

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

Authors

Maria L. Roemhildt, Ph.D.

Kathryn M. Coughlin, M.S.

Glenn D. Peura, M.S.

Gary J. Badger, M.S.

Dave Churchill, Ph.D. *

Braden C. Fleming, Ph.D.**

Bruce D. Beynnon, Ph.D.

University of Vermont, Burlington, VT USA

* Microstrain, Williston, VT USA

**Warren Alpert Medical School at Brown University, Providence, RI USA

Maria L. Roemhildt, Ph.D.

Department of Orthopaedics & Rehabilitation

95 Carrigan Drive

Burlington, VT 05405 USA

Tel: 802-656-3823

Fax: 802-656-4247

E-mail: maria.roemhildt@med.uvm.edu

Running title: Effects of in-vivo altered loading of the rabbit knee

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, material properties and thickness of the cartilage were determined 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.

1 I. Introduction

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

15 Commonly used animal models to investigate cartilage degeneration alter joint contact
16 mechanics through ligament transection and meniscectomy, or induce pathology through blunt
17 impact (Pritzker, 1994). These animal models typically disrupt the joint capsule and result in
18 rapid, degenerative changes. Other animal models utilize osteotomy or external loading devices
19 (Gu et al., 2009; Novotny et al., 2009). The magnitude of altered load applied to the AC in
20 existing animal models remains challenging to control and quantify.

21 Metabolic and biochemical changes are likely early events in cartilage degeneration;
22 however, the clinical symptoms of OA may not arise until the tissue's mechanical properties
23 have been altered such that the tissue can no longer perform its load-bearing function. The
24 intrinsic material properties of the AC are indicators of collagen network integrity and

1 proteoglycan content (Buckwalter et al., 2001, Mow et al., 1989). Material properties change
2 with the onset of degeneration and can be determined through mechanical testing such as the
3 creep-indentation test (Buckwalter, 2001).

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

11

12 **2. Methods**

13 ***2.1. Experimental Device***

14 The VLD applied a varus moment to the knee while allowing normal joint function (Fig.
15 1). The VLD allows moments to be applied to the knee only during the designated treatment
16 period and can subsequently be disengaged. The magnitude of the spring torque, T , required to
17 generate a desired change in load, ΔP , can be calculated knowing the moment arm lengths (Fig.
18 1, Equation 1). A description of the VLD and results from an *in vitro* validation study are
19 presented in Appendix 1.

20 ***2.2. In Vivo Application of the VLD***

21 Thirty-one female New-Zealand-White rabbits, 12 months of age, were randomized into
22 one of four treatment groups. Three groups of animals were exposed to one of three levels of
23 varus moment producing additional loads on the medial compartment equal to 0% (Sham),

1 +22%, or +44% of the animal's body weight (BW) (n=8/group). A fourth group served as
2 unoperated controls (n=7). NIH guidelines for use of animals were observed.

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

19 Loading was initiated after 7 days of recovery. The calculated torque for the VLD was
20 set using a torque wrench (T5165, FUTEK, Irvine, CA USA). The VLD was engaged 12 hours
21 per day, 5 days per week, for 12 weeks (Fig. 2). At the end of the daily treatment, the VLD was
22 disengaged so that no external load was applied to the joint. All animals ambulated normally
23 with normal range of motion. No difference in activity level was detected between loaded and

1 control animals, as measured by the number of hops taken during a 10-minute daily exercise
2 period.

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

6 The material properties and thicknesses of the AC were determined through mechanical
7 testing. Permeability, aggregate modulus, and Poisson's ratio were evaluated using the biphasic
8 creep-indentation test (Mak et al., 1987; Mow, 1989). Cartilage thickness was determined using
9 the needle probe test (Athanasίου et al., 1991). Central and posterior sites were tested in the
10 medial and lateral compartments of the femoral and tibial condyles (Fig. 3) in experimental and
11 contralateral legs using a custom materials testing device (Roemhildt et al., 2006). The central
12 sites were selected to represent cartilage with direct contact between the tibia and femur when
13 the rabbit knee is $\sim 120^{\circ}$ from full extension (FFE), coinciding with the flexion angle at which the
14 highest peak loads of the joint are observed during gait (Gushue et al., 2005; Mansour et al.,
15 1998). The posterior sites reflect contact points between the femur and tibia when the knee is in
16 full flexion as occurs when a rabbit is sitting. Specimens were thawed in a bath of lactated
17 Ringer's Solution approximately 30 minutes prior to testing. Indentation testing of the tibia used
18 a cylindrical, plane-ended, porous, 1.0 mm diameter indenter tip as previously described
19 (Roemhildt, 2006). Femoral specimens were tested in a similar manner using a 0.75 mm
20 diameter indenter tip because of the higher radius of curvature of the specimen. An arthroscope
21 was used to confirm the positioning and alignment of the specimen relative to the indenter.
22 Following application of a tare load (0.437 MPa) for 15 minutes, the indentation test proceeded
23 with the application of the test load (0.1249 MPa) until displacement reached equilibrium with
24 data sampled at 1 Hz (Athanasίου, 1991). Following a period of recovery equal to the test

1 duration, the thickness of the AC at the testing site was determined using a needle probe test
2 (Roemhildt, 2006). Material properties of the AC were determined by curve-fitting the load-
3 displacement response with the biphasic indentation creep solution via a nonlinear regression
4 procedure (Mow, 1989).

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

10 Site specific analyses of covariance were used to evaluate differences in mean
11 permeability, aggregate modulus, Poisson's ratio, and thickness across the four treatment
12 conditions (Control, 0% BW, +22% BW, and +44% BW). For each outcome measure, the site
13 matched observation obtained from the contralateral limb was used as a covariate when
14 evaluating treatment effects in the intervention limb. Separate analyses were performed for
15 central and posterior sites of each compartment (medial and lateral) for both the tibia and femur.
16 Results from one animal (+22% BW group) were excluded from analyses due to device
17 malfunction during mechanical testing. Pairwise comparisons between the four treatment
18 conditions were performed using Fisher's LSD procedure. These comparisons were based on
19 least square means which were adjusted for the covariate. Statistical analyses were performed
20 using SAS statistical Software Version 8.2 (SAS Institute, Cary, NC USA). Sample size
21 calculations were determined *a priori* to have power $(1-\beta) = 0.80$ to detect an effect size
22 (Δ^2) (Winer et al., 1991) of 0.5 for aggregate modulus and permeability (Goodman and Berlin,
23 1994).

24

1 **3. Results**

2 Site and group specific raw means for the material properties and thickness of the AC are
3 presented for the tibia (Table 1) and the femur (Table 2). Load-induced alterations in AC
4 properties were site specific and most pronounced in the medial-posterior site of the tibia. At
5 this site, significant differences across treatment conditions were observed for permeability,
6 thickness and Poisson's ratio ($p= 0.04, 0.02, 0.05$, respectively; Fig. 4). Permeability in the
7 +44% BW group was increased 128% compared to the 0% BW group ($p=0.02$) and 160%
8 compared to the +22% BW group ($p=0.02$). Thickness in the +44% BW group was 26% greater
9 than the 0% BW ($p =0.03$) and 39% greater than the +22% BW group ($p = 0.01$). Poisson's ratio
10 was increased 46% for the 0% BW and 35% for the +22% BW groups as compared to the
11 Control ($p= 0.01$ and $p=0.04$, respectively). There were no significant differences across groups
12 on aggregate modulus ($p=0.24$); however, pairwise comparisons indicated a 37% reduction in the
13 aggregate modulus in the +44% BW group compared to the +22% BW group ($p=0.05$).

14 In the medial-central site of the tibia, differences across treatment conditions for
15 permeability, thickness, aggregate modulus, and Poisson's ratio were less pronounced (Fig. 5).

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

20 Histological sections indicated mild fibrillation of the medial compartment of the
21 experimental limb and a decreased number of chondrocytes in the +22 and +44% BW groups as
22 compared to 0% BW group (Fig. 6).

23

24 **4. Discussion**

1 The response of AC to chronic-load alteration in the rabbit knee was investigated by
2 evaluation of material properties and thickness. The AC responded to increased loading of
3 +22% and +44% BW for 12 weeks without gross erosion. AC material properties and thickness
4 were sensitive to loading at select sites. The most prominent treatment effects occurred in the
5 medial-posterior site in the tibia of the experimental leg. This site corresponds to the contact
6 area between the femur and tibia when the rabbit is sitting, a posture in which alert laboratory
7 rabbits spend the majority of time.

8 In the medial-posterior site of the tibia, permeability values for the +44% BW group were
9 128% and 160% greater than values for the 0% BW and +22% BW groups (Fig. 4). An increase
10 in permeability is indicative of proteoglycan loss as occurs with early degenerative changes in
11 AC. Our results are similar to permeability increases of ~150% observed in the rabbit 2 weeks
12 after meniscectomy (Hoch, 1983).

13 The thickness of AC in the tibia (medial-posterior) increased 26% and 39% in the +44%
14 BW group as compared to the 0% BW and +22% BW groups, respectively (Fig. 4). Although a
15 decrease in cartilage thickness occurs with OA progression, the present findings are consistent
16 with AC swelling observed in the early stages of cartilage degeneration (Calvo et al., 2004).

17 A decrease in aggregate modulus results with the progression of degenerative changes in
18 AC (Setton et al., 1999), while an increase in stiffness and thickness was observed with increased
19 loading resulting from moderate and strenuous exercise (Jurvelin et al., 1990; Kiviranta et al.,
20 1988). In a long-term canine study, no change in cartilage material properties or thickness
21 resulted from lifelong exercise while animals wore weighted vests (Newton et al., 1997);
22 whereas with increased running distance (≤ 40 km/day), decreases in glycosaminoglycan content
23 and shear modulus were observed (Arokoski et al., 1993; Arokoski et al., 1994). These studies
24 illustrate the sensitivity of AC to the magnitude and duration of loading. In the medial

1 compartment of the tibia, the aggregate modulus of the +22% BW group was elevated 19% in
2 comparison to the 0% BW group; whereas with increased load level, the aggregate modulus was
3 decreased 26% in the +44% BW compared to 0% BW group. The nonlinearity of the aggregate
4 modulus and permeability responses indicate that the loading treatments used in the present
5 study may bracket a threshold between anabolic and catabolic responses.

6 Load levels used in this study were based on *in vivo* measurements which found an
7 average peak load of ~40% BW in the medial compartment of the rabbit knee during hopping
8 (Coughlin, 2005). Additionally, a 43% increase in load has been reported clinically with varus
9 malalignment of the knee (Noyes, 1992). Gushue calculated a peak load of 262-285% BW in the
10 medial compartment of the rabbit knee during hopping (Gushue, 2005). Therefore the levels of
11 altered load used may only represent an 8-16% change in peak load. These results demonstrate
12 that even small changes in chronic loading induced changes in the AC.

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

21 Changes in vertical force during gait have been observed in animal models. In a canine
22 ACL-transection model the vertical force in the experimental limb decreased to ~36% of normal
23 2 weeks following surgery and recovered to only ~50% of normal at 12 weeks while the
24 contralateral limb was unaltered (O'Connor et al., 1989). Although altered weight bearing

1 resulting from the surgical procedure may contribute to the experimental effects observed, this
2 effect is thought to be small in comparison to the effect of increased loading, as the VLD was
3 applied without disrupting the joint capsule or musculature and no gross changes in animal gait
4 or muscle mass were observed. By close observation, animals were fully weight bearing with
5 full range of motion (~0-180° flexion); however, in-depth kinematic and kinetic analyses of gait
6 would be required to estimate the total joint contact forces in the knee.

7 For select outcome measures, a difference between the Control and 0% BW group was
8 observed indicating a sham effect (Fig. 4). This pattern was evident in both experimental and
9 contralateral legs. In the tibia, the 0% BW group showed a 30-40% decrease in permeability, a
10 15% decrease in thickness, and a 30-40% increase in Poisson's ratio as compared to Control in
11 experimental and contralateral legs (Tables 1 & 2). Anesthesia/analgesia, application of the
12 device (<1% BW), undetected differences in animal activity, altered distribution of weight-
13 bearing across limbs, or systemic factors such as inflammation may contribute to this effect. In
14 an ACL-transection model, compositional changes were observed in the contralateral limb that
15 were not due to changes in peak vertical force (McDevitt and Muir, 1976; O'Connor, 1989).
16 Furthermore, in rabbit studies the metabolic response of AC in the contralateral limb following
17 meniscectomy or sham surgery followed the same response as in the experimental and was
18 attributed to systemic factors (Moskowitz et al., 1981, Floman et al., 1980).

19 The observed AC response to loading demonstrates a slower development of
20 degenerative changes as compared to established transection-based models of OA in which gross
21 changes occur within 2-12 weeks of surgery (Hoch, 1983; Kiviranta, 1988; Setton, 1999).
22 Quantitative measures of compressive or shear force alterations resulting from meniscectomy or
23 ACL-transection have yet to be published for these frequently used animal models preventing
24 direct comparison to the load levels used in this work. The rapid onset of gross-degenerative

1 changes in these models differs from OA progression in humans that develops over many years.
2 An animal model in which degenerative changes develop more gradually may be more relevant
3 to OA in humans. The VLD model may be useful to study early events in the disease process.
4 The VLD model allows application of controlled, quantifiable load alteration, modulation of
5 altered load, and unconstrained use of the joint, without compromising the joint capsule.

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

1 **References**

- 2 Arokoski, J., Kiviranta, I., Jurvelin, J., Tammi, M., and Helminen, H.J., 1993. Long-distance
3 running causes site-dependent decrease of cartilage glycosaminoglycan content in the knee joints
4 of beagle dogs. *Arthritis & Rheumatism* 36 (10), 1451-1459.
- 5 Arokoski, J., Jurvelin, J., Kiviranta, I., Tammi, M., and Helminen, H.J., 1994. Softening of the
6 lateral condyle articular cartilage in the canine knee joint after long distance (up to 40 km/day)
7 running training lasting one year. *International Journal of Sports Medicine* 15 (5), 254-260.
- 8 Athanasiou, K.A., Rosenwasser, M.P., Buckwalter, J.A., Malinin, T.I., and Mow, V.C., 1991.
9 Interspecies comparisons of in situ intrinsic mechanical properties of distal femoral cartilage.
10 *Journal of Orthopaedic Research*. 9 (3), 330-340.
- 11 Brouwer, G.M., Tol, A.W.V., Bergink, A.P., Belo, J.N., Bernsen, R.M.D., Reijman, M., Pols,
12 H.A.P., and Bierma-Zeinstra, S.M.A., 2007. Association between valgus and varus alignment
13 and the development and progression of radiographic osteoarthritis of the knee. *Arthritis &*
14 *Rheumatism* 56 (4), 1204-1211.
- 15 Calvo, E., Palacios, I., Delgado, E., Sanchez-Pernaute, O., Largo, R., Egido, J., and Herrero-
16 Beaumont, G., 2004. Histopathological correlation of cartilage swelling detected by magnetic
17 resonance imaging in early experimental osteoarthritis. *Osteoarthritis & Cartilage* 12 878-886.
- 18 Coughlin, K.M., Peura, G.D., Fleming, B.C., Hallock, S., and Beynon, B.D., 2005. In vivo
19 loads in the medial compartment of the rabbit knee. *Clinical Biomechanics* 20 (9), 1007-1009.
- 20 Felson, D.T., Anderson, J.J., Naimark, A., Walker, A.M., and Meenan, R.F., 1988. Obesity and
21 knee osteoarthritis. The Framingham Study. *Annals of Internal Medicine* 109 (1), 18-24.
- 22 Floman, Y., Eyre, D.R., and Glimcher, M.J., 1980. Induction of osteoarthrosis in the rabbit knee
23 joint: biochemical studies on the articular cartilage. *Clinical Orthopaedics & Related Research*
24 (147), 278-286.

1 Goodman, S.N., and Berlin, J.A., 1994. The Use of Predicted Confidence Intervals When
2 Planning Experiments and the Misuse of Power When Interpreting Results. *Annals of Internal*
3 *Medicine* 121 (3), 200-206.

4 Grelsamer, R.P., 1995. Unicompartamental osteoarthritis of the knee. *J Bone Joint Surg Am* 77
5 (2), 278-292.

6 Gushue, D.L., Houck, J., and Lerner, A.L., 2005. Rabbit knee joint biomechanics: Motion
7 analysis and modeling of forces during hopping. *Journal of Orthopaedic Research* 23 (4), 735.

8 Hellio Le Graverand, M.-P., Mazzuca, S., Duryea, J., and Brett, A., 2009. Radiographic-Based
9 Grading Methods and Radiographic Measurement of Joint Space Width in Osteoarthritis.
10 *Radiologic Clinics of North America* 47 (4), 567-579.

11 Hoch, D.H., Grodzinsky, A.J., Koob, T.J., Albert, M.L., and Eyre, D.R., 1983. Early changes in
12 material properties of rabbit articular cartilage after meniscectomy. *Journal of Orthopaedic*
13 *Research*. 1 (1), 4-12.

14 Jurvelin, J., Kuusela, T., Heikkila, R., Pelttari, A., Kiviranta, I., Tammi, M., and Helminen, H.J.,
15 1983. Investigation of articular cartilage surface morphology with a semiquantitative scanning
16 electron microscopic method. *Acta Anatomica*. 116 (4), 302-311.

17 Jurvelin, J.S., Kiviranta, I., Saamanen, A.-M., Tammi, M., and Helminen, H.J., 1990. Indentation
18 stiffness of young canine knee articular cartilage- Influence of strenuous joint loading. *Journal of*
19 *Biomechanics* 23 (12), 1239-1246.

20 Jurvelin, J.S., Rasanen, T., Kolmonen, P., and Lyyra, T., 1995. Comparison of optical, needle
21 probe and ultrasonic techniques for the measurement of articular cartilage thickness. *Journal of*
22 *Biomechanics* 28 (2), 231-235.

1 Kiraly, K., Lammi, M., Arokoski, J., Lapvetelainen, T., Tammi, M., Helminen, H., and
2 Kiviranta, I., 1996. Safranin O reduces loss of glycosaminoglycans from bovine articular
3 cartilage during histological specimen preparation. *Histochemical Journal*. 28 (2), 99-107.
4 Kiviranta, I., Tammi, M., Jurvelin, J., Saamanen, A.M., and Helminen, H.J., 1988. Moderate
5 running exercise augments glycosaminoglycans and thickness of articular cartilage in the knee
6 joint of young beagle dogs. *Journal of Orthopaedic Research* 6 (2), 188-195.
7 Mak, A.F., Lai, W.M., and Mow, V.C., 1987. Biphasic indentation of articular cartilage--I.
8 Theoretical analysis. *Journal of Biomechanics* 20 (7), 703-714.
9 Mansour, J.M., Wentorf, F.A., and Degoede, K.M., 1998. In vivo kinematics of the rabbit knee
10 in unstable models of osteoarthritis. *Annals of Biomedical Engineering* 26 (3), 353-360.
11 McDevitt, C.A., and Muir, H., 1976. Biochemical changes in the cartilage of the knee in
12 experimental and natural osteoarthritis in the dog. *Journal of Bone & Joint Surgery - British*
13 *Volume* 58 (1), 94-101.
14 Moskowitz, R.W., Goldberg, V.M., and Malesud, C.J., 1981. Metabolic responses of cartilage
15 in experimentally induced osteoarthritis. *Annals of the Rheumatic Diseases* 40 (6), 584-592.
16 Mow, V.C., Gibbs, M.C., Lai, W.M., Zhu, W.B., and Athanasiou, K.A., 1989. Biphasic
17 indentation of articular cartilage--II. A numerical algorithm and an experimental study. *Journal*
18 *of Biomechanics* 22 (8-9), 853-861.
19 Newton, P.M., Mow, V.C., Gardner, T.R., Buckwalter, J.A., and Albright, J.P., 1997. Winner of
20 the 1996 Cabaud Award. The effect of lifelong exercise on canine articular cartilage. *American*
21 *Journal of Sports Medicine* 25 (3), 282-287.
22 Noyes, F.R., Schipplein, O.D., Andriacchi, T.P., Suddemi, S.R., and Weise, M., 1992. The
23 Anterior Cruciate Ligament-Deficient Knee with Varus Alignment: An Analysis of Gait
24 Adaptations and Dynamic Joint Loadings. *American Journal of Sports Medicine* 20 (6), 707-716.

1 O'Connor, B.L., Visco, D.M., Heck, D.A., Myers, S.L., and Brandt, K.D., 1989. Gait alterations
2 in dogs after transection of the anterior cruciate ligament. *Arthritis & Rheumatism* 32 (9), 1142-
3 1147.

4 Pritzker, K.P., 1994. Animal models for osteoarthritis: processes, problems and prospects. *Ann*
5 *Rheum Dis* 53 (6), 406-420.

6 Roemhildt, M.L., Coughlin, K.M., Peura, G.D., Fleming, B.C., and Beynon, B.D., 2006.
7 Material properties of articular cartilage in the rabbit tibial plateau. *Journal of Biomechanics* 39
8 (12), 2331-2337.

9 Setton, L.A., Mow, V.C., Muller, F.J., Pita, J.C., and Howell, D.S., 1997. Mechanical behavior
10 and biochemical composition of canine knee cartilage following periods of joint disuse and
11 disuse with remobilization. *Osteoarthritis & Cartilage* 5 (1), 1-16.

12 Setton, L.A., Elliott, D.M., and Mow, V.C., 1999. Altered mechanics of cartilage with
13 osteoarthritis: human osteoarthritis and an experimental model of joint degeneration.
14 *Osteoarthritis & Cartilage* 7 2-14.

15 Vanwanseele, B., Lucchinetti, E., and Stüssi, E., 2002. The effects of immobilization on the
16 characteristics of articular cartilage: current concepts and future directions. *Osteoarthritis and*
17 *Cartilage* 10 (5), 408-419.

18

19

1 **Figure Legends**

2

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

9

10 Figure 2. Rabbit with VLD attached and engaged.

11

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

14

15 Figure 4. Mechanical testing results for the **Tibia - Medial compartment - Posterior**: Least
16 square means for material properties and thickness from covariate analysis. P-values indicate
17 overall significance of treatment group based on analyses of covariance. Means not sharing a
18 common letter are significantly different based on Fishers LSD procedure ($p < 0.05$).

19

20 Figure 5. Mechanical testing results for the **Tibia - Medial compartment - Central**: Least
21 square means for material properties and thickness from covariate analysis. P-values indicate
22 overall significance of treatment group based on analyses of covariance. Means not sharing a
23 common letter are significantly different based on Fishers LSD procedure ($p < 0.05$).

24

1 Figure 6. Representative histological sections of the medial tibia of experimental limbs from A)
2 0% BW Sham and B) 44% BW (Safarin O staining, original image 4x, ∂ cutting artifact, *
3 indicates cartilage fibrillation). Mild surface erosion/fibrillation was present in the 22% and 44%
4 BW sections as compared to 0% BW. Representative sections of the medial tibia taken at area of
5 maximum cartilage thickness: C) 0% BW and D) 44% BW (H&E staining, 10x, \diamond indicates a
6 typical chondrocyte). Fewer chondrocytes were observed in sections from loaded animals
7 compared to 0% BW (Chondrocyte number = 0%: 345, 22%: 251, 44%: 220).

Figure 1

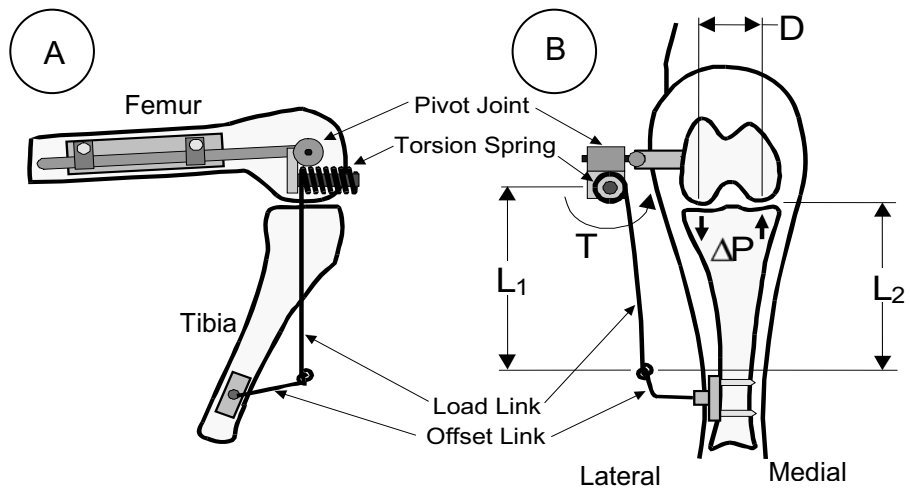


Figure 2



Figure 3

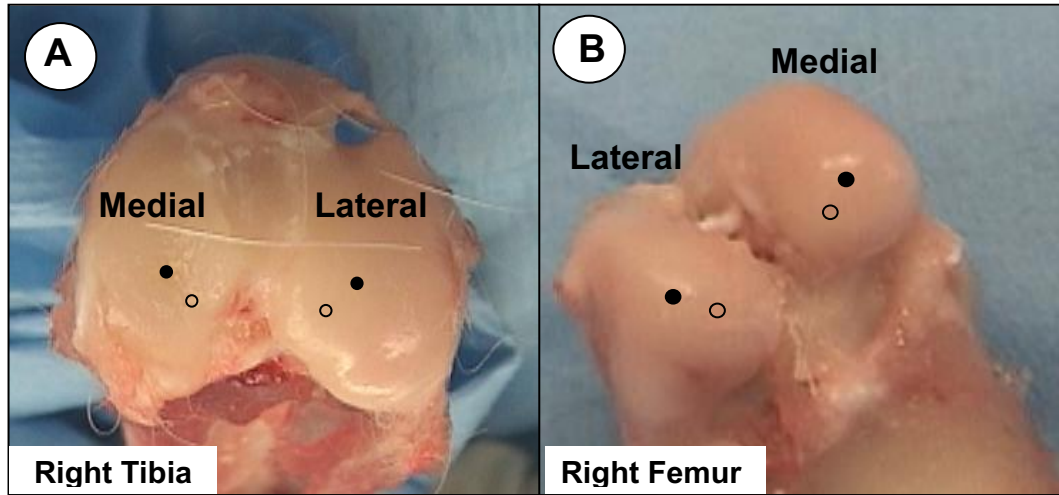
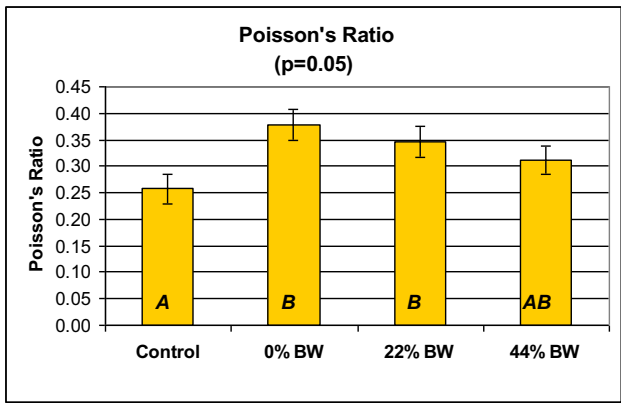
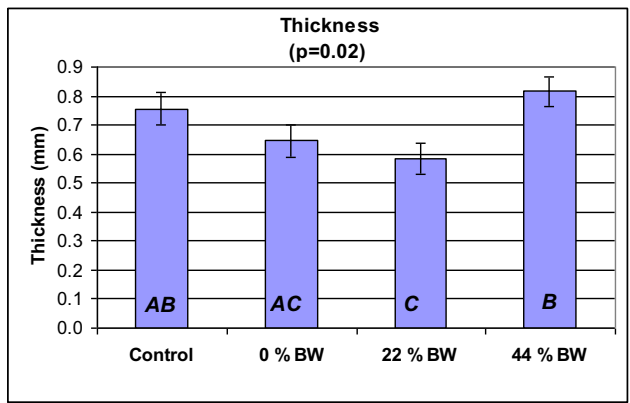
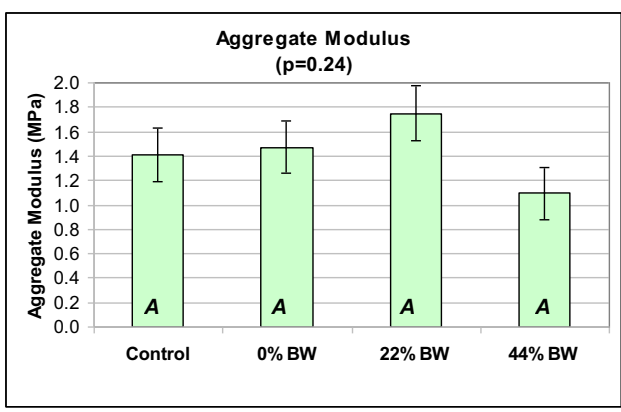
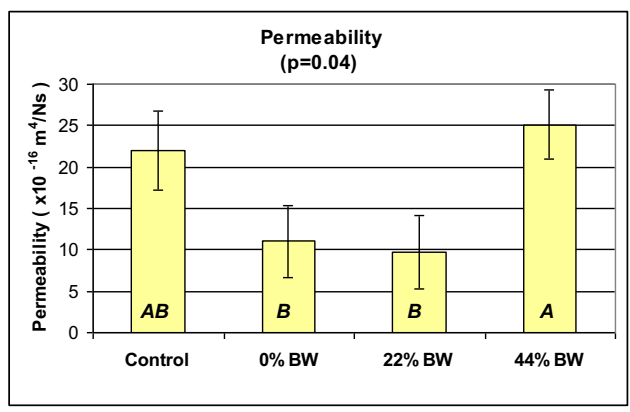


Figure 4

Tibia: Medial-posterior



Tibia: Medial-Central

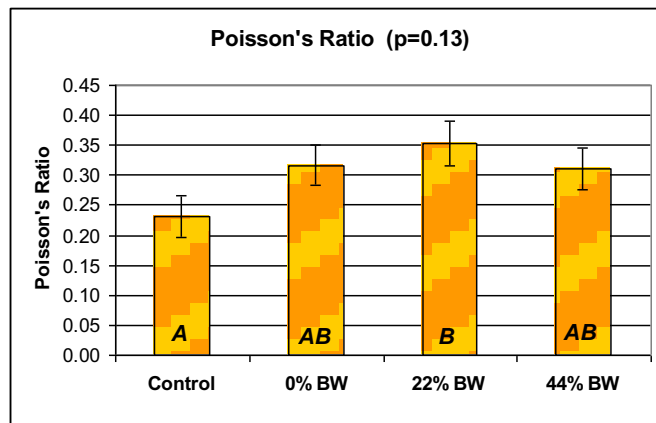
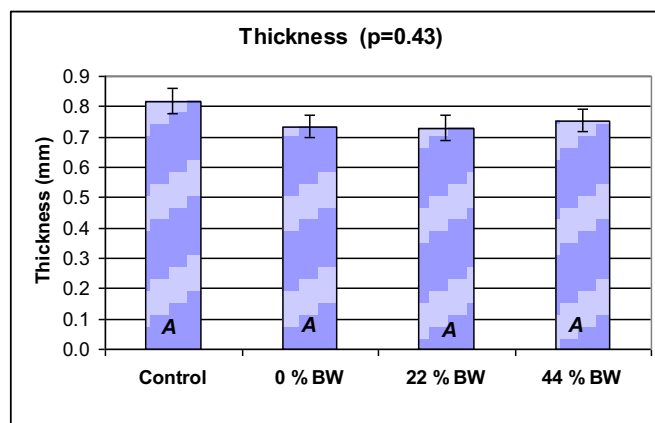
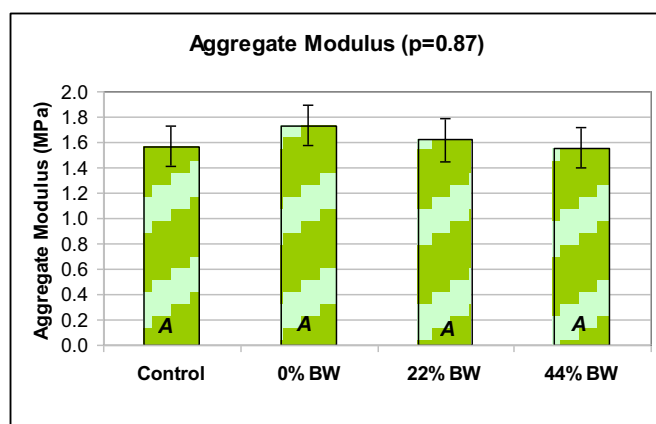
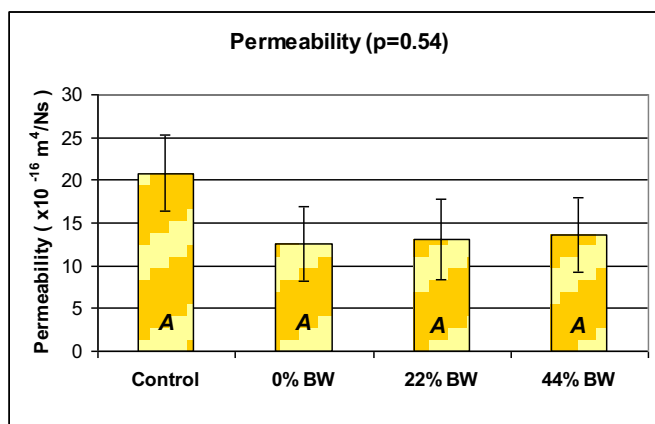


Figure 6

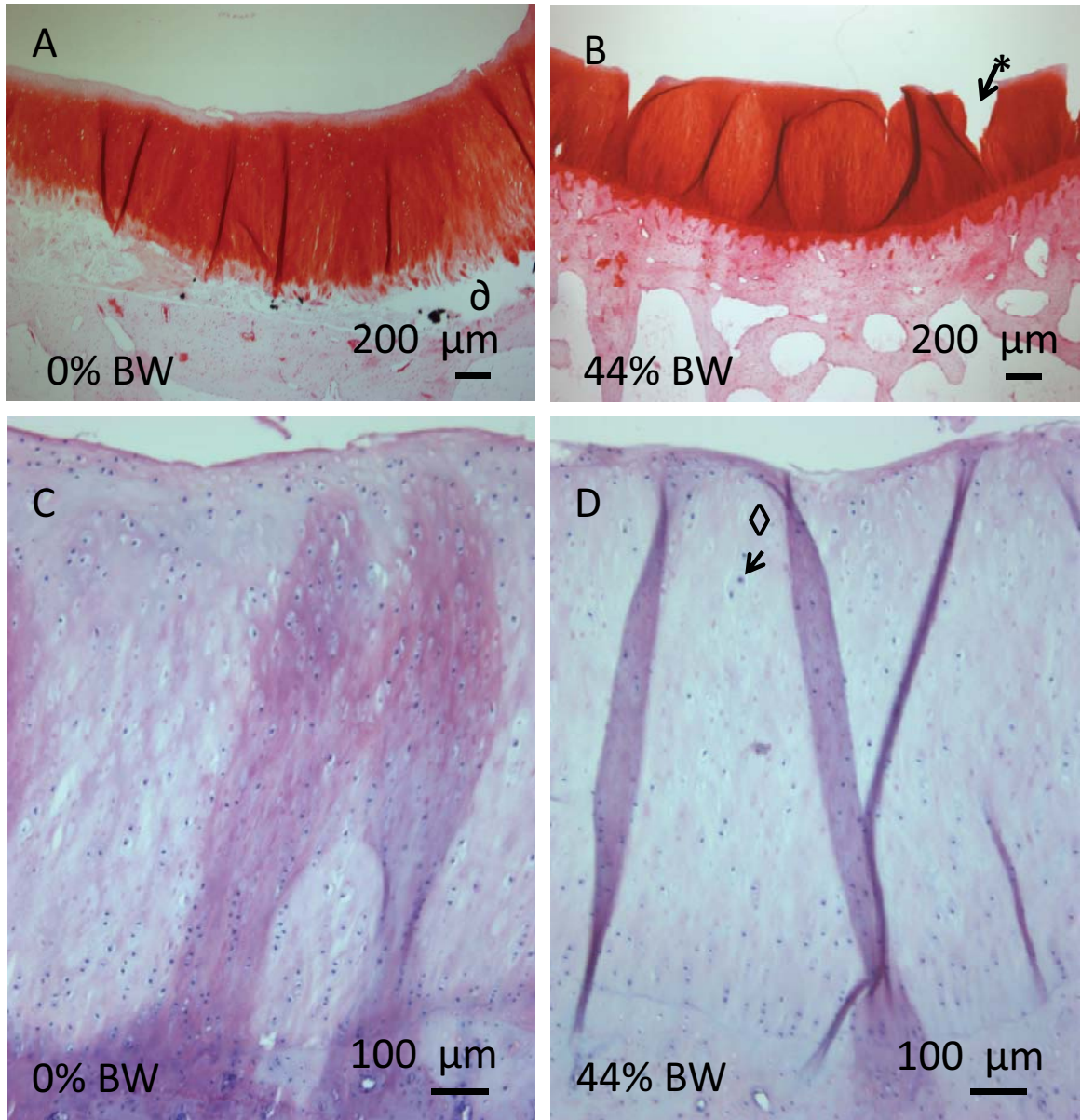


Table 1. Material Properties of Articular Cartilage of the Tibial Plateau

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)
		Lateral	Posterior	1.80 (0.42)	5.4 (2.1)	0.34 (0.06)	0.49 (0.10)
			Central	1.68 (0.38)	6.1 (4.1)	0.29 (0.06)	0.55 (0.10)
	Experimental	Medial	Posterior	1.50 (0.72)	20.5 (13.3)	0.26 (0.11)	0.78 (0.15)
			Central	1.57 (0.34)	20.9 (19.6)	0.23 (0.13)	0.84 (0.12)
		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)
0% BW (n=7)	Contralateral	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)
		Lateral	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	Medial	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)
		Lateral	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)
22% BW (n=6)	Contralateral	Medial	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)
		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)
	Experimental	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)
		Lateral	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)
44% BW (n=7)	Contralateral	Medial	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)
		Lateral	Posterior	1.70 (0.57)	7.4 (5.3)	0.33 (0.06)	0.51 (0.11)
			Central	1.56 (0.23)	5.4 (1.4)	0.33 (0.06)	0.51 (0.06)
	Experimental	Medial	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)
		Lateral	Posterior	1.92 (0.60)	6.6 (2.4)	0.31 (0.10)	0.57 (0.13)
			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 ($10^{-16} \text{ m}^4/\text{Ns}$)	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)
		Lateral	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)
0% BW (n=7)	Contralateral	Medial	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)
		Lateral	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)
	Experimental	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)
22% BW (n=6)	Contralateral	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)
		Lateral	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)
	Experimental	Medial	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)
		Lateral	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)
			Central	1.45 (0.66)	2.6 (1.8)	0.25 (0.13)	0.27 (0.13)
	Experimental	Medial	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

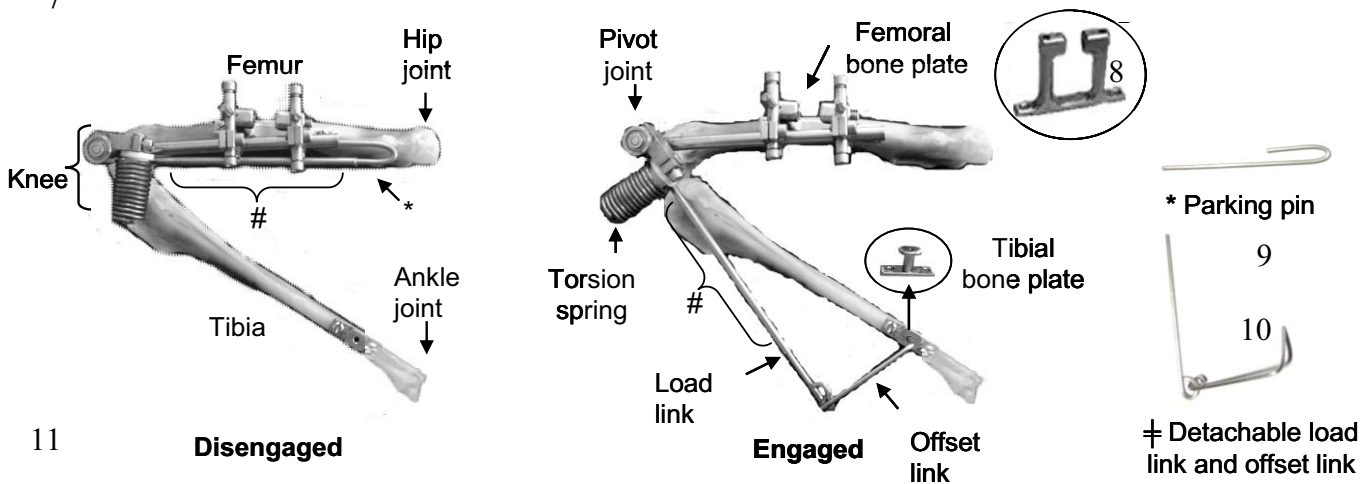
1 **APPENDIX 1: Design of a Varus Loading Device for Application to a Rabbit Hind Limb and**
2 ***In Vitro* Validation**

3
4 **A. Design of a Varus Loading Device (VLD)**

5 The Varus Loading Device (VLD) was designed to apply a varus moment to the knee
6 while allowing normal joint function. Its main beam is clamped to two pins which insert into
7 posts of a transcutaneous bone plate that is attached to the lateral aspect of the femur (Fig. A1).
8 The clamps are adjustable in all six degrees of freedom. The torsion spring, which generates the
9 varus moment, is mounted via a pivot joint to the distal end of the beam. The pivot axis is
10 aligned with the knee's flexion axis. The "fixed" end of the torsion spring is mounted to a
11 mandrel on the pivot joint housing. The mandrel can be rotated to adjust the spring tension, and
12 hence the applied varus moment results in an increased load in the medial compartment and
13 decreased load in the lateral compartment without constraining normal knee function. A
14 cannulated swingarm is mounted to the "free" end of the torsion spring. The swingarm assembly
15 consists of a load link and an offset link, connected by a ball joint. The ball joint allows the
16 angle between the load link and offset link to vary to accommodate minor changes in the length
17 of L_1 which may result from superior-inferior and anterior-posterior translations of the knee.
18 The plane of the tibial plateau in the rabbit has a posterior directed slope of approximately 25°
19 (Grover et al., 2007, Messner et al., 2001). In order to avoid inducing an internal/external torque
20 about the long axis of the tibia, the varus moment vector must act parallel to this plane.
21 Therefore, the offset link is used to align the load link at an angle of 25° relative to the tibia's
22 mechanical axis. The distal end of the offset link is inserted into a tapped hole in the
23 transcutaneous bone plate attached to the distal tibia, and delivers the applied varus moment to

APPENDIX 1. VARUS LOADING DEVICE: DESIGN AND VALIDATION

1 the tibia. The bone plates and bone screws (2.0 mm diameter) are made from 316L stainless
 2 steel. The total weight of the VLD is less than 25 g. The VLD allows moments to be applied to
 3 the knee only during the designated treatment period. At all other times, the moment can be
 4 “disengaged” by detaching the distal portion of the load link along with the offset link (Fig. A1).
 5 The proximal portion of the load link is rotated into alignment with the femur and fixed to the
 6 main beam and secured with a parking pin.



13 Figure A1. Lateral view of VLD applied to a rabbit femur and tibia. Posts of the VLD are
 14 inserted into tapped holes of the transcutaneous bone plates and secured with set screws. The
 15 offset link and a portion of the load link (‡) detach from the VLD. The residual load link (#) is
 16 aligned with the main beam and secured with a parking pin (*) to disengage the device.

17

18 The magnitude of the spring torque, T , required to generate a desired change in load, ΔP , can
 19 be calculated knowing the appropriate moment arm lengths (Manuscript Fig. 1, Equation 1). The
 20 intercompartmental moment arm, D , is measured from an anterior-posterior (A-P) radiograph taken
 21 with the knee in 30° flexion. The other moment arms are measured from a lateral radiograph. The

APPENDIX 1. VARUS LOADING DEVICE: DESIGN AND VALIDATION

1 torsion spring has a constant of $0.78 \times 10^{-3} \text{ Nm/}^\circ$ and is designed to be deflected between 360° and
2 540° to reach target torque. Small changes in deflection ($\sim 10^\circ$) would result in small changes
3 ($< 2\%$) in the torque developed. In practice it is difficult to precisely align the VLD's pivot axis
4 with the knee's flexion axis and any angular misalignment would cause the spring deflection to
5 vary throughout the flexion cycle. Misalignments on the order of 3 mm and 10° , however, can be
6 readily accommodated. Any position misalignment would cause the tibial moment arm, L_2 , to vary
7 throughout the flexion cycle. The magnitude of L_2 is approximately 45 mm and small variations
8 ($\sim 3\text{mm}$) would result in small changes in applied moment ($< 6\%$). The change in contact load, ΔP ,
9 with application of the VLD represents an increase in compressive load in addition to the normal
10 physiologic loads produced by muscle activity, gravity, and inertial effects and does not quantify
11 the total load experienced by the medial compartment. A reduction in contact load in the lateral
12 compartment of magnitude ΔP would also occur. If the lateral compartment undergoes distraction,
13 then the tensile load will be taken by the lateral collateral ligament, joint capsule, and cruciate
14 ligaments. In addition to altering the compressive joint load, the VLD generates a medial/lateral
15 directed shear load at the joint line. Based on equilibrium analysis, the magnitude of the shear load
16 is estimated to be less than $\frac{1}{8} \Delta P$ acting in the coronal plane.

17

18 **B. *In Vitro* Validation of the VLD**

19 The increase in medial compartment compressive load generated by the VLD was measured
20 directly *in vitro* and compared to the predicted value. In addition, the altered load was evaluated
21 over the range of flexion in the rabbit knee.

22 The VLD was installed on five mature New Zealand White rabbit hind limb specimens.
23 The medial joint capsule was opened and the medial tibial plateau, including the medial meniscus,

APPENDIX 1. VARUS LOADING DEVICE: DESIGN AND VALIDATION

1 cartilage and subchondral bone, was resected down to a level 6 mm below the joint line. The tibial
2 attachments of the cruciate ligaments and medial collateral ligaments were left intact. A miniature
3 load cell, 6 mm dia. x 6 mm tall, (ALD Micro, ALD Design, Buffalo, NY USA) was inserted in
4 the resection cavity and cemented in place, maintaining the original level of the joint line (Fig. A2).
5 The medial femoral condyle contacted the load sensor ensuring that the entire compressive load
6 developed in the medial compartment was measured directly by the load cell.

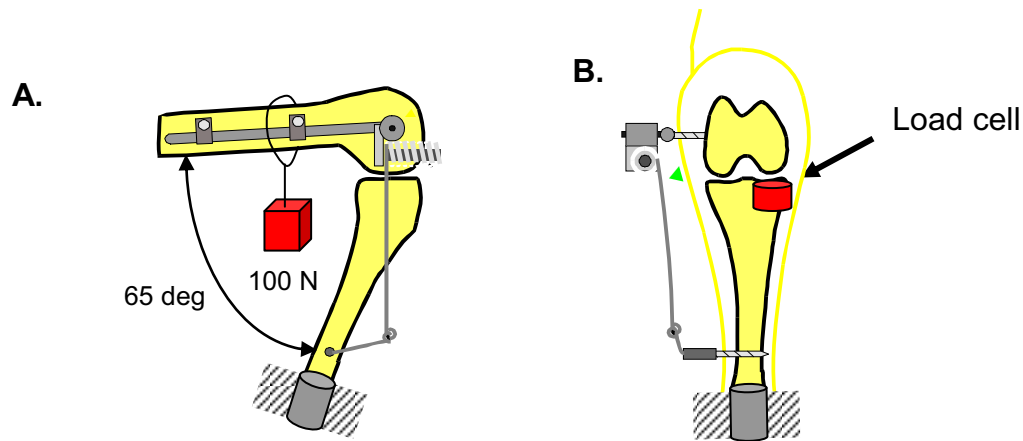


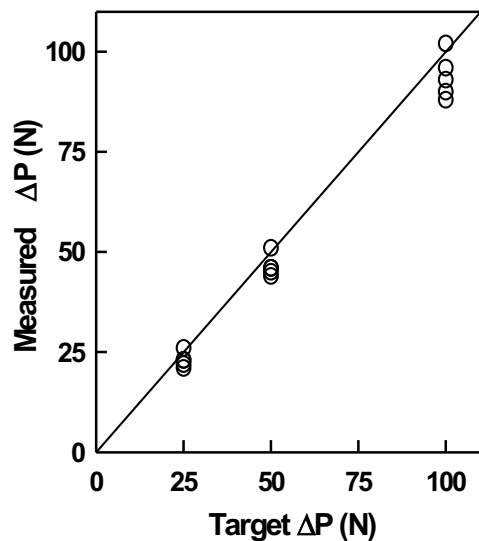
Figure A2. Sketch of rabbit hind limb specimen for *in vitro* testing. A). Lateral view of hind limb with VLD applied and 100 N applied to simulate physiologic joint load. B). Anterior-posterior view of hind limb illustrating excision of the medial tibial plateau and placement of the load cell.

7
8
9 The femoral head was mounted in a ball joint and the distal tibia was fixed in a clamp,
10 positioned such that the femur was horizontal, and the flexion angle set to approximately 115° to
11 simulate the flexion angle in the rabbit knee during gait. This oriented the tibial plateau
12 horizontally. A 100 N weight was hung on a cable attached to the femur just proximal to the knee
13 joint. This generated a compressive tibio-femoral load approximately equally divided between the
14 medial and lateral compartments. This was intended to simulate the physiologic joint load

APPENDIX 1. VARUS LOADING DEVICE: DESIGN AND VALIDATION

1 normally present in the knee due to muscle activity, gravity and inertial loads. The compression
2 load cell was zeroed with this static load in place. Moment arms were measured. The target torque
3 level was set and measured using a spring scale acting over the moment arm L_1 . Three values of
4 ΔP were targeted: 25 N, 50 N, and 100 N. Using equation 1, the spring torque required to generate
5 each of these was calculated (Manuscript Fig. 1). These torques were applied to the VLD in
6 succession and the actual ΔP generated, as measured by the load cell, was recorded.

7 For all data points, the measured ΔP was within 14% of the target ΔP (Fig. A3). The slope
8 of the least squares fit line through the data points was not significantly different than 1. One
9 principal source of variability was in setting the target spring torque and a more accurate torque
10 sensor will be used in future work. These results demonstrate the overall feasibility of applying a
11 known compressive overload, ΔP , to the medial compartment of the knee using the VLD.



12
13 Figure A3. Results of experiment evaluating the feasibility of applying a known ΔP with the
14 VLD. Scatter plot shows the measured vs. target values of ΔP for five *in vitro* test specimens.

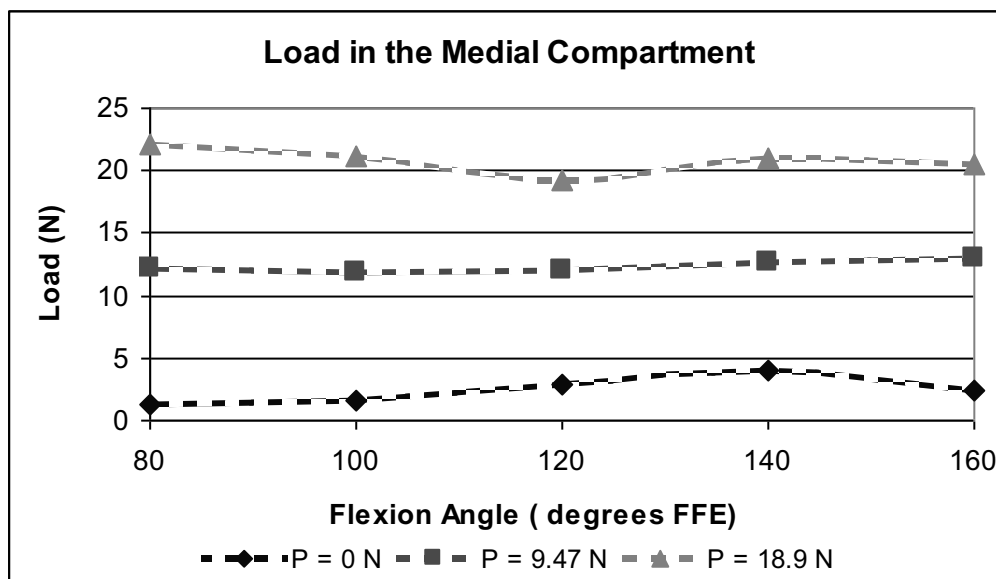
15

APPENDIX 1. VARUS LOADING DEVICE: DESIGN AND VALIDATION

1 To evaluate the altered load applied over the range of flexion in the rabbit knee, a thin
2 flexible sensor was used to measure compressive force in the medial compartment (Flexiforce
3 A201, Tekscan, thickness = 0.13 mm). The soft tissue of the hind limb was excised leaving the
4 joint capsule and ligaments of the knee intact. Medial-anterior and posterior incisions of the
5 joint capsule were made and the sensor was inserted under the medial meniscus. Suture taped to
6 the anterior or posterior edges of the sensor was anchored to the tibia to prevent migration of the
7 sensor during testing. Data was collected for 1 minute followed by a 20 minute unloading period
8 between readings. The force in the medial compartment of the knee was measured at 80, 100,
9 120, 140, and 160° from full extension with the VLD engaged at 0, +22 and +44% BW. The
10 lateral compartment femoral condyle was then excised and the sensor output was recorded with
11 the application of known weights to calibrate the sensor.

12 The measured loads in the medial compartment were uniformly increased over baseline
13 values with VLD application for knee flexion of 80-160° with an RMS error <10% (Fig A4).

14



15

APPENDIX 1. VARUS LOADING DEVICE: DESIGN AND VALIDATION

1 Fig. A4. In vitro measurement of increased load (ΔP) in the medial compartment of the knee
2 with application of the VLD over flexion angles representing normal range of motion in the
3 rabbit. Measured load levels in the medial compartment of the knee were uniformly increased
4 over baseline values ($\Delta P=0$) over 80-160° of flexion for ΔP -values representing +22% and 44%
5 BW.

6
7 This work presents a method to apply controlled overloads to the medial compartment of
8 the rabbit knee via a varus loading device to study the effects of altered loading on articular
9 cartilage without disrupting the joint capsule. The ability to set the desired load level as well as
10 remove the altered loading allows the investigation of the effects of load level and loading
11 exposure.

12

13 **Appendix References:**

14 Grover, D. M., Chen, A. A. & Hazelwood, S. J. (2007) Biomechanics of the rabbit knee and
15 ankle: Muscle, ligament, and joint contact force predictions. *J Biomech*, 40, 2816-2821.

16 Messner, K., Fahlgren, A., Persliden, J. & Andersson, B. M. (2001) Radiographic joint space
17 narrowing and histologic changes in a rabbit meniscectomy model of early knee
18 osteoarthritis. *American Journal of Sports Medicine.*, 29, 151-60.