

MRI Evaluation of Spontaneous Intervertebral Disc Degeneration in the Alpaca Cervical Spine

Dean K. Stolworthy,¹ Anton E. Bowden,¹ Beverly L. Roeder,² Todd F. Robinson,³ Jacob G. Holland,⁴ S. Loyd Christensen,⁴ Amanda M. Beatty,¹ Laura C. Bridgewater,⁴ Dennis L. Eggett,⁵ John D. Wendel,⁶ Susanne M. Stieger-Vanegas,⁷ Meredith D. Taylor⁸

¹Department of Mechanical Engineering, Brigham Young University, Provo, Utah, ²Department of Biology, Brigham Young University, Provo, Utah, ³Department of Plant and Wildlife Sciences, Brigham Young University, Provo, Utah, ⁴Department of Microbiology and Molecular Biology, Brigham Young University, Provo, Utah, ⁵Department of Statistics, Brigham Young University, Provo, Utah, ⁶Diversified Radiology, Denver, Colorado, ⁷College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, ⁸Department of Electrical Engineering, Brigham Young University, Provo, Utah

Received 6 January 2015; accepted 16 June 2015

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.22968

ABSTRACT: Animal models have historically provided an appropriate benchmark for understanding human pathology, treatment, and healing, but few animals are known to naturally develop intervertebral disc degeneration. The study of degenerative disc disease and its treatment would greatly benefit from a more comprehensive, and comparable animal model. Alpacas have recently been presented as a potential large animal model of intervertebral disc degeneration due to similarities in spinal posture, disc size, biomechanical flexibility, and natural disc pathology. This research further investigated alpacas by determining the prevalence of intervertebral disc degeneration among an aging alpaca population. Twenty healthy female alpacas comprised two age subgroups (5 young: 2–6 years; and 15 older: 10+ years) and were rated according to the Pfirrmann-grade for degeneration of the cervical intervertebral discs. Incidence rates of degeneration showed strong correlations with age and spinal level: younger alpacas were nearly immune to developing disc degeneration, and in older animals, disc degeneration had an increased incidence rate and severity at lower cervical levels. Advanced disc degeneration was present in at least one of the cervical intervertebral discs of 47% of the older alpacas, and it was most common at the two lowest cervical intervertebral discs. The prevalence of intervertebral disc degeneration encourages further investigation and application of the lower cervical spine of alpacas and similar camelids as a large animal model of intervertebral disc degeneration. © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res

Keywords: animal model; intervertebral disc degeneration; spine; alpaca

The human spine operates under a mechanically harsh environment, which plays a significant role in the development of degenerative disc disease (DDD) and low back pain.^{1–4} A large percentage of the aging human population is afflicted with this seemingly irreversible condition:^{5,6} approximately 40% of people under 30 years of age and upwards of 90% of people 55 years of age or older are afflicted with moderate-to-severe levels of DDD in their lumbar spine.⁶ The increasing incidence of DDD with age⁷ and its correlation with lower back pain⁸ is an alarming trend in modern society as it is already the leading cause of disability for people younger than 45-years,⁵ with financial and emotional effects that severely strain the modern society.^{8,9}

Our understanding of the causes of disc degeneration is limited due to the difficult nature of obtaining and testing appropriate, live human material.¹⁰ Thus, much of our current understanding of spinal biomechanics has been obtained using cadaveric, ex-vivo testing methodologies.^{11–14} Recently, in-vitro models have emerged that use human or animal tissue that is sustained using advanced bioreactors and cell culture techniques.^{15–17} These techniques hold great promise, but are currently limited in scope (e.g., a single spinal disc without adjacent tissue or bone), mechanics (e.g.,

simple loading conditions), and availability (e.g., only a few specialized sites have demonstrated long-term survival of human discs).¹⁸

A likely step in accelerating research and forging breakthroughs in disc degeneration and lower back pain may rely on our ability to identify more accurate animal models.^{10,19} Animal models have historically provided an appropriate benchmark for understanding human spinal biology,²⁰ and mechanics,^{21,22} along with their relation to injury,^{23,24} pathology,^{4,10,25,26} and healing.^{27,28} However, few animals are known to naturally develop intervertebral disc degeneration, and it remains difficult to characterize the presence or severity of accompanying pain. Currently available models of disc degeneration have important limitations in regards to the type and mechanism of degeneration.^{4,10,25,28–33} For example, specific dog breeds (e.g., dachshunds, beagles, and bulldogs) develop DDD^{32,34} after losing their notochordal cells, whereas other dog breeds have not been found to experience DDD; the degeneration found in the above mentioned canines has been attributed to a chondrodystrophy condition that is not present in humans. Some non-human primates have also demonstrated spontaneous disc degeneration.^{31,35,36} Like all animals, the use of primates as research animals is limited by ethical, regulatory, and cost considerations.^{18,37} Animal models have been developed that rely on injury-based methods to trigger DDD,^{30,38} but there is substantial concern as to whether this accurately models disc degeneration in aging humans.³⁷

Grant sponsor: National Science Foundation; Grant number: CMMI-0952758.

Correspondence to: Anton E. Bowden, (T: + 801 422-4760; F: +801 422-05-16; E-mail: Abowden@byu.edu)

© 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc.

The lower cervical spine of camelids, specifically alpacas, and llamas, have recently been suggested as a potential large animal model of disc degeneration and regeneration due to similarities in spinal posture, intervertebral disc size, and biomechanical flexibility.³⁹ Additionally there have been two published papers (of which we are aware) reporting post-mortem identification of disc degeneration and herniation in camelids: a single llama that exhibited clinical signs of pain,⁴⁰ and an alpaca with spondylitis.⁴¹ From a biomechanics standpoint, the camelid cervical spine has a vertically oriented spinal posture and is unsupported near the end in an open kinetic chain, thus providing multiple mechanical parallels with the human lumbar spine. The camelid cervical intervertebral disc size is more similar to the human lumbar intervertebral disc than other currently used large animals, such as porcine, ovine, bovine, and baboons. Average flexibility (range of motion) of a camelid spinal motion segment showed similarities in all modes of loading. With over 1,800 registered alpaca farms located throughout the USA,⁴² and even more located throughout the world, this potential animal model is particularly exciting for the research community.

Alpacas are typically sexually mature between 14 months and 18 months for females and 30–36 months for males, both reaching skeletal maturity between 30 and 36 months of age. Alpacas have an average lifespan of 15–20 years. Alpacas are divided into two breeds based on minor appearance considerations: huacaya and suri. Worldwide, approximately ninety percent of alpacas are huacaya and the remainder are suri.

The alpaca cervical spine consists of seven vertebrae, which are relatively long compared to their

transverse geometry, although the atlas (C1) and C7-vertebra are noticeably shorter⁴³ (Fig. 1). Note the differences in the shape of the atlas and axis, compared to the mid- to caudal-cervical vertebrae (C3–C6): C3 is tall and thin, and C6 is shorter and wider. Also, note the significantly shorter vertebral height of the C7-vertebra compared to C2–C6. The extended lengths of the vertebral bodies of the cervical spine create a long moment-arm that further exacerbates the bending stresses, particularly in the lower segments.^{1,3} The camelid second to seventh cervical (C2–C7) vertebrae have two sets (one upper and one lower) of bilateral transverse processes that protrude ventrally. The mid-cervical vertebral bodies are hour-glass shaped with the mid-transverse section being thinner and expanding outward towards the endplates. Similar to the human lumbar IVDs, the camelid cervical IVDs get progressively larger with lower segments,⁴³ which are likely a response to elevated mechanical cell-signaling from the increased stresses⁴⁴ of supporting the head and upper cervical mass. The supporting musculature and large bifurcated nuchal ligament in the alpaca appear to play a significant role in limiting flexion of the neck as they provide stabilization of the neck in the vertical orientation.

The purpose of the present work was to quantify the prevalence and severity of intervertebral disc degeneration in the cervical spine of a small alpaca population with hopes of identifying a large animal model to better understand disc degeneration in the human lumbar spine. If alpacas develop spontaneous disc degeneration with a similar prevalence and graded severity as humans, the case is strengthened to further investigate the camelid family to better understand their limitations in regards to the types and mechanisms of DDD. This study will potentially

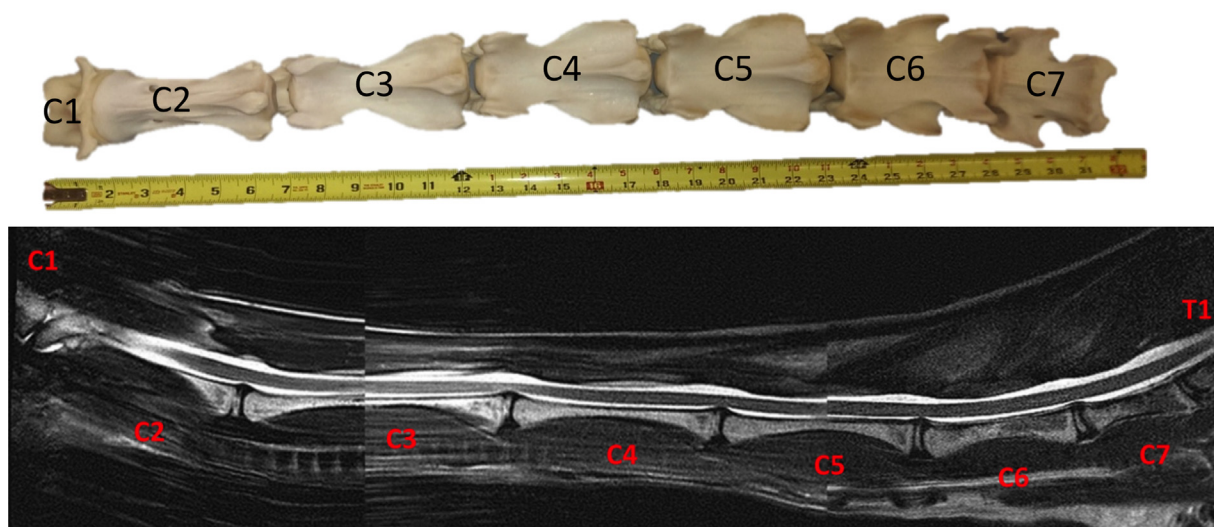


Figure 1. (Top) Alpaca cervical spine: cervical vertebra (C) #1 (C1, atlas), C2 (axis), C3–C7. (Bottom) Composite MRI of an alpaca cervical spine (12-yr-old, 55-kg), with the cervical vertebrae labeled. Note the differences in the shape of the atlas and axis, compared to the mid- to lower-cervical vertebrae (C3–C6): C3 is tall and thin, and C6 is shorter and wider. Also, note the significantly shorter vertebral height of the C7-vertebra compared to C2–C6.

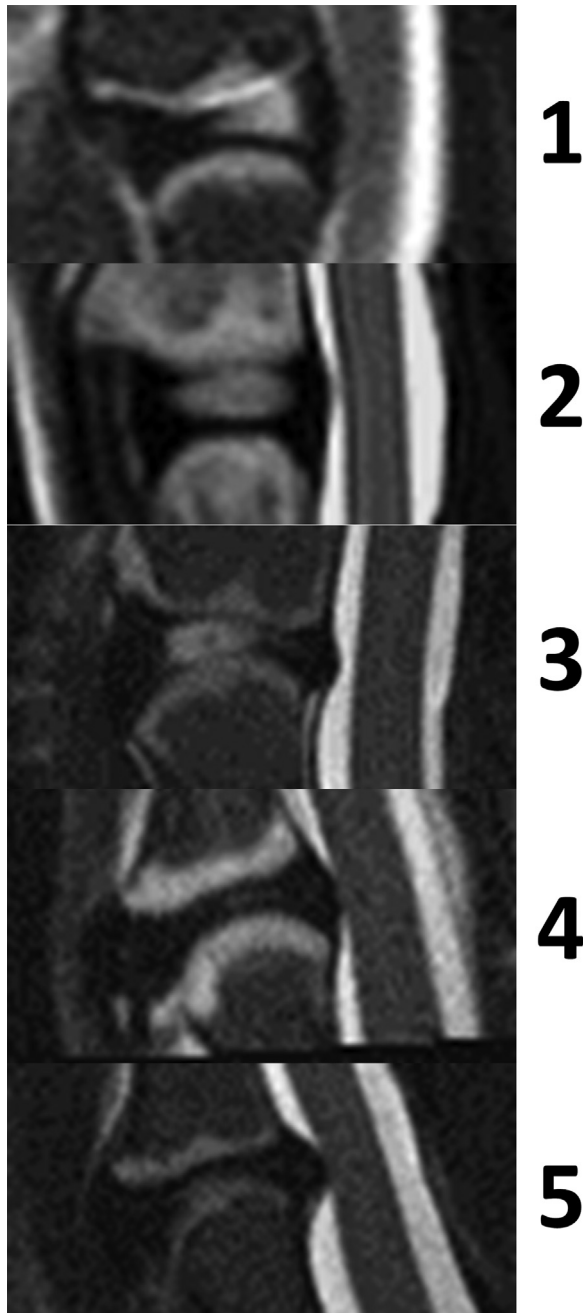


Figure 2. Exemplar Pfirrmann grades for various IVDs.

open the way for future research using camelids to study DDD and for pre-clinical testing of spinal implants and other therapeutics.^{18,25,29,45}

METHODS

Twenty clinically normal, pasture-raised, female alpacas (*Vicugna pacos*) were randomly chosen from a herd of 175 alpacas and transported to the imaging facility. Each alpaca was observed at a normal walking pace prior to MR imaging by the study veterinarian (BLR) to assess its gait for symmetry and lateral sequence to detect any neurologic or

orthopedic abnormality. All alpacas were deemed to be in good health based on clinical assessment by the caretaker (TFR) and examination by the study veterinarian (BLR). All animals had a normal gait with no obvious clinical signs of pain. Demographic information for the alpacas included in this study are available as part of the online supplemental content. All study parameters were approved by the Institutional Animal Care and Use Committee.

All alpacas were fasted for 18 h and had no access to water for 12 h prior to the imaging study to minimize the risk of regurgitation, aspiration, and bloating, during sedation and imaging. Following a physical exam, each alpaca was sedated with an intramuscular (IM) injection of “BKK” solution mixed 1:1 by volume. BKK was prepared by adding 1 ml of butorphanol (10 mg/ml; made in Germany for Merck Animal Health; Summit, NJ) and 1 ml of xylazine (100 mg/ml; Putney; Portland, ME) to a 10 ml vial of ketamine (100 mg/ml, Putney; Portland, ME); BKK solution was dosed at 1 ml/18.2 kg body weight (0.04 mg/kg butorphanol, 4–5 mg/kg ketamine, and 0.4–0.5 mg/kg xylazine combined in a single syringe and administered by IM injection to induce recumbency within 3–8 min and to achieve up to 45 min of sternal recumbency. Alpaca of the Suri breed received an additional 0.5 ml of BKK solution, as recommended in the literature.⁴⁶

Immediately after administration of the BKK sedative, aseptic preparation of a jugular venipuncture site was performed prior to placement of an intravenous (IV) catheter for additional sedation during the MRI scan (if needed). Following sedation, alpacas were placed in a kushed position (sternal recumbency) on the MRI table. The vital signs of each alpaca, including rectal body temperature, heart rate (HR), respiratory rate (RR), capillary refill time (CRT), color of mucous membranes (CMM), and reflexes (jaw tone, palpebral, panniculus, pinnae, tail), were monitored at 5 min intervals during sedation and until the animal was conscious, exhibiting purposeful movements, and lifting its head up while in sternal recumbency, then every 15 min until it was standing in the trailer. The sedated alpacas maintained a normal respiratory rate of 15–30 breaths/min breathing supplemental oxygen via nasal insufflation at a rate of 5 L/min during the MRI procedure. If the animal portrayed spontaneous movement of the ventral eyelid, which is correlated with insufficient anesthesia depth, during the MRI scan, additional sedation was administered IV using 0.125 mg/kg diazepam (Hospira, Inc.; Lake Forest, IL) and 0.3 mg/kg ketamine (Putney; Portland, ME).^{46,47}

Magnetic resonance (MR) imaging of the cervical spine was performed using a standard clinical 3.0-T MR unit (Siemens 3T Trio System; Siemens Healthcare, Erlangen, Germany) with an eight channel spine array and a four channel neck coil to acquire sagittal T2-weighted Turbo Spin Echo images [Scan 1 (C1–C3): repetition time (TR)=3500-ms, echo time (TE)=111-ms; Scan 2 (C3–C5): TR=4,000-ms TE=87-ms; Scan 3 (C5–T1): TR=3500-ms TE=99-ms] (Fig. 1). DDD was evaluated using the Pfirrmann grading system,⁴⁸ which is a 5-point grading scale for classification of degeneration of the intervertebral disc where a Pfirrmann grade of “1 (one)” is healthy and “5 (five)” is severely degenerated. The Pfirrmann grade is based on disc structure, distinction between the nucleus pulposus and annulus fibrosus, MR signal intensity, and disc height.⁴⁸ MR images of the Pfirrmann grade are presented in Figure 2 for study/comparison, with Figure 3 showing exemplar lower cervical

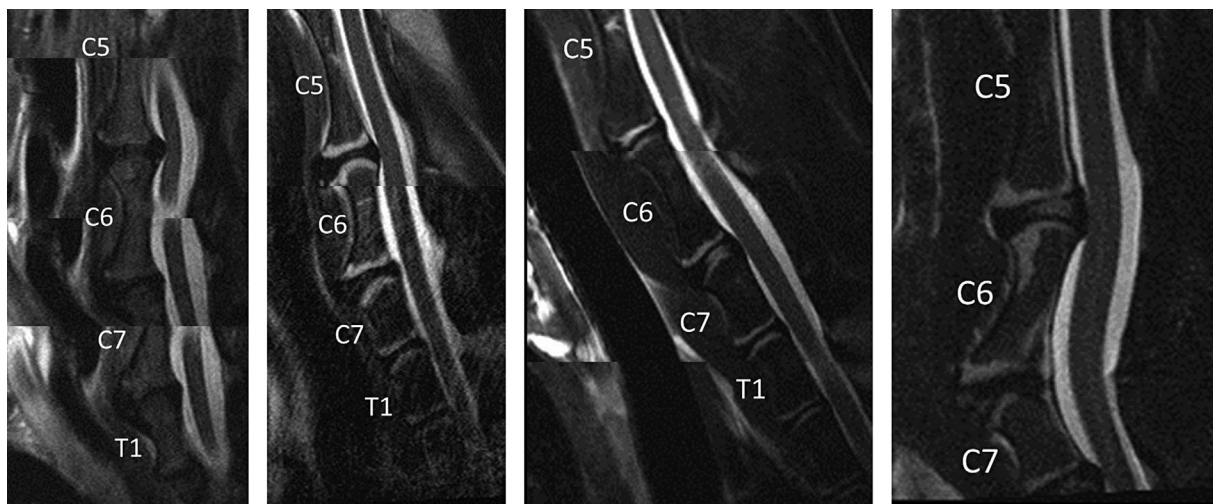


Figure 3. Composite MRI of several alpaca lower cervical spines labeled to show the specific spinal levels. Morphologic signs of degeneration are present in C6–C7 (low signal intensity) and C7–T1 (spinal cord impingement) in the far right, for example.

spines. Each intervertebral disc in each alpaca was evaluated independently by a board-certified veterinary radiologist (SMS) and a board-certified human radiologist (JDW).

Data Analysis

For the present work, the alpacas were divided into two subgroups according to age. The younger subgroup (3–6 years) consisted of five alpacas (mean age 4.8 ± 1.1 years; mean body weight 54.8 ± 6.6 kg), and the older subgroup (10–18 years) consisted of 15 alpacas (mean age 13.1 ± 2.2 years; mean body weight 61.5 ± 6.3 kg). The alpaca in this study included four Suri, and 16 Huacaya; each of the younger subgroup was Huacaya, with the four Suri being in the older subgroup. The sample size was not sufficient to determine if alpaca breed was a significant factor with the incidence of DDD. The effects of IVD level (e.g., C3–C4) and the age subgroup were analyzed for statistical significance

using a mixed-model analysis of variance with $\alpha = 0.05$ blocking on the randomized alpaca group, which was nested with the age subgroup. Least-square means values were calculated to demonstrate the combined effect of age and IVD level, and linear trendlines were determined according to the sum of least-squares methods. The radiologists were assumed to not be a factor with DDD grading, and both observations were weighted equally for analysis and determination of least-squares effects. Statistical significances for each effect (i.e., p -values) were determined with a post-hoc t -test from the mixed-model analysis of the Statistical Analysis Software System (SAS Institute Inc., Cary, North Carolina).

RESULTS

All the alpacas in this study were anecdotally found to be asymptomatic for pain and did not exhibit obvious

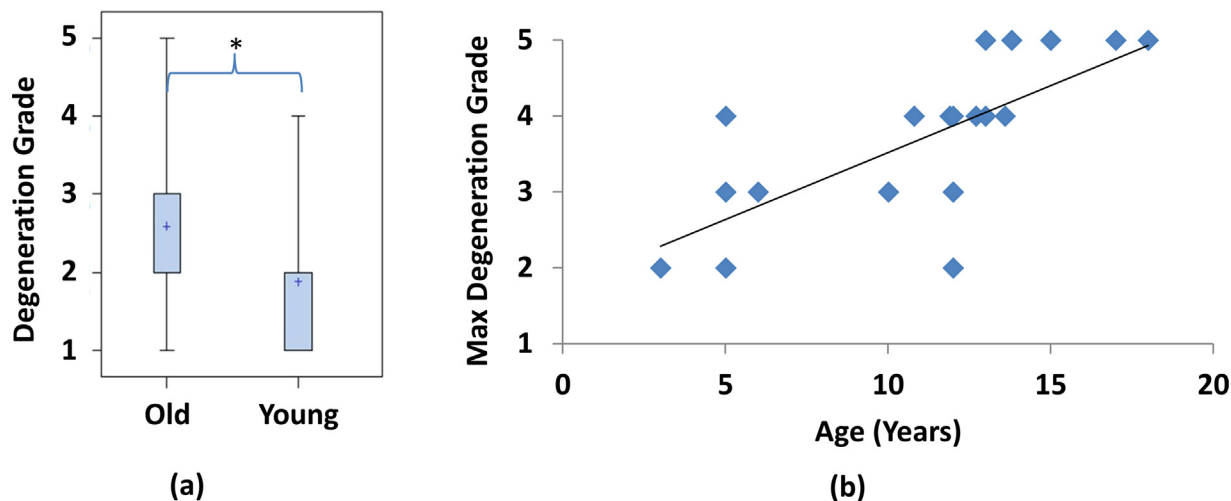


Figure 4. (a) A boxplot comparison of the Pfirrmann grades between the old and young subgroups, with the mean indicated by the marker inside the box, which indicates the first and third quartiles. Whiskers indicate the range of data. The asterisk (*) indicates that the two groups are significantly different ($p = 0.0042$). (b) The maximum Pfirrmann-grade for each alpaca is plotted with the animal age. The maximum degeneration in the alpaca spine is positively correlated with age, as indicated by the linear trendline ($R^2 = 0.51$).

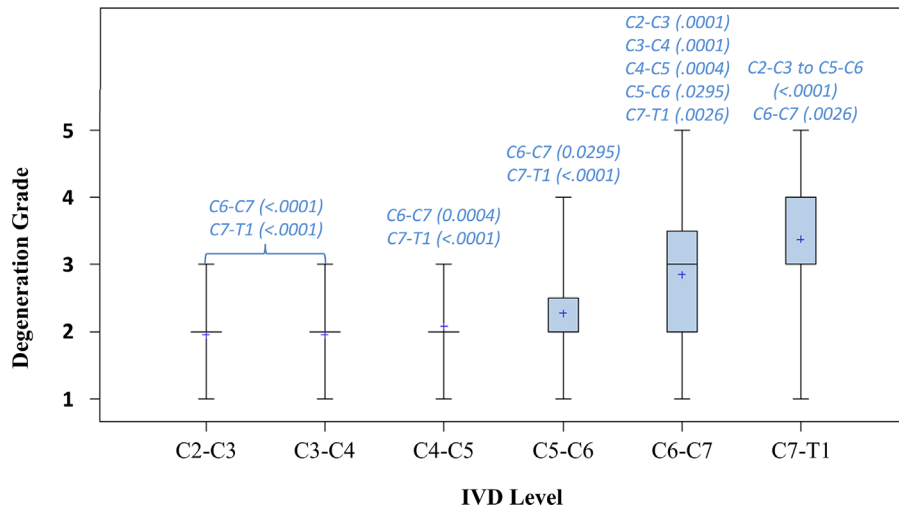


Figure 5. Boxplots of the Pfirrmann grades for all alpacas, grouped according to IVD level, show the positive correlation between DDD and IVD Level. Data mean is indicated by the “+” marker inside the box, which is bounded by the first and third quartiles, and the line inside the box represents the median. Where there is no box (e.g., C2–C3–C4–C5), the median, and the first and third quartiles were identical. Whiskers indicate the maximum and minimum range of the dataset. *P*-values are shown above each whisker where the differences between the degeneration grade for the different IVD levels showed strong significance ($p < 0.05$).

clinical signs of pain or abnormality of gait or neck movement. Each alpaca was observed to have a symmetrical gait with a lateral sequence footfall pattern when walking that was assessed to be sound, clinically normal, and not suffering from any orthopedic disease.⁴⁹ Several IVDs presented decreased MR signal intensity and had visible defects associated with the disc structure, including disc protrusions ($n = 9$), disc prolapse ($n = 5$), spinal cord impingement ($n = 1$), and mild spinal cord deviation ($n = 3$), as shown in Figure 3.

Analysis of the imaging results showed that DDD was more likely to occur in the older subgroup ($p = 0.042$), with a positive correlation shown by the linear trendline in Figure 4. A strong positive correlation also was present between the location (IVD level) of the intervertebral disc in the cervical spine and the degree (or severity) of DDD ($p < 0.0001$), which was more likely to occur in the lower cervical segments

(e.g., C6–C7 and/or C7–T1) (Fig. 5), with C7–T1 being the most commonly affected disc.

There was an interaction with age and IVD level ($p = 0.062$) as the differences between the two subgroups (young vs. old) increased with lower IVD levels (Fig. 6). In older alpacas, both moderate (Pfirrmann grade 3) and advanced (Pfirrmann grade 4 or higher) DDD was more prevalent in the lower cervical IVDs (e.g., C6–C7 and C7–T1) and non-existent in the higher cervical IVDs (e.g., C2–C3–C4–C5), as shown in Figure 7. Prevalence of moderate DDD was 0% in the higher cervical IVDs of younger alpaca, but the lower cervical vertebrae of the older subgroup showed moderate DDD prevalence that approached 90%. While non-existent in younger alpacas, nearly half of older alpacas had advanced DDD in the lower cervical IVDs.

The human and veterinary radiologist evaluation of the IVDs using the Pfirrmann-grade resulted in consensus on 82% (98/120) of IVDs; they differed by 1 grade on 18% (21/120) of IVDs, and differed by 2 grades on 0.8% (1/120) of IVDs. There was no difference greater than two. Analysis of the differences between the radiologists showed that in the cases where Pfirrmann grade differed, the veterinary radiologist generally rated the IVD as healthier than the human radiologist ($p = 0.0642$).

DISCUSSION

To the authors' knowledge, this study marks the first time the prevalence of naturally occurring disc degeneration has been evaluated for alpacas. The present work identified features on MR images of the lower alpaca cervical spine that indicate that alpacas may experience cervical disc degeneration at high enough rates that make alpacas potentially feasible for evaluating treatments of DDD in humans. Similar to

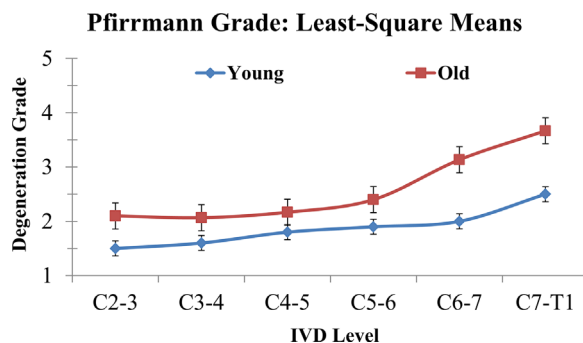


Figure 6. Least-square means degeneration lines for the old and young age subgroup by disc segment. Note that the difference in disc degeneration between the old and young subgroups increases with the IVD level.

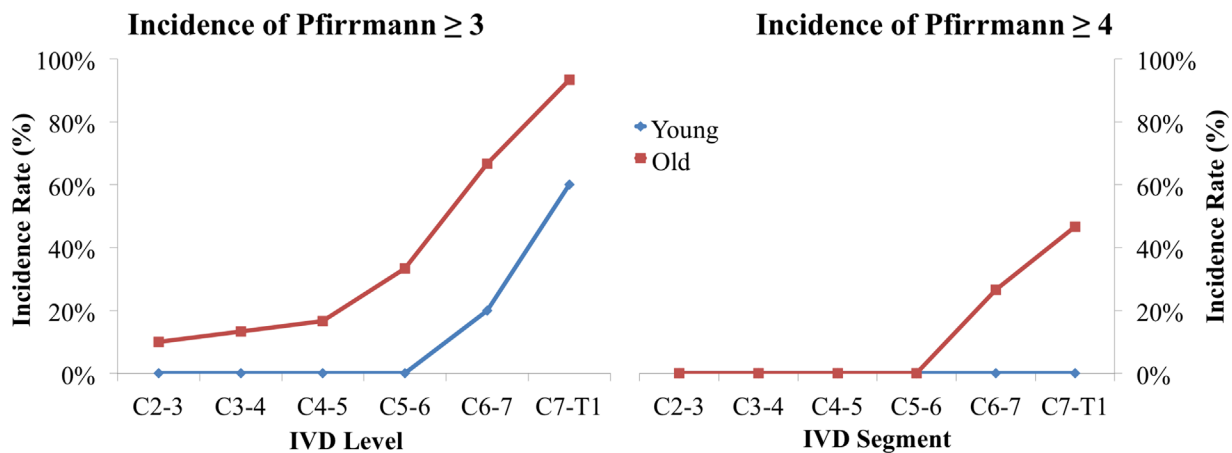


Figure 7. Incidence rate of degeneration by disc segment for the old and young age subgroups: (left) moderate disc degeneration, Pfirrmann ≥ 3 ; (right) advanced disc degeneration, Pfirrmann ≥ 4 .

humans, the present work demonstrates that increasing age was correlated with a higher likelihood of DDD and that progression of DDD in aging camelids may be similar to the human condition in regards to prevalence and severity.⁴⁰ This, and other parallels that were identified in this study in regards to age and location of degeneration, strengthens the case to further investigate alpacas and other members of the camelid family (e.g., llama) to better understand the strengths and limitations of this potential large animal model of disc degeneration in regards to types and mechanism of degeneration. An item that is particularly interesting is the observation that all the alpacas in this study were asymptomatic in that they did not exhibit obvious clinical signs of pain or abnormality of gait. This outcome is not inconsistent with humans, in that while the correlation between symptomatic spine patients and incidence of disc degeneration is high, the reverse is not. Still, we note that our understanding of how an alpaca might manifest cervical spine pain is quite limited, and there has been at least one published case of painful disc herniation noted in a llama,⁴⁰ and one case of painful disc herniation in an alpaca.⁴¹ Since the alpaca cervical spine does not support a significant load during gait, future work may be needed to identify a more biomechanically appropriate means (e.g., neck range of motion, etc.) of quantifying whether an animal is symptomatic. Further research is also needed investigate the prevalence of this condition across other species within the camelid family.

There were several limitations of the present work that remain to be addressed in future work. This study utilized female alpacas, which is ideal for production animals as an average alpaca breeding farm keeps a ratio of one male per 10–20 females. However, lack of gender diversity may miss insights available based on behavioral, structural, and hormonal differences between the sexes. Cost considerations for using an

alpaca as a preclinical model have not yet been evaluated, but are likely in line with those of other small ruminant animal models. Longitudinal evaluation of alpaca degeneration with age may also identify important similarities and differences with the human condition and will be key to identifying the specific age at which alpacas might lose notochordal cells. The scope of this study did not involve the sacrifice of any alpacas, but post-mortem inspection and biomechanical analysis of the spine may offer substantial histological and morphological details regarding the progression of disc degeneration in camelids. Finally, an increased sample size could allow improved accuracy in regards to investigation of all potential effects and their correlation between the severity of disc degeneration and animal age (e.g., increasing the number of age subgroups, or use age as a continuous variable), breed, gender, genetics, weight, and more. These unique parallels may yield further understanding with the correlation between disc degeneration and other disc properties, such as disc size, biomechanics, nutrition, and posture.

From a biomechanics standpoint, there are significant differences between both the kinetics and the kinematics of quadrupeds and humans. Those animals that are currently used in spinal research have a horizontal spine orientation for the thoracic and lumbar spine while standing; however this is vertical in humans when standing.^{50,51} The passive compression provided by spinal musculature in the thoracic and lumbar spine is unlikely to capture the kinetic/kinematic effects of gravity and loading when the rostral and caudal ends are supported by the front and hind limbs.⁵¹ However, in the majority of animals the cervical spine is loaded in an open-kinetic chain and more vertically oriented. In fact, the posture of the cervical spine directed over the front legs of a quadruped is markedly similar to the bipedal structure of the human. Despite the cervical spine supporting a

reduced load, pressures, or mechanical stresses may still be similar and warrant investigation.

In summary, the prevalence and severity of spontaneous intervertebral disc degeneration in the alpaca cervical spine, as evaluated using MRI, suggests that the alpaca lower cervical spine may be a potential large animal model for studying DDD and its treatment using therapeutics or surgical interventions.

AUTHORS' CONTRIBUTIONS

All authors have read and approved the final submitted manuscript, made substantial contributions to research design, or the acquisition, analysis, or interpretation of data, drafting the paper or revising it critically, and approval of the submitted and final versions.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the BYU MRI Research Facility (Provo, UT), especially the medical imaging expertise of Dr. Neal Bangerter, Kevin Perkins, Haonan Wang, Michelle Nash, and Danny Park. Alpaca subjects were provided by The Camelid Center (Moroni, UT). Research support for this project was provided in part by the National Science Foundation (CMMI-0952758).

REFERENCES

- Adams MA, Freeman BJ, Morrison HP, et al. 2000. Mechanical initiation of intervertebral disc degeneration. *Spine (Phila Pa 1976)* 25:1625–1636.
- Adams MA, Roughley PJ. 2006. What is intervertebral disc degeneration, and what causes it? *Spine (Phila Pa 1976)* 31:2151–2161.
- Schnake KJ, Putzier M, Haas NP, et al. 2006. Mechanical concepts for disc regeneration. *Eur Spine J* 15:S354–S360.
- Singh K, Masuda K, An HS. 2005. Animal models for human disc degeneration. *Spine J* 5:267S–279S.
- Andersson GB. 1999. Epidemiological features of chronic low-back pain. *Lancet* 354:581–585.
- Cheung KM, Karppinen J, Chan D, et al. 2009. Prevalence and pattern of lumbar magnetic resonance imaging changes in a population study of one thousand forty-three individuals. *Spine (Phila Pa 1976)* 34:934–940.
- Buckwalter JA. 1995. Aging and degeneration of the human intervertebral disc. *Spine (Phila Pa 1976)* 20:1307–1314.
- Katz JN. 2006. Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. *J Bone Joint Surg Am* 88:21–24.
- Gore M, Sadosky A, Stacey BR, et al. 2012. The burden of chronic low back pain: clinical comorbidities, treatment patterns, and health care costs in usual care settings. *Spine (Phila Pa 1976)* 37:E668–E677.
- Alini M, Eisenstein SM, Ito K, et al. 2008. Are animal models useful for studying human disc disorders/degeneration? *Eur Spine J* 17:2–19.
- Kikkawa J, Cunningham BW, Shirado O, et al. 2010. Biomechanical evaluation of a posterolateral lumbar disc arthroplasty device: an in vitro human cadaveric model. *Spine (Phila Pa 1976)* 35:1760–1768.
- Panjabi MM, Goel V, Oxland T, et al. 1992. Human lumbar vertebrae. Quantitative three-dimensional anatomy. *Spine (Phila Pa 1976)* 17:299–306.
- Stolworthy DK, Zirbel SA, Howell LL, et al. 2014. Characterization and prediction of rate-dependent flexibility in lumbar spine biomechanics at room and body temperature. *Spine J* 14:789–798.
- Zirbel SA, Stolworthy DK, Howell LL, et al. 2013. Intervertebral disc degeneration alters lumbar spine segmental stiffness in all modes of loading under a compressive follower load. *Spine J*.
- Gawri R, Mwale F, Ouellet J, et al. 2011. Development of an organ culture system for long-term survival of the intact human intervertebral disc. *Spine (Phila Pa 1976)* 36:1835–1842.
- Jim B, Steffen T, Moir J, et al. 2011. Development of an intact intervertebral disc organ culture system in which degeneration can be induced as a prelude to studying repair potential. *Eur Spine J* 20:1244–1254.
- Risbud MV, Izzo MW, Adams CS, et al. 2003. An organ culture system for the study of the nucleus pulposus: description of the system and evaluation of the cells. *Spine (Phila Pa 1976)* 28:2658–2659.
- An HS, Masuda K. 2006. Relevance of in vitro and in vivo models for intervertebral disc degeneration. *J Bone Joint Surg Am* 88:88–94.
- Vo N, Niedernhofer LJ, Nasto LA, et al. 2013. An overview of underlying causes and animal models for the study of age-related degenerative disorders of the spine and synovial joints. *J Orthop Res*.
- An HS, Masuda K, Inoue N. 2006. Intervertebral disc degeneration: biological and biomechanical factors. *J Orthopaedic Sci: Off J Jap Orthopaedic Assoc* 11:541–552.
- Wetzel FT, Panjabi MM, Pelker RR. 1989. Biomechanics of the rabbit cervical spine as a function of component transection. *J Orthop Res* 7:723–727.
- Wilke HJ, Kettler A, Claes LE. 1997. Are sheep spines a valid biomechanical model for human spines? *Spine (Phila Pa 1976)* 22:2365–2374.
- Winkelstein BA, Myers BS. 1997. The biomechanics of cervical spine injury and implications for injury prevention. *Med Sci Sports Exercise* 29:S246–S255.
- Zhang Y, Drapeau S, An HS, et al. 2011. Histological features of the degenerating intervertebral disc in a goat disc-injury model. *Spine (Phila Pa 1976)* 36:1519–1527.
- Kroeber MW, Unglaub F, Wang H, et al. 2002. New in vivo animal model to create intervertebral disc degeneration and to investigate the effects of therapeutic strategies to stimulate disc regeneration. *Spine (Phila Pa 1976)* 27:2684–2690.
- Korecki CL, Kuo CK, Tuan RS, et al. 2009. Intervertebral disc cell response to dynamic compression is age and frequency dependent. *J Orthop Res* 27:800–806.
- Schimandle JH, Boden SD. 1994. Spine update. The use of animal models to study spinal fusion. *Spine (Phila Pa 1976)* 19:1998–2006.
- Kettler A, Liakos L, Haegele B, et al. 2007. Are the spines of calf, pig and sheep suitable models for pre-clinical implant tests? *Eur Spine J* 16:2186–2192.
- An YH, Friedman RJ. 1999. Animal models in orthopaedic research. Boca Raton: CRC Press. p 604.
- Keorochana G, Johnson JS, Taghavi CE, et al. 2010. The effect of needle size inducing degeneration in the rat caudal disc: evaluation using radiograph, magnetic resonance imaging, histology, and immunohistochemistry. *Spine J* 10:1014–1023.
- Nuckley DJ, Kramer PA, Del Rosario A, et al. 2008. Intervertebral disc degeneration in a naturally occurring primate model: radiographic and biomechanical evidence. *J Orthop Res* 26:1283–1288.
- Sether LA, Nguyen C, Yu SN, et al. 1990. Canine intervertebral disks: correlation of anatomy and MR imaging. *Radiology* 175:207–211.
- Detiger SE, Hoogendoorn RJ, van der Veen AJ, et al. 2013. Biomechanical and rheological characterization of mild

- intervertebral disc degeneration in a large animal model. *J Orthop Res* 31:703–709.
34. Seiler G, Hani H, Scheidegger J, et al. 2003. Staging of lumbar intervertebral disc degeneration in nonchondrolytic dogs using low-field magnetic resonance imaging. *Vet Radiol Ultrasound: Official J Am Coll Vet Radiol Int Vet Radiol Assoc* 44:179–184.
 35. Kramer PA, Newell-Morris LL, Simkin PA. 2002. Spinal degenerative disk disease (DDD) in female macaque monkeys: epidemiology and comparison with women. *J Orthop Res* 20:399–408.
 36. Lauerma WC, Platenberg RC, Cain JE, et al. 1992. Age-related disk degeneration: preliminary report of a naturally occurring baboon model. *J Spinal Disord* 5:170–174.
 37. Lotz JC. 2004. Animal models of intervertebral disc degeneration: lessons learned. *Spine (Phila Pa 1976)* 29:2742–2750.
 38. Korecki CL, Costi JJ, Iatridis JC. 2008. Needle puncture injury affects intervertebral disc mechanics and biology in an organ culture model. *Spine (Phila Pa 1976)* 33:235–241.
 39. Stolworthy DK, Fullwood RA, Merrell TM, et al. 2015. Biomechanical Analysis of the Camelid Cervical Intervertebral Disc. *J Orthopaedic Transl* 3:34–43.
 40. Valentine BA, Saulez MN, Cebra CK, et al. 2006. Compressive myelopathy due to intervertebral disk extrusion in a llama (*Lama glama*). *J Vet Diagn Invest: Off Pub Am Assoc Vet Lab Diagn, Inc* 18:126–129.
 41. Zanolari P, Konar M, Tomek A, et al. 2006. Paraparesis in an adult alpaca with discospondylitis. *J Vet Int Med/Am Coll Vet Int Med* 20:1256–1260.
 42. Online A. 2014. Online alpaca directory for AOBA farm members. p. North American Alpaca Catalog.
 43. Pacheco Torres VcR, Altamirano Enciso AJ, Guerra Porras ES. 1986. The osteology of South American camelids: institute of Archaeology. Los Angeles: University of California. ix,32p.
 44. Mow VC, Huiskes R. 2005. Basic orthopaedic biomechanics and mechano-biology, 3rd ed. Philadelphia: Lippincott Williams and Wilkins. xvi 720.
 45. Neyt JG, Buckwalter JA, Carroll NC. 1998. Use of animal models in musculoskeletal research. *Iowa orthopaedic J* 18:118–123.
 46. Abrahamsen EJ. 2009. Chemical Restraint, Anesthesia, and Analgesia for Camelids. *The Veterinary Clinics of North America. Food Anim Pract* 25:455–494.
 47. Garcia Pereira FL, Greene SA, McEwen MM, et al. 2006. Analgesia and anesthesia in camelids. *Small Ruminant Res* 61:227–233.
 48. Pfirrmann CW, Metzendorf A, Zanetti M, et al. 2001. Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine (Phila Pa 1976)* 26:1873–1878.
 49. Pfau T, Hinton E, Whitehead C, et al. 2011. Temporal gait parameters in the alpaca and the evolution of pacing and trotting locomotion in the Camelidae. *J Zool* 283:193–202.
 50. Hamilton L, Franklin RJ, Jeffery ND. 2007. Development of a universal measure of quadrupedal forelimb-hindlimb coordination using digital motion capture and computerised analysis. *BMC Neurosci* 8:77.
 51. RJ Konz, S Fatone, RL Stine, et al. 2006. A kinematic model to assess spinal motion during walking *Spine (Phila Pa 1976)* 31 ; E898–E906

Supporting Information

Additional supporting information may be found in the online version of this article.