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A biomechanical assessment to evaluate breed differences in normal porcine medial collateral ligaments

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ABSTRACT

Little information is available on the role of genetic factors and heredity in normal ligament behaviour and their ability to heal. Assessing these factors is challenging because of the lack of suitable animal models. Therefore, the purpose of this study was to develop a porcine model in order to evaluate and compare the biomechanical differences of normal medial collateral ligaments (MCLs) between Yorkshire (YK) and red Duroc (RD) breeds. It was hypothesized that biomechanical differences would not exist between normal YK and RD MCLs. Comparisons between porcine and human MCL were also made. A biomechanical testing apparatus and protocol specific to pig MCL were developed. Ligaments were subjected to cyclic and static creep tests and then elongated to failure. Pig MCL morphology, geometry, and low- and high-load mechanical behaviour were assessed. The custom-designed apparatus and protocol were sufficiently sensitive to detect mechanical property differences between breeds as well as inter-leg differences. The results reveal that porcine MCL is comparable in both shape and size to human MCL and exhibits similar structural and material failure properties, thus making it a feasible model. Comparisons between RD and YK breeds revealed that age-matched RD pigs weigh more, have larger MCL cross-sectional area, and have lower MCL failure stress than YK pigs. The effect of weight may have influenced MCL geometrical and biomechanical properties, and consequently, the differences observed may be due to breed type and/or animal weight. In conclusion, the pig serves as a suitable large animal model for genetic-related connective tissue studies.

1. Introduction

Historically, *in vivo* animal models have been used to better understand the anatomy and biomechanics of the human knee. Methods of how to clinically manage ligament and tendon healing and repair were achieved by characterizing these processes using animal models. While numerous strategies, such as joint immobilization, surgical repair, grafting, and tissue engineering have been used to promote healing, it still remains true that the original properties and function of healing ligaments and tendons cannot fully be restored. For this reason, animal models are continually being developed and studied to better understand healing mechanisms and potential treatment strategies.

One of the most commonly studied and frequently injured knee ligaments is the medial collateral ligament (MCL). The MCL is easily accessible and identifiable, and following major trauma, can heal without surgical treatment. Biomechanical properties of MCL have been previously examined in mice, rats, rabbits, goats, sheep, mini-swine, and dogs (Abramowitch et al., 2003; Frank et al., 1983; Gijssen et al., 2004; Swapnil, 2005; Woo et al., 1983, 1987; Yiannakopoulos et al., 2005). These models have been beneficial; however, extrapolating biomechanical results from several of the smaller, less comparable models to adult humans is difficult because of known and unknown differences between species (Nunamaker, 1998; Xerogeanes et al., 1998). Porcine knees have been previously used to characterize the anterior cruciate ligament and are described as being most comparable to human knees because of the similar *in situ* loading (Livesay et al., 1997; Xerogeanes et al., 1998; Yeow et al., 2008). The pig's larger knees and ligaments make joint manipulation easy, arthroscopic surgery possible, and more tissue available for collection and analysis. Therefore, it is imperative to explore the domestic pig as a larger, more comparable animal model.

Currently, little is understood on the role of genetic factors and heredity in normal ligament behaviour and ligament healing (Wang et al., 2006). The domestic pig may be a suitable model for studies of genetic differences in healing connective tissues, like healing ligaments, because genetic differences were observed when comparing healing of dermal tissue of the Yorkshire (YK) and red Duroc (RD) breeds (Gallant-Behm and Hart, 2006; Gallant-Behm et al., 2007). In addition, the availability of transgenic (Hao et al., 2006; Klassen et al., 2008) and knockout

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(Griesemer et al., 2009) pigs, as well as stem cells from skin (Zhao et al., 2009) and synovial membranes (Ando et al., 2007; Shimomura et al., 2010) greatly expand the application and use of a suitable pig model.

This study was conducted to determine whether the domestic pig would be a suitable animal model for evaluating the biomechanical properties of the MCL. The objectives were to develop a biomechanical testing apparatus, to establish a biomechanical testing protocol specific for the porcine knee, and to identify the low-load (laxity and creep) and high-load (failure) mechanical properties of porcine MCL. In addition, our findings were used to draw comparisons between pig and human MCL. To assess genetic variability in normal ligament, a breed comparison of biomechanical properties of YK and RD porcine MCL was performed. We hypothesized that biomechanical differences would not exist between normal YK and RD MCLs.

2. Methods

2.1. Animal experiments

Six YK and six RD female adolescent (6–8 month old) pigs were purchased from Pig Improvement Canada Ltd. (Airdrie, AB) and used in this study (Fig. 1). All animal procedures were approved by the University of Calgary Animal Care Committee in accordance with Canadian Council on Animal Care guidelines. All animals acclimated for approximately one week at the University of Calgary Veterinary Sciences Research Station before being euthanized with an intracardiac overdose of sodium pentobarbital (Euthanyl, 0.5 ml/kg, Bimeda-MTC Animal Health Inc., Cambridge, ON). The hind limbs were disarticulated at the hip and ankle, wrapped in saline soaked gauze, sealed in plastic bags, and stored at −20 °C because prolonged freezing did not change the biomechanical properties of rabbit MCL (Woo et al., 1986). Twenty-four hours prior to biomechanical testing, limbs were thawed at 4 °C. Bones were transected approximately 15 cm from the joint line. Keeping the joint moist, muscle and fascia were removed, while the menisci and collateral and cruciate ligaments remained intact. Then, the dissected joints were wrapped in saline soaked gauze until they were mechanically tested.

2.2. Testing apparatus and set-up

The apparatus was modified from a pre-existing ovine MCL testing apparatus (Swapnil, 2005) with the cooperation of the University of Calgary Schulich School of Engineering Machine Shop, to accommodate the anatomy of the porcine knee. The apparatus consisted of an upper clamp attached to a 5 kN load cell on a hydraulic actuator of an MTS system (MTS Systems Corporation, Minneapolis, MN). A lower clamp was attached to a stainless steel base plate on a stainless steel test table which was fixed to a load floor (Fig. 2). Each clamp included an aluminum pot for securing the tibia and femur, an aluminum arc for facilitating an appropriate knee flexion angle, and various dove-tail wedge tracks for aligning the ligament. The keys, inserted into the dove-tail wedge sliders lock and key mechanism, were constructed of brass. Teflon inserts were added to the bone pots to adjust their volume from that of the ovine apparatus.

To mount the joint in the apparatus, the tibia was secured in its bone pot using screws and polymethylmethacrylate (PMMA) (Westan Dental Products Group,

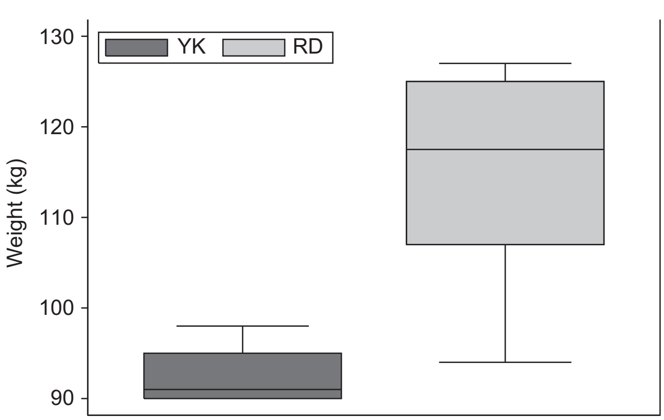

Fig. 1. Box plot illustrating the body weights of the YK and RD pigs ($p < 0.01$).

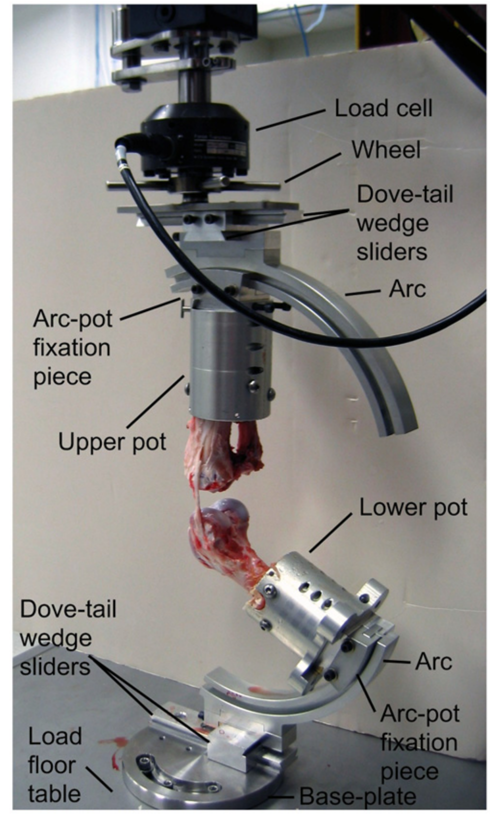

Fig. 2. Image of the modified custom-built porcine MCL testing apparatus. The attached porcine FMTC has been elongated to failure.

Calgary, AB) which was added in stages. Once the PMMA was set, the tibia bone pot was attached to its arc on the upper clamp thus allowing the femur to hang freely. Using a goniometer and a straight-edged ruler with a built-in level, the knee was placed at approximately 80° of flexion, while ensuring the MCL was aligned with the load axis of the actuator. At this angle, the ligament midsubstance had even tautness which was determined by touching the anterior and posterior borders of the midsubstance with a pair of forceps (Germscheid, 2008). Once positioned at this angle, the femur was secured in the bone pot of the lower clamp. The MCL was kept at physiological conditions (∼37 °C and 99% relative humidity) throughout the mechanical testing protocol, using a uniquely modified humidifier and kettle with PVC tubing to create a humidity-blower (Germscheid, 2008), because MCLs exhibit temperature-dependent viscoelastic properties (Woo et al., 1986, 1987).

2.3. Protocol development

The well-established rabbit MCL creep protocol served as a guideline for the pig MCL (Thornton et al., 1997, 2002). Pig knees obtained from an abattoir (Red Deer Lake Meats, Calgary, AB) revealed that pig MCLs were substantially larger in size and experienced failure loads which were approximately four times larger than rabbit MCLs (Germscheid, 2008; Thornton et al., 2005). Thus, several protocol modifications were required. Peak loads for compression–tension cycles, used to measure laxity and establish 'ligament zero', were increased four-fold for pig MCL compared to rabbit MCL. MCL length measurements were recorded before 'ligament zero' was established. Digital calipers were used to measure width and thickness, and porcine MCL was assumed to have a rectangular cross-section (Woo et al., 1983). While rabbit MCL was creep tested at loads corresponding to various stresses in the toe- and linear-region of the stress–strain curve (Thornton et al., 2002), pig MCL was creep tested at one load in the toe-region of the load–deformation curve. Applying a 100 N creep load was deemed appropriate for pig MCL because a gradual creep deformation was observed and step increases in deformation and/or failures did not occur. This load corresponds to the normal *in vivo* loads experienced by ligaments which are typically 5–10% of their ultimate strength (Butler et al., 2003).

2.4. Testing protocol

Once the joint was mounted in the apparatus, the whole joint underwent 2 cycles from −20 N to +8 N at 1 mm/min. Then, menisci and cruciate and lateral collateral ligaments were removed, thereby isolating the femur-MCL-tibia-complex (FMTC). Following this, two additional compression–tension cycles were performed to