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SPONTANEOUSLY ARISING DISEASE

The Temporomandibular Joint of the Domestic Dog (*Canis lupus familiaris*) in Health and Disease

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Summary

This study aimed to characterize the histological, biomechanical and biochemical properties of the temporomandibular joint (TMJ) of the domestic dog in health and disease. In addition, we sought to identify structure–function relationships and to characterize TMJ degenerative lesions that may be found naturally in this species. TMJs ($n = 20$) from fresh cadaver heads ($n = 10$) of domestic dogs were examined macroscopically and microscopically and by cone-beam computed tomography. The TMJ discs were evaluated for their mechanical and biochemical properties. If TMJ arthritic changes were found, pathological characteristics were described and compared with healthy joints. Five (50%) dogs demonstrated macroscopically normal fibrocartilaginous articular surfaces and fibrous discs and five (50%) dogs exhibited degenerative changes that were observed either in the articular surfaces or the discs. In the articulating surfaces, these changes included erosions, conformational changes and osteophytes. In the discs, degenerative changes were represented by full-thickness perforations. Histologically, pathological specimens demonstrated fibrillations with or without erosions, subchondral bone defects and subchondral bone sclerosis. Significant anisotropy in the TMJ discs was evident on histology and tensile mechanical testing. Specifically, the discs were significantly stiffer and stronger in the rostrocaudal direction compared with the mediolateral direction. No significant differences were detected in compressive properties of different disc regions. Biochemical analyses showed high collagen content and low glycosaminoglycan (GAG) content. No significant differences in biochemical composition, apart from GAG, were detected among the disc regions. GAG concentration was significantly higher in the central region as compared with the caudal (posterior) region. The TMJ of the domestic dog exhibits similarities, but also differences, compared with other mammals with regards to structure–function relationships. The TMJ articular surfaces and the disc exhibit degenerative changes as seen in other species, including perforation of the disc as seen in man. The degenerative changes had greater effects on the mechanical properties compared with the biochemical properties of the TMJ components. Translational motion of the TMJ does occur in dogs, but is limited.

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Keywords: cartilage; dog; osteoarthritis; temporomandibular joint degeneration

Introduction

The temporomandibular joint (TMJ) is a bilateral synovial joint found in all mammalian species with an important role in mastication, communication and

the survival of animals (Murphy *et al.*, 2013b). The joint constituents are the mandibular fossa of the squamous temporal bone, the intra-articular disc and its attachments and the condylar process of the mandible (Herring, 2003; Willard *et al.*, 2012). The fibrocartilaginous disc reduces friction, dissipates loads and compensates for incongruity between the

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mandibular fossa and condylar process (Tanaka and van Eijden, 2003). The structure of the TMJ disc reflects its function (Kalpakci *et al.*, 2011). Previous studies of bovine and porcine discs have determined the biochemical composition to be between 83 and 96% collagen and 0.6–10% glycosaminoglycan (GAG) of the dry weight (Nakano and Scott, 1989; Kalpakci *et al.*, 2011; Vapniarsky *et al.*, 2017). The construction of GAGs within the interstices of mostly type I collagen fibres gives the disc its viscoelastic properties crucial for withstanding tension, compression and shear during normal physiological function (Tanaka and van Eijden, 2003). The disc is somewhat biconcave in shape, with thicker rostral (anterior) and caudal (posterior) bands encompassing a thinner central region. Additionally, disc composition is reported to be anisotropic in most species and with collagen fibres oriented primarily in the anteroposterior (man) and rostrocaudal (pigs, carnivores) direction centrally and mediolaterally in the rostral (anterior) and caudal (posterior) bands. In ruminants, the orientation of the fibres in the central location is mediolateral (Vapniarsky *et al.*, 2017); however, the distribution of GAGs among the species is unclear (Detamore and Athanasiou, 2003).

TMJ disorders (TMDs) are common in man, affecting 25–60% of the human population worldwide (Solberg *et al.*, 1979; Gatchel *et al.*, 2006). TMDs are defined clinically as a group of conditions that cause pain and dysfunction in the jaw joint and muscles that control jaw movement. The pathogenesis of TMDs, however, is vague and currently considered multifactorial involving structural, behavioural and hormonal components (Aryaei *et al.*, 2016). Given its ill-defined pathological mechanism and variable symptoms, instances of TMDs are likely underreported. In man, the most common pathological change in TMD is degenerative joint disease, also clinically referred to as osteoarthritis, often caused by disruption of the articular disc or associated TMJ components. Advanced degenerative TMD can result in morphological and functional defects, including impaired and painful mastication (Zarb and Carlsson, 1999; Tanaka *et al.*, 2008).

Domestic dogs (*Canis lupus familiaris*) also experience TMD. However, extensive studies characterizing TMD in this species are lacking. A retrospective computed tomographic (CT) study determined that TMD occurs frequently in combination with other diseases (i.e. osteoarthritis, fractures, dysplasia, tumours), with TMJ osteoarthritis (TMJ-OA) being the most common disorder in the TMJ of dogs (Arzi *et al.*, 2013). There is general consensus that the canine TMJ moves predominantly in a

hinge-like motion with limited lateral movement (Lantz, 2012). However, translational movement (i.e. movement of the condylar process in a forward and downward sliding motion when the mouth is open) of the canine TMJ was suspected previously, but has not been revisited (Vollmerhaus and Roos, 1996). Identifying any translational capabilities of the canine TMJ may help further elucidate the structure–function relationship and its possible role in TMJ disease processes.

Therefore, the aim of this study was to characterize the structure–function relationship of the TMJ of the domestic dog in health and disease, based on macroscopic, microscopical, biochemical, biomechanical and cone-beam computer tomography (CBCT) findings.

Materials and Methods

Specimens

The TMJs of 10 skeletally mature mesaticephalic (i.e. skull configuration with the cranium and nasal cavity about equal lengths) domestic dogs were obtained from client-owned cadavers donated through the Veterinary Medical Teaching Hospital, University of California, Davis. The dogs were from the following breeds: Labrador retriever ($n = 3$), Australian cattle dog ($n = 1$), mixed breed ($n = 5$) and Airedale terrier ($n = 1$). Subjects were humanely destroyed for reasons unrelated to the study and consisted of four females and six males ranging from 3 to 16 years of age (mean 10.4 years) and weighing 12.5–40 kg (mean 27.96 kg). All specimens were obtained fresh (post-mortem interval <24 h) and kept frozen at -20°C until dissection and analysis. None of the 10 subjects had reported clinical signs of historical known pathology involving the TMJ prior to death.

Gross Evaluation

Prior to arthrotomy, the anatomical and structural features of the TMJ were evaluated using CBCT (NewTom 5G, Verona, Italy) in open- and closed-mouth positions. Open-mouth images were obtained with the jaws opened at 45° to evaluate the articulation mechanism. Reference points to measure possible translation were the shortest distance between the retroarticular process of the temporal bone and the junction of the head and neck of the condylar process of the mandible in closed- and open-mouth positions. CBCT images were evaluated by a board-certified veterinary radiologist (DDC) and a human oral radiologist (DH) using Invivo5 software (Anatomage, San Jose, California, USA). De-identified images of human CBCT scans with the mouth closed and

open were courtesy of Dr. D. C. Hatcher and served as an example for comparison of the translation motion of the TMJ.

Following CBCT, the TMJs were dissected open and assessed for integrity of the articular surfaces and TMJ disc and for the presence of degenerative changes. The discs were excised *en bloc* from their attachments, with the mandibular fossa and the condylar process removed subsequently. The discs were washed with phosphate buffered saline (PBS; Sigma, St. Louis, Missouri, USA), wrapped in gauze, soaked with PBS containing protease inhibitors (10 mM *N*-ethylmaleimide and 1 mM phenylmethylsulphonyl fluoride; Sigma) and stored at -20°C until testing (Murphy *et al.*, 2013a; Arzi *et al.*, 2015). The excised mandibular fossa and mandibular heads of the condylar process were fixed in 10% neutral buffered formalin and subsequently decalcified in 10% formic acid before further processing for routine histology.

Microscopical Evaluation

For routine histology, formalin-fixed and paraffin wax-embedded TMJ discs and decalcified articulating TMJ components were sectioned (5 μm) and stained with haematoxylin and eosin (HE) according to standard procedures. Additional sections were stained with safranin O/fast green and picro-Sirius red for assessment of GAGs and collagen components, respectively. All histological specimens were assessed by a veterinary pathologist (NV) for evidence of disease.

For scanning electron microscopy (SEM) fresh samples from varying anatomical regions of the disc were fixed in 2.5% glutaraldehyde and cacodylate buffer (Sigma) for 96 h at 4°C . Following dehydration, using increasing ethanol concentrations, the specimens were exposed to liquid nitrogen and cryo-fractured. The cryofractured specimens were then critical point dried, sputter coated with gold and mounted. The sections were viewed with a Philips XL30 TMP SEM (Phillips, North Billerica, Massachusetts, USA) and images were captured at $15,000\times$ magnification. Image J software (National Institutes of Health, Bethesda, Maryland, USA) was used to assess fibre thickness and alignment in different anatomical regions.

Biomechanical Characterization

Tensile and compressive testing was performed using an Instron 5965 (Instron, Norwood, Massachusetts, USA). For tensile properties analyses, dog-bone-shaped samples were obtained from three distinct

anatomical locations on the disc (i.e. rostral band [anterior], central region [central] and caudal band [posterior]). The samples from the rostral (anterior) and caudal (posterior) bands were tested under uniaxial tension in the mediolateral (ML) direction only, while samples from the central region were subjected to uniaxial tensile testing in two perpendicular directions rostrocaudal (anteroposterior, AP) and ML. Strain to failure was performed at a strain rate of 1% of the gauge length per second. Stress–strain curves were generated based on the load–displacement curve and analyzed with a custom MATLAB program; the Young's modulus (E_Y , i.e. tensile stiffness) and ultimate tensile strength (UTS) were calculated from the linear portion of the curve (Murphy *et al.*, 2013a).

For unconfined stress-relaxation compressive testing, 3 mm diameter circular samples representing rostral (anterior), central and caudal (posterior) regions of the disc were obtained. The samples were kept in isotonic saline at room temperature throughout testing. Step strains of 10% and 20% were applied at a rate of 10% per second, with platen position (i.e. metal portion of the Instron machine via which the sample is compressed) maintained for 5 min for each relaxation to reach equilibrium. Instantaneous modulus (E_i), relaxation modulus (E_r) and coefficient of viscosity (μ) at each strain level were calculated using a custom MATLAB code programmed to fit the data to the standard linear solid viscoelastic model (Vapniarsky *et al.*, 2017).

Biochemical Characterization

Tissue samples for biochemical evaluation were obtained from rostral, caudal and central regions of the TMJ disc. The weights of the specimens were obtained before and after lyophilization. Specimens were then digested in 27.8 mg/ml papain (Sigma) in phosphate buffer (pH 6.5) containing 5 mM *N*-acetyl cysteine (Sigma) and 5 mM ethylenediaminetetraacetic acid for 18 h at 60°C . GAG content was measured with Blyscan GAG assay (Bicolor, Westbury, New York, USA) based on 1, 9-dimethylmethyl blue binding. Total collagen content was measured through hydrolyzing samples with 4N NaOH for 20 min at 110°C , using a chloramine-T hydroxyproline assay with Sircol™ collagen standards (Bicolor). Total DNA content was assessed using a PicoGreen assay (Invitrogen).

Immunohistochemistry

For immunohistochemistry (IHC), formalin-fixed and paraffin wax-embedded specimen sections were

labelled with anti-human collagen type I (ab35710, 1 in 500 dilution; Abcam, Cambridge, Massachusetts, USA), anti-bovine collagen type II (ab34712, 1 in 500; Abcam) primary antibodies. Antigen retrieval was performed with 0.4% papain for 20 min at 37°C. Vectastain rabbit secondary antibodies (1 in 2,000 dilution) and avidin–biotin labelling were subsequently used (Vecta stain ABC NC213702; Fisher Scientific, Burlingame, California, USA) following the manufacturer's instructions. The colour was developed with 3, 3' diaminobenzidine (DAB) peroxidase horseradish peroxidase (HRP) substrate kit (SK-4100; Vector Laboratories, Burlingame, California, USA). Canine tendon and articular cartilage sections were used as positive and negative controls for immunolabelling of collagen type I and type II, respectively.

Statistical Analysis

The effects of individual dog, joint (right versus left) and disc region on biochemical and mechanical properties were assessed by ANOVA for a split–split plot design using statistical analysis software (JMP Pro 13, SAS Institute, Cary, North Carolina, USA). Individual dog was treated as a random effect, with disc region nested within joint and both region and joint nested within dog to account for repeated measures. For analysis of tensile test results, four levels of disc region were considered: rostral (anterior)-ML (AML), caudal (posterior)-ML (PML), central-ML (CML) and central-AP (CAP). After initial ANOVA, residual terms were assessed for normality and constancy of variance. If indicated, response variables were log transformed and ANOVA repeated. Significant differences between disc regions were assessed by Tukey's *post hoc* test when indicated. Potential differences between healthy and diseased discs were assessed by least square means contrast for all regions/areas. Statistical significance was defined by $P < 0.05$.

Results

CBCT Evaluation of the Hinge-like Articulation

The TMJs of all dogs exhibited hinge-like articulation (Fig. 1). In addition, a translation motion (i.e. rostral and ventral movement) was observed between the open- and closed-mouth positions in five dogs (50%), which consisted of one healthy and four diseased TMJs. In these five dogs, translation of the mandibular condylar process varied from medial to lateral, with more translation of its lateral aspect ($1.95 \text{ mm} \pm 0.95$) as compared with the medial aspect ($0.67 \text{ mm} \pm 0.93$).

Gross Evaluation and Histology

Normal Healthy Joints. In five dogs (three females and two males) with an age range of 3–16 years (mean 10.8 years), the TMJs were found to be without macroscopically visible abnormalities (Figs. 2 and 3). The normal mandibular heads were concave and elliptical with smooth, tan and glistening articulating surfaces. Histologically, the articular surfaces were lined by a compact fibrous connective tissue that almost immediately transitioned into subchondral bone (Fig. 2). Multifocally, one cell layer-thick cartilaginous regions were observed, but these lacked a GAG rich matrix and, therefore, were difficult to discern from the fibrous layer above and the subchondral bone beneath (Fig. 2). Subchondral bone transitioned abruptly into trabecular bone with marrow cavities filled with adipose tissue and rare bone marrow cells. The normal TMJ discs had an approximately oval perimeter, were biconcave and semitransparent in the centre (Fig. 3). The central region of the discs was thinner than the periphery. The thickness at the rostral region was 1.23–2.14 mm (mean 1.5 mm), at the central region 0.21–1.14 mm (mean 0.57 mm) and at the caudal region 1.13–2.2 mm (mean 1.8 mm). Histologically, the discs were paucicellular and composed of tightly packed eosinophilic collagen fibres interspersed by occasional regions of basophilic matrix (Fig. 3). These basophilic regions were more prominent on horizontal sections of the discs. The central regions of the discs were avascular and only small calibre vessels were observed at the periphery. Rare small islands of adipose tissue were located along the periphery of the disc, separating amidst the collagen bundles. Plain histology and SEM of the disc demonstrated antero-posterior orientation of fibres in the central regions and predominantly lateromedial orientation in the rostral and caudal bands (Fig. 3). The thickness of the individual collagen fibres was $0.108 \pm 0.013 \mu\text{m}$ in the central regions and $0.115 \pm 0.009 \mu\text{m}$ and $0.142 \pm 0.029 \mu\text{m}$ in the rostral and caudal bands, respectively. The fibre diameter in the caudal regions was significantly thicker than in the rostral or central regions ($P = 0.0068$ and $P = 0.0001$, respectively). Meanwhile, no significant difference was detected in fibre diameters between the rostral and central areas.

Diseased Joints. In five dogs (three females and two males) with age range of 7–15 years (mean 10 years), gross pathology was observed at the TMJ either at the mandibular head ($n = 2$), mandibular fossa or the disc ($n = 1$), or both ($n = 2$). The affected articular surfaces were irregular with areas of eroded fibrocartilage exposing the subchondral bone (Fig. 2).

Fig. 1. Cone-beam computer tomography images of canine (A and C) and human (B and D) TMJ discs in open (C and D) – and closed (A and B) – mouth positions. Note the translation (sliding) of the mandibular head rostrally (anteriorly) and under the articular eminence in a human subject on mouth opening (D). Minimal translation is observed in the dog (C). The human CBCT images are courtesy of D. C. Hatcher.

Surface deformities and osteophytes were also observed. Histologically, the affected fibrocartilage exhibited fibrillation and often complete erosion with subchondral bone exposure (Fig. 2). Subchondral bone sclerosis was often, but not always, associated with the eroded areas. These changes were consistent with degenerative joint disease. One dog had bilateral perforation of the disc. In both discs the perforation was central and measured on average 9×6.8 mm.

Histologically, the perforated discs had frazzled and occasionally clubbed collagen bundles that faced the perforation. The affected bundles contained completely acellular regions and surfaces facing the synovial space were occasionally lined by a thin layer of cells resembling synoviocytes (Fig. 4). In other diseased discs, segmental fibrillation and separation of the

collagen bundles was observed. These areas often corresponded to the areas of erosion or osteophyte formation of the articulating bony components. Mild synovial hyperplasia was present in the diseased joints.

Mechanical Characterization

Tensile properties of the TMJ discs were assessed in healthy and diseased joints and results are shown in Fig. 5. Significant differences were detected in the tensile properties between the regions within the discs. Specifically, within the dog population examined in this study (diseased and healthy joints combined) AML (52.1 ± 46.2), PML (36.5 ± 16.9) and CAP (40.9 ± 42.5) were significantly stronger and stiffer ($P < 0.0001$) than CML (4.9 ± 3.8).

Fig. 2. Articular surfaces of the mandibular head and the mandibular fossa in healthy and diseased TMJs from domestic dogs. **In health:** (a) and (b) Mandibular fossa gross and histological morphology. The articulating surfaces are smooth and glistening. Thin layer of subchondral bone transitions into trabecular bone with marrow spaces containing primarily adipose tissue. (c) and (d) Mandibular head gross and histological morphology. The mandibular head is fusiform in shape with a smooth and glistening articulating surface. (e) Histology of the sagittal section through the TMJ demonstrates biconcave shape of the TMJ disc. (f) Higher magnification of the mandibular fossa. Note the compact fibrous layer subtended by subchondral bone with minimal to non-cartilage layer. (g) Higher magnification of the mandibular head. Note the compact fibrous layer subtended by one to two cell layer cartilage zone. The cartilage staining is pink to amphophilic indicating very low GAG content. Dashed white line indicates the plane of section shown on corresponding microscopical morphology images. AS, articulating surface; M, medial; L, lateral. **In disease:** (a) and (b) Mandibular fossa gross and corresponding histological morphology with degenerative changes. Note deep excavation (*) extending deep into subchondral bone. The excavation is filled with loose fibrous connective tissue. The mandibular fossa is wider with an altered, irregular outline. (c) and (d) Mandibular head gross and histological morphology with degenerative changes. Note the widened and flattened articulating surface with triangular osteophyte projecting from the surface (#). This triangular expansion of the bone corresponds with the cavitation (*) shown in (b). (e) and (f) Articular fossa gross and corresponding histological morphology with degenerative changes. Note the grossly altered surface that corresponds to the areas of subchondral bone sclerosis (SBS). (g) and (h) Mandibular head gross and histological morphology with degenerative changes. Note the osteophyte (OP) extending into the articular space (AS). Also observe the separation and splitting of the collagen bundles in the fibrous layer. (i) and (j) Mandibular head gross and histological morphology with degenerative changes. Note the irregular and rough articulating surface grossly. Histologically, the bundles forming the fibrous layer are swollen, separated and showing fibrillation. The fibrous layer is overlaid by a cellular layer resembling synovial lining.

Discs from healthy joints were significantly stiffer in tension than discs from diseased joints ($P = 0.0132$), but there was no difference in UTS between the healthy and diseased groups in all regions of tensile testing.

The viscoelastic compressive properties of healthy and diseased discs at 10% and 20% strain are shown in Fig. 5. There were no significant differences in compressive properties between healthy and diseased

populations or between different anatomical regions of the disc ($P > 0.05$).

Biochemical Content

The biochemical content of the TMJ discs in health and disease is shown in Fig. 6.

Within the entire sample population (healthy and diseased dogs combined) GAG content in the central

Healthy TMJ disc

Fig. 3. Gross morphology and histology of a healthy canine TMJ disc. (a) and (b) Superior and inferior surface of the TMJ is shown; R, rostral (anterior); C, caudal (posterior); CE, central; M, medial; L, lateral. Note the unique shape of the disc and almost transparent centre in the medial aspect. (c) Cross section of the disc is shown. Note the concave shape. (d) Low magnification of the disc histology. The disc was sectioned in rostrocaudal (anteroposterior) direction. (e), (f) and (g) Higher magnification of the rostral (anterior), central and caudal (posterior) regions, respectively. Note almost exclusively rostrocaudal (anteroposterior) orientation of collagen fibres in the central region. Mixed pattern of fibre orientation is present in the anterior region. Bar, 100 μm. (h) Safranin O/fast green counterstain of the central region of the TMJ disc. Note the minimal GAG content evident by absence of orange staining. Bar, 100 μm (i) PicroSirius red staining of the TMJ disc demonstrates abundance of collagen in this tissue. Bar, 100 μm. (j) Collagen type 1 IHC demonstrates strong immunoreactivity. Bar, 100 μm. (k) Collagen type 2 IHC demonstrates minimal immunoreactivity. Bar, 100 μm. Canine tendon was used as positive and negative tissue control for collagen type 1 and collagen type 2, respectively. Canine articular cartilage was used as positive and negative tissue control for collagen type 2 and collagen type 1, respectively. (l), (m) and (n) SEM images obtained from rostral (anterior), central and caudal (posterior) regions of the disc, respectively. Note the thicker fibres of the caudal (posterior) region. Bar, 2 μm.

Diseased TMJ disc

Fig. 4. Gross and histological morphology of the diseased canine TMJ disc. (a) and (b) Right and left TMJ disc gross morphology. Note the large central perforations of the disc bilaterally. (c) Zoomed-in capture demonstrating rounded and slightly hyperaemic margin of the perforation. (d) Low-magnification histology of the disc sectioned in rostrocaudal (anteroposterior) direction. Note the pale eosinophilic staining of the stoma adjacent to the perforation, which may indicate oedema or cellular loss. (e) and (f) Higher magnification of the disc areas marked with rectangles in (d). Note the increased cellularity alternating with acellular areas. Also note the lining of the disrupted collagen bundles facing the perforation by synovial-like cells. (g) and (h) SEM images of the central area of the disc. Note the disarray in collagen fibre orientation. (i) and (j) Collagen type 1 and collagen type 2 IHC, respectively.

Fig. 5. Biomechanical characteristics of healthy and diseased canine TMJ discs at the rostral (anterior), central and caudal (posterior) regions. (A) and (B) demonstrate the compressive relaxation moduli of the disc tissue at 10% and 20% strain (displacement), respectively. (C) and (D) demonstrate the compressive instantaneous moduli of the disc tissue at 10% and 20% strain (displacement), respectively. (E) and (F) demonstrate the coefficient of viscosity of the TMJ disc tissue at 10% and 20% strain (displacement), respectively. (G) Young's moduli represent tensile stiffness of the TMJ disc by area and by direction of testing (AP, anteroposterior direction of testing; ML, mediolateral direction of testing). Significant differences (*) were detected in the tensile stiffness between the regions within the discs relative to the direction of testing. Specifically, the central region was significantly stiffer than the rostral and caudal regions when tested in the anteroposterior direction in both healthy and diseased groups. The central region had significantly greater stiffness in the anteroposterior direction than in the mediolateral direction. (H) Ultimate tensile strength of the TMJ disc by region and direction of testing. Significant differences (*) were detected in the tensile strength between the regions within the discs relative to the direction of testing. Specifically, the central region was significantly stronger than the rostral and caudal regions when tested in the anteroposterior direction in healthy groups, but not in diseased groups. The central region had significantly greater strength in the anteroposterior direction than in the mediolateral direction. Healthy disc values are shown in dotted columns, the diseased discs values are demonstrated in dark grey. Tabulated raw values are available in the [Supplemental materials](#).

Fig. 6. Biochemical characteristics of healthy and diseased canine TMJ discs at the rostral (anterior), central and caudal (posterior) regions. (A) and (B) GAG content normalized to dry and wet weight, respectively. The X axis indicates the anatomical region of the disc and the Y axis represents the % of GAG per dry and wet weight, respectively. (*) The GAG content was significantly higher in the central than in the caudal regions. (C) and (D) Collagen content normalized to dry and wet weight, respectively. The X axis indicates the anatomical regions of the disc and the Y axis represents the % of collagen per dry and wet weight, respectively. Healthy disc values are shown in the dotted columns, the diseased discs values are indicated in dark grey. Tabulated raw values are available in the [Supplemental materials](#).

region (1.79 ± 0.9) was significantly higher than in the caudal (posterior) region (1.0 ± 0.36) ($P < 0.0001$). However, GAG content in the rostral (anterior) region (1.14 ± 0.42) did not differ significantly from the central and caudal (posterior) regions. There were no significant differences in all of the examined biochemical properties between healthy and diseased TMJ discs (i.e. GAG, collagen and hydration).

Discussion

To our knowledge, this is the first comprehensive insight into the canine TMJ, which characterizes the histological, biomechanical and biochemical properties of the TMJ and its disc in health and disease. In addition, this study sought to identify structure–function relationships and characterize TMJ arthritic lesions found in this species. This study illuminated several cardinal aspects of the canine TMJ. Firstly, we found that dogs exhibit a variety

of degenerative changes in the TMJ, ranging from osteophytes and subchondral bone defects to disc perforations. Furthermore, the degenerative changes had significant effects on the mechanical tensile properties, but no effects of disease were observed on compressive or biochemical properties. Finally, although the TMJ of dogs functions as a hinge, a translational motion was identified in 50% of the dogs at 45° open-mouth position.

Degenerative joint disease (DJD) appears to be the most common TMD in dogs ([Arzi *et al.*, 2013](#)). The term osteoarthritis has been used interchangeably with the term DJD in both the human and the veterinary nomenclature. In that context, it is possible that inflammatory changes (i.e. osteoarthritis) were present at the initial event that lead to development of degenerative changes; with lack of inflammatory infiltrates in our cohort of dogs we support the use of DJD rather than arthritis ([Zarb and Carlsson, 1999](#); [Tanaka *et al.*, 2008](#); [Kristensen *et al.*, 2011](#)). In the present study, TMJ DJD was observed in 50% of

the middle-aged to older dogs. In addition, a previous study evaluating TMD in dogs using CT found that 32 of out of 41 (78%) dogs with TMD also have degenerative changes (Arzi *et al.*, 2013). In agreement with the present study, a human autopsy study found that articular remodelling was present in 57 of 102 (56%) of middle-aged and older individuals (Oberg *et al.*, 1971). The historical prevalence of TMJ degeneration across species likely reflects its characteristic thin layer of fibrocartilage, as opposed to the hyaline cartilage, which covers most synovial joints (Arzi *et al.*, 2012, 2013; Nanci, 2017). This may be attributed to the TMJ's role in bearing load, rather than weight, during mastication (Allen and Athanasiou, 2006; Arzi *et al.*, 2012; Nanci, 2017). In addition, remodelling is a normal physiological process necessary in load-bearing joints for adaptation to stress. While remodelling helps maintain proper joint function and stress distribution, excessive wear and tear on the joint overwhelms the natural remodelling process, manifesting as degenerative changes (Moffett *et al.*, 1964; de Bont *et al.*, 1986). This study has demonstrated that the TMJ of dogs undergoes degenerative changes as seen in man and with relatively similar occurrence.

The finding of TMJ disc perforation was surprising, as it has not been reported in dogs previously. Disc perforations are difficult to diagnose clinically because magnetic resonance imaging (MRI) or double contrast radiography is required to visualize this change. The very thin, biconcave shape of the canine disc further makes visualization of small defects challenging. In a human study, TMJ disc perforation appears to have a more prevalent, yet low, occurrence with 207 of 2,524 joints (8%) identified as having disc perforations using MRI and arthroscopy (Dijkgraaf *et al.*, 1999; Shen *et al.*, 2014). Furthermore, the clinical implications of disc perforation remain unclear, as disc perforations, as well as other TMJ OA lesions, may not manifest in pain or disability. For example, a CT study evaluating TMD in dogs has found that only four of 15 (27%) dogs and two of four (50%) cats with degenerative changes had clinical signs of pain (Arzi *et al.*, 2013). In agreement with this, human studies demonstrate a similar pattern, with 12 of 34 (35%) patients with TMJ arthritic changes exhibiting or reporting no clinical signs (Brooks *et al.*, 1992). With that said, there is marked variability in pain sensation among individuals and it often correlates poorly with the severity of degenerative changes (Arzi *et al.*, 2013).

The TMJ of dogs has been described as a highly congruent joint functioning in a hinge-like motion (Lantz, 2012; Evans and DeLahunta, 2012). Although minor laterotrusion of mandibular motion is present in

some dogs and can be palpated under general anaesthesia, the findings that some translational motion is present in 50% of the specimens were intriguing (Lantz, 2012). We found that more translational motion occurred in the lateral aspect of the TMJ as compared with the medial aspect. That is surprising, as the lateral aspect of the TMJ is reinforced not only with a fibrous capsule, but also with a lateral ligament (Evans and DeLahunta, 2012). Unlike the human TMJ, mild translation was only found in 50% of the dogs, which suggests translational movement in dogs is not a consistent feature and may be minimal. However, a recent study illuminated shape variation within the canine TMJ, with some domestic dogs diverging from typical hinge-like TMJ morphology towards a sledge-like shape as seen in omnivorous mammals (Curth *et al.*, 2017). This may also explain the minor translation seen in 50% of our specimens. Importantly, dogs in the present study had mesaticephalic skull morphology. It is possible that alteration of the skull (and hence, TMJ morphology) towards brachycephalic or dolichocephalic may have different level of congruency or translation.

Furthermore, this has implications for TMJ translational research as various therapeutics aimed to improve TMJ OA utilize dogs as a surgical experimental model (Brown *et al.*, 2012). Therefore, from functional aspects, the TMJ of dogs is unlikely to represent a good surgical experimental model for translational studies. Further clinical and kinematic studies are required to determine the extent and clinical implications of TMJ translation in dogs.

The biochemical analysis of sulphated GAGs demonstrated significant differences in GAG content by dry weight between caudal (posterior) and central regions of the disc. This may be attributed to a physiological distribution of GAGs, as seen in other species, or may be the result of new synthesis and redistribution in the diseased discs (Sindelar *et al.*, 2000; Vapniarsky *et al.*, 2017). Our findings contradict the often reported structure–function relationship of articular cartilage that relates GAG content to compressive stiffness (Nakano and Scott, 1989; Ficklin *et al.*, 2007). There were no significant differences in the compressive properties of the canine TMJ disc in central regions despite the presence of higher GAG content in that region. This suggests additional factors besides GAGs may influence disc compressive properties. For example, in the present study, the caudal region of the disc possessed the thickest fibril diameters without similar correlating compressive properties, which was also observed in other species. This may be the result of various intrinsic and extrinsic factors that influence mechanical properties, such as ageing, injury and pathology (Tanaka and

van Eijden, 2003; Vapniarsky *et al.*, 2017). Slight disruptions of collagen fibres may also lead to significant mechanical softening of the TMJ disc, altering its reinforcement and mechanical properties under compression (Fazaeli *et al.*, 2016). To further understand and characterize the structure–function relationship of the canine TMJ disc, such factors require further examination.

Bidirectional tensile testing of the TMJ discs demonstrated anisotropy (i.e. property of being directionally dependent), with greater values present in the central region in the rostrocaudal direction relative to the ML direction (Shengyi and Xu, 1991; Detamore and Athanasiou, 2003). The discs also demonstrated higher tensile strength and stiffness in the rostrocaudal direction than in the ML direction, which matches the typical vertical mandibular movement of these species for mastication (McDonald *et al.*, 2015). Healthy and diseased discs showed significantly different results during tensile testing. Healthy discs were significantly stiffer than diseased discs. Our findings agree with an earlier study that observed reduced tensile biomechanical integrity in deformed porcine TMJ discs as compared with morphologically normal discs (Matuska *et al.*, 2016). Decreases in tensile stiffness and strength render diseased discs less effective in withstanding physiological loading forces and contributes to the progression of joint dysfunction (Matuska *et al.*, 2016).

In conclusion, this study characterizes cardinal aspects of the TMJ of the domestic dog in health and disease. We found that degenerative changes in the TMJ of dogs influence the mechanical properties of the disc more so than the biochemical changes. Additionally, dogs exhibit a variety of degenerative changes similar to those reported in man, including TMJ disc perforations. Finally, translational motion of the TMJ does occur in dogs, but is minimal. This study provides the foundation for future studies on naturally occurring TMJ arthritic changes in dogs.

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Supplementary data

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