rotating the stage 30°.

Unexpectedly, tensile properties were preserved among disease groups. Though this finding was surprising, it was supported by our findings that collagen content was not significantly different between disease groups. Histologically, collagen fiber alignment was largely preserved despite an increase in GAG content in discs from diseased joints. The hypothesis that there would be a loss of tensile properties was based on the findings of the TMJ disc characterization in two tigers (Murphy et al., 2013a). One of the specimens in that study had severe evidence of OA and a loss in tensile properties. Comparison of visibly deformed and grossly normal porcine TMJ discs also found loss of tensile properties in deformed discs (Matuska et al., 2016). In contrast, another study found increasing tensile stiffness in bovine TMJ discs associated with age, but the presence or absence of TMJ OA was not reported in that study (Tanaka et al., 2001). In our study, 21 of 32 equine TMJ's had gross evidence of OA. The greater prevalence of disease than previously reported (Carmalt et al., 2016) is likely due to bias inherent to obtaining post-mortem samples and the potentially greater sensitivity of directly visualizing the articular surfaces compared to CT. To assess the effect of joint disease on disc properties, we attempted to control for individual variation and age by comparing disc properties between joints from horses with asymmetrical OA. Only eight of sixteen horses had asymmetrical OA, and it is possible that there was not sufficient statistical power to elucidate small changes in tensile stiffness associated with joint disease. Additionally, none of the horses in this study had a history of clinical signs associated with TMJ disease, and specimens in our study may not have had disease severe enough to cause a loss in tensile properties.

The biochemical composition of equine TMJ discs is similar to other species, and particularly other herbivores. The mean TMJ disc collagen content was measured to be 94% of the tissue dry weight in this study, similar to collagen content of bovine discs when tissue hydration is taken into account (Kalpakci et al., 2011). Elastin has been reported to comprise as much as 7% of the dry weight of juvenile bovine TMJ discs (Keith, 1979), but we did not assay for elastin in this study. Elastin contains the amino acid hydroxyproline and may have slightly inflated our measurements of collagen content. GAG content in the lateral, central, and medial regions of TMJ discs was similar between horses and cows, goats, and rabbits (Kalpakci et al., 2011). Our results found that increasing GAG content directly related to increasing compressive stiffness in the equine TMJ disc, and other relationships among TMJ disc biochemical and mechanical properties are discussed further in the Appendix. The similar GAG content among herbivores may reflect their diet and the compressive forces generated during mastication to properly break down their feed. In contrast, omnivorous pigs and carnivorous tigers are reported to have much less GAG throughout their TMJ discs (Kalpakci et al., 2011; Murphy et al., 2013a). Interestingly, horses in this study had greater GAG in the rostral region of the disc than other species, a finding that was consistent regardless of horse age. The greater GAG content in the rostral region of the equine TMJ disc may reflect unique mechanical demands, even among other species of herbivores.

In conclusion, the equine TMJ disc is highly anisotropic and its GAG content and compressive stiffness vary between disc regions. The disc also exhibits increasing GAG content and compressive stiffness with increasing age, and a region-specific decrease in compressive stiffness associated with joint disease. While equine TMJ disc properties are generally similar to other herbivores, greater compressive relaxation modulus throughout the disc and greater GAG content in its rostral and caudal regions suggest mechanical demands on the TMJ disc differ between horses and other species. The clinical significance of changes in the TMJ disc is not known in horses, but decreases in compressive stiffness in the disc's caudal region may be associated with erosion and ulceration of the caudal aspect of the underlying mandibular head.

Data statement

Data in support of the manuscript titled, "Regional and disease-related differences in properties of the equine temporomandibular joint disc," are available via the following URL: https://doi.org/10.25338/B86W22.

Conflict of interest statement

The authors have no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiomech.2018.10.017.

References

Adams, K., Schulz-Kornas, E., Arzi, B., Failing, K., Vogelsberg, J., Staszyk, C., 2016. Functional anatomy of the equine temporomandibular joint: collagen fiber texture of the articular surfaces. Vet. J. 217, 58–64.

Allen, K.D., Athanasiou, K.A., 2006. Viscoelastic characterization of the porcine temporomandibular joint disc under unconfined compression. J. Biomech. 39, 312–322.

Almarza, A.J., Bean, A.C., Baggett, L.S., Athanasiou, K.A., 2006. Biochemical analysis of the porcine temporomandibular joint disc. Br. J. Oral Maxillofac. Surg. 44, 124–128.

Almarza, A.J., Brown, B.N., Arzi, B., Angelo, D.F., Chung, W., Badylak, S.F., Detamore, M., 2018. Preclinical animal models for temporomandibular joint tissue engineering. Tissue Eng. Part B Rev. 24, 171–178.

Arzi, B., Cissell, D.D., Verstraete, F.J., Kass, P.H., DuRaine, G.D., Athanasiou, K.A., 2013. Computed tomographic findings in dogs and cats with temporomandibular joint disorders: 58 cases (2006–2011). J. Am. Vet. Med. Assoc. 242, 69–75.

Arzi, B., Willard, V.P., Huey, D.J., Verstraete, F.J.M., Vapniarsky-Arzi, N., Athanasiou, K.A., 2011. The temporomandibular joint disc of Asian elephant (Elephas maximus) and African elephant (Loxodonta africana). Eur. J. Wildlife Res. 58, 451–459.

Baker, G.J., 2002. Equine temporomandibular joints (TMJ): morphology, function and clinical disease. AAEP Proc. 48, 442–447.

Bonin, S.J., Clayton, H.M., Lanovaz, J.L., Johnson, T.J., 2006. Kinematics of the equine temporomandibular joint. Am. J. Vet. Res. 67, 423–428.

Carmalt, J., 2014. Equine temporomandibular joint (TMJ) disease: fact or fiction? Equine Vet. Educ. 26, 64–65.

Carmalt, J.L., Kneissl, S., Rawlinson, J.E., Zwick, T., Zekas, L., Ohlerth, S., Bienert-Zeit, A., 2016. Computed tomographic appearance of the temporomandibular joint in 1018 asymptomatic horses: a multi-institution study. Vet. Radiol. Ultrasound 57, 237–245.

Carmalt, J.L., Simhofer, H., Bienert-Zeit, A., Rawlinson, J.E., Waldner, C.L., 2017. The association between oral examination findings and computed tomographic appearance of the equine temporomandibular joint. Equine Vet. J. 49, 780–783.

Cissell, D.D., Link, J.M., Hu, J.C., Athanasiou, K.A., 2017. A modified hydroxyproline assay based on hydrochloric acid in Ehrlich's solution accurately measures tissue collagen content. Tissue Eng. Part C Methods 23, 243–250.

Cordes, V., Lüpke, M., Gardemin, M., Seifert, H., Staszyk, C., 2012. Periodontal biomechanics: finite element simulations of closing stroke and power stroke in equine cheek teeth. BMC Vet. Res. 8, 60.

Detamore, M.S., Athanasiou, K.A., 2003. Tensile properties of the porcine temporomandibular joint disc. J. Biomech. Eng. 125, 558.

Embree, M.C., Iwaoka, G.M., Kong, D., Martin, B.N., Patel, R.K., Lee, A.H., Nathan, J.M., Eisig, S.B., Safarov, A., Koslovsky, D.A., Koch, A., Romanov, A., Mao, J.J., 2015. Soft tissue ossification and condylar cartilage degeneration following TMJ disc perforation in a rabbit pilot study. Osteoarthritis Cartilage 23, 629–639.

Haskin, C.L., Milam, S.B., Cameron, I.L., 1995. Pathogenesis of degenerative joint disease in the human temporomandibular joint. Crit. Rev. Oral Biol. Med. 6, 248–277.

Herring, S.W., 2003. TMJ anatomy and animal models. J. Musculoskeletal Neuronal Interactions 3, 391.

Kalpakci, K.N., Willard, V.P., Wong, M.E., Athanasiou, K.A., 2011. An interspecies comparison of the temporomandibular joint disc. J. Dent. Res. 90, 193–198.

Keith, D., 1979. Elastin in the bovine mandibular joint. Arch. Oral Biol. 24, 211–215.

Kol, A., Arzi, B., Athanasiou, K.A., Farmer, D.L., Nolta, J.A., Rebhun, R.B., Chen, X., Griffiths, L.G., Verstraete, F.J., Murphy, C.J., Borjesson, D.L., 2015. Companion animals: translational scientist's new best friends. Sci. Transl. Med. 7, 308ps321.

Liu, F., Steinkeler, A., 2013. Epidemiology, diagnosis, and treatment of temporomandibular disorders. Dent. Clin. North Am. 57, 465–479.

Marguelles-Bonnet, R.E., Carpentier, P., Yung, J.P., Defrennes, D., Pharaboz, C., 1995. Clinical diagnosis compared with findings of magnetic resonance imaging in 242 patients with internal derangement of the TMJ. J. Orofac Pain 9, 244–253.

Matuska, A.M., Muller, S., Dolwick, M.F., McFetridge, P.S., 2016. Biomechanical and biochemical outcomes of porcine temporomandibular joint disc deformation. Arch. Oral Biol. 64, 72–79.

Moncada, G., Cortes, D., Millas, R., Marholz, C., 2014. Relationship between disk position and degenerative bone changes in temporomandibular joints of young subjects with TMD. An MRI study. J. Clin. Pediatr. Dent 38, 269–276.

Murphy, M.K., Arzi, B., Vapniarsky-Arzi, N., Athanasiou, K.A., 2013a. Characterization of degenerative changes in the temporomandibular joint of the bengal tiger (Panthera tigris tigris) and siberian tiger (Panthera tigris altaica). J. Comp. Pathol. 149, 495–502.

Murphy, M.K., MacBarb, R.F., Wong, M.E., Athanasiou, K.A., 2013b. Temporomandibular disorders: a review of etiology, clinical management, and tissue engineering strategies. Int. J. Oral Maxillofac Implants 28, e393–414.

Ramzan, P., 2006. The temporomandibular joint: component of clinical complexity. Equine Vet. J. 38, 102–104.

Smyth, T.T., Carmalt, J.L., Treen, T.T., Lanovaz, J.L., 2016. The effect of acute unilateral inflammation of the equine temporomandibular joint on the kinematics of mastication. Equine Vet. J. 48, 523–527.

Tanaka, E., Tanaka, M., Hattori, Y., Aoyama, J., Watanabe, M., Sasaki, A., Sugiyama, M., Tanne, K., 2001. Biomechanical behaviour of bovine temporomandibular articular discs with age. Arch. Oral Biol. 46, 997–1003.

Warren, M.P., Fried, J.L., 2001. Temporomandibular disorders and hormones in women. Cells Tissues Organs 169, 187–192.

Wright, G.J., Coombs, M.C., Hepfer, R.G., Damon, B.J., Bacro, T.H., Lecholop, M.K., Slate, E.H., Yao, H., 2016. Tensile biomechanical properties of human temporomandibular joint disc: effects of direction, region and sex. J. Biomech. 49, 3762–3769.