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Effects of Increased Chronic Loading on Articular Cartilage Material Properties in the Lapine Tibio-Femoral Joint

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Abstract

Methods of producing relevant and quantifiable load alterations *in vivo* with which to study load-induced cartilage degeneration analogous to osteoarthritis are limited. An animal model was used to investigate the effects of increased chronic loads on articular cartilage. Mature rabbits were randomized into one of three experimentally-loaded groups and a fourth unoperated control group. A mechanical-loading device was skeletally fixed to the hind limb of animals in the loaded groups. Engaging the device resulted in an additional load of 0, +22 or +44% body weight to the medial compartment of the experimental knee while allowing normal joint function. Following a 12-week loading protocol, a creep indentation test and needle probe test were used to determine the biphasic material properties and thickness of the cartilage at four locations of each femoral and tibial condyle of the experimental and contralateral limbs. Analyses of covariance were performed to compare outcome measures across treatment groups. The effect of increased load was site and load-level specific with alterations of material properties and thickness most prominent in the posterior region of the medial compartment of the tibia. At this site, permeability increased 128% and thickness increased 28% in the +44% body weight group relative to the 0% body weight group. This model of altered chronic loading initiated changes in material properties to the articular cartilage at the sites of increased load over 12-weeks that were consistent with early degenerative changes suggesting that increased tibio-femoral loading may be responsible for the alterations. This work begins to elucidate the chronic load threshold and the time course of cartilage degeneration at different levels of altered loading.

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I. Introduction

Little is known regarding the sequella leading up to the onset of degenerative changes in articular cartilage (AC) of the knee, or if early interventions may modify the course of disease progression. Osteoarthritis (OA) is a degenerative disease affecting joint tissues, including AC, subchondral bone, and synovium. Abnormal mechanical loads are likely a primary component of the pathogenic mechanism (Grelsamer, 1995; Pritzker, 1994). For example, joint malalignment and increased body mass, both of which modify the inter-segmental compressive loads produced across the tibio-femoral joint, have been shown to be primary risk factors in the development of OA (Brouwer et al., 2007; Felson et al., 1988). Despite acceptance of the role of mechanical loads in the progression of OA, *in-vivo* quantitative assessments of how AC responds to different magnitudes of sustained load are limited. Identification of a threshold for chronic stress beyond which degenerative changes are initiated *in vivo* and elucidation of the dose-response relationship between abnormal contact stress and AC degeneration may provide the foundation for developing improved treatments for OA.

Commonly used animal models to investigate cartilage degeneration alter joint contact mechanics through ligament transection and meniscectomy, or induce pathology through blunt impact (Oegema and Visco, 1998; Pritzker, 1994) or injury as in the groove model (Marijnissen et al., 2002) and may relate more directly to secondary (post-traumatic) OA. These animal models typically disrupt the joint capsule and result in more rapid, degenerative changes than occurs in humans. Other animal models have focused on risk factors for primary (idiopathic) OA by utilizing osteotomy or external loading devices (Gu et al., 2009; Novotny et al., 2009; Ogata et al., 1977). The magnitude of altered load applied to the AC in existing animal models remains challenging to control and quantify. The present work focuses on altered loading, a known risk factor in the development of primary OA.

Metabolic and biochemical changes are likely early events in cartilage degeneration; however, the clinical symptoms of OA may not arise until the tissue's mechanical properties have been altered such that the tissue can no longer perform its load-bearing function. The intrinsic material properties of the AC are indicators of collagen network integrity and proteoglycan content (Buckwalter et al., 2001; Mow et al., 1989). Material properties change with the onset of degeneration and can be determined through mechanical testing such as the creep-indentation test (Buckwalter, 2001). Similar techniques have been used extensively to study rabbit AC (Ewers et al., 2000; Julkunen et al., 2009; Lane et al., 1979; Parsons and Black, 1987; Rasanen and Messner, 1999; Wei et al., 1997).

The objective of this study was to apply compressive overloads to the medial compartment of the knee and determine the relationship between the applied load and resulting biphasic cartilage material properties determined by creep indentation testing. A varus-loading device (VLD) was applied to the hind limb of a rabbit to deliver a controlled overload to the medial compartment of the tibio-femoral joint *in vivo*. We hypothesized that deleterious changes in the material properties and thickness of AC can be initiated by chronic, increased tibio-femoral compressive loads resulting from an applied varus moment, and that the extent of these changes are correlated with the magnitude of applied load.

2. Methods

2.1. Experimental Device

The VLD applied a varus moment to the knee while allowing normal joint function (Fig. 1). The VLD allows moments to be applied to the knee only during the designated treatment period and can subsequently be disengaged. The magnitude of the spring torque, T , required to generate a desired change in load, ΔP , can be calculated knowing the moment arm lengths (Fig. 1, Equation 1). A description of the VLD and results from an *in vitro* validation study are presented in Appendix 1.

2.2. In Vivo Application of the VLD

Thirty-one female New-Zealand-White rabbits, 12 months of age, were randomized into one of four treatment groups. Three groups of animals were exposed to one of three levels of varus moment producing additional loads on the medial compartment equal to 0% (Sham), +22%, or +44% of the animal's body weight (BW) ($n=8/\text{group}$). A fourth group served as unoperated controls ($n=7$). NIH guidelines for use of animals were observed.

2.3. Surgical Procedure

Animals in the 0, +22, and +44% BW groups underwent unilateral surgery to implant custom bone plates on the femur and tibia. Following anesthetization, an incision was made over the intermuscular interval running along the femur posterior to the tensor *fascia lata* and this interval was dissected to expose the lateral aspect of the femur. Two guide holes were created in the femur. The femoral bone plate was positioned and secured with two stainless steel 2 mm diameter, transcortical bone screws. Following closure of the incision, stab incisions were created and the skin was fit over the posts of the transcutaneous bone plate. A similar technique was used to implant the tibial bone plate. The VLD was attached to the bone plates and adjusted to fit the animal. The pivot axis of the VLD was aligned with the femoral transepicondylar axis using palpation and visual inspection. The alignment of the load link was adjusted so that no internal/external torque was induced about the long axis of the tibia when a spring load was engaged. Lateral and anterior-posterior radiographs were taken with the knee positioned at 30° flexion to confirm proper positioning of the VLD. Load link moment arms (L_1 and L_2) and the intercompartmental moment arm (D) were measured from the radiographs and the spring torque (T) required to generate $\Delta P = +22\%$ or +44% BW was calculated (Fig. 1). No torque was applied to the 0% BW group. Fracture of the femur occurred in three animals following installation of the bone plates. These animals were removed from the study and replaced with animals that completed the experimental protocol.

2.4. Loading Protocol

Loading was initiated after 7 days of recovery. The calculated torque for the VLD was set using a torque wrench (T5165, FUTEK, Irvine, CA USA). The VLD was engaged 12 hours per day, 5 days per week, for 12 weeks (Fig. 2). At the end of the daily treatment, the VLD was disengaged so that no external load was applied to the joint. All animals ambulated normally with normal range of motion. No difference in activity level was detected between loaded and control animals, as measured by the number of hops taken during a 10-minute daily exercise period.

Following 12 weeks of loading, animals were euthanized. Femoral and tibial condyles were excised and stored at -80°C until testing. Gross observation revealed no overt erosion of the AC in any of the experimental groups.

2.5. Mechanical Testing

The material properties and thicknesses of the AC were determined through mechanical testing. Permeability, aggregate modulus, and Poisson's ratio were evaluated using the biphasic creep-indentation test (Mak et al., 1987; Mow, 1989). Cartilage thickness was determined using the needle probe test (Athanasίου et al., 1991). Central and posterior sites were tested in the medial and lateral compartments of the femoral and tibial condyles (Fig. 3) in experimental and contralateral legs using a custom materials testing device (Roemhildt et al., 2006). The central sites were selected to represent cartilage with direct contact between the tibia and femur when the rabbit knee is $\sim 120^\circ$ from full extension (FFE), coinciding with the flexion angle at which the highest peak loads of the joint are observed during gait (Gushue et al., 2005; Mansour et al., 1998). The posterior sites reflect contact points between the femur and tibia when the knee is in full flexion as occurs when a rabbit is sitting. Specimens were thawed in a bath of lactated Ringer's Solution approximately 30 minutes prior to testing. Indentation testing of the tibia used a cylindrical, plane-ended, porous, 1.0 mm diameter indenter tip as previously described (Roemhildt, 2006). Femoral specimens were tested in a similar manner using a 0.75 mm diameter indenter tip because of the higher radius of curvature of the specimen. An arthroscope was used to confirm the positioning and alignment of the specimen relative to the indenter. Following application of a tare load (0.0437 MPa) for 15 minutes, the indentation test proceeded with the application of the test load (0.1249 MPa) until displacement reached equilibrium with data sampled at 1 Hz (Athanasίου, 1991). Following a period of recovery equal to the test duration, the thickness of the AC at the testing site was determined using a needle probe test (Roemhildt, 2006). Material properties of the AC were determined by curve-fitting the load-displacement response with the biphasic indentation creep solution via a nonlinear regression procedure (Mow, 1989). Material properties from the tibia of the unoperated control group have been previously reported in a study examining the effect of testing site and age (Roemhildt, 2006).

2.6. Glycosaminoglycan (GAG) content

Following mechanical testing, AC was removed from each specimen. Compartment specific tibial and femoral AC was combined to ensure adequate tissue. Samples were digested with α -chymotrypsin and Proteinase K (Billinghurst et al., 1997). GAG content of each digest was determined using the dimethylmethylene blue (DMMB) assay (Farndale et al., 1986). GAG measures were normalized by DNA content as determined fluorometrically by the bisbenzimidazole method (Labarca and Paigen, 1980).

2.7. Histology

Tibial and femoral specimens from one animal from the 0, +22, and +44% BW groups were prepared for qualitative histological analysis. Specimens were serially sectioned at 2mm, formalin fixed, decalcified using 10% EDTA, dehydrated, and embedded in paraffin (Király et al., 1996). Serial 5 μ m sections were cut, deparaffinized, and stained with Safranin O or Hematoxylin and Eosin (H&E) prior to examination under light microscopy.

2.8. Statistics

Site specific analyses of covariance were used to evaluate differences in mean permeability, aggregate modulus, Poisson's ratio, thickness, and GAG across the four treatment conditions (Control, 0% BW, +22% BW, and +44% BW). For each outcome measure, the site matched observation obtained from the contralateral limb was used as a covariate when evaluating treatment effects in the intervention limb. Separate analyses were performed for central and posterior sites of each compartment (medial and lateral) for both the tibia and femur. Results from one animal (+22% BW group) were excluded from analyses due to device malfunction.

during mechanical testing. Pairwise comparisons between the four treatment conditions were performed using Fisher's LSD procedure. These comparisons were based on least square means which were adjusted for the covariate. Statistical analyses were performed using SAS statistical Software Version 8.2 (SAS Institute, Cary, NC USA). Sample size calculations were determined *a priori* to have power $(1-\beta) = 0.80$ to detect an effect size (Δ^2) (Winer et al., 1991) of 0.5 for aggregate modulus and permeability (Goodman and Berlin, 1994)

3. Results

Site and group specific raw means for the material properties and thickness of the AC are presented for the tibia (Table 1) and the femur (Table 2). Load-induced alterations in AC properties were site specific and most pronounced in the medial-posterior site of the tibia. At this site, significant differences across treatment conditions were observed for permeability, thickness and Poisson's ratio ($p = 0.04, 0.02, 0.05$, respectively; Fig. 4). Permeability in the +44% BW group was increased 128% compared to the 0% BW group ($p=0.02$) and 160% compared to the +22% BW group ($p=0.02$). Thickness in the +44% BW group was 26% greater than the 0% BW ($p=0.03$) and 39% greater than the +22% BW group ($p = 0.01$). Poisson's ratio was increased 46% for the 0% BW and 35% for the +22% BW groups as compared to the Control ($p = 0.01$ and $p=0.04$, respectively). There were no significant differences across groups on aggregate modulus ($p=0.24$); however, pairwise comparisons indicated a 37% reduction in the aggregate modulus in the +44% BW group compared to the +22% BW group ($p=0.05$).

In the medial-central site of the tibia, differences across treatment conditions for permeability, thickness, aggregate modulus, and Poisson's ratio were less pronounced (Fig. 5). Data for +22 % and +44 BW% groups as a function of the site provenience are shown in Fig 6.

When considering the femur, no significant treatment related differences for permeability, thickness, aggregate modulus, or Poisson's ratio were observed in the medial-posterior or medial-central sites. In the lateral compartment, central and posterior sites of tibia and femur, no significant differences across the experimental groups were observed.

GAG content in the medial compartment was not different across groups ($p=0.38$). An effect of group was observed in the lateral compartment ($p=0.02$), with mean GAG decreased in the 0% BW group as compared to Control and +22% BW groups ($p=0.01$ and 0.01 , respectively, Fig. 7).

Histological sections indicated mild fibrillation of the medial compartment of the experimental limb and a decreased number of chondrocytes in the +22 and +44% BW groups as compared to 0% BW group (Fig. 8).

4. Discussion

The response of AC to chronic-load alteration in the rabbit knee was investigated by evaluation of material properties and thickness. The AC responded to increased loading of +22% and +44% BW for 12 weeks without gross erosion. AC material properties and thickness were sensitive to loading at select sites. The most prominent treatment effects occurred in the medial-posterior site in the tibia of the experimental leg. This site corresponds to the contact area between the femur and tibia when the rabbit is sitting, a posture in which alert laboratory rabbits spend the majority of time.

In the medial-posterior site of the tibia, permeability values for the +44% BW group were 128% and 160% greater than values for the 0% BW and +22% BW groups (Fig. 4). Our

results are similar to permeability increases of ~150% observed in the rabbit 2 weeks after meniscectomy (Hoch et al., 1983). An increase in permeability suggests decreased proteoglycan content; however, this was not confirmed by GAG analysis of compartment specific samples pooled from femoral and tibial AC. However, these pooled GAG results do not carry the same site sensitivity that the mechanical measurements had.

The thickness of AC in the tibia (medial-posterior) increased 26% and 39% in the +44% BW group as compared to the 0% BW and +22% BW groups, respectively (Fig. 4). The present findings are consistent with AC swelling observed in the early stages of OA (Calvo et al., 2001; Calvo et al., 2004). In the development of OA, an increase in AC thickness results from swelling of the matrix stemming from a decrease in proteoglycan content and disruption of the collagen fibrils which resist the swelling pressure within the AC (Hollander et al., 1994; McDevitt and Muir, 1976).

A decrease in aggregate modulus results with the progression of degenerative changes in AC (Setton et al., 1999), while an increase in stiffness and thickness was observed with increased loading resulting from moderate and strenuous exercise (Jurvelin et al., 1990; Kiviranta et al., 1988). In a long-term canine study, no change in cartilage material properties or thickness resulted from lifelong exercise while animals wore weighted vests (Newton et al., 1997); whereas with increased running distance (≤ 40 km/day), decreases in GAG content and shear modulus were observed (Arokoski et al., 1993; Arokoski et al., 1994). These studies illustrate the sensitivity of AC to the magnitude and duration of loading. In the present study, the aggregate modulus of the loaded groups did not differ significantly from the Control or 0% BW groups.

Load levels used in this study were based on *in vivo* measurements which found an average peak load of ~40% BW in the medial compartment of the rabbit knee during hopping (Coughlin et al., 2005). Additionally, a 43% increase in load has been reported clinically with varus malalignment of the knee (Noyes et al., 1992).

Altered loading was applied for 12 hours, followed by 12 hours of “normal” loading, with no altered loading applied on weekends. The 12-hour exposure period was selected to provide a moderate exposure to increased loading. The daily and weekly rest periods may obscure the response of AC to chronic altered loading. These periods of normal loading were sufficient to maintain the cartilage material properties in the lateral compartment of the +22% and +44% BW groups which experienced a decrease in load with engagement of the VLD. Similarly, animal studies of AC unloading through immobilization found no changes in compressive modulus up to 8 weeks (Setton et al., 1997; Vanwanseele et al., 2002).

Changes in vertical force during gait have been observed in animal models. In a canine ACL-transection model the vertical force in the experimental limb decreased to ~36% of normal 2 weeks following surgery and recovered to only ~50% of normal at 12 weeks while the contralateral limb was unaltered (O'Connor et al., 1989). Altered weight bearing resulting from the surgical procedure may contribute to the experimental effects observed, even though the VLD was applied without disrupting the joint capsule or musculature and no gross changes in animal gait or muscle mass were observed. By close observation, animals were fully weight bearing with full range of motion (~0–180° flexion); however, in-depth kinematic and kinetic analyses of gait would be required to estimate the total joint contact forces in the knee. A detailed assessment of animal activity level throughout the course of the study was not performed and activity level may have varied between animals or across groups.

For select outcome measures, a difference between the Control and 0% BW group was observed indicating a sham, or surgery, effect (Fig. 4). This pattern was evident in both

experimental and contralateral legs. In the tibia, the 0% BW group showed a 30–40% decrease in permeability, a 15% decrease in thickness, and a 30–40% increase in Poisson's ratio as compared to Control in experimental and contralateral legs (Tables 1 & 2). Anesthesia/analgesia, application of the device (<1% BW), undetected differences in animal activity, altered distribution of weight-bearing across limbs, or systemic factors such as inflammation may contribute to this effect. In an ACL-transection model, compositional changes were observed in the contralateral limb that were not due to changes in peak vertical force (McDevitt and Muir, 1976; O'Connor, 1989). Furthermore, in rabbit studies the metabolic response of AC in the contralateral limb following meniscectomy or sham surgery followed the same response as in the experimental and was attributed to systemic factors (Floman et al., 1980; Moskowitz et al., 1981).

The reduced GAG content in the lateral compartment of the 0% BW group compared to Control further demonstrates a sham effect. GAG content normalized by wet weight exhibited a similar pattern. GAG levels observed in this study were elevated in comparison to values reported for rabbit tibiofemoral cartilage (Kojima et al., 2001).

Regional variations in the material properties of AC have previously been studied in the rabbit (Rasanen and Messner, 1999; Roemhildt, 2006; Thambyah et al., 2006). As many intervention methods to initiate cartilage degeneration alter the contact mechanics of AC in a site specific manner, the location of the greatest treatment effect may be model and animal specific. In a rabbit ACL-transection model, localized cartilage degeneration was found predominantly in posteromedial aspects of the joint in areas covered by the meniscus as assessed by surface scoring (Chang et al., 1997).

The observed AC response to loading with the VLD demonstrates a slower development of degenerative changes as compared to established transection-based models in which gross changes occur within 2–12 weeks of surgery (Hoch, 1983; Setton et al., 1999). Quantitative measures of compressive or shear force alterations resulting from meniscectomy or ACL-transection have yet to be published for these frequently used animal models preventing direct comparison to the load levels used in this work. The rapid onset of gross-degenerative changes in these models differs from the development of OA in humans which occurs over many years. An animal model in which degenerative changes develop more gradually may be more relevant to primary OA in humans. The VLD model allows application of controlled, quantifiable load alteration, modulation of altered load, and unconstrained use of the joint, without compromising the joint capsule. The VLD model may be particularly useful to study the contribution of altered loading to the early events occurring with cartilage degeneration.

This study illustrates the sensitivity of AC to different levels of chronic loading and explores the dose-response relationship for altered contact stress in the initiation of cartilage degeneration. The establishment of such a dose-response relationship would be useful in identifying what mechanical conditions are likely to predispose an individual to primary OA. These results demonstrate the potential of the VLD to initiate alterations in AC as evidenced by changes in material properties and thickness and give insight into the early response of AC to altered loading which may be indicative of initial changes in the degenerative process. However, the level and duration of chronic loading required to initiate changes consistent with degenerative processes that are not self-mitigating remains to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Arokoski J, Kiviranta I, Jurvelin J, Tammi M, Helminen HJ. Long-distance running causes site-dependent decrease of cartilage glycosaminoglycan content in the knee joints of beagle dogs. *Arthritis & Rheumatism*. 1993; 36 (10):1451–1459. [PubMed: 7692860]
- Arokoski J, Jurvelin J, Kiviranta I, Tammi M, Helminen HJ. Softening of the lateral condyle articular cartilage in the canine knee joint after long distance (up to 40 km/day) running training lasting one year. *International Journal of Sports Medicine*. 1994; 15 (5):254–260. [PubMed: 7960320]
- Athanasίου KA, Rosenwasser MP, Buckwalter JA, Malinin TI, Mow VC. Interspecies comparisons of in situ intrinsic mechanical properties of distal femoral cartilage. *Journal of Orthopaedic Research*. 1991; 9(3):330–340. [PubMed: 2010837]
- Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, Mitchell P, Hambor J, Diekmann O, Tschesche H, Chen J, Wart HV, Poole AR. Enhanced Cleavage of Type II Collagen by Collagenases in Osteoarthritic Articular Cartilage. *J Clin Invest*. 1997; 99(7):1534–1545. [PubMed: 9119997]
- Brouwer GM, Tol AWV, Bergink AP, Belo JN, Bernsen RMD, Reijman M, Pols HAP, Bierma-Zeinstra SMA. Association between valgus and varus alignment and the development and progression of radiographic osteoarthritis of the knee. *Arthritis & Rheumatism*. 2007; 56 (4):1204–1211. [PubMed: 17393449]
- Buckwalter, JA.; Mow, VC.; Hunziker, E. Concepts of cartilage repair in osteoarthritis. In: Moskowitz, RW., et al., editors. *Osteoarthritis: Diagnosis and Medical/Surgical Management*. W.B. Saunders Company; Philadelphia, PA: 2001.
- Calvo E, Palacios I, Delgado E, Ruiz-Cabello J, Hernandez P, Sanchez-Pernaute O, Egido J, Herrero-Beaumont G. High-resolution MRI detects cartilage swelling at the early stages of experimental osteoarthritis. *Osteoarthritis and Cartilage*. 2001; 9 (5):463–472. [PubMed: 11467895]
- Calvo E, Palacios I, Delgado E, Sanchez-Pernaute O, Largo R, Egido J, Herrero-Beaumont G. Histopathological correlation of cartilage swelling detected by magnetic resonance imaging in early experimental osteoarthritis. *Osteoarthritis & Cartilage*. 2004; 12:878–886. [PubMed: 15501403]
- Chang DG, Iverson EP, Schinagl RM, Sonoda M, Amiel D, Coutts RD, Sah RL. Quantitation and localization of cartilage degeneration following the induction of osteoarthritis in the rabbit knee. *Osteoarthritis & Cartilage*. 1997; 5(5):357–372. [PubMed: 9497942]
- Coughlin KM, Peura GD, Fleming BC, Hallock S, Beynon BD. In vivo loads in the medial compartment of the rabbit knee. *Clinical Biomechanics*. 2005; 20 (9):1007–1009. [PubMed: 16099082]
- Ewers BJ, Newberry WN, Haut RC. Chronic softening of cartilage without thickening of underlying bone in a joint trauma model. *Journal of Biomechanics*. 2000; 33 (12):1689–1694. [PubMed: 11006394]
- Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochimica et Biophysica Acta*. 1986; 883(2):173–177.
- Felson DT, Anderson JJ, Naimark A, Walker AM, Meenan RF. Obesity and knee osteoarthritis. The Framingham Study. *Annals of Internal Medicine*. 1988; 109 (1):18–24. [PubMed: 3377350]
- Floman Y, Eyre DR, Glimcher MJ. Induction of osteoarthrosis in the rabbit knee joint: biochemical studies on the articular cartilage. *Clinical Orthopaedics & Related Research*. 1980; (147):278–286. [PubMed: 7371310]
- Goodman SN, Berlin JA. The Use of Predicted Confidence Intervals When Planning Experiments and the Misuse of Power When Interpreting Results. *Annals of Internal Medicine*. 1994; 121 (3):200–206. [PubMed: 8017747]
- Grelsamer RP. Unicompartamental osteoarthrosis of the knee. *J Bone Joint Surg Am*. 1995; 77 (2):278–292. [PubMed: 7844137]
- Gu XI, Leong DJ, Guzman F, Mahamud R, Li YH, Majeska RJ, Schaffler MB, Sun HB, Cardoso L. Development and validation of a motion and loading system for a rat knee joint in vivo. *Ann Biomed Eng*. 2010 Mar; 38(3):621–31. Epub 2009 Dec 16. [PubMed: 20013312]

- Gushue DL, Houck J, Lerner AL. Rabbit knee joint biomechanics: Motion analysis and modeling of forces during hopping. *Journal of Orthopaedic Research*. 2005; 23 (4):735. [PubMed: 16022984]
- Hoch DH, Grodzinsky AJ, Koob TJ, Albert ML, Eyre DR. Early changes in material properties of rabbit articular cartilage after meniscectomy. *Journal of Orthopaedic Research*. 1983; 1(1):4–12.
- Hollander AP, Heathfield TF, Webber C, Iwata Y, Bourne R, Rorabeck C, Poole AR. Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. *Journal of Clinical Investigation*. 1994; 93(4):1722–1732. [PubMed: 7512992]
- Julkunen P, Harjula T, Iivarinen J, Marjanen J, Seppänen K, Närhi T, Arokoski J, Lammi MJ, Brama PA, Jurvelin JS, Helminen HJ. Biomechanical, biochemical and structural correlations in immature and mature rabbit articular cartilage. *Osteoarthritis and Cartilage*. 2009; 17 (12):1628–1638. [PubMed: 19615962]
- Jurvelin JS, Kiviranta I, Saamanen AM, Tammi M, Helminen HJ. Indentation stiffness of young canine knee articular cartilage- Influence of strenuous joint loading. *Journal of Biomechanics*. 1990; 23 (12):1239–1246. [PubMed: 2292603]
- Kiraly K, Lammi M, Arokoski J, Lapveteläinen T, Tammi M, Helminen H, Kiviranta I. Safranin O reduces loss of glycosaminoglycans from bovine articular cartilage during histological specimen preparation. *Histochemical Journal*. 1996; 28(2):99–107. [PubMed: 8737291]
- Kiviranta I, Tammi M, Jurvelin J, Saamanen AM, Helminen HJ. Moderate running exercise augments glycosaminoglycans and thickness of articular cartilage in the knee joint of young beagle dogs. *Journal of Orthopaedic Research*. 1988; 6 (2):188–195. [PubMed: 3278079]
- Kojima T, Mwale F, Yasuda T, Girard C, Poole AR, Lavery S. Early degradation of type IX and type II collagen with the onset of experimental inflammatory arthritis. *Arthritis & Rheumatism*. 2001; 44(1):120–127. [PubMed: 11212149]
- Labarca C, Paigen K. A simple, rapid, and sensitive DNA assay procedure. *Analytical Biochemistry*. 1980; 102(2):344–352. [PubMed: 6158890]
- Lane JM, Chisena E, Black J. Experimental knee instability: early mechanical property changes in articular cartilage in a rabbit model. *Clinical Orthopaedics & Related Research*. 1979; (140):262–265. [PubMed: 477081]
- Mak AF, Lai WM, Mow VC. Biphasic indentation of articular cartilage--I. Theoretical analysis. *Journal of Biomechanics*. 1987; 20 (7):703–714. [PubMed: 3654668]
- Mansour JM, Wentorf FA, Degoede KM. In vivo kinematics of the rabbit knee in unstable models of osteoarthrosis. *Annals of Biomedical Engineering*. 1998; 26 (3):353–360. [PubMed: 9570218]
- Marijnissen AC, van Roermund PM, Verzijl N, Tekoppele JM, Bijlsma JW, Lefeber FP. Steady progression of osteoarthritic features in the canine groove model. *Osteoarthritis & Cartilage*. 2002; 10(4):282–289. [PubMed: 11950251]
- McDevitt CA, Muir H. Biochemical changes in the cartilage of the knee in experimental and natural osteoarthritis in the dog. *Journal of Bone & Joint Surgery - British Volume*. 1976; 58(1):94–101. [PubMed: 131804]
- Moskowitz RW, Goldberg VM, Malesud CJ. Metabolic responses of cartilage in experimentally induced osteoarthritis. *Annals of the Rheumatic Diseases*. 1981; 40 (6):584–592. [PubMed: 7332379]
- Mow VC, Gibbs MC, Lai WM, Zhu WB, Athanasiou KA. Biphasic indentation of articular cartilage--II. A numerical algorithm and an experimental study. *Journal of Biomechanics*. 1989; 22 (8–9): 853–861. [PubMed: 2613721]
- Newton PM, Mow VC, Gardner TR, Buckwalter JA, Albright JP. Winner of the 1996 Cabaud Award. The effect of lifelong exercise on canine articular cartilage. *American Journal of Sports Medicine*. 1997; 25 (3):282–287. [PubMed: 9167804]
- Novotny, J.; Chiravuri, V.; Docimo, J.; Holcombe, P.; Wang, L. Altered Knee Loading Produces Structural and Mechanical Changes in Articular Cartilage and Subchondral Bone. *Proceedings of the 55th Annual Meeting of the Orthopaedic Research Society*; Las Vegas, NV. 2009.
- Noyes FR, Schipplein OD, Andriacchi TP, Saddemi SR, Weise M. The Anterior Cruciate Ligament-Deficient Knee with Varus Alignment: An Analysis of Gait Adaptations and Dynamic Joint Loadings. *American Journal of Sports Medicine*. 1992; 20 (6):707–716. [PubMed: 1456365]

- O'Connor BL, Visco DM, Heck DA, Myers SL, Brandt KD. Gait alterations in dogs after transection of the anterior cruciate ligament. *Arthritis & Rheumatism*. 1989; 32 (9):1142–1147. [PubMed: 2775322]
- Oegema, TR., Jr; Visco, D. Animal models of osteoarthritis. In: An, HS., editor. *Animal Models in Orthopaedic Research*. CRC Press; Boca Raton, FL: 1998. p. 349-368.
- Ogata K, Whiteside L, Lesker P, Simmons D. The effect of varus stress on the moving rabbit knee joint. *Clinical Orthopaedics & Related Research*. 1977; 129:313–318. [PubMed: 608292]
- Parsons JR, Black J. Mechanical behavior of articular cartilage quantitative changes with enzymatic alteration of the proteoglycan fraction. *Bulletin of the Hospital for Joint Diseases Orthopaedic Institute*. 1987; 47(1):13–30. [PubMed: 3038223]
- Pritzker KP. Animal models for osteoarthritis: processes, problems and prospects. *Ann Rheum Dis*. 1994; 53 (6):406–420. [PubMed: 8037500]
- Rasanen T, Messner K. Articular cartilage compressive stiffness following oophorectomy or treatment with 17beta-estradiol in young postpubertal rabbits. *Acta Obstetrica et Gynecologica Scandinavica*. 1999; 78(5):357–362. [PubMed: 10326876]
- Roemhildt ML, Coughlin KM, Peura GD, Fleming BC, Beynnon BD. Material properties of articular cartilage in the rabbit tibial plateau. *Journal of Biomechanics*. 2006; 39 (12):2331–2337. [PubMed: 16168420]
- Setton LA, Mow VC, Muller FJ, Pita JC, Howell DS. Mechanical behavior and biochemical composition of canine knee cartilage following periods of joint disuse and disuse with remobilization. *Osteoarthritis & Cartilage*. 1997; 5 (1):1–16. [PubMed: 9010874]
- Setton LA, Elliott DM, Mow VC. Altered mechanics of cartilage with osteoarthritis: human osteoarthritis and an experimental model of joint degeneration. *Osteoarthritis & Cartilage*. 1999; 7:2–14. [PubMed: 10367011]
- Thambyah A, Nather A, Goh J. Mechanical properties of articular cartilage covered by the meniscus. *Osteoarthritis and Cartilage*. 2006; 14 (6):580. [PubMed: 16621620]
- Vanwanseele B, Lucchinetti E, Stüssi E. The effects of immobilization on the characteristics of articular cartilage: current concepts and future directions. *Osteoarthritis and Cartilage*. 2002; 10 (5):408–419. [PubMed: 12027542]
- Wei X, Gao J, Messner K. Maturation-dependent repair of untreated osteochondral defects in the rabbit knee joint. *Journal of Biomedical Materials Research*. 1997; 34(1):63–72. [PubMed: 8978654]
- Winer, BJ.; Brown, DR.; Michels, KM. *Experimental Design*. McGraw-Hill; 1991. *Statistical Principles*.

Figure 1.

Schematic of the varus-loading device applied to an animal hind limb: (A) lateral view and (B) anterior view. Application of the VLD results in an increase in compressive load in the medial compartment by an amount ΔP . The change in load is quantifiable and can be modulated in a controlled manner by setting the spring torque as shown by Equation 1 where: ΔP = change in contact load, T = torque setting of the spring, D = intercompartmental moment arm, L_1 = load link moment arm, and L_2 = tibia moment arm. Equation 1: $\Delta P = T/D * L_2/L_1$.

Figure 2.
Rabbit with VLD attached and engaged.

Figure 3.
Mechanical testing sites on: (A) tibial plateau and (B) femoral condyles; • Central, °
Posterior.

Figure 4.

Mechanical testing results for the **Tibia - Medial compartment - Posterior**: Least square means for material properties and thickness from covariate analysis. P-values indicate overall significance of treatment group based on analyses of covariance. Means not sharing a common letter are significantly different based on Fishers LSD procedure ($p < 0.05$).

Figure 5.

Mechanical testing results for the **Tibia - Medial compartment - Central**: Least square means for material properties and thickness from covariate analysis. P-values indicate overall significance of treatment group based on analyses of covariance. Means not sharing a common letter are significantly different based on Fishers LSD procedure ($p < 0.05$).

Figure 6.

Location specific mechanical testing results for the tibia of the experimental limb normalized by Control values: MP=medial posterior, MC=medial central, LP=lateral posterior, LC=lateral central.

Figure 7.

Total glycosaminoglycan (GAG) content for medial and lateral compartments: Least square means for GAG from covariate analysis. P-values indicate overall significance of treatment group based on analyses of covariance. Means not sharing a common letter are significantly different based on Fishers LSD procedure ($p < 0.05$)

Figure 8.

Representative histological sections of the medial tibia of experimental limbs from A) 0% BW Sham and B) +44% BW (Safarin O staining, original image 4x, ∂ cutting artifact, * indicates cartilage fibrillation). Mild surface erosion/fibrillation was present in the +22% and +44% BW sections as compared to 0% BW. Representative sections of the medial tibia taken at area of maximum cartilage thickness: C) 0% BW and D) +44% BW (H&E staining, 10x, ◇ indicates a typical chondrocyte). Fewer chondrocytes were observed in sections from loaded animals compared to 0% BW (Chondrocyte number = 0%: 345, +22%: 251, +44%: 220).

Table 1

Material Properties of Articular Cartilage of the Tibial Plateau

Group	Leg	Comp	Site	H _A (MPa)	k (10 ⁻¹⁶ m ⁴ /Ns)	ν	h (mm)
Control (n=7)	Contralateral	Medial	Posterior	1.24 (0.43)	18.6 (6.1)	0.25 (0.10)	0.77 (0.07)
			Central	1.79 (0.96)	15.8 (5.8)	0.27 (0.10)	0.82 (0.08)
		Posterior	Posterior	1.80 (0.42)	5.4 (2.1)	0.34 (0.06)	0.49 (0.10)
	Experimental	Lateral	Central	1.68 (0.38)	6.1 (4.1)	0.29 (0.06)	0.55 (0.10)
			Posterior	1.50 (0.72)	20.5 (13.3)	0.26 (0.11)	0.78 (0.15)
		Medial	Central	1.57 (0.34)	20.9 (19.6)	0.23 (0.13)	0.84 (0.12)
0% BW (n=7)	Contralateral	Lateral	Posterior	1.87 (0.73)	6.9 (2.9)	0.30 (0.07)	0.54 (0.16)
			Central	1.60 (0.74)	7.3 (3.5)	0.31 (0.06)	0.53 (0.10)
		Medial	Posterior	1.58 (0.44)	9.8 (3.8)	0.38 (0.03)	0.57 (0.11)
			Central	1.48 (0.56)	11.8 (5.5)	0.28 (0.13)	0.72 (0.10)
		Posterior	Posterior	1.73 (0.51)	5.2 (2.5)	0.29 (0.10)	0.44 (0.16)
			Central	1.86 (0.52)	5.2 (1.5)	0.32 (0.05)	0.53 (0.06)
	Experimental	Lateral	Posterior	1.46 (0.48)	11.8 (5.8)	0.38 (0.05)	0.62 (0.14)
			Central	1.72 (0.51)	12.6 (5.6)	0.32 (0.07)	0.73 (0.10)
		Medial	Posterior	1.76 (0.38)	5.8 (2.7)	0.33 (0.04)	0.53 (0.08)
			Central	1.80 (0.44)	5.6 (1.7)	0.29 (0.08)	0.59 (0.04)
		Posterior	Posterior	1.63 (0.45)	12.1 (4.6)	0.30 (0.11)	0.73 (0.10)
			Central	1.73 (0.43)	12.6 (8.7)	0.32 (0.04)	0.69 (0.14)
22% BW (n=6)	Contralateral	Lateral	Posterior	2.27 (0.91)	5.3 (1.9)	0.27 (0.14)	0.54 (0.18)
			Central	1.65 (0.67)	5.6 (2.5)	0.30 (0.14)	0.52 (0.10)
		Medial	Posterior	1.72 (0.65)	9.9 (6.6)	0.35 (0.05)	0.60 (0.10)
			Central	1.62 (0.41)	13.1 (7.5)	0.35 (0.05)	0.72 (0.08)
		Posterior	Posterior	2.08 (0.53)	4.8 (1.8)	0.35 (0.08)	0.49 (0.14)
			Central	1.75 (0.77)	4.6 (1.0)	0.34 (0.02)	0.54 (0.06)
	Experimental	Lateral	Posterior	1.71 (0.46)	11.5 (6.0)	0.29 (0.12)	0.65 (0.16)
			Central	1.92 (0.58)	11.8 (5.9)	0.29 (0.08)	0.70 (0.11)
		Medial	Posterior	1.70 (0.57)	7.4 (5.3)	0.33 (0.06)	0.51 (0.11)
			Central				
		Posterior	Posterior				
			Central				

Group	Leg	Comp	Site	H _A (MPa)	k (10 ⁻¹⁶ m ² /Ns)	ν	h (mm)
	Experimental		Central	1.56 (0.23)	5.4 (1.4)	0.33 (0.06)	0.51 (0.06)
			Posterior	1.04 (0.31)	25.5 (14.0)	0.31 (0.05)	0.81 (0.13)
			Central	1.57 (0.37)	13.5 (4.1)	0.31 (0.08)	0.74 (0.11)
			Posterior	1.92 (0.60)	6.6 (2.4)	0.31 (0.10)	0.57 (0.13)
		Lateral	Central	1.79 (0.65)	6.4 (3.9)	0.30 (0.09)	0.56 (0.14)

Values given as mean (standard deviation). k = permeability, H_A = aggregate modulus, ν = Poisson's ratio, and h = cartilage thickness

Table 2

Material Properties of Articular Cartilage of the Femoral Condyles

Group	Leg	Comp	Site	H _A (MPa)	k (10 ⁻¹⁶ m ⁴ /Ns)	ν	h (mm)
Control (n=7)	Contralateral	Medial	Posterior	2.34 (0.71)	4.0 (2.0)	0.36 (0.08)	0.39 (0.08)
			Central	2.26 (0.90)	2.9 (0.9)	0.29 (0.15)	0.31 (0.10)
		Lateral	Posterior	1.83 (0.75)	2.3 (1.8)	0.35 (0.14)	0.26 (0.09)
			Central	1.98 (1.57)	1.9 (0.7)	0.31 (0.16)	0.24 (0.08)
	Experimental	Medial	Posterior	2.34 (1.05)	4.7 (2.4)	0.33 (0.10)	0.38 (0.14)
			Central	2.64 (0.94)	1.8 (1.3)	0.31 (0.17)	0.26 (0.09)
0% BW (n=7)	Contralateral	Medial	Posterior	2.16 (0.33)	2.2 (1.1)	0.38 (0.03)	0.26 (0.05)
			Central	1.17 (0.38)	2.4 (0.6)	0.30 (0.08)	0.22 (0.07)
		Lateral	Posterior	2.74 (0.74)	3.6 (2.9)	0.39 (0.04)	0.38 (0.12)
			Central	3.25 (1.15)	1.8 (1.0)	0.39 (0.05)	0.29 (0.06)
	Experimental	Medial	Posterior	1.47 (0.69)	1.4 (1.0)	0.32 (0.15)	0.21 (0.06)
			Central	1.37 (0.75)	1.7 (1.2)	0.29 (0.17)	0.21 (0.04)
22% BW (n=6)	Contralateral	Medial	Posterior	2.21 (0.81)	3.6 (1.2)	0.36 (0.06)	0.41 (0.08)
			Central	1.66 (0.94)	2.3 (1.9)	0.29 (0.13)	0.29 (0.16)
		Lateral	Posterior	2.76 (1.34)	1.8 (1.0)	0.35 (0.11)	0.27 (0.05)
			Central	1.70 (0.60)	2.5 (1.2)	0.31 (0.11)	0.26 (0.09)
	Experimental	Medial	Posterior	2.52 (0.33)	4.9 (2.3)	0.34 (0.05)	0.46 (0.07)
			Central	2.85 (1.96)	2.4 (1.0)	0.38 (0.03)	0.34 (0.12)
44% BW (n=7)	Contralateral	Medial	Posterior	2.48 (0.84)	2.3 (1.1)	0.38 (0.04)	0.30 (0.10)
			Central	1.61 (0.85)	3.0 (1.7)	0.31 (0.07)	0.31 (0.13)
		Lateral	Posterior	2.92 (0.84)	4.6 (3.1)	0.32 (0.09)	0.46 (0.08)
			Central	2.71 (0.94)	1.3 (0.3)	0.40 (0.03)	0.26 (0.05)
	Experimental	Medial	Posterior	1.93 (0.70)	2.7 (1.2)	0.35 (0.07)	0.29 (0.13)
			Central	1.24 (0.61)	1.5 (0.9)	0.26 (0.15)	0.22 (0.11)
44% BW (n=7)	Contralateral	Medial	Posterior	2.82 (1.20)	3.5 (2.3)	0.40 (0.04)	0.36 (0.09)
			Central	1.87 (0.60)	2.5 (2.4)	0.34 (0.15)	0.25 (0.09)
		Lateral	Posterior	2.15 (0.94)	1.7 (0.8)	0.34 (0.16)	0.27 (0.07)

Group	Leg	Comp	Site	H _A (MPa)	k (10 ⁻¹⁶ m ⁴ /Ns)	ν	h (mm)
	Experimental		Central	1.45 (0.66)	2.6 (1.8)	0.25 (0.13)	0.27 (0.13)
			Posterior	2.48 (0.89)	5.2 (4.7)	0.33 (0.10)	0.40 (0.07)
			Central	2.48 (1.32)	1.4 (0.6)	0.34 (0.15)	0.22 (0.05)
		Lateral	Posterior	2.77 (1.74)	1.5 (0.8)	0.35 (0.16)	0.25 (0.07)
			Central	1.58 (0.77)	1.9 (1.1)	0.34 (0.11)	0.25 (0.09)

Values are presented as mean (standard deviation). k = permeability, H_A = aggregate modulus, ν = Poisson's ratio, and h = cartilage thickness