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BLUNT FORCE INJURY TO CARTILAGE: SOME EFFECTS OF EXERCISE AND A NUTRACEUTICAL

presented by

LYNN MICHELLE MARTIN

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M.S. degree in Mechanical Engineering

Roger C. Haut
Major Professor's Signature

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Date

BLUNT FORCE INJURY TO CARTILAGE: SOME EFFECTS OF EXERCISE AND A NUTRACEUTICAL

By

Lynn Michelle Martin

A THESIS

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in partial fulfillment of the requirements
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ABSTRACT

BLUNT FORCE INJURY TO CARTILAGE:
SOME EFFECTS OF EXERCISE AND A NUTRACEUTICAL

By

Lynn Michelle Martin

Osteoarthritis is a degenerative joint disease characterized by loss of articular cartilage and alterations to the underlying subchondral bone. There is evidence of hereditary defects that may predispose to osteoarthritis, yet other factors such as age, excessive joint loading, and joint injury increase the risk for development of this disease. The current study uses an *in vivo* post-traumatic animal model to investigate blunt impacts on the patello-femoral joint. Treatment options for the relief of pain due to chronic joint disease are currently limited. In Chapter 1 the role of two nutraceuticals, glucosamine and chondroitin sulfate, taken before and after a 6.0 joule blunt impact to the patello-femoral joint are examined in a regularly exercising animal model. Regular exercise has been shown to have beneficial effects on preserving articular cartilage in the knee after a blunt force trauma. Chapter 1 documented that pre-trauma exercise may play a role in strengthening the cartilage to protect it from severe trauma due to a blunt impact. Chapter 2 investigates the effects of mechanically stressing cartilage, with intermittent cyclic compressive loading of chondral explants. This regular loading tends to increase the mechanical properties of the cartilage. Finally, this investigation of stressed tissue, or "exercise" versus "no-exercise" is further examined with use of an animal model in Chapter 3. In this chapter chronic joint degeneration is accelerated in an animal model by increasing the amount of impact energy to the patello-femoral joint to 10.0 joules.

DEDICATION

I would like to thank my parents for their never ending support and encouragement throughout my life. Without their support I would never have had the drive to accomplish all that I have in my life and academic career.

ACKNOWLEDGEMENTS

I would like to acknowledge and thank my professor, mentor, and good friend Dr. Roger Haut for all he has taught me in the past two years. I am extremely grateful to Dr. Wright and Dr. Orth for serving on my committee. I would like to extend a sincere thank you to Clifford Becket, for never hesitating to take the time to what I needed to know and more, and for always helping to find "the answer" if it wasn't already known. I would also like to thank Jane Walsh and Jean Atkinson, for all of their hard work and dedication. I would like to thank all of the undergraduate students for all of their hard work and assistance: Zach Kaltz, Austin McPhillamy, and Nurit Golenburg. Last but not least, I would like to thank my fellow graduate students for their help and friendship: Eugene Kepich, Mike Sinnott, Eric Meyer, Derek Baars, Steve Rundell.

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INTRODUCTION

Each year in the United States over 775,000 children under the age of 15 are treated in hospital emergency rooms for sports-related injuries. While apparently 5 percent of these injuries involve broken bones, some of these sports-related injuries can have more lasting effects to the individual. A single knee injury can put a person at five times the risk for adulthood osteoarthritis (Arthritis Foundation, 2005). It is estimated that 60% of the population will have symptoms of osteoarthritis by the age of 65 (Green, 2001).

Osteoarthritis is a degenerative joint disease characterized by loss of articular cartilage and alterations to the underlying subchondral bone. Articular cartilage is a tough, elastic connective tissue covering the ends of joints. Its purpose is to distribute load and provide a near frictionless surface for the movement of joint surfaces against one another. Cartilage is composed of chondrocytes (cells) surrounded by a matrix of water, collagen (fibrous proteins), and proteoglycans (Figure 1). Proteoglycans are protein aggregates having polysaccharide side-chain units known as glycosaminoglycans (GAGs). As the joint is subjected to load, the cartilage will deform in order to distribute the load, causing compressive, tensile, and shear stresses throughout the cartilage (Mow and Setton, 1998). The function of the collagen is to provide the cartilage with tensile strength (Mow

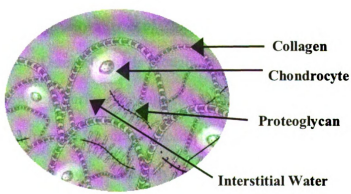

Figure 1. The extracellular matrix of articular cartilage is composed mainly of collagen fibers, proteoglycans, and water.

and Setton, 1998), whereas the proteoglycans are associated more with the stiffness properties of the cartilage in compression (Helminen *et al.*, 1992). The content and structure of proteoglycans and collagen fibers varies throughout the depth of the cartilage. The matrix can be divided into three regions: a superficial tangential zone, a middle zone, and a deep zone (Figure 2).

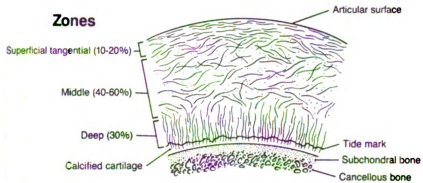

Figure 2. A sketch showing the cross section of cartilage, illustrating the collagen network and the three distinct regions of this tissue.

Hereditary defects may predispose to osteoarthritis, yet other risk factors such as age, excessive joint loading, and joint injury increase the risk for development of this disease (Buckwalter *et al.*, 2004; Helminen *et al.*, 1992; Gelber *et al.*, 2000; Marsh *et al.*, 2002). Osteoarthritis is thought to be initiated by fibrillation (the unbinding of collagen fibrils and surface fraying) and swelling of the cartilage matrix due to the influx of fluid. This increased hydration leads to a softening of the articular cartilage, which increases the pressure on the underlying subchondral bone (Radin *et al.*, 1996). These early stages of osteoarthritis may initiate an increase in the subchondral bone thickness, and lead to changes such as osteophyte formations (bony outgrowths) and erosion of the articular cartilage, eventually causing complete loss of this soft tissue (Figure 3).

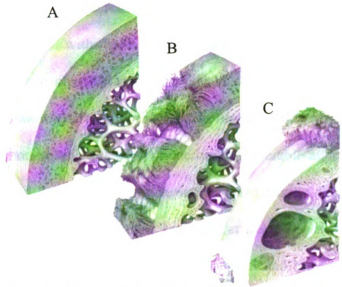

Figure 3. The progressive stages of OA. **A)** Normal articular cartilage and bone. **B)** Cartilage surface becomes fibrillated and the subchondral bone thickens. **C)** Total loss of cartilage with bone cyst formation.

Our laboratory has developed a post-traumatic animal model to study the degenerative joint changes *in vivo* using a Flemish Giant rabbit (Haut *et al.*, 1995). Methods of evaluating changes in joint tissue include stress-relaxation testing via mechanical indentation to determine changes in mechanical properties of articular cartilage (Garcia, 1998; Hayes *et al.*, 1972), histological sectioning to assess structural and cellular changes throughout the depth of the cartilage, and biochemical testing to measure alterations in tissue composition, such as in the content of proteoglycans. Previous studies by our laboratory have shown significant softening of the cartilage, thickening of the subchondral plate, and histological degradation by 7.5 months post-impact on the patello-femoral joint of rabbits (Newberry *et al.*, 1997; Newberry *et al.*, 1998; Ewers and Haut, 2000; Ewers *et al.*, 2002). End-stage disease with complete loss of articular cartilage has eluded various studies by this laboratory.

Treatment options for the relief of osteoarthritis pain are very limited. Mild cases are commonly treated with non-steroidal anti-inflammatory drugs (NSAIDs). Unfortunately NSAIDs can have side effects, such as stomach ulcers and kidney damage, and do nothing to slow the progression of the disease. However, the nutraceuticals glucosamine and chondroitin sulfate have received considerable attention as a treatment for relieving pain and delaying the progression of osteoarthritis. (Braham *et al.*, 2003; Pavelka *et al.*, 2002; Richy *et al.*, 2003; Tiraloche *et al.*, 2005). Little is known about these nutraceuticals and their effects on the mechanical and biochemical properties of cartilage *in vivo*. Chapter 1 evaluates the efficacy of glucosamine and chondroitin sulfate on enhancing the mechanical and biochemical properties of cartilage in an animal model prior to and after a blunt impact to its patello-femoral joint. These studies describe the results of both acute and chronic studies on the tissue after a severe (6.0 J) impact to the joint.

In vivo experiments on the response of a joint to blunt force trauma using animal models are very expensive and time consuming (Parkkinen *et al.*, 1989). *In vivo* models also create difficulties in controlling the loading situation and the cellular response of joint tissues following trauma (Parkkinen *et al.*, 1989). Many new mechanical explant testing systems have been developed to better control loading of the joint tissues by using chondral or osteochondral cartilage explants (Torzilli *et al.*, 1997; Sah *et al.*, 1989; Sauerland *et al.*, 2003). Our laboratory has recently created a "cartilage exerciser" to cyclically load chondral explants. Chapter 2 examines the effects of intermittent cyclic loading and the differences in mechanical properties and cell death between loaded and non-loaded chondral explants in a series of pilot studies with this newly developed

device. The role of exercise in the rehabilitation of a joint after trauma has been a subject of controversy over the years. Numerous studies have shown beneficial effects of regular exercise (Jurvelin *et al.*, 1986; Otterness *et al.*, 1998; Weaver and Haut, 2005), however, evidence has also demonstrated a deleterious effect of excessive (repetitive) joint loading in normal and injured joints (Buckwalter *et al.*, 2004).

In a previous study by another laboratory using a high intensity 10.0 J blunt impact animal model, advanced signs of cartilage degeneration were documented as early as 3 months post-impact (Mazieres *et al.*, 1987). The model showed a thickening of the subchondral bone at 3 months post-trauma, and exposure of subchondral bone by 6 months. In contrast, in a similar study by our laboratory using Flemish Giant rabbits with a 10.0 J impact showed there were few signs of advanced disease by 7.5 months post trauma (Weaver, 2001). However, a major difference noted between the studies by Mazieres *et al.* and Weaver was the level of post-trauma exercise. The rabbits in the Mazieres study were confined to cage activity, whereas the rabbits in the Weaver study were subjected to a daily exercise regimen. Chapter 3 addresses the issue of regular exercise versus normal cage activity in a high intensity (10.0 J) impact animal model.

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CHAPTER ONE

ADMINISTRATION OF A NUTRACEUTICAL AND EXERCISE TO HELP PROTECT JOINT CARTILAGE FROM TRAUMA

ABSTRACT

Blunt force trauma to the patello-femoral joint has been shown to cause degradation of cartilage, often times leading to degenerative disease of the joint such as osteoarthritis. Treatment options for the relief of pain due to degeneration have been very limited. New chondroprotective agents have been introduced to help normalize the cartilage matrix, and possibly strengthen the cartilage by increasing the synthesis of proteoglycans. Two studies were executed, where we supplemented the daily feed of Flemish Giant rabbits with 2% Cosamin®DS (containing glucosamine and low molecular weight chondroitin sulfate) before impacting the patello-femoral joint with a 6.0 Joule impact. All animals were exercised regularly, and a non-diet supplemented group was used for a controlled comparison. Mechanical, histological, and biochemical observations were made in the first study immediately after impact. The second study consisted of a short-term supplemented group (supplementation only before impact), a long-term supplemented group (supplementation before and after impact), and a non-supplemented control group. All specimens underwent an additional 24 weeks of exercise after impact. Results of the current studies revealed less of a difference in fissuring between impacted and non-impacted limbs, a slight increase in stiffness, and a trend for a decrease in permeability in the Cosamin®DS treated groups.

INTRODUCTION

Each year in the U.S. an estimated 30 million children and adolescents participate in organized sports (NIH, 1991), and approximately 150 million adults participate in non work-related physical activities (CDC, 2003). With a recent societal emphasis on a healthy lifestyle, more people are beginning to exercise and are becoming involved in sports activities. Yet participation in sports has a risk of injury and has evolved as a cause of osteoarthritis (OA), especially in hip and knee joints (Gelber *et al.*, 2000).

Severe impact trauma to a joint has been shown to damage the articular cartilage matrix and kill cells (Lewis *et al.*, 2003), and is also a suspected factor in the initiation of progressive disease, such as osteoarthritis (Gelber *et al.*, 2000; Marsh *et al.*, 2002). Our laboratory has developed a model using a single blunt impact to the flexed patello-femoral (PF) joint of Flemish Giant rabbits (Haut *et al.*, 1995), involving regular treadmill exercise of the animals (Oyen-Tiesma *et al.*, 1998). This model shows a significant softening of the retro-patellar cartilage, and an increase in the number of fissures, average fissure depth, and total fissure length on the impacted limb at 4.5 months post-trauma (Ewers *et al.*, 2002). At 7.5 months post-trauma, a significant increase in permeability, thickness of the subchondral plate (Ewers *et al.*, 2002), and loss of proteoglycans has been documented (Ewers and Haut, 2000).

Various interventions have been introduced as possible treatments to mitigate the development of clinical OA. Polysulfated glycosaminoglycan treatments have been shown to inhibit the degradation of articular cartilage both clinically (Howell *et al.*, 1986; May *et al.*, 1988), and also *in vivo* using a post-trauma animal model (Ewers and Haut, 2000). These treatments have helped to limit softening of the articular cartilage, without

changes in underlying bone and fissure depth (Ewers and Haut, 2000). Regular treadmill exercise of the animal model, as opposed to cage activity, has also shown beneficial effects by limiting the amount of histological degradation of the cartilage such as ossification/calcification and erosion (Weaver and Haut, 2005).

Chondroprotective therapeutic agents have been introduced as a method of slowing cartilage degeneration, normalizing the cartilage matrix, and possibly stimulating the synthesis of glycosaminoglycans (Lippiello *et al.*, 2000). Two of these agents, glucosamine and chondroitin sulfate, have shown some degree of efficacy in the relief of joint pain and reduction of joint space narrowing in patients with clinically diagnosed osteoarthritis (Richy *et al.*, 2003). *In vitro* and *in vivo* studies have shown that this nutraceutical enhances the synthesis of cartilage matrix proteoglycans (PG's); (Oegema *et al.*, 2002; Tiraloche *et al.*, 2005), especially in mechanically stressed tissue (Lippiello, 2003). However, little is known about this nutraceutical and its effects on the mechanical properties in the *in vivo* setting.

Two separate studies will be discussed in this chapter. In the first study, the acute study, the hypothesis was that 2 months administration of a commercial nutraceutical, Cosamin®DS (containing glucosamine and low molecular weight chondroitin sulfate), to an exercising animal model would be effective in enhancing the tolerance of joint tissue cartilage to acute blunt impact, by increasing the level of tissue proteoglycans (PG's). In theory, this increase in PG's would stiffen the retro-patellar cartilage and reduce the extent of acute damage under a defined impact load.

After the results of the acute study, it was hypothesized that more time for degradation may be necessary for the supplement to have a significant effect, so a chronic

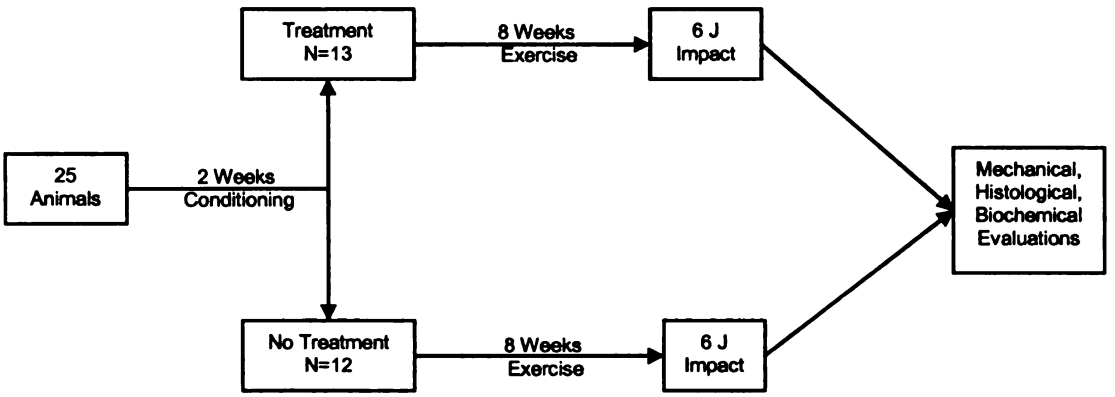
study was conducted with another group of animals. The hypothesis of the chronic study was that continued diet supplementation after blunt impact trauma to the joint would limit the degree of chronic degeneration in the joint cartilage, based on its biomechanical properties and histological appearance after 6 months post blunt impact trauma.

METHODS

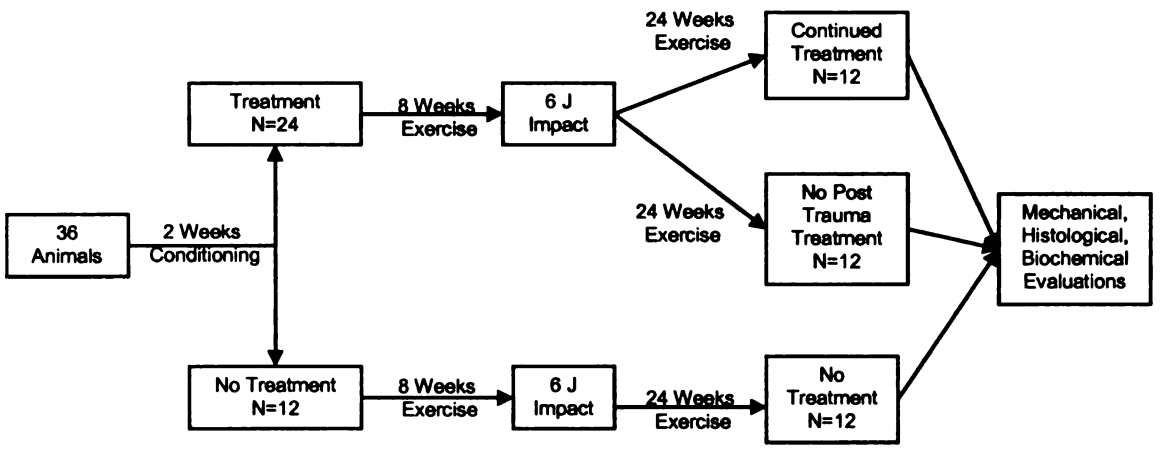
A total of sixty-one mature Flemish Giant rabbits were used in two separate studies. Twenty-five of the rabbits (5.7 ± 0.5 kg, 6-8 months of age) were used in an acute study. Another group of animals, thirty-six rabbits (5.7 ± 0.6 kg, 6-8 months of age) were purchased from the same breeder at the end of the acute study, and used for a chronic study. Animal experiments were conducted with the approval of the All-University Committee on Animal Use and Care. For the acute study the rabbits were randomly split into two groups: a control group with no dietary supplementation (n=12), and a group that had their 200g of daily feed supplemented with 2% Cosamin®DS (Nutramax Laboratories, Inc., Edgewood, MD) (n=13) (Figure 1a). For a two-month pre-impact period, all animals were exercised 10 minutes a day, 5 days a week at 0.3 mph on a treadmill (Oyen-Tiesma *et al.*, 1998). When not exercising, all animals were housed individually in cages (122 cm x 61 cm x 49 cm). At the end of the two-month exercising period, both diet-supplemented and normal diet animals were euthanized with a lethal injection of Pentabarbitol (85.9 g/kg) and within 5 minutes, received a single blunt impact to the right patello-femoral joint (discussed later).

For the chronic study the rabbits were first randomly divided into two groups: a control group with no dietary supplementation (n=12); and a group that had their 200g of

daily feed supplemented with 2% Cosamin®DS (Nutramax Laboratories, Inc., Edgewood, MD) (n=24). For the two-month pre-impact period all animals were exercised 10 minutes a day, 5 days a week at 0.3 mph on a treadmill. When not exercising, all animals were housed individually in cages (122 cm x 61 cm x 49 cm). After the 8 weeks of exercise, all animals were anesthetized (2% Isoflurane and oxygen) and received a single blunt impact to the right patello-femoral joint. The animals receiving dietary supplementation were then split into two groups of 12. One group continued to receive daily dietary supplementation for 24 weeks, while the other group had a normal diet. Post trauma, all animals were allowed a 5-day period of rest before continuing their daily exercise regimen for 24 weeks (Figure 1b). At this time all animals were euthanized with a lethal injection of Pentabarbitol (85.9 g/kg) (Weaver, 2001).



(a)



(b)

Figure 1. (a) Acute study schedule; **(b)** chronic study schedule.

Impacts were administered with a gravity drop fixture, which has been used in previous studies by this laboratory. Blunt impact was administered to the right hind patello-femoral joint (Newberry *et al.*, 1998) (Figure 2) (see Appendix D for standard operating procedure). Each animal was placed in a specially designed chair that held the right hind limb rigid, while flexed at 120° with the animal supine and the femur aligned vertically. A strap was placed across the left hind limb, which prevented the pelvis from rotating during impact. Six joules of impact energy was administered by dropping a 1.33

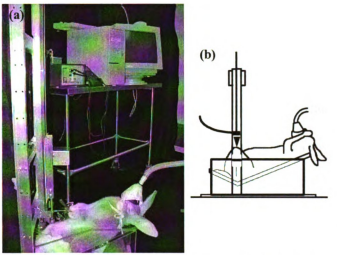


Figure 2. (**a**) Photograph of the impact set-up; (**b**) Sketch illustrating the impact load directed onto the patella.

kg mass from a height of 0.46 m with a rigid impact interface. The impact did not result in bone fracture. The dropped mass was arrested electronically after the first impact to prevent multiple impactions. A load transducer (model 31/1432: Sensotec, Columbus, OH, U.S.A.) with a 2.2 kN capacity was attached behind the impact head to record the impact loads. Experimental data were collected at 10 kHz by a personal computer equipped with an analog-to-digital board. The peak load and time to peak were recorded from the load versus time curves (Figure 3).

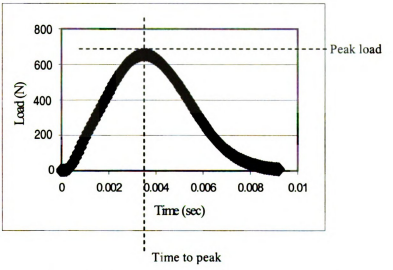


Figure 3. Typical plot of load-time data collected during impact.

Immediately after sacrifice, impacted and un-impacted patellae were excised and matrix damage was assessed. Retro-patellar surfaces were wiped with India ink, photographed at 25X under a dissection microscope (Wild M5A, Wild Heerbrugg Ltd., Switzerland), and evaluated in terms of total surface fissure length using digital image software (SigmaScan, SPSS Inc., Chicago, IL) (Ewers *et al.*, 2002). The patellae were then immersed in room-temperature, phosphate buffered saline (pH 7.2) for mechanical indentation tests on the retro-patellar cartilage (see Appendix E for standard operating procedure). Briefly, each patella was placed in a clamp attached to a camera mount (Bogen, Ramsey, NJ) which was secured to the base of a custom made mounting frame, which allowed three degrees of movement for precision placement of the patella under the indenter tip (Figure 4). The camera mount allowed rotation of the patella, while the mounting plate allowed translation. The mounting insured indentation tests were performed perpendicular to flat locations on the patella. The tests were performed using a computer controlled stepper motor (Physik Instruments, Waldbrom, Germany: model M-168.30), at two different sites on the lateral retro-patellar facet (Figure 5). A 1.0 mm diameter flat, non-porous probe was pressed 0.1 mm into the cartilage in 30 ms and held for 150 seconds while resistive loads of relaxation were measured (Data Instruments, Acton, MA: model JP-25, 25 lb capacity), amplified, and collected at 1000 Hz for the first second and 20 Hz thereafter. The cartilage was then allowed to recover for 5 minutes, and the test was repeated with a 1.5 mm diameter flat, non-porous probe. After another 5-minute recovery, the thickness of the indentation site was determined by depressing a needle probe into the cartilage.

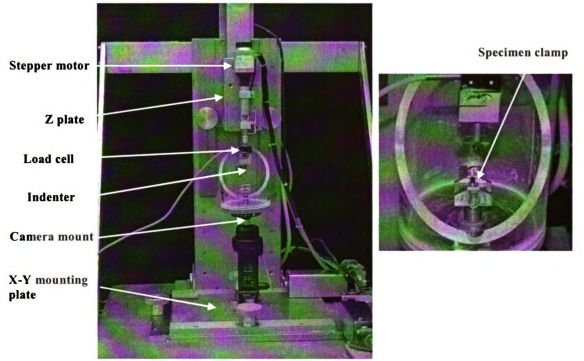


Figure 4. Photograph of the indentation test fixture. The X-Y mounting plate allows for left/right or forward/backward placement, and the Z plate allows for up.down placement. The camera mount allowed for rotation of the sample to set the surface perpendicular to the indenter.

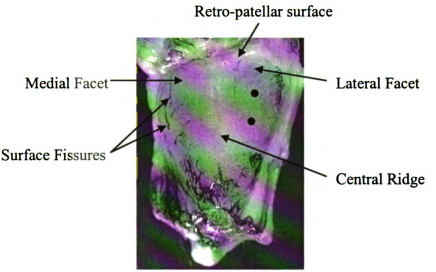


Figure 5. Photograph of excised rabbit patella. (Original photograph = 25X). ● Indicates two sites where mechanical indentation tests are performed.

Cartilage has two phases (solid and liquid) with a superficial zone formed by sheets of tightly woven collagen fibrils (Figure 2, pg 2), which suggests a model with a Young's modulus in the plane (E_{11}) different than that in the direction perpendicular to the surface (E_{33}) (Garcia *et al.*, 1998) (Figure 6). Therefore, mechanical data were analyzed using a biphasic (poroelastic) model having a transversely isotropic (TI) solid structure. Four elastic parameters (E_{11}, E_{33}, G_{13}, and ν_{31}) and two permeability measures (k_1 and k_3) were computed using a curve-fitting algorithm (Garcia *et al.*, 1998). Poisson's ratio ν_{12} was assumed equal to:

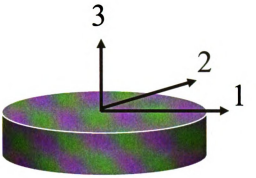


Figure 6. Illustration of coordinate system designated to cartilage. Isotropy is assumed in the plane 1-2.

$$\nu_{12} = 1 - 0.5\,(E_{11} / E_{33}) \qquad (1)$$

After completion of the mechanical indentation tests, the patellae were split in half for evaluation of tissue proteoglycan content using the DMB assay (Farndale *et al.*, 1982) and histological sectioning using standard methods (Atkinson *et al.*, 1998). All patellae were histologically processed by placing them in 10% buffered formalin for seven days, followed by decalcification in 20% formic acid for another seven days. Tissue blocks were cut transversely across the patella in areas of high contact pressure (Haut et al., 1995). Histological sections (six) were cut 8 microns thick and stained with Safranin O-Fast Green and examined in light microscopy at 12–100X. Each histologic section was analyzed to determine the area most affected by impact. A histopathalogic scoring system was developed based on the literature (Colombo *et al.*, 1983; Mazieres *et*

al., 1987). This system was used to quantify the progression of degenerative changes in the cartilage (examples shown in Figures 7-9). Each aspect was graded from 0 (normal or absent) to +4 using the guidelines in Table 1. One independent blinded reader (JW) examined each of six slides for each sample to find the most representative slide. This slide was then assessed for each parameter at three locations on the patella: medial, central, and lateral, and index scores were recorded. The scores of each parameter were then summed across these locations. The mean and range of each parameter were documented for the impacted and non-impacted limb of both impact groups. The thickness of the subchondral bone plate underlying the retro-patellar cartilage was measured at 25X for all six histology sections with a calibrated eye-piece at the center of each facet (medial, central, and lateral) by a single investigator (J.W.) using established protocols (Newberry *et al.*, 1998) (Figure 10).

	0	+1	+2	+3	+4
Surface Integrity	Regular	Slightly irregular	Moderately irregular	Focally severe	Extensively severe
Proteoglycan staining (Figure 8)	Normal	Slight loss	Moderate loss (to mid zone)	Focally severe (loss beyond mid zone)	Total loss
Fissures	Absent	1-2 (small) (just under the surface)	3 (small) or 1 (mid zone)	4 (small) or 2 (mid zone)	5 or more (small), 3 or more (mid zone) or 1 (full thickness)
Clones	1-2 (small)[a]	3-4 (small) or 1-2 (medium)[b]	5-6 (small) or 3-4 (medium) or 1-2 (large)[c]	7 or more (small) or 5-6 (medium) or 3-4 (large)	7 or more (medium) or 5 or more (large)
Disruptions (Figure 9)	Absent	----------	Compression ridges	----------	Horizontal or Vertical splits
Ossification	Absent	----------	----------	Present	----------
Exposure of Subchondral Bone	Absent	----------	----------	Present	----------
Erosion (Figure 8)	Absent	Detectable	Moderate	Focally severe	Extensively severe
Zone of Calcified Cartilage Thickness (at 100x)	0-10 units	11-13 units	14-17 units	18-23 units	24 or more units
Zone of Calcified Cartilage Cells	Normal	Slight	Moderate	Focally excessive	Excessive
Subchondral Bone Thickness (at 40x)	Less than 20 units	20-29 units	30-39 units	40-49 units	50 or more units

[a]Small = 2-4 cells [b]Medium = 5-8 cells [c]Large = 9 or more cells

1 unit = 0.02 mm at 40x = 0.008 mm at 100x

Table 1. Histopathologic scoring system.

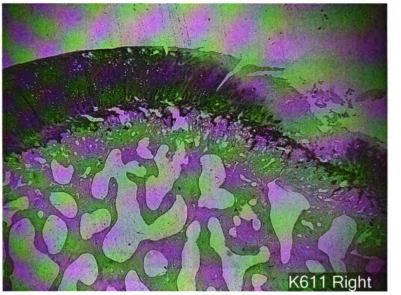


Figure 7. Section from a chronic non-supplemented animal showing local erosion and loss of proteoglycans in the vicinity of impact-induced lesions. (Original photograph = 40X).

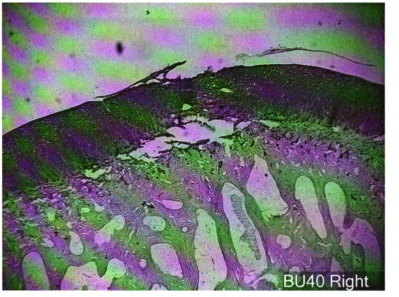


Figure 8. Section from a chronic short-term supplemented animal showing horizontal disruption. (Original photograph = 40X).

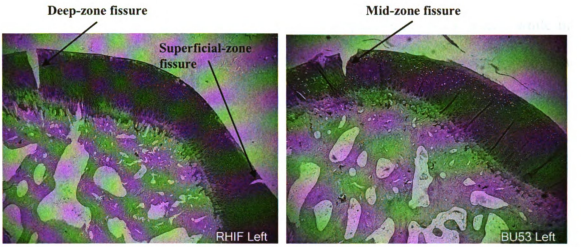


Figure 9. (a) Section from an acute supplemented animal showing surface and deep-zone fissuring. **(b)** Section from a chronic control animal showing mid-zone fissuring. (Original photographs = 40X).

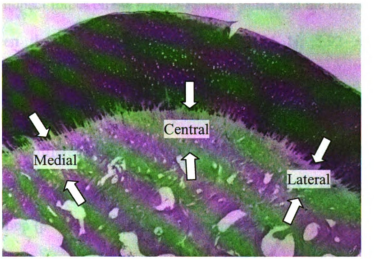


Figure 10. Histologic section of patella: arrows indicate the subchondral bone thickness measurements.

A two way (limb, group) repeated factor analysis of variance (ANOVA) with a Student-Newman-Keuls post hoc test was used to test for statistical differences between impacted and non-impacted limbs within a group, and to test differences between groups.

The Wilcoxon-Signed Rank test was used to test for differences between impacted and non-impacted limbs in all groups based on the histopathologic index scores, while the Mann-Whitney Rank Sum test was used to test for differences between groups. The Pearson Product Moment Correlation test was used to study statistical correlations between mechanical parameters and proteoglycan content of the tissue. Statistical significance was set at $p < 0.05$.

RESULTS

Part A: Acute Study

In the acute study no significant differences were noted by the veterinary technician (J.A.) in the gait or health of rabbits on the supplemented or non-supplemented diets. The blunt impact forces on the patello-femoral joints and the times to peak were not different between the non-supplemented (654 ± 154 N; 5.92 ± 0.48 ms) and supplemented (649 ± 204 N; 5.55 ± 0.42 ms) groups.

Gross photographs of the retro-patellar cartilage surface were studied and revealed significant differences in the total length of surface fissures between the non-impacted (4.93 ± 6.57 mm) and the impacted (11.33 ± 11.53 mm) limbs of the non-supplemented group ($p=0.044$, Figure 11). The same trend was present between the non-impacted (5.65 ± 8.28 mm) and the impacted (11.20 ± 6.04 mm) limbs of the supplemented group ($p=0.074$), yet the difference was not significant (Figure 11).

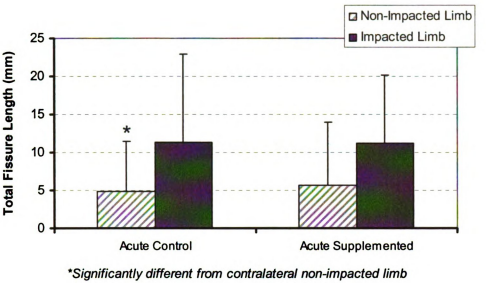


Figure 11. Bar graph of total surface fissure lengths of impacted and non-impacted contralateral limbs of each group in the acute study. Lengths were measured using digital imaging software.

Analyses of the indentation relaxation data revealed no significant differences between contralateral limbs for E_{11} (p=0.93), E_{33} (p=0.53), k_1 (p=0.56), and k_3 (p=0.38) of the non-supplemented group, or for E_{11} (p=0.26), E_{33} (p=0.18), or k_3 (p=0.23) in the supplemented group. There was however, a significant difference between limbs for the k_1 permeability in the supplemented group (p=0.04). No differences between groups were found for E_{11} (p=0.93), E_{33} (p=0.69), and k_3 (p=0.63), yet there was a significant difference between groups in the k_1 permeability in the impacted limbs (p=0.04) (Table 3, Figures 12-13).

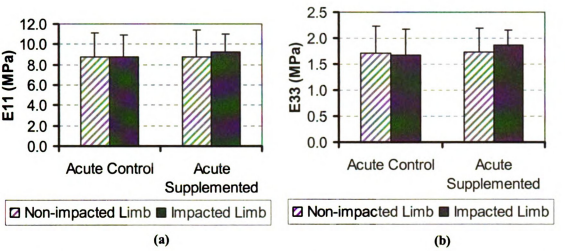


Figure 12. **(a)** Bar chart of the E_{11} modulus for impacted and non-impacted limbs of each group in the acute study. **(b)** Bar chart of the E_{33} modulus for impacted and non-impacted limbs.

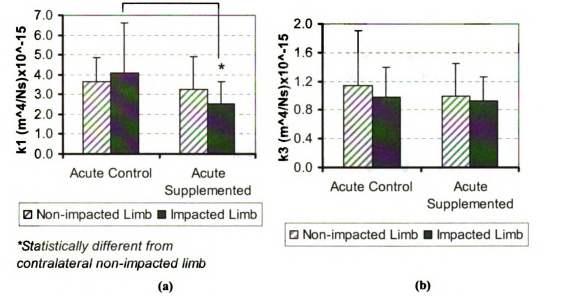


Figure 13. **(a)** Bar chart of the k_1 permeability for impacted and non-impacted limbs of each group in the acute study. **(b)** Bar chart of the k_3 permeability for impacted and non-impacted limbs.

The tissue proteoglycan (PG) content was significantly greater in the impacted limb of the supplemented group (21.8 ± 5.5 μg/mg W.W.) compared to the non-impacted limb (18.3 ± 6.1 μg/mg W.W., p=0.023). Conversely, PG content was not different between the impacted (22.0 ± 7.7 μg/mg W.W.) and non-impacted (20.9 ± 7.7 μg/mg

W.W.) limbs of the non-supplemented group (p=0.364, Figure 14). There was also no difference found between non-supplemented (21.5 ± 7.5 μg/mg W.W.) and supplemented (20.4 ± 5.7 μg/mg W.W.) groups (p=0.672, Figure 14). The correlation analyses revealed a significant, negative correlation between the proteoglycan content of retro-patellar cartilage and k_1 in the non-supplemented group (p=0.037, Figure 15). No other significant correlations were documented between proteoglycan content and any of the other mechanical parameters for either group (Figures 16-18), however, there did seem to be a positive trend in the non-supplemented group between proteoglycan content and both the E_{11} (p=0.168, Figure 16) and the E_{33} moduli (p=0.072, Figure 17).

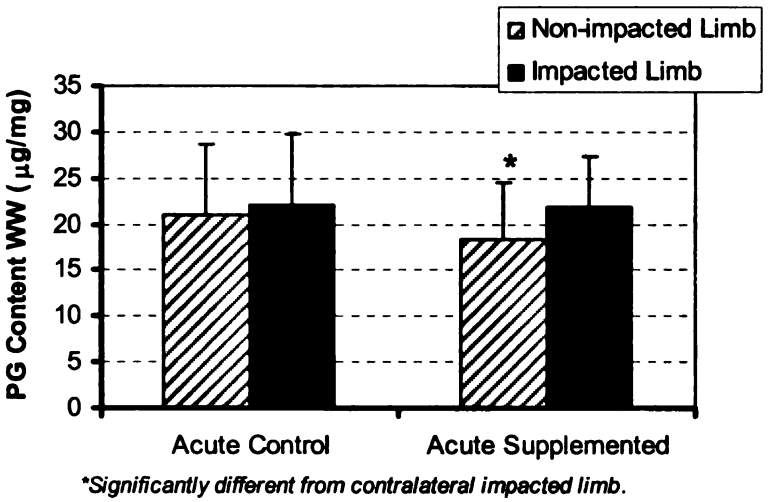


Figure 14. Bar chart of proteoglycan contents, measured in wet weight, for contralateral limbs in each group of the acute study.

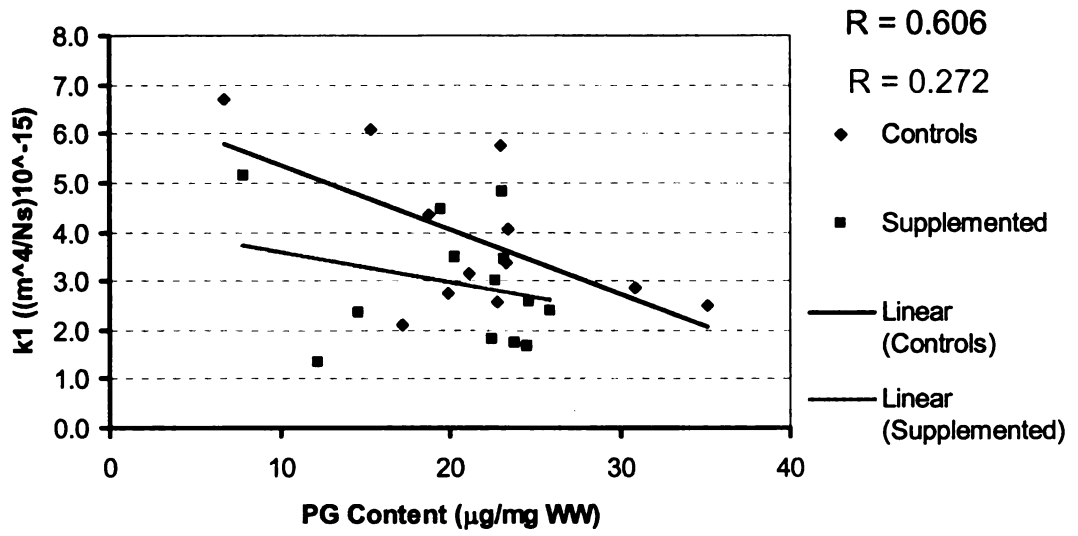


Figure 15. Correlation plot of the k_1 permeability vs. the proteoglycan content for the supplemented vs. non-supplemented groups of the acute study. Right and left limbs were averaged for each group.

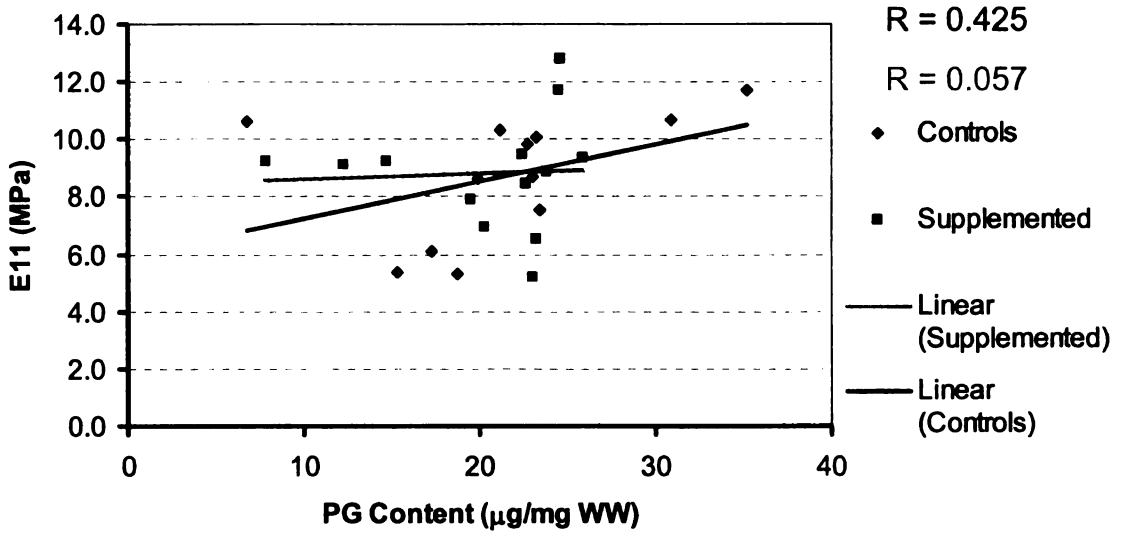


Figure 16. Correlation plot of the E_{11} modulus vs. the proteoglycan content for the supplemented vs. non-supplemented groups of the acute study. Right and left limbs were averaged for each group.

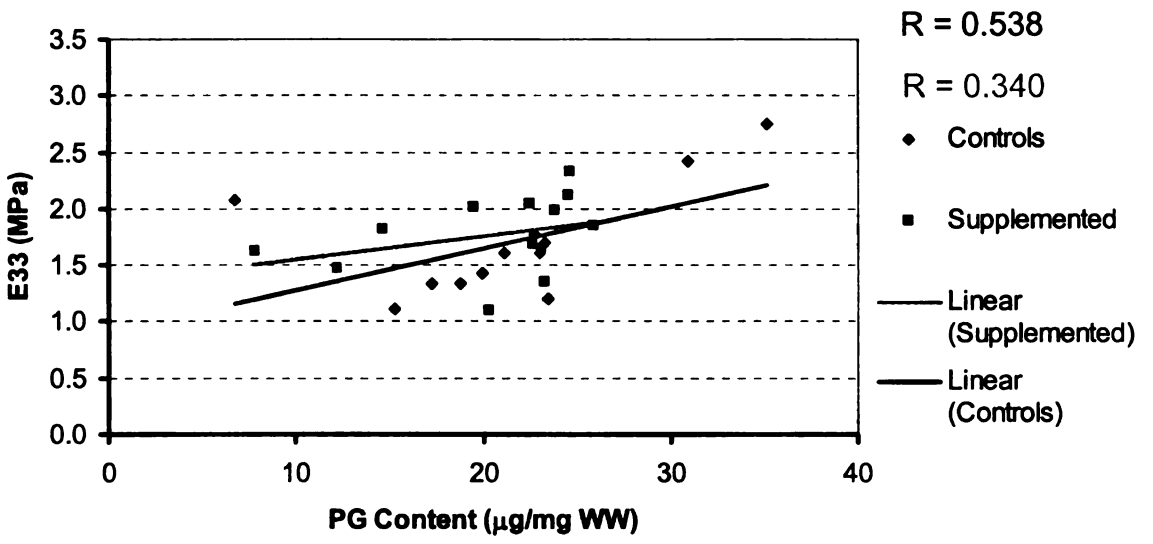


Figure 17. Correlation plot of the E_{33} modulus vs. the proteoglycan content for the supplemented vs. non-supplemented groups of the acute study. Right and left limbs were averaged for each group.

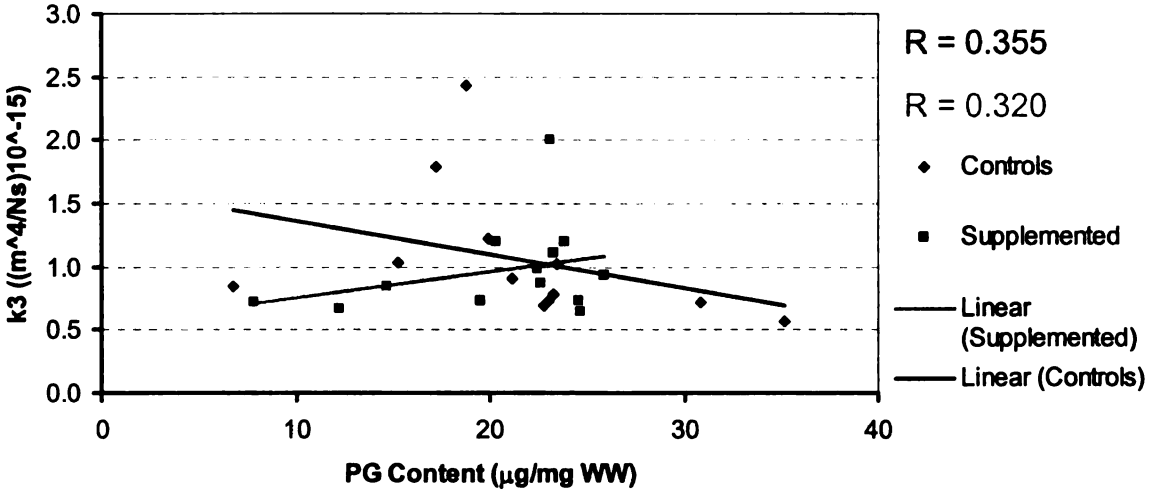


Figure 18. Correlation plot of the k_3 permeability vs. the proteoglycan content for the supplemented vs. non-supplemented groups of the acute study. Right and left limbs were averaged for each group.

Part B: Chronic Study

In the chronic study no significant differences were noted by the veterinary technician (J.A.) in the gait or health of rabbits on the supplemented or non-supplemented diets. The blunt impact forces on the patello-femoral joints and the times to peak were not

different between the non-supplemented (633 ± 114 N; 4.4 ± 1.0 ms), short-term supplemented (576 ± 118 N; 3.5 ± 1.9 ms), and long-term supplemented (593 ± 96 N; 4.6 ± 1.1 ms) groups.

Gross photographs of the retro-patellar cartilage surface were studied and revealed significant increases in the total length of surface fissures in the non-impacted limb (11.09 ± 8.00 mm) versus the impacted limb (17.56 ± 13.34 mm) of the non-supplemented group (p=0.047) (Figure 19). Conversely, no differences were documented between impacted (18.11 ± 13.09 mm) and non-impacted (21.97 ± 16.30 mm) limbs of the short-term supplemented group (p=0.295), or between impacted (22.72 ± 11.95 mm) and non-impacted (20.38 ± 12.26 mm) limbs of the long-term supplemented group (p=0.517).

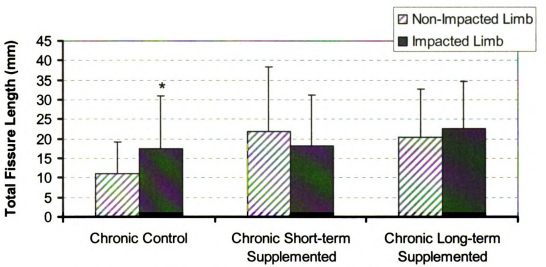


Figure 19. Bar graph of total surface fissure lengths of impacted and non-impacted contralateral limbs of each group in the chronic study. Lengths were measured using digital imaging software.

Analyses of the indentation relaxation data revealed no significant differences between contralateral limbs for E_{11} (p=0.668), E_{33} (p=0.680), k_1 (p=0.846), and k_3 (p=0.507) of the non-supplemented group, for k_1 (p=0.640), or k_3 (p=0.141) in the short-

term supplemented group, or for E_{11} (p=0.446), E_{33} (p=0.123), k_1 (p=0.069), or k_3 (p=0.284) in the long-term supplemented group (Figures 20-21). There was however, a significant increase in the E_{11} (p=0.050) and E_{33} (p=0.020) modulii in the impacted limb of the short-term supplemented group. No differences between groups were found for any of the mechanical parameters, with p values of 0.817, 0.634, 0.457, and 0.834, for E_{11}, E_{33}, k_1, and k_3, respectively (Table 2, Figures 20-21).

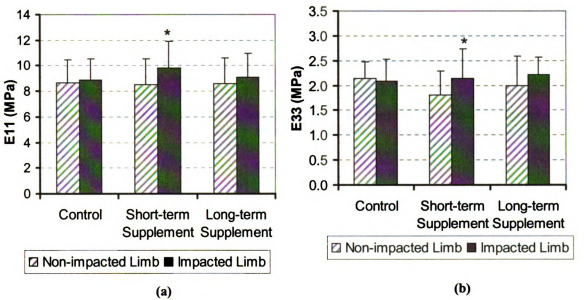


Figure 20. **(a)** Bar chart of the E_{11} modulus for impacted and non-impacted limbs of each group in the chronic study. **(b)** Bar chart of the E_{33} modulus for impacted and non-impacted limbs.

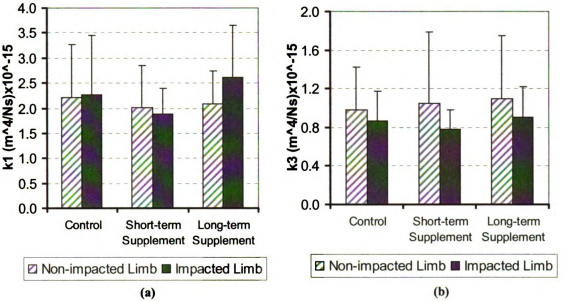


Figure 21. **(a)** Bar chart of the k_1 permeability for impacted and non-impacted limbs of each group in the chronic study. **(b)** Bar chart of the k_3 permeability for impacted and non-impacted limbs.

Group	Limb	Peak Load (N)	Time to Peak (ms)	E_{11} (MPa)	E_{33} (MPa)	G_{13} (MPa)	ν_{31}	k_1 $(m^4/Ns)10^{-15}$	k_3 $(m^4/Ns)10^{-15}$
Acute Controls	Non-impacted[a]	----------	----------	8.72 ± 2.38	1.72 ± 0.52	0.22 ± 0.05	0.13 ± 0.03	3.63 ± 1.23	1.14 ± 0.76
	Impacted[a]	654 ± 154	3.9 ± 1.4	8.76 ± 2.21	1.67 ± 0.50	0.23 ± 0.05	0.12 ± 0.04	4.08 ± 2.55	0.99 ± 0.41
Acute Supplemented	Non-impacted[b]	----------	----------	8.78 ± 2.62	1.74 ± 0.46	0.21 ± 0.06	0.12 ± 0.03	3.26 ± 1.63	0.99 ± 0.46
	Impacted[b]	649 ± 204	3.5 ± 1.5	9.24 ± 1.81	1.87 ± 0.29	0.22 ± 0.04	0.11 ± 0.03	2.54 ± 1.13*,+	0.93 ± 0.34
Chronic Controls	Non-impacted[a]	----------	----------	8.63 ± 1.85	2.14 ± 0.33	0.33 ± 0.26	0.15 ± 0.04	2.20 ± 1.07	0.98 ± 0.44
	Impacted[a]	633 ± 114	4.4 ± 1.0	8.91 ± 1.65	2.09 ± 0.45	0.20 ± 0.07	0.15 ± 0.03	2.26 ± 1.20	0.86 ± 0.31
Chronic Short-term Supplemented	Non-impacted[a]	----------	----------	8.48± 2.07*	1.80 ± 0.49*	0.52 ± 0.58	0.12 ± 0.04	2.02 ± 0.83	1.05 ± 0.75
	Impacted[a]	576 ± 118	3.5 ± 1.9	9.81 ± 2.11	2.13 ± 0.60	0.33 ± 0.24	0.13 ± 0.04	1.88 ± 0.50	0.78 ± 0.20
Chronic Long-term Supplemented	Non-impacted[a]	----------	----------	8.57 ± 2.02	1.99 ± 0.60	0.66 ± 0.73	0.11 ± 0.06	2.07 ± 0.66	1.09 ± 0.66
	Impacted[a]	593 ± 96	4.6 ± 1.1	9.07 ± 1.92	2.21 ± 0.37	0.36 ± 0.47	0.15 ± 0.04	2.60 ± 1.06	0.90 ± 0.32

[a] N=12

[b] N=13

* Significantly different from contralateral non-impacted limb.

+ Significantly different from impacted limb in the acute control group.

Table 2. Transversely isotropic material properties of non-impacted and impacted patellae of the acute and chronic studies (Avg ± stdev).

The tissue proteoglycan (PG) content was not different between the impacted (39.7 ± 5.4 μg/mg W.W.) and non-impacted (39.8 ± 6.1 μg/mg W.W.) limbs of the non-supplemented group (p=0.964, Figure 22). There was a slight trend for an increase in PG content in the impacted limb (37.6 ± 7.4 μg/mg W.W.) compared to the non-impacted limb (35.5 ± 7.4 μg/mg W.W.) of the short-term supplemented group (p=0.470), however the difference was not significant (Figure 22). No difference was found between impacted (35.5 ± 6.2 μg/mg W.W.) and non-impacted (36.5 ± 8.8 μg/mg W.W.) limbs of the long-term supplemented group (p=0.577, Figure 22). PG content was nearly the same between the non-supplemented (39.7 ± 5.4 μg/mg W.W.), short-term supplemented (36.5 ± 6.5 μg/mg W.W.) and long-term supplemented (36.0 ± 7.0 μg/mg W.W.) groups. The correlation analyses revealed no significant correlations between proteoglycan content and any of the mechanical parameters for the non-supplemented group (Figures 23-26). However, in the long-term supplemented group there was a significant, positive correlation documented between proteoglycan content and each of the following parameters: in-plane modulus (E_{11}) (p=0.026); thickness direction modulus (E_{33}) (p=0.002); and a negative correlation for in-plane permeability (k_1) (p=0.005).

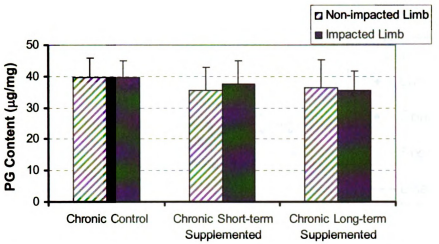


Figure 22. Bar chart of proteoglycan contents, measured in wet weight, for contralateral limbs in each group of the chronic study.

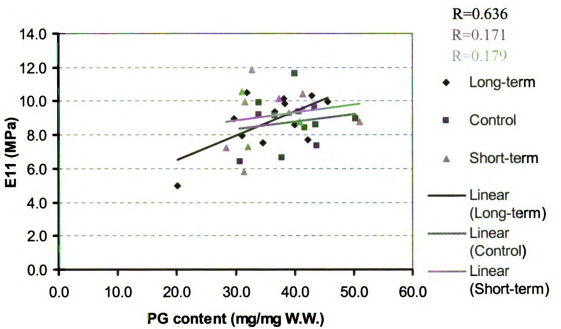


Figure 23. Correlation plot of the E_{11} modulus vs. the proteoglycan content for the supplemented vs. non-supplemented groups of the chronic study. Right and left limbs were averaged for each group.

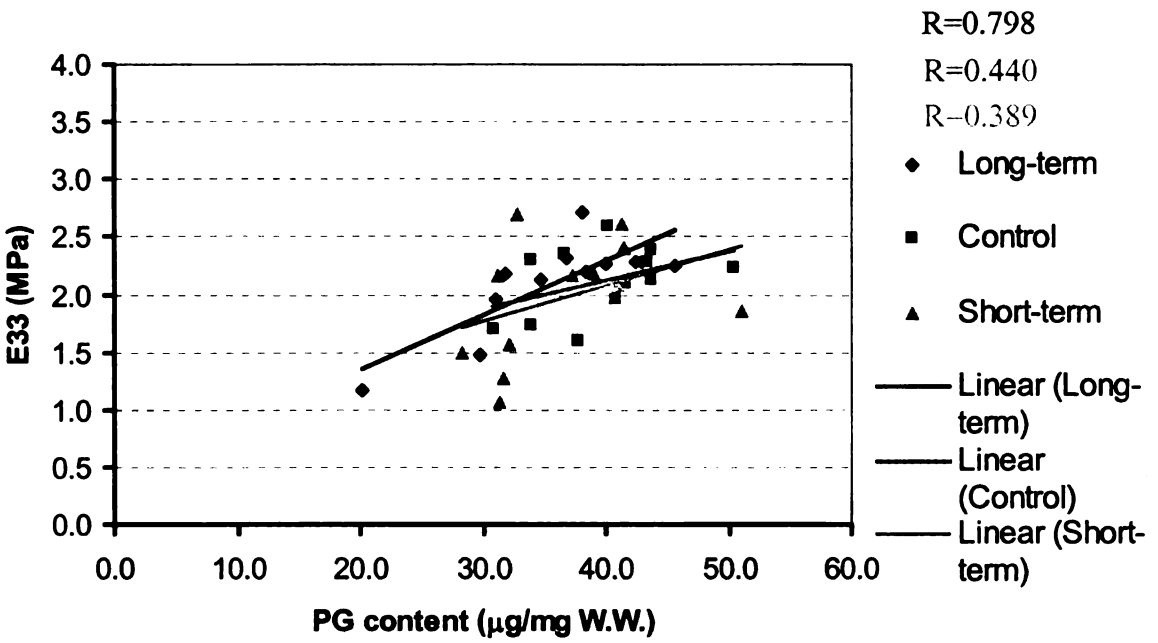


Figure 24. Correlation plot of the E_{33} modulus vs. the proteoglycan content for the supplemented vs. non-supplemented groups of the chronic study. Right and left limbs were averaged for each group.

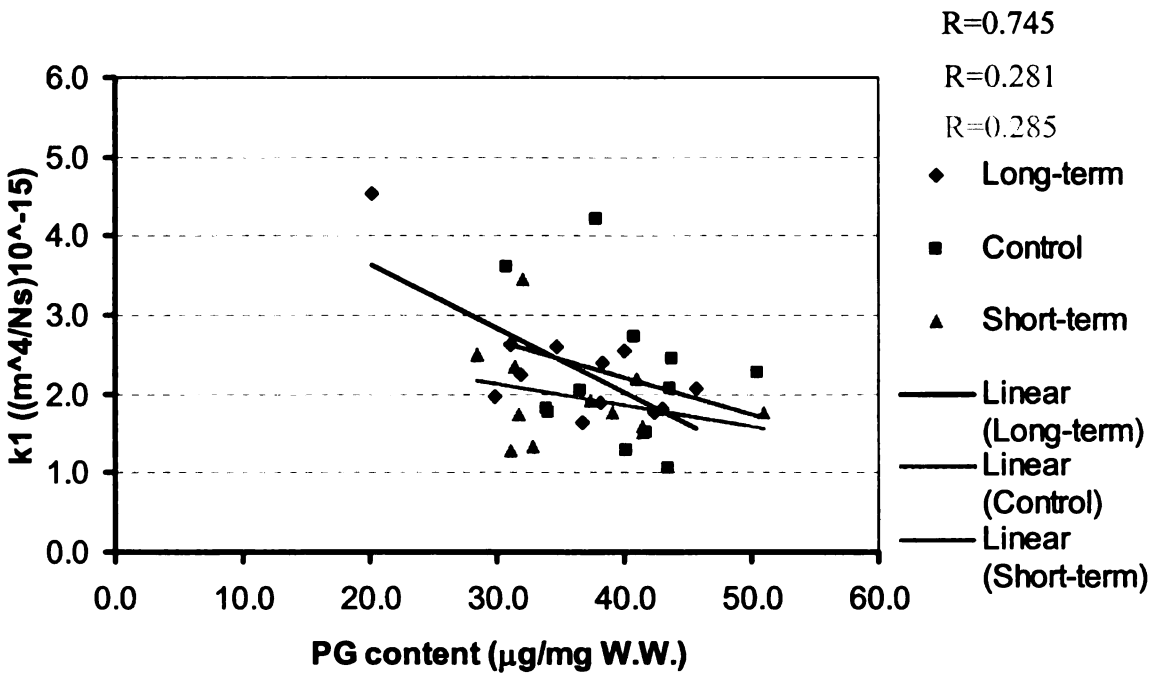


Figure 25. Correlation plot of the k_1 permeability vs. the proteoglycan content for the supplemented vs. non-supplemented groups of the chronic study. Right and left limbs were averaged for each group.

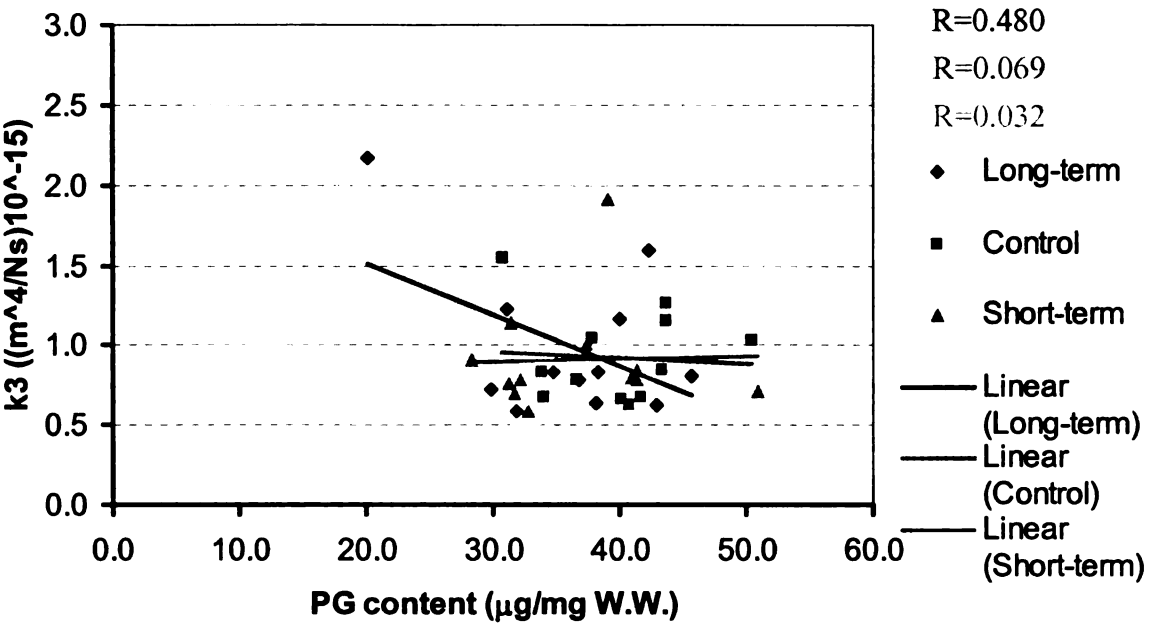


Figure 26. Correlation plot of the k_3 permeability vs. the proteoglycan content for the supplemented vs. non-supplemented groups of the chronic study. Right and left limbs were averaged for each group.

Part C: Acute vs. Chronic

A significantly greater amount of surface fissuring was documented in the short-term supplemented group in the chronic study (20.04 ± 14.69 mm) compared to the acute supplemented group (8.43 ± 8.86 mm, p=0.02). There was also a significant difference in fissuring in the chronic long-term supplemented group (21.55 ± 11.90 mm) compared to the acute supplemented group (8.43 ± 8.86 mm, p=0.03).

Analyses of the indentation relaxation data revealed a significant decrease in k_1 in the chronic control group (2.23 ± 1.13 $(m^4/Ns)*10^{-15}$) compared to the acute control group (3.86 ± 1.89 $(m^4/Ns)*10^{-15}$, p=0.005). No differences were found, however, between these groups for the k_3 permeability. In comparing the overall effects of each study (combining groups within a study), a 16% increase in the E_{33} modulus (p=0.004), a

35% decrease in the k_1 permeability ($p<0.001$), and a 45% increase in proteoglycan content ($p<0.001$) were documented in the chronic study versus the acute study. No differences between groups were found for E_{11} ($p = 0.779$) or k_3 ($p=0.499$), (Table 3).

Group	PG Content (μg/mg W.W.)	E_{11} (MPa)	E_{33} (MPa)	k_1 $(m^4/Ns)10^{-15}$	k_3 $(m^4/Ns)10^{-15}$
Acute[a]	20.69 ± 6.29	8.76 ± 2.10	1.73 ± 0.42	3.35 ± 1.51	0.86 ± 0.73
Chronic[b]	37.42 ± 6.95*	8.91 ± 1.93	2.06 ± 0.49*	2.17 ± 0.91*	0.94 ± 0.48

[a] N=25

[b] N=36

**Statistically different from Acute group*

Table 3. Average proteoglycan content, stiffness, and permeability for all groups combined in the acute and chronic studies.

Histologically, the chronic control group had a trend for a greater loss of proteoglycans than the acute control group, yet the difference was not significant ($p=0.068$). The acute control group had a significantly greater loss of tidemark than the chronic control group ($p=0.021$). Neither the articular cartilage thickness nor the subchondral bone thicknesses were different between impacted and non-impacted patellae, or between groups within a study (Table 4). However, a significant decrease in subchondral bone thickness from the acute to the chronic study was documented. The acute non-supplemented group was significantly different from the chronic non-supplemented group at both the central ($p=0.001$) and lateral ($p=0.014$) locations of the impacted limb. The acute supplemented group was significantly different from the chronic short-term supplemented group at the central ($p<0.001$) and lateral ($p=0.02$) locations, and also from the chronic long-term supplemented group at the central ($p<0.001$) and lateral ($p=0.001$) locations.

Group	Limb	Subchondral bone			Cartilage
		Medial	Central	Lateral	
Acute Controls	Non-impacted[a]	0.52 ± 0.12	0.86 ± 0.33	0.68 ± 0.14	0.61 ± 0.09
	Impacted[a]	0.59 ± 0.22	1.02 ± 0.38*	0.70 ± 0.24*	0.57 ± 0.06
Acute Supplemented	Non-impacted[b]	0.54 ± 0.11	0.97 ± 0.18[+,x]	0.69 ± 0.15[x]	0.58 ± 0.09
	Impacted[b]	0.59 ± 0.16	0.95 ± 0.27[+,x]	0.76 ± 0.21[+,x]	0.62 ± 0.08
Chronic Controls	Non-impacted[a]	0.51 ± 0.10	0.65 ± 0.26	0.55 ± 0.12	0.60 ± 0.06
	Impacted[a]	0.52 ± 0.09	0.56 ± 0.18	0.54 ± 0.10	0.59 ± 0.07
Chronic Short-term Supplemented	Non-impacted[a]	0.53 ± 0.14	0.65 ± 0.26	0.57 ± 0.25	0.59 ± 0.06
	Impacted[a]	0.53 ± 0.14	0.63 ± 0.20	0.57 ± 0.17	0.61 ± 0.07
Chronic Long-term Supplemented	Non-impacted[a]	0.49 ± 0.10	0.54 ± 0.18	0.52 ± 0.1218	0.61 ± 0.06
	Impacted[a]	0.54 ± 0.13	0.62 ± 0.26	0.55 ± 0.1618	0.64 ± 0.08

[a] N=12

[b] N=13

* Significantly different from impacted limb of the chronic control group.

[+] Significantly different from impacted limb of the chronic short-term supplemented group.

[x] Significantly different from impacted limb of the chronic long-term supplemented group.

Table 4: Subchondral bone thickness (mm) was measured at 40X with a calibrated eye-piece at the central region of the patella and midline of the lateral and medial facets (average ± S.D.); the cartilage thickness was measured at the sites of biomechanical testing on the lateral facet (average ± S.D.).

DISCUSSION

One objective of the acute study was to test the hypothesis that administration of Cosamin®DS over a period of two months prior to a blunt impact would increase the level of tissue proteoglycans. The study did show a significant increase in the amount of proteoglycans in the impacted limb compared to the non-impacted limb in the diet

supplemented animals. However, the supplement was not able to increase the level of PG's in comparison with the non-supplemented group. While the non-supplemented group, and others from the literature, have shown positive correlations between tissue proteoglycan content and aggregate modulus (Mizrahi *et al.*, 1986), and negative correlations between proteoglycan content and permeability (Mow and Hayes, 1991), the acute study did not show similar correlations in the diet supplemented group. Analyses of the correlation plots suggest that while some diet supplemented animals have relatively low PG contents, the mechanical properties of retro-patellar cartilage were relatively high. Whereas subjects in the normal diet group with similarly low PG contents showed rather poor mechanical properties. These data showing an increase in mechanical properties in supplemented animals with low PG contents may also agree with recent clinical data suggesting that those patients with early stages of disease also seem to benefit the most from this nutraceutical (Das and Hammad, 2000).

Another objective of the acute study was to test the hypothesis that two months of Cosamin®DS would stiffen the cartilage and reduce the extent of acute damage under impact. It was expected that an increase in tissue proteoglycan content would result in an increase in the stiffness of the cartilage (Mizrahi *et al.*, 1986, Helminen *et al.*, 1992). The supplement was able to significantly decrease the k_1 permeability in the impacted limb compared to both the contralateral non-impacted limb, and the non-impacted limb of the non-supplemented group. There was also a trend for an increase in the E_{33} modulus in the impacted limb of the supplemented group, however it was not significant. While previous studies show that resultant degenerative effects cannot be expected immediately after impact (Ewers *et al.*, 2002; Newberry *et al.*, 1998), these changes in k_1 and E_{33} suggested

that the Cosamin®DS was beginning to show positive effects, but possibly needed more time for cartilage degradation to have a more significant effect.

One more objective of the acute study was to test the hypothesis that two months of Cosamin®DS would reduce the extent of acute damage under impact. In comparison with the non-supplemented group, the acute study showed reduced differences in the amount of surface fissuring between limbs in those animals treated with Cosamin®DS. One possible explanation could be that the nutraceutical had a slight effect on minimizing the amount of impact-induced fissuring. However, since the p-value for the difference between limbs of the non-supplemented group was 0.044, and the p-value for the difference between limbs of the supplemented group was only 0.074, it is possible that with the small number of samples, the low power and the increased variance in the supplemented group caused the difference to be insignificant. Also, the difference between supplemented and non-supplemented groups was not different, agreeing with a recent study which demonstrated that a two month administration of glucosamine *after* induced injury in a rabbit ACL transaction model did not prevent fibrillation of the articular cartilage (Tiraloche *et al.*, 2005). However, the chronic animals had more surface damage, even in the non-impacted limbs compared to the acute study. It is believed that it is difficult to create further extensive surface damage on animals that already have a considerable amount of baseline damage (Silyn-Roberts and Broom, 1990), which could be an alternative explanation for the reduced difference in fissuring between limbs in the chronic animals. The results for the control groups of each study compare well with those of an earlier study using this model, such that there were significant differences between limbs both at time 0 and at 7.5 months post-trauma

(Ewers *et al.*, 2002). However, in the previous study no significant time-dependent changes were found in the total length of surface fissures, which gives reason to believe that the significant increase in the amount of fissuring over time in the current study could be due simply to more initial baseline damage in these chronic animals which came from a different population than the acute animals.

The mechanical data from the transversely isotropic biphasic analysis of the traumatized retro-patellar cartilage revealed a significant decrease in the k_1 permeability, and a subtle trend for an increase in the E_{33} modulus in the acute study after just two months of feeding Cosamin®DS. In the chronic short-term supplemented group, where the animals also received two months of pre-impact treatment (before waiting six more months to be sacrificed), similar effects were seen. This time there was a significant increase in both the E_{11} and the E_{33} moduli, and a trend for a decrease in both the k_1 and k_3 permeabilities. These effects however, were not significant in the long-term supplemented group. Previous studies have shown that high doses of glucosamine over long periods of time can have a toxic effect (De Mattei *et al.*, 2002). The recommended daily dosage for humans is 20 mg/kg a day. The rabbits in the current study were fed 2 % of their daily 200 g feed, which is equal to 4 g/day. With an average weight of 5.7 kg, this is approximately equal to 700 mg/kg a day. This is approximately 35 times the recommended daily dosage per bodyweight for humans. The long-term supplemented animals received this diet supplementation for a total of 8 months. Lippiello *et al.* (2000) used the same 2% concentration in a rabbit ACL transaction model, and saw positive effects of the Cosamin®DS. However, those animals only received supplementation for 4 months. The long-term supplemented animals in the current chronic study may have

received too high of a dose for too long of a period of time. This may have begun to have a slightly negative effect that was reversing any positive effects shown by the 2 months of supplementation.

The current study produced some results different from those of other investigations. A study by Ewers et al. (2002) showed that at 7.5 months post-impact there was a significant reduction in stiffness and an increase in cartilage permeability in the impacted limb versus the non-impacted limb (although not significant compared to time 0). Other studies by our laboratory also documented a softening of the cartilage in the impacted limb after 6 months and 7.5 months, respectively, following a blunt force impact at 6.0 J (Newberry *et al.*, 1998; Ewers and Haut, 2000). Yet in the current study, 6 months after impact there were no significant changes in the impacted limb of the non-supplemented and long-term supplemented groups, and in the short-term supplemented group the cartilage actually appeared stiffer and less permeable in the impacted than non-impacted limbs. Also, the chronic groups overall seemed stiffer and less permeable than the acute results (Ewers *et al.*, 2002). One aspect of the current model that has not been investigated by our laboratory was the exercise regimen that included 8 weeks of exercise prior to the impact on the knee. This regimen may have had a protective effect on the cartilage, defending it from extensive damage in the impact situation, helping to explain why the severe degenerative effects normally seen with this model did not occur in the current study. Other researchers believe that glucosamine has no effect on normal tissue homeostasis (Oegema *et al.*, 2002; Deniz *et al.*, 2003). If we were unable to cause severe damage to the cartilage in the current model due to the pre-impact exercise, this would explain why the supplement appeared to have no major effects on the tissue.

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CHAPTER TWO

MECHANICAL RESPONSE OF CARTILAGE EXPLANTS TO CYCLIC COMPRESSIVE LOADING

ABSTRACT

As a way to examine more controlled loads an articular cartilage in a more cost effective manner than our *in vivo* animal models, our laboratory has been developing a mechanical testing apparatus to load cartilage explants *in vitro*. The objective of the current study was to determine whether, and to what extent, regular cyclic compressive loading alters the biomechanical properties of articular cartilage. Chondral explants were taken from the bovine metacarpophalangeal joint and exposed to 10 cycles of loading every hour at a magnitude of 0.5 MPa for 0, 1, 2, 3, or 6 days. Stiffness of the cartilage was measured by mechanical indentation, and the tissue was evaluated for cell viability. Results indicated a general trend for a stiffening of cartilage explants when exposed to regular cyclic loading at low intensities, along with a decrease in fluid gain and an increase in deep zone cell death.

INTRODUCTION

Numerous *in vivo* animal models have been utilized to examine changes in joint tissue, along with effects of regular exercise in contrast with normal cage activity or joint immobilization (Newberry *et al.*, 1997; Ewers *et al.*, 2002; Weaver and Haut, 2005; Helminen *et al.*, 1992; Jurvelin *et al.*, 1986). While immobilization has been shown to soften the articular cartilage (Helminen *et al.*, 1992), regular exercise has been shown to have a stiffening effect (Jurvelin *et al.*, 1986). Mechanical stiffness of articular cartilage

has been associated with proteoglycan content (Helminen *et al.*, 1992; Mizrahi *et al.*, 1986), and *in vivo* models have shown that increased weight-bearing and regular exercise have an upregulating effect on tissue proteoglycans (PGs) in articular cartilage in the knee (Kiviranta *et al.*, 1987; Helminen *et al.*, 1992). However, *in vivo* animal models limit the degree of control on factors such as amplitude and distribution of physical forces, and create difficulties in directly relating the cellular response to various loading situations. As a result, *in vitro* mechanical explant test systems have been developed to address these issues (Torzilli *et al.*, 1997; Sah *et al.*, 1989; Sauerland *et al.*, 2003).

Various studies have attempted to quantify the effects of static (constant) or dynamic (cyclic) compressive stresses on cartilage explants *in vitro* (Quinn *et al.*, 1998); (Sah *et al.*, 1989; Torzilli *et al.*, 1997). Although some researchers were not able to show an increase in proteoglycan biosynthesis (Torzilli *et al.*, 1997), the general consensus is that static stress tends to decrease proteoglycan synthesis while cyclic stresses tend to increase the synthesis of tissue PGs (Palmoski and Brandt, 1984; Parkkinen *et al.*, 1989; Quinn *et al.*, 1998).

Our laboratory has developed a "cartilage exerciser" to cyclically load chondral explants *in vitro*, simulating normal loading or exercise on the joint. The cartilage exerciser was designed to be load controlled, representing the physical conditions cartilage might experience in a synovial joint. A low level of stress ($\leq$ 0.5 MPa) and a higher frequency of compression (0.01 - 1 Hz) were chosen based on a study by Sah showing a threshold of biosynthesis at these levels (Sah *et al.*, 1989).

Other studies have investigated the *in vitro* mechanical response on cartilage explants to injurious compression (Loening *et al.*, 2000; Morel *et al.*, 2005; Rundell and

Haut, 2005; Thibault *et al.*, 2002). These researchers have found that an increase in fluid gain corresponds to a decrease in cartilage stiffness (Jurvelin *et al.*, 1986) and an increase in surface fissuring when subjected to high compressive loads (Loening *et al.*, 2000; Morel *et al.*, 2005; Rundell and Haut, 2005; Thibault *et al.*, 2002). Other laboratories have used mechanical explant testing systems to focus primarily on the response of proteoglycan synthesis to applied static compressive stresses or cyclical strains (Torzilli *et al.*, 1997; Sah *et al.*, 1989; Sauerland *et al.*, 2003). Unfortunately, few studies have investigated the direct correlation between regular cyclic loading of chondral or osteochondral explants and mechanical stiffness in non-injurious compression. The hypothesis of the current study was that regular, intermittent *in vitro* cyclic loading of cartilage explants would cause an increase in the mechanical stiffness of the cartilage.

Since the cartilage exerciser was a newly developed device in our laboratory, pilot studies were executed in order to define the desired loading parameters for the apparatus. This chapter will discuss both these initial results from pilot studies, as well as results obtained from experimental studies with a regular intermittent loading regimen.

METHODS

Six pairs of skeletally mature (12-24 months) bovine forelegs were obtained from a local abattoir within 3 hours of slaughter. The legs were cut proximal to the metacarpophalangeal surface leaving the joint intact. The legs were rinsed with distilled water, skinned, and rinsed again prior to opening the joint under a laminar flow hood. A 6-mm biopsy punch was used to make 154 cartilage plugs. These plugs were removed from the underlying bone with a scalpel, thickness measurements were taken (described later), and the explants were divided into two groups, exercise and no-exercise.

All explants were washed three times in bovine media with Dulbecco's Modified Eagle's Medium (Gibco, USA #12500-039) (see Appendix F for stock recipe), and then placed in the medium supplemented with 10% fetal bovine serum and antibiotics (penicillin 100 units/ml, streptomycin 1μg/ml, amphotericin B 0.25 μg/ml) in a 24 well plate (see Appendix G for media handling standard operating procedure). The bovine media was replaced with fresh media every 2 days throughout a study. The well plate was placed in a mechanical loading device (the "cartilage exerciser", Figure 1) inside of a humidity-controlled incubator (37° C, 5% CO_2, NuAire, Plymouth, MN). The cartilage exerciser consists of 12 loading chambers simultaneously powered by air. Pneumatic cylinders force the pistons downward to apply a compressive load to the specimens through 14.60 mm diameter nonporous Teflon® platens (Figure 2). The cartilage exerciser was designed to hold a 24 well culture plate, so that 12 cartilage samples would be mechanically loaded and 12 unloaded control explants would be subjected to an identical culture environment (Figure 3).

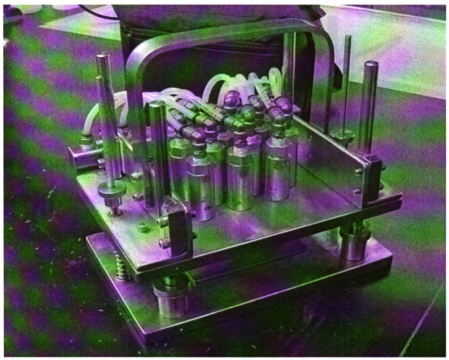


Figure 1. The "cartilage exerciser" mechanical loading device with 12 cylinders to apply compressive loads to the cartilage explants.

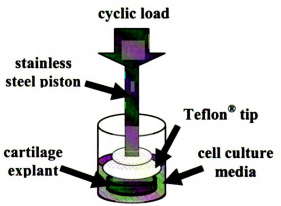


Figure 2. Each piston of the cartilage exerciser is powered by air to simultaneously load the explant with a nonporous Teflon® platen.

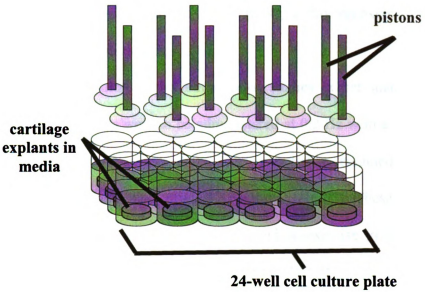


Figure 3. The cartilage exerciser consists of 12 pistons and is designed to simultaneously load 12 explants in a 24 well plate, leaving 12 explants as non-loaded controls.

Pilot studies involved various loading conditions in order to determine the most optimal parameters. In each of the three pilot studies, intermittent uniaxial cyclic loads were administered using a sinusoidal waveform of 1 Hz at a peak stress of 0.05 MPa. Ten cycles of load were applied once every hour starting at day 0 (right off the joint) and ending at 1, 2, or 7 days. Between loading cycles the pistons rise above the cartilage so that zero load is applied to the samples. A total of twenty-seven samples were used for the first pilot study (6/14/05), where explants were separated into 5 groups: time zero (n=4), 2 day exercise (n=6), 2 day no-exercise (n=6), 7 day exercise (n=5), and 7 day no-exercise (n=6). Thirty explants were used for the following two pilot studies (6/28/05) and (7/12/05), with 6 samples in each of the 5 groups: time zero, 2 day exercise, 2 day no-exercise, 7 day exercise, and 7 day no-exercise. In the final pilot study (11/1/05) the peak load was raised from 0.05 MPa to 0.5 MPa, at a frequency of 0.2 Hz, and the samples were again loaded with 10 cycles every hour. This study included the following

five groups: time zero (n=9), 2 day exercise (n=4), 2 day no-exercise (n=4), 3 day exercise (n=4), and 3 day no-exercise (n=4).

Based on the results of the initial pilot studies, a set loading pattern was determined. The cyclic loads were to be administered at 0.2 Hz with a peak stress of 0.5 MPa for ten cycles applied once every hour. These parameters remained constant for each of the two final experimental studies: (11/8/05) and (11/16/05). These two studies each consisted of time 0 (n=8), 1 day exercise (n=4) and no-exercise (n=4), 3 day exercise (n=4) and no-exercise (n=4), and 6 day exercise (n=4) and no-exercise (n=4) groups. After a desired loading time of 1, 3, or 6 days the specimens were then evaluated in terms of mechanical stiffness, cell viability, and proteoglycan content.

Structural integrity of the metacarpophalangeal cartilage was determined using indentation stress relaxation tests (see Appendix F for standard operating procedures). After allowing a minimum of 30 minutes in media for the explants to swell, mechanical indentation tests were performed at one of six times: 0, 1, 2, 3, 6, or 7 days of exercise after removal from the joint. Cartilage explant thickness was measured immediately off the joint, and again before mechanical indentation testing. The percentage in thickness change due to swelling was found by dividing the change in thickness (final minus initial) by the initial (off the bone) thickness. Thickness was measured at two perpendicular sites across the center of the explant using a digital Vernier caliper (Mitutoyo Corp.:

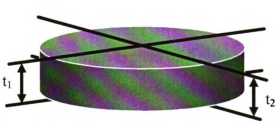


Figure 4. Explant thickness taken at two perpendicular sites across the explant using digital calipers.

Abosolute Digimatic, Model No. CD-6" CS) with a resolution of 0.01 mm (Figure 4). The two thickness values were then averaged together and recorded. The explants were placed on a flat level surface so that the face of the explant was perpendicular to the indenter tip. A magnet with a 4.5 mm diameter hole was placed on top of the explant to secure the edges to help resist curling of the explants (Figure 5). The explant and fixture were then submerged in a room temperature phosphate buffered solution (pH 7.2) (Figure 5). A 1.0 mm diameter flat, non-porous probe was lowered into the cartilage until a preload of 0.03 N was attained and held for 60s. After the 60s preload, the indenter was pressed into the cartilage 15% of the total thickness in 2s and maintained for 360s while resistive loads of relaxation were measured (Data Instruments, Acton, MA: model JP-25, 25 lb capacity), amplified, and collected at 1000 Hz for the first second and 20 Hz for the remainder. Note that this protocol differs slightly from that in Chapter 1 due to the nature of the specimens being tested. In Chapter 1, a 0.02 N preload was applied and immediately the indenter was pressed 0.01 mm into the cartilage. Due to the curling nature of the cartilage explants in the current study, a slightly larger (0.03 N) preload was used to ensure that the entire tip of the indenter was in contact with the explant. Since a larger preload was applied, the cartilage was allowed 60 seconds to relax in order to verify that the cartilage was not in a stressed state at the time of indentation. Also, it was decided to indent 15 % of the cartilage thickness, rather than a prescribed distance, due to the variability of the explant thicknesses.

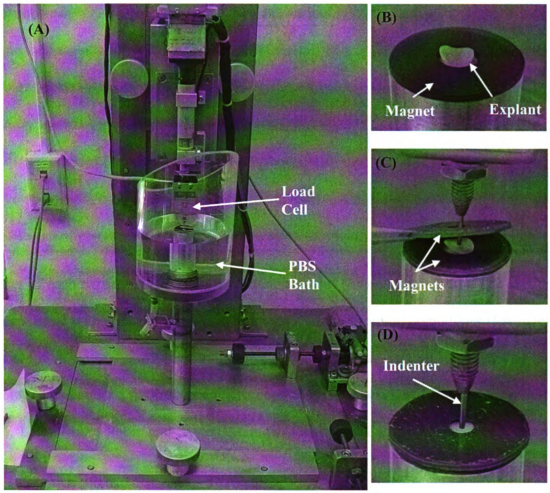


Figure 5. **(A)** Explant indentation test system and fixture. **(B)** First, explant is placed in hole of bottom magnet on flat steel surface. **(C)** Second, a top magnet is lowered over the top of the explant to hold down edges. **(D)** Finally, the indenter tip is lowered to a preload of 0.03 N.

The stiffness of the cartilage was determined from the results of the indentation and thickness test by a calculation of the shear modulus from an assumed elastic layer on a rigid half space (unbonded) (Lebedev and Ufliand, 1958). The instantaneous shear modulus (G_u) and the relaxed shear modulus (G_r) were calculated based on the peak load at 62 s (60 s of preload, and 2 s for indentation application) and the relaxed load at 360 s, respectively, using equation 1.

$$G = \frac{P(1-\nu)}{4 * a * K\left(\frac{a}{h}\right) * \omega} \quad (1)$$

Where P = the measured load
ν = Poisson's ratio (Assumed to be 0.5 for G_U and 0.4 G_R)
a = Indenter radius
h = layer thickness
ω = penetration depth
G = Elastic shear modulus
K = scaling factor.

For cell viability, three 0.5-mm slices were taken through the thickness at the center of each explant using a customized cutting tool (Ewers *et al.*, 2001). The sections were stained with a kit containing calcein and ethidium bromide homodimer (Live/Dead &Viability/Cytotoxity, Molecular Probes, Oregon). Each section was viewed and photographed under a fluorescence microscope at 100X (Lecia DM LB (frequency: 50-60 Hz), Lecia Mikroskopie and Systeme GmgH, Germany). Full thickness, digital images were taken of a 2.5-mm length at the center of each explant. The percentage of dead cells (red) to viable cells (green) were manually determined by taking a measurement of the thickness of the dead zone and dividing it by the full thickness of the sample using digital imaging software (Sigma Scan, SPSS Inc., Chicago, IL). This was done at three sites on each of three slices from every explant (Figure 6), and the percentages were averaged together and recorded.

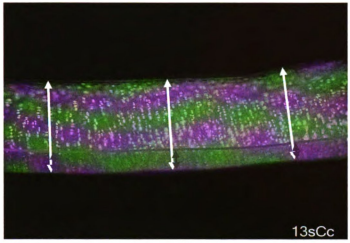


Figure 6. Gross photographs were used to determine percentage of viable cells in the cartilage explants. The thickness of the death zone and the total thickness of the explant were measured at three sites.

After completion of the mechanical indentation and cell viability testing, the remaining end pieces of the explant samples were evaluated for tissue proteoglycan content using the DMB assay (Farndale *et al.*, 1982).

A one way ANOVA with Student-Newman-Keuls post hoc tests was used to compare the mechanical stiffness, proteoglycan contents, and percentage of cell death between exercised and non-exercised groups. All data are reported as mean ± standard deviation. Statistical significance was indicated at $p<0.05$.

RESULTS

Pilot Studies

Mechanical stiffness results from the first pilot study (6/14/05) revealed a significant increase in both the instantaneous shear modulus G_u ($p=0.005$) and the relaxed shear modulus G_r ($p=0.009$) in the exercise group compared to the non-exercise control

group at 2 days (Figure 7). There was also a slight trend for an increase in G_u and G_r after 7 days of exercise compared to the non-exercise group, yet neither was statistical (Figure 7).

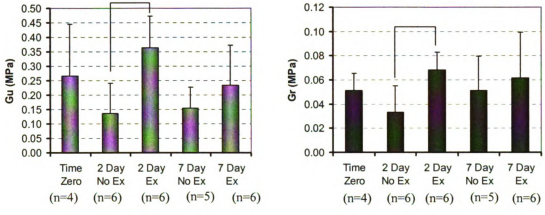
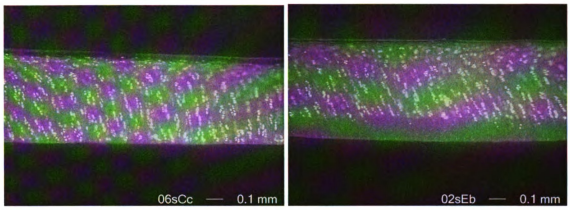


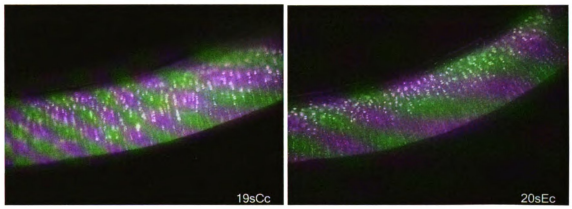
Figure 7. **(a)** Bar chart of the instantaneous modulus *(G_u)* and **(b)** the relaxed shear modulus *(G_r)* for no-exercise and exercise samples from each group in the 6/14/05 study.

Staining for cell viability showed a band of death in the deep zone of the cartilage, as well as a smaller band of death in the superficial zone in both non-exercise (7.7 ± 7.8 % cell death) and exercise (26.1 ± 11.1 % cell death) groups after 2 days (Figure 8). The same effect, only more extensive, was seen in the exercise (44.4 ± 9.7 % cell death) and no-exercise (26.6 ± 21.5 % cell death) groups after 7 days (Figure 9). These increases in cell death in the exercised explants in comparison with the non-exercised controls were found to be significant at both 2 days ($p=0.032$) and 7 days ($p=0.047$). Over time there was also a trend for an increase in cell death from 2 days to 7 days in non-loaded controls ($p=0.069$), and from 2 days of exercise to 7 days of exercise ($p=0.098$), although these results were not quite significant (Figure 10).



(a) **(b)**

Figure 8. Representative cell viability photographs for **(a)** the 2 day no-exercise group and **(b)** the 2 day exercise group in the 6/14/05 study. Green cells are viable and red cells are dead.



(a) **(b)**

Figure 9. Representative cell viability photographs for **(a)** the 7 day no-exercise group and **(b)** the 7 day exercise group in the 6/14/05 study. Green cells are viable and red cells are dead.

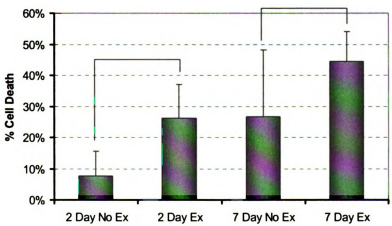


Figure 10. Percentage of cell death in each of the no-exercise and exercise groups in the 6/14/05 study.

The second set of experiments (6/28/05) revealed a trend for an increase in G_u in the exercise group (0.52 ± 0.35 MPa) versus the non-exercise group (0.45 ± 0.42 MPa) after 2 days (Figure 11). There was also a trend for an increase in G_r in the exercise (0.09 ± 0.06 MPa) group compared to the non-exercise (0.08 ± 0.04 MPa) group after 2 days, however, the differences in G_u and G_r were not statistically significant. The 7 day groups showed no change in G_u and a slight decrease in G_r in the exercise group (0.11 ± 0.05 MPa) compared to the non-exercise (0.13 ± 0.05 MPa) group (Figure 11).

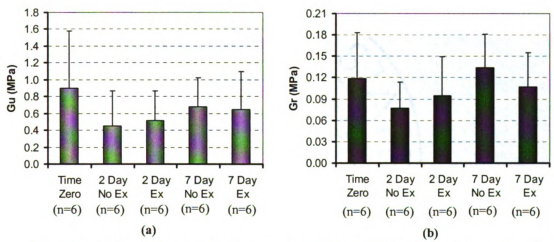
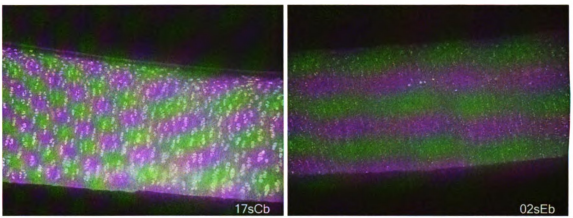


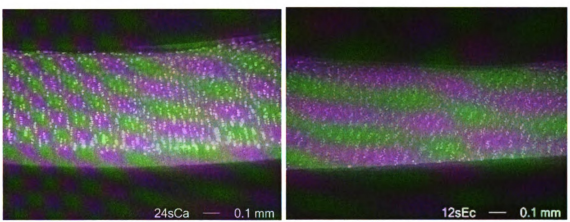
Figure 11. (**a**) Bar chart of the instantaneous modulus *(G_u)* and (**b**) the relaxed shear modulus *(G_r)* for no-exercise and exercise samples from each group in the 6/28/05 study.

Cell viability staining revealed complete death in all exercise specimens after 2 days (Figure 12) and 7 days (Figure 13) of loading. Minimal death occurred in the deep zone of the control samples after 2 days (Figure 12). However, the band of death was increased in the 7 day no-exercise group (Figure 13).



(a) (b)

Figure 12. Representative cell viability photographs for **(a)** the 2 day no-exercise group and **(b)** the 2 day exercise group in the 6/28/05 study. Green cells are viable and red cells are dead.



(a) (b)

Figure 13. Representative cell viability photographs for **(a)** the 7 day no-exercise group and **(b)** the 7 day exercise group in the 6/28/05 study. Green cells are viable and red cells are dead.

The (7/12/05) pilot study indicated a significant increase in G_u (p=0.020) and G_r (p=0.014) in the no-exercise group compared to exercise group after 2 days of loading (Figure 14). The same result was found in G_u after 7 days of loading in comparison with non-loaded controls (p=0.02) (Figure 14). There was also a trend for 7 days of loading to increase G_r, yet the result was not quite significant (p=0.064) (Figure 14). However, cell viability revealed 100% death in all exercised specimens, and no death in the non-exercised controls.

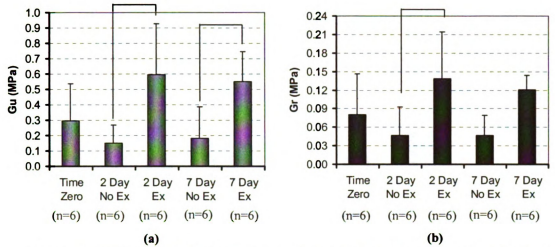


Figure 14. **(a)** Bar chart of the instantaneous modulus (G_u) and **(b)** the relaxed shear modulus (G_r) for no-exercise and exercise samples from each group in the 7/12/05 study.

Proteoglycan analysis showed a trend for an increase in PG content after 2 days of exercise compared to the non-loaded control group, however the difference was not significant (Figure 15). An overall decrease was seen from time zero to 2 day no-exercise and both 7 day groups (Figure 15). The 2 day and 7 day exercise groups showed an increase in percent thickness change in comparison to the no-exercise group from initial reading off the joint to 2 or 7 days in media (Figure 16).

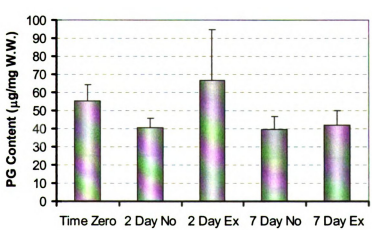


Figure 15. Proteoglycan contents for no-exercise and exercise samples from each group in the 7/12/05 study.

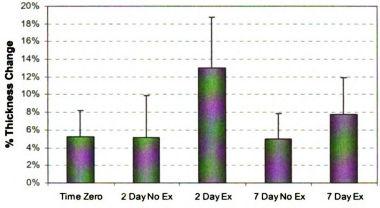


Figure 16. Increases in thickness due to swelling in the 7/12/05 study are plotted here. Explant thickness was measured directly off the joint, and again after 30 min, 2 days, or 7 days of equilibration.

In the final pilot study (11/1/05) a slight decrease in G_u was found in the 2 day exercise group (1.07 ± 0.56 MPa) compared to the 2 day no-exercise group (1.43 ± 0.52 MPa), as well as a slight decrease in G_r in the exercise (0.14 ± 0.03 MPa) vs. no-exercise (0.19 ± 0.07 MPa) group (Figure 17). A very subtle decrease in G_u and G_r of the exercised explants was also found after 3 days of loading compared to the non-exercised control group, although the differences were not significant (Figure 17).

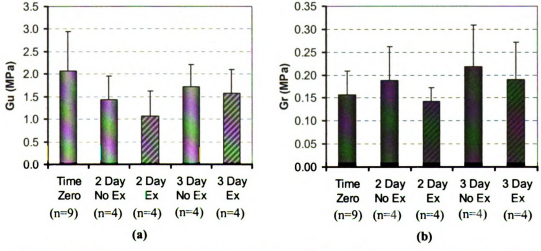


Figure 17. **(a)** Bar chart of the instantaneous modulus *(G_u)* and **(b)** the relaxed shear modulus *(G_r)* for no-exercise and exercise samples from each group in the 11/1/05 study.

Cell viability showed a slight increase in cell death in the exercise compared to non-exercise group after 2 days (Figure 18). This effect was increased after 3 days of loading, and a larger band of death was seen in the exercised explants (Figure 18). Death in the control specimens mainly occurred in the superficial zone, whereas death in the exercised samples occurred mostly in the deep zone with a small amount of death in the superficial zone (Figures 19-20).

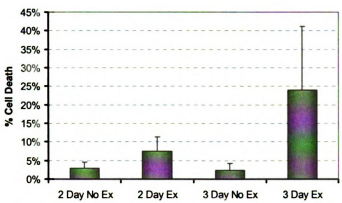
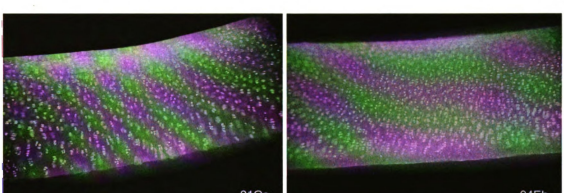
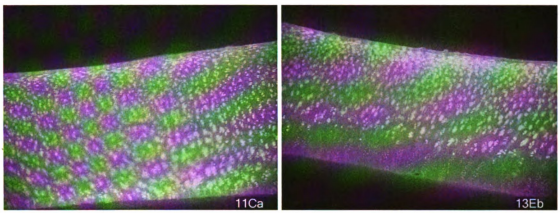


Figure 18. Percentage of cell death in each of the no-exercise and exercise groups in the 11/1/05 study.



(a) **(b)**

Figure 19. Representative cell viability photographs for **(a)** the 2 day no-exercise group and **(b)** the 2 day exercise group in the 11/1/05 study. Green cells are viable and red cells are dead.

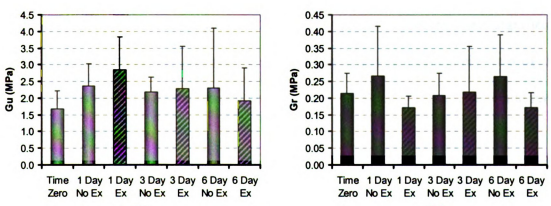


(a) **(b)**

Figure 20. Representative cell viability photographs for **(a)** the 7 day no-exercise group and **(b)** the 7 day exercise group in the 11/1/05 study. Green cells are viable and red cells are dead.

Experimental Studies

In the first of the experimental studies, there was a trend for an increase in G_u after 1 day of exercise, compared to the 1-day control group (Figure 21). The opposite trend was seen for the G_r values after 1 day (Figure 21). Differences between exercise and no-exercise were very minimal at 3 days, yet after 6 days an increase in both G_u and G_r was found in the no-exercise group compared to the exercised specimens (Figure 21). No differences between limbs or between groups were statistically significant.



(a) **(b)**

Figure 21. **(a)** Bar chart of the instantaneous modulus (G_u) and **(b)** the relaxed shear modulus (G_r) for no-exercise and exercise samples from each group in the 11/8/05 study. In the time zero group n=8. In all other groups (no-exercise and exercise) n=4.

After 1 day of loading there was a decrease in percent thickness change compared to the non-loaded group (Figure 22). The trend after 6 days was similar, yet less evident, however at 3 days the trend was reversed, with a slight increase in percent thickness change in the exercised samples (Figure 22). A decrease in percent thickness change seemed to correspond with an increase in G_u at 1 and 6 days (Figure 22).

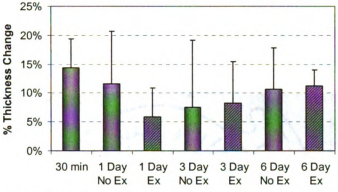


Figure 22. Increases in thickness due to swelling in the 11/8/05 study are plotted here. Explant thickness was measured directly off the joint, and again after 30 min, 1 day, 3 days, or 6 days of equilibration.

Cell death after 1 day (exercise and no-exercise samples) was negligible (Figure 23). Although cell death was still minimal after 3 days, there was a slight increase in death in the exercised specimens (3.4 ± 1.1 % cell death) compared to the non-exercised group (1.5 ± 0.6 % cell death) (Figure 23). However, after 6 days there was a significant increase in cell death in the no-exercise group (Figure 23). Unfortunately, two of the control samples were contaminated (Figure 24), so it is unknown if exercise would have caused more death otherwise.

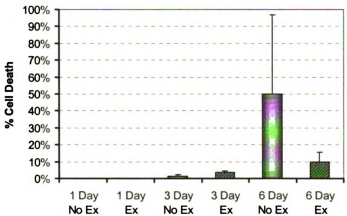
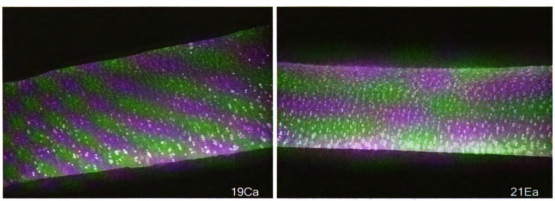


Figure 23. Percentage of cell death in each of the non-exercised and exercised groups in the 11/8/05 study.



(a) **(b)**

Figure 24. Representative cell viability photographs for (**a**) the 6 day no-exercise group and **(b)** the 6 day exercise group in the 11/8/05 study. Green cells are viable and red cells are dead.

Results of the final study (11/16/05) revealed a consistent trend for an increase in G_u after 1, 3, and 6 days of loading in comparison with the non-loaded controls (Figure 25). This trend remained the same for G_r at 3 and 6 days, however at 1 day the exercise group actually saw a slight decrease in G_r compared to the no-exercise group (Figure 25). Percent thickness change was consistent throughout the study, with a decrease in the exercise groups at 1, 3, and 6 days (Figure 26), corresponding to the increases in G_u.

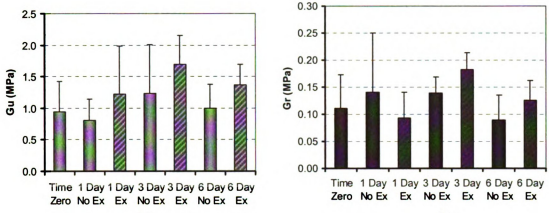


Figure 25. (a) Bar chart of the instantaneous modulus *(G_u)* and **(b)** the relaxed shear modulus *(G_r)* for exercise and no-exercise samples of each group in the 11/16/05 study. In the time zero group n=8. In all other groups (no-exercise and exercise) n=4.

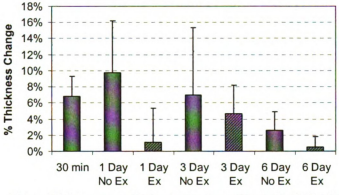


Figure 26. Increases in thickness due to swelling in the 11/16/05 study are plotted here. Explant thickness was measured directly off the joint, and again after 30 min, 1 day, 3 days, or 6 days of equilibration.

No significant differences in cell death were found between groups (Figure 27). There was a slight trend for an increase in cell death in the no-exercise group after 1 day. However, after 3 and 6 days, more cell death was found in the exercise samples (Figures 28-30). Cell death in the control specimens was mostly confined to the superficial zones, whereas death in the exercised specimens was in both the superficial and deep zones.

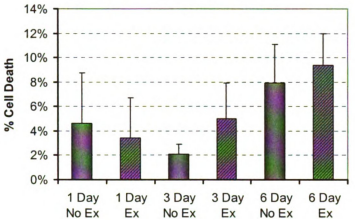
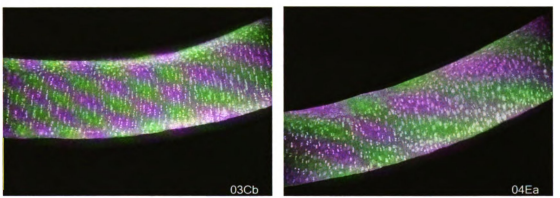
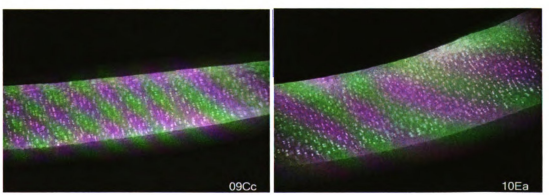


Figure 27. Percentage of cell death in each of the no-exercise and exercise groups in the 11/16/05 study.



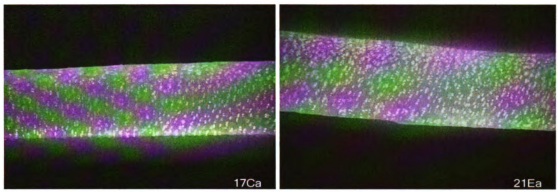
(a) **(b)**

Figure 28. Representative cell viability photographs for **(a)** the 1 day no-exercise group and **(b)** the 1 day exercise group in the 11/16/05 study. Green cells are viable and red cells are dead.



(a) **(b)**

Figure 29. Representative cell viability photographs for **(a)** the 3 day no-exercise group and **(b)** the 3 day exercise group in the 11/16/05 study. Green cells are viable and red cells are dead.



(a) **(b)**

Figure 30. Representative cell viability photographs for **(a)** the 6 day no-exercise group and **(b)** the 6 day exercise group in the 11/16/05 study. Green cells are viable and red cells are dead.

Proteoglycan analysis showed a trend for an increase in PG content after 3 and 6 days of exercise compared to the non-loaded control group, however the difference was not significant (Figure 31). A significant correlation was found between the relaxed shear modulus (G_r) and the tissue proteoglycan content (p=0.036, Figure 31).

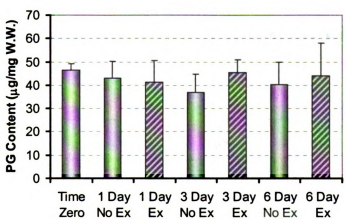


Figure 31. Proteoglycan contents for no-exercise and exercise samples from each group in the 11/16/05 study.

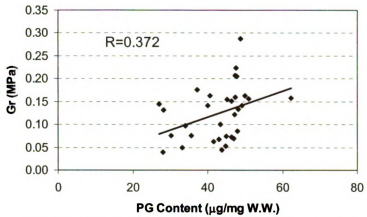


Figure 32. Correlation between relaxed shear modulus G_r and proteoglycan contents for no-exercise and exercise samples from each group in the 11/16/05 study.

Two experimental studies combined

When combining the two final studies, there was a trend for increased G_u at 1 and 3 days, and an increased G_r at 3 days in the exercise groups (Figure 33). However, the no-exercise groups demonstrated an increase in G_r at 1 and 6 days (Figure 33). There was also a trend for an increase in thickness change in the no-exercise samples at 1, 3, and 6 days (Figure 34).

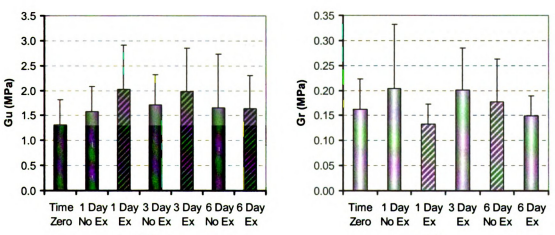


Figure 33. **(a)** Bar chart of the instantaneous modulus (G_u) and **(b)** the relaxed shear modulus (G_r) for no-exercise and exercise samples for the 11/8/05 and 11/16/05 studies combined.

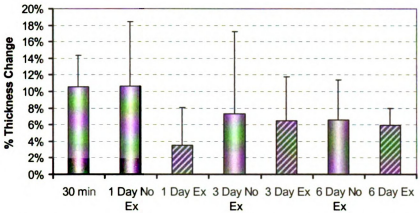


Figure 34. Increases in thickness due to swelling in the 11/8/05 and 11/16/05 studies combined are plotted here. Explant thickness was measured directly off the joint, and again after 30 min, 1 day, 3 days, or 6 days of equilibration.

DISCUSSION

In a previous study by our laboratory the mechanical response to injurious compression was examined in equilibrated and non-equilibrated chondral explants (Rundell and Haut, 2005). The results of the previous study demonstrated that the stiffness of the equilibrated specimens was less than that of the non-equilibrated specimens. Unfortunately, few studies investigate the mechanical response to regularly loaded explants at non-injurious levels. The hypothesis of the current study was that regular cyclic loading of chondral explants would cause an increase in mechanical stiffness of the cartilage.

The initial three pilot studies (6/14/05; 6/28/05; 7/12/05) showed a significant loss of viable cells, with 26 – 100 % cell death occurring in the exercised samples, and 8 – 27 % in the non-loaded controls. These large amounts of cell death can be explained by contamination in the system. Twelve diaphragms are used inside the cartilage exerciser chambers to assist in the vertical plunging action of the pistons. After eliminating numerous factors, it was determined that the diaphragm material (neoprene) was

biologically unsafe. This material had more direct contact with the exercised wells, and was causing complete death in these specimens. It is also possible that this material was contaminating the air, causing some death in the control specimens. To eliminate this problem, the diaphragms were replaced with new biologically safe (silicone) diaphragms. In these pilot studies, major increases in mechanical stiffness were seen in exercised specimens compared to non-exercised controls. With minimal amounts of viable cells remaining in the exercised specimens, it can be concluded that the stiffening effect seen in these initial pilot studies was strictly due to a mechanical response rather than a cellular response.

In the final pilot study and the following two experimental studies, slightly more cell death occurred in the exercised samples compared to the non-exercised controls. When cell death did exist in the control specimens, it was typically located in the superficial zone. Conversely, there was a consistent band of death in the deep zone of the exercised samples. These studies also showed a trend for an increase in fluid gain in the non-loaded explants compared to the exercised samples. These data agree with Rundell's study, where he documented an increase in superficial zone cell death, and a decrease in deep zone cell death, with an increase in water gain (Rundell and Haut, 2005).

One limitation of the current study was that the cartilage explants were not weighed before and after equilibration in media. The percentage change in thickness was attributed to fluid gain, and this was used as a comparison to previous equilibration studies (Rundell and Haut, 2005). However, a better measure of fluid gain would be increase in wet weight of the tissue. Another limitation of the current study was the method of measuring the percentage of cell death. The method used assumed a

uniformity in cell density throughout the depth of the explant. If more time were available, a better measure of cell death would be to individually count live and dead cells in each explant.

A consistent association between an increase in fluid content and a decrease in the instantaneous shear modulus G_u was shown in the current study. These results agree with other researchers, who have also shown a decrease in cartilage stiffness with an increase in fluid gain (Armstrong and Mow, 1982; Morel *et al.*, 2005; Rundell and Haut, 2005). In Chapter 1, both diet supplemented and non-supplemented animals showed no softening of the articular cartilage in impacted limbs (short-term or long-term) versus non-impacted limbs. These data were not characteristic of earlier studies by this laboratory (Newberry *et al.*, 1997; Newberry *et al.*, 1998; Ewers and Haut, 2000; Ewers *et al.*, 2002). Recall that a major difference between the Chapter 1 study and previous studies by our laboratory was that the Chapter 1 animals involved a two-month period of pre-impact exercising. Morel believes that increased matrix swelling may cause the cartilage to be more susceptible to injury (Morel *et al.*, 2005). Therefore, if exercise can help reduce fluid content in joint cartilage, as shown in the current study, the cartilage may be stiffer and better able to withstand a blunt impact with less damage than a non-exercising specimen.

ACKNOWLEDGEMENT

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CHAPTER THREE

EFFECTS OF EXERCISE ON JOINT TRAUMA IN A HIGH ENERGY IMPACT MODEL

ABSTRACT

High intensity impacts (10.0 J) have been shown to accelerate the degradation of articular cartilage in the patello-femoral joint of rabbits in both exercising and non-exercising models. However, no studies have directly investigated the effects of exercise versus no-exercise in an impact model at this high intensity. In this study we impacted the rabbit patello-femoral joint with a severe impact (10.0 J) and compared a group of regularly exercised animals to animals restricted to cage activity only. The rabbits were sacrificed at one of three times: 0, 12, and 24 months post-impact, and the retro-patellar cartilage was biomechanically and histologically examined. Stiffness and permeability of the cartilage were measured by indentation, pathology was scored histologically, and the thickness of the zone of calcified cartilage and subchondral bone were measured. The data indicated that a restriction to cage activity accelerated the histological degeneration of the cartilage in comparison with the regularly exercised animals, yet no loss of articular cartilage was found in any of the groups.

INTRODUCTION

Osteoarthritis (OA) is a chronic joint disease which can be characterized by loss of articular cartilage. OA affects over 20 million Americans, and costs the United States economy more that $60 billion per year (Buckwalter *et al.*, 2004). The incidence of OA is known to rise with age, yet age is not the only factor contributing to OA. Impact trauma

to the joint can cause severe articular deterioration by damaging the cells and disrupting the cartilage matrix (Marsh *et al.*, 2002).

Our laboratory has previously developed a post-traumatic animal model using the rabbit (Flemish Giant) to examine the development of osteoarthritis (Haut *et al.*, 1995). It has been shown that a high severity (6.0 J) impact with a rigid interface causes a softening of the retropatellar cartilage adjacent to superficial lesions, and thickening of underlying subchondral bone at 12 months post-impact in an exercising rabbit model (Ewers *et al.*, 2002). In another study thickening of the subchondral bone was documented at 3 months post impact in a non-exercising rabbit model (New Zealand White), using a higher intensity 10.0 J impact (Mazieres *et al.*, 1987). Histologically this study showed significantly higher scores for degradation of articular cartilage in the contusive knees compared to the opposite control knees, with increasing scores over time in the impacted knees. Softening of the cartilage on the first day post-contusion was also documented, persisting for the first month yet disappearing by 3 months. Based on the Mazieres study, it appears that a higher-intensity impact (> 6.0 J) may be able to accelerate the onset of post-traumatic osteoarthritis. However, few studies explore these effects in an exercising animal model, to examine whether or not exercise will help delay degeneration. One study by our laboratory showed advanced histological degradation in a non-exercising compared to a regularly exercised animal model in a 6.0 J blunt impact to the patello-femoral joint, although there was interestingly an increase in cartilage stiffness in the more degraded non-exercised animals, which was thought to be a factor of increased ossification (Weaver and Haut, 2005).

The role of activity or rest in the treatment of joint injury has been a subject of controversy (Buckwalter, 1995), yet many researchers have shown that regular exercise helps to prevent cartilage degeneration by maintaining normal articular cartilage metabolism (Otterness *et al.*, 1998), and that reduced joint loading can be more or less deleterious to the cartilage (Helminen *et al.*, 1992; Newton *et al.*, 1997). A strong correlation between exercise and cartilage proteoglycans has been shown. It is believed that proteoglycans are the main contributor to cartilage stiffness (Mizrahi *et al.*, 1986), and researchers have demonstrated that low-intensity training increases proteoglycan content in knee articular cartilage (Helminen *et al.*, 1992).

The hypothesis of the current study was that a high-energy impact (10.0 J) would accelerate the onset and progression of osteoarthritis, showing visible degenerative results in less time than noted in previous studies by our laboratory where a lower energy (6.0 J) impact was administered. It was also hypothesized that regular treadmill exercise would help delay the onset of osteoarthritis compared to a non-exercising model, by possibly stiffening the cartilage to protect it from severe histological damage and major changes in the underlying subchondral bone.

METHODS

A total of sixty-three mature Flemish Giant rabbits were used in two separate studies. Thirty rabbits (5.7 ± 0.8 kg, 6-8 months of age) were used in the first study. Another group of animals, thirty-three rabbits (6.1 ± 1.0 kg, 6-8 months of age), were purchased from the same breeder at the end of the first study and used in a second study. Animal experiments were conducted with the approval of the All-University Committee

on Animal Use and Care. For the first study the rabbits were randomly split into three groups: a time zero impact group (n=10), an exercise control group (no impact) (n=11), and an impacted exercise group (n=9). All impacted animals received a single blunt impact to the right patello-femoral joint. The blunt impact protocol has been described with detail in Chapter 1, although the intensity of the impact was increased from 6.0 J to 10.0 J for this study. Briefly, the animals were maintained under general anesthesia (2% Isoflurane and oxygen) with the right hind limb flexed approximately 120°, and the femur aligned vertically with the animal supine. A 10.0 J impact was administered by dropping a 1.0 kg mass from a height of 1.0 m onto the right patello-femoral joint. The dropped mass was arrested electronically after the first impact in order to prevent multiple impactions.

All impacted animals received one injection of Butorphenol (0.2 mg/kg) for early, post-surgical pain after trauma. Following a 5-day period of rest, the exercise group was run on a treadmill 10 minutes a day, 5 days a week, running at 0.3 mph, using an established protocol (Oyen-Tiesma *et al.*, 1998). When not exercising, the animals were housed individually in cages (122 cm x 61 cm x 49 cm). A licensed veterinary technician (J.A.) exercised and cared for the animals in the study. After 12 months both treadmill-exercised and cage-activity animals were euthanized with a legal injection of Pentabarbitol (85.9 g/kg).

The second study also consisted of three randomized groups: a 1 year no-exercise impacted group (n=12), a 2 year exercise control group (no impact) (n=10), and a 2 year impacted exercise group (n=11). All impacted animals were anesthetized (2% Isoflurane and oxygen) and received a single 10.0 J blunt impact to the right patello-femoral joint.

After a 5 day period of rest the exercised groups of animals were run (hopped) 10 minutes a day, 5 days a week at 0.3 mph on a treadmill (Oyen-Tiesma *et al.*, 1998), whereas the remaining animals were allowed routine cage activity. When not exercising, the animals were housed individually in cages (122 cm x 61 cm x 49 cm). After 12 months, the cage-activity animals were euthanized, and after 24 months the treadmill-exercised animals were euthanized with a legal injection of Pentabarbitol (85.9 g/kg).

Immediately after the animals were sacrificed, the patellae were excised, stained with India ink, examined and photographed for permanent records. Structural integrity of the retro-patellar cartilage was determined using indentation stress relaxation tests described with detail in Chapter 1. Briefly, the patellae were placed in a phosphate buffered solution (pH 7.2) and clamped into a custom-built test fixture. A 1.0 mm flat, non-porous probe was pressed into the cartilage 0.1 mm in 50 ms and maintained for 150s in the first study. For the second study it was decided to extend this holding time to 300s. After allowing 5 minutes for the cartilage to recover, the test was repeated with a 1.5 mm diameter flat, non-porous probe. After another 5 minute recovery, the thickness of the indentation site was determined by depressing a needle probe into the cartilage. These indentation tests were repeated at two different sites on the lateral retropatellar facet, away from surface lesions.

Mechanical data were analyzed using a biphasic (poroelastic) model having a transversely isotropic (TI) solid structure. Four elastic parameters (E_{11}, E_{33}, G_{13}, *and* ν_{31}) and two permeability measures (k_1, k_3) were computed using a curve-fitting algorithm (Garcia, 1998). Poisson's ratio ν_{12} was assumed to be equal to the following:

$$\nu_{12} = 1 - 0.5\,(E_{11} / E_{33}) \qquad (1).$$

All patellae from the time zero and 1 year groups were histologically processed. Patellae were placed in 10% buffered formalin for seven days and decalcified in 20% formic acid for another seven days. Tissue blocks were cut transversely across the patella in areas of high contact pressure (Haut et al., 1995). Six sections, eight microns thick were stained with Safranin O-Fast Green and examined in light microscopy at 12–400X.

All patellae were scored based on the histopathologic index scoring system described in Chapter 1. A blinded reader (J.W.) read one representative slide from each patella. The scores were recorded over three different locations on the patella: medial, central, and lateral. The mean and range were documented for all aspects for each group of animals. The zone of calcified cartilage (ZCC) was also measured over the three locations (medial, central, and lateral) by finding the area of the ZCC over a measured distance using digital imaging software (SigmaScan, SPSS Inc., Chicago, IL) (Figure 1).

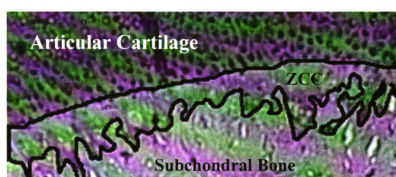

Figure 1. Example of a section of the zone of calcified cartilage outlined in black. Average ZCC thickness measured with use of digital imaging software.

A one-factor ANOVA was used to test for differences between groups based on the impact force and time to peak. A two-factor repeated measures analysis of variance (ANOVA) with Student-Newman Keuls (SNK) *post-hoc* test was used to evaluate the differences in transversely isotropic properties between limbs within groups, and between groups. Limb was the repeated factor, with group being the independent measure. The

Wilcoxon-Signed Rank test (designed to compare two factors) was used to test for differences in histopathologic index scores between impacted and non-impacted limbs in all groups, while the Kruskal-Wallis ANOVA on Ranks test (designed to compare 3 or more groups) was used to check for differences between groups. Statistical significance in this study was set at $p < 0.05$.

RESULTS

First Study: Time Zero, 1 Year Exercise (with Impact), and 1 Year Exercise Control (No Impact) Groups

No significant differences were noted in the gait or health of rabbits between groups. The blunt impact forces on the patello-femoral joint and the times to peak were not different between the time zero (992 ± 205N; 2.4 ± 0.9 ms) and 1 year exercise impact (964 ± 223N; 2.3 ± 0.4 ms) groups.

Gross visual examination of the impacted joints revealed surface fissuring running proximal to distal in 6 out of 10 impacted patellae in the time zero group, and in 8 of 8 in the one year exercise impact group (Figure 2). All joints showed cartilage degeneration distally on the retropatellar surface.

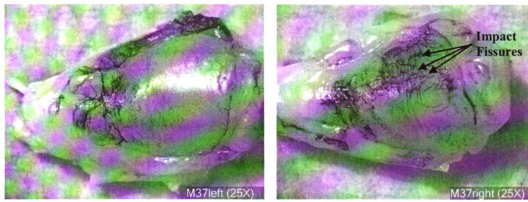

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Figure 2. Photographs of (**a**) left patella, and (**b**) right patella, of an animal from the 1 year exercise impact group. Photographs were taken at 25 x.

The total fissure length on the retro-patellar cartilage in the impacted limb (33.23 ± 15.55 mm) was significantly different from the non-impacted limb (15.78 ± 21.03 mm) in the 1 year exercise with impact group ($p=0.005$). No differences were documented between contralateral limbs in the time zero or 1 year exercise control groups. There was however, a significant difference between impacted limbs of the time zero group (7.19 ± 6.74 mm) and the 1 year exercise impacted group (24.50 ± 20.02 mm, $p<0.001$), and also between right limbs of the 1 year exercise impact group (33.23 ± 15.55 mm) and the 1 year exercise control group (10.96 ± 10.47 mm, $p<0.001$) (Figure 3).

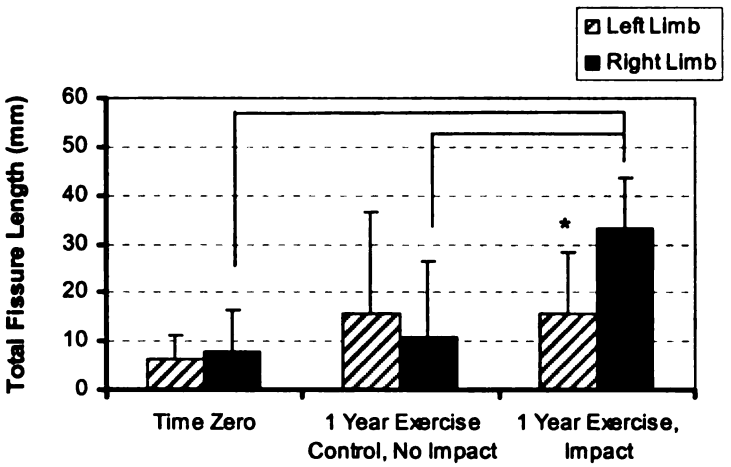


Figure 3. Bar graph of total surface fissure lengths of impacted and non-impacted contralateral limbs of each group in the first study. Lengths were measured using digital imaging software. *Indicates a significant difference compared to the contralateral non-impacted limb. ⌐¬ indicates a significant difference between groups.

Analysis of the indentation relaxation data revealed an overall increase in the E_{11} and the E_{33} moduli over time with one year of exercise. Significant differences were found in E_{11} between the time zero group (2.69 ± 1.18 MPa) and the 1 year exercise control group (4.83 ± 2.26 MPa, p=0.004), and also between the time zero group and the 1 year exercise impact group (4.03 ± 1.48 MPa, p=0.005, Figure 4, Table 1). Significant differences were also found in E_{33} in the time zero group (0.76 ± 0.20 MPa) vs. the 1 year exercise control group (1.10 ± 0.03 MPa, p=0.002), and also between the time zero group and the 1 year exercise impact group (1.02 ± 0.17 MPa, p=0.011, Figure 5, Table 1). In comparing the two exercise groups, an overall decrease in stiffness (E_{11} and E_{33}) was seen in the impacted group, yet these differences were not statistically significant. The results for tissue permeability revealed an overall decrease over time with exercise. A significant difference was found in the k_1 permeability between the time zero group (1.52 ± 0.78 $m^4/Ns \times 10^{-14}$) and the 1 year exercise control group (1.00 ± 0.70 $m^4/Ns \times 10^{-14}$,

p=0.008, Figure 6, Table 1). There was also a significant difference in k_3 between the time zero group (1.27 ± 1.56 $m^4/Ns \times 10^{-14}$) vs. the 1 year exercise control group (0.31 ± 0.20 $m^4/Ns \times 10^{-14}$, p=0.045, Figure 7, Table 1). Between exercise groups, a trend for an increase in permeability (k_1 and k_3) in the impacted group was noted, although neither of these differences was significant. Interestingly, while differences in mechanical parameters were noted between groups, no significant differences were found between contralateral limbs of any group.

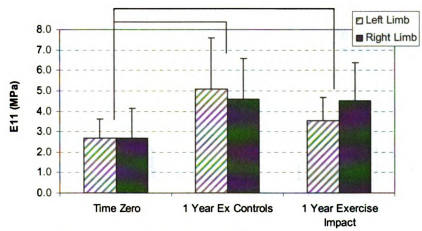


Figure 4. Bar chart of the E_{11} modulus for impacted and non-impacted limbs of each group in the first study.

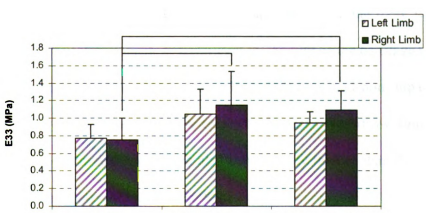


Figure 5. Bar chart of the E_{33} modulus for impacted and non-impacted limbs of each group in the first study.

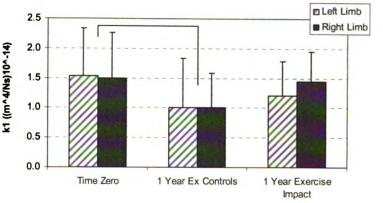


Figure 6. Bar chart of the k_1 permeability for impacted and non-impacted limbs of each group in the first study.

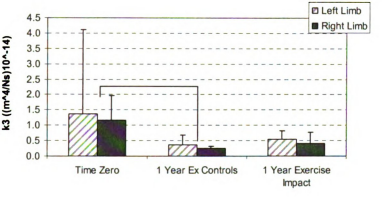


Figure 7. Bar chart of the k_3 permeability for impacted and non-impacted limbs of each group in the first study.

Examination of the articular cartilage thickness revealed that there was no major loss of cartilage 1 year after impact. A significant thickening of the cartilage was found in the time zero group between the impacted (0.70 ± 0.20 mm) and non-impacted (0.59 ± 0.19 mm, p=0.05) limbs, possibly as a result of impact-induced swelling. The right (impacted) limb in the time 0 group was also significantly thicker than the right limb in the 1 year control group (p=0.034) (Figure 8).

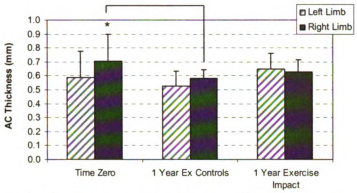
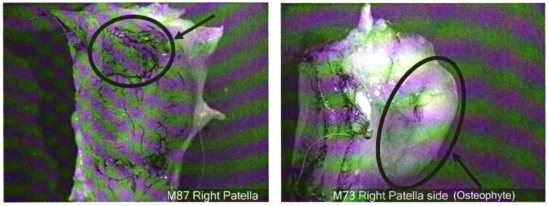


Figure 8. Bar chart of the articular cartilage thickness for impacted and non-impacted limbs of each group in the first study. * Indicates a significant difference compared to the contralateral non-impacted limb. ⌐¬ indicates a significant difference between groups.

Second Study: 1 Year No Exercise and 2 Year Exercise (control and impact) Groups

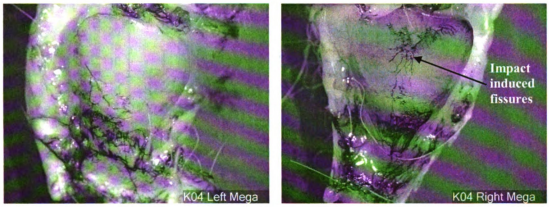
No significant differences were noted in the gait or health of rabbits between groups. The blunt impact forces on the patello-femoral joints and the times to peak were not different between the 1 year no exercise (1048 ± 156N; 3.1 ± 0.6 ms) and the 2 year exercise (1087 ± 127N; 3.0 ± 0.5 ms) groups.

Gross visual examination of the impacted joints revealed 3 cases of osteophyte formations in the 1 year no exercise group (Figure 9), and none in the 2 year groups. Also, fissure patterns on the impacted patella of the 1 year no-exercise group were very consistent, with the majority of the fissuring running proximal to distal near the central ridge of the patella in all 12 samples (Figure 10). However, in the two-year exercise impact group the fissure pattern was very diffuse, with little indication of fissuring caused directly by the impact. In fact, the non-impacted limbs seem to have the same diffuse pattern of fissuring as the impacted limbs (Figure 11), where only 1 out of 11 of the impacted patellae showed indication of the impact site with fissures running proximal to distal along the center ridge.



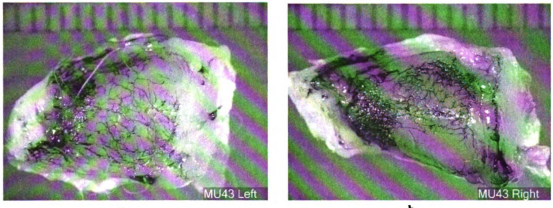
a b

Figure 9. Photographs of (**a**) an osteophyte at the proximal tip of the patella and (**b**) an osteophyte on the anterior side of the patella on animals from the 1 year no exercise group. Photographs were taken at 25 x.



a b

Figure 10. Photographs of (**a**) left patella and (**b**) right impacted patella of an animal from the 1 year no exercise group showing the proximal to distal orientation of impact fissures. Photographs were taken at 25 x.



a b

Figure 11. Photographs of (**a**) left patella and (**b**) right impacted patella of an animal from the 2 year exercise impact group showing a diffuse pattern of fissures across surfaces of both limbs. Photographs were taken at 25 x.

The total fissure length on the retro-patellar cartilage of the impacted limb (29.93 ± 14.58 mm) was significantly different from the non-impacted limb (7.46 ± 6.44 mm) for the 1 year no exercise group ($p<0.001$), and also between impacted (24.76 ± 11.95) and non-impacted (16.43 ± 9.49) limbs of the 2 year exercise impacted group ($p=0.031$) (Figure 12).

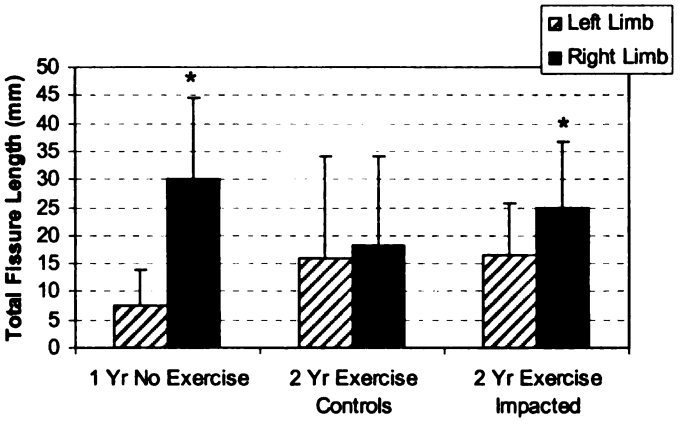


Figure 12. Bar graph of total surface fissure lengths of impacted and non-impacted contralateral limbs of each group in the second study. Lengths were measured using digital imaging software. *Indicates a significant difference compared to the contralateral non-impacted limb.

Analysis of the indentation relaxation data revealed no significant differences in the in-plane modulus (E_{11}), or the thickness direction modulus (E_{33}) between limbs or between groups (Figures 13-14, Table 1). However, in comparing 2 year exercise groups, an overall increase was noted in the impacted group (E_{11}: 10.24 ± 3.62 MPa; E_{33}: 2.35 ± 1.00 MPa) vs. the non-impacted control group (E_{11}: 9.24 ± 2.44 MPa; E_{33}: 2.05 ± 0.65 MPa). A trend for an increase in stiffness was also found in the impacted limb of the 2 year exercise impact group (E_{11}: 10.50 ± 3.92 MPa; E_{33}: 2.42 ± 0.96 MPa) compared to the 1 year no-exercise impact group (E_{11}: 9.60 ± 2.83 MPa; E_{33}: 2.2 ± 0.41 MPa). The k_1 permeability showed a trend for a decrease in the 2 year exercise impacted group (1.58 ±

0.76 $m^4/Ns\times10^{-14}$) vs. the 2 year non-impacted control group (1.79 ± 1.14 $m^4/Ns\times10^{-14}$) (Figure 15, Table 1). Conversely, a trend for an increase in k_3 was found in the 2 year impact group (0.97 ± 0.56 $m^4/Ns\times10^{-14}$) vs. the 2 year non-impact group (0.83 ± 0.37 $m^4/Ns\times10^{-14}$), where the difference in the right (impacted) limb alone of the 2 year impact group (1.10 ± 0.73 $m^4/Ns\times10^{-14}$) was significantly different from the right limb of the control group (0.83 ± 0.37 $m^4/Ns\times10^{-14}$, p=0.04) (Figure 16, Table 1). The 2 year exercise impact group had a trend for a decrease in k_1 (1.58 ± 0.76 $m^4/Ns\times10^{-14}$) in comparison with the 1 year no-exercise impact group (2.19 ± 1.25 $m^4/Ns\times10^{-14}$), which was statistically significant in the impacted limb alone (p=0.05). A trend was also documented for an increase in k_3 (0.97 ± 0.56 $m^4/Ns\times10^{-14}$) of the 2 year exercise impact group in comparison with the 1 year no-exercise impact group (0.75 ± 0.32 $m^4/Ns\times10^{-14}$).

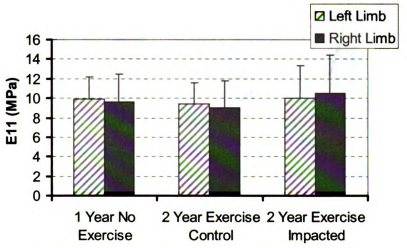


Figure 13. Bar chart of the E_{11} modulus for impacted and non-impacted limbs of each group in the second study.

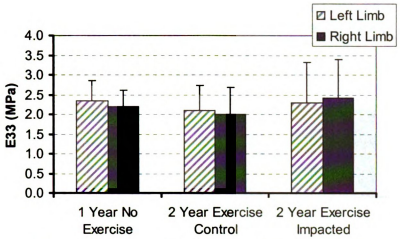


Figure 14. Bar chart of the E_{33} modulus for impacted and non-impacted limbs of each group in the second study.

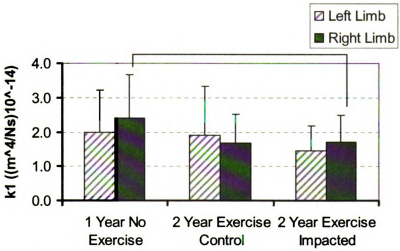


Figure 15. Bar chart of the k_1 permeability for impacted and non-impacted limbs of each group in the second study.

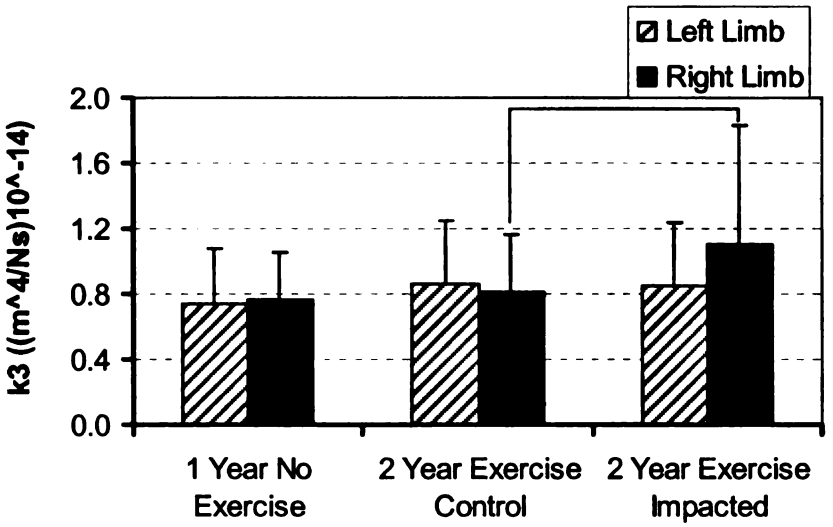


Figure 16. Bar chart of the k_3 permeability for impacted and non-impacted limbs of each group in the second study.

Examination of the articular cartilage (AC) thickness revealed no major loss of cartilage at 1 or 2 years post-impact, regardless of exercise. In fact, there was a trend for an increase in AC thickness in the 2 year exercise impact group (0.63 ± 0.09 mm) compared to the 2 year exercise non-impact group (0.56 ± 0.09 mm), which was statistically significant in the impacted limb alone (p=0.04) (Figure 17). There was also a trend for a thickening of the AC in the 2 year exercise impact group (0.63 ± 0.09 mm) compared to the 1 year no-exercise impact group (0.58 ± 0.07 mm), which again was significant in the impacted limb alone (p=0.02) (Figure 17). A trend was found for a thickening of the articular cartilage in the impacted limb of the 2 year exercise impact group vs. its contralateral non-impacted limb, yet the difference was not significant (p=0.080) (Figure 17).

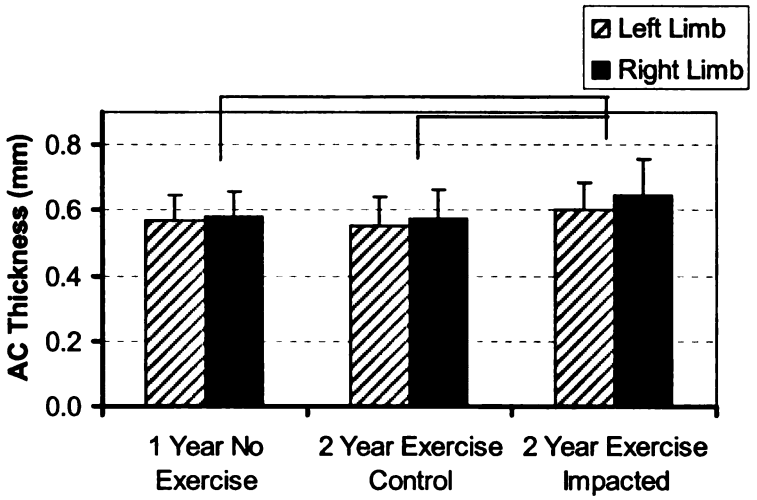


Figure 17. Bar chart of the articular cartilage thickness for impacted and non-impacted limbs of each group in the second study.

		Peak Load (N)	Time to Peak (ms)	Thickness (mm)	E_{11} (MPa)	E_{33} (MPa)	G_{13} (MPa)	ν_{31}	k_1 $(m^4/Ns)10^{-15}$	k_3 $(m^4/Ns)10^{-15}$
Time Zero	Non-imp[a]	----------	----------	0.59 ± 0.11	2.70 ± 1.25	0.77 ± 0.32	0.19 ± 0.06	0.13 ± 0.02	1.53 ± 0.74	1.37 ± 2.76
	Impacted[a]	992 ± 205	2.4 ± 0.9	0.70 ± 0.20*,x	2.69 ± 1.46	0.75 ± 0.25	0.22 ± 0.10	0.12 ± 0.04	1.50 ± 0.76	1.18 ± 0.78
1 Year Exercise Controls	Left[b]	----------	----------	0.53 ± 0.11	5.07 ± 2.53	1.05 ± 0.28	0.31 ± 0.35	0.11 ± 0.04	1.01 ± 0.82	0.37 ± 0.32
	Right[b]	----------	----------	0.58± 0.06	4.59 ± 1.98	1.15 ± 0.39	0.17 ± 0.05	0.13 ± 0.04	1.00 ± 0.59	0.25 ± 0.08
1 Year Exercise Impact	Non-imp[c]	----------	----------	0.65 ± 0.11	3.54 ± 1.11	0.95 ± 0.13	0.19 ± 0.02	0.13 ± 0.04	1.20 ± 0.58	0.55 ± 0.28
	Impacted[c]	964 ± 223	2.3 ± 0.4	0.63 ± 0.09	4.52 ± 1.84	1.09 ± 0.22	0.19 ± 0.04	0.14 ± 0.03	1.44 ± 0.51	0.42 ± 0.36
1 Year No Exercise Impact	Left[d]	----------	----------	0.57 ± 0.07	9.87 ± 2.28	2.34 ± 0.51	0.97 ± 0.85	0.09 ± 0.05	1.98 ± 1.24	0.73 ± 0.35
	Right[d]	1048 ± 156	3.1 ± 0.6	0.58 ± 0.08	9.60 ± 2.83	2.20 ± 0.41	0.60 ± 0.62	0.11 ± 0.06	2.40 ± 1.26	0.73 ± 0.30
2 Year Exercise Impact	Non-imp[b]	----------	----------	0.60 ± 0.08	9.99 ± 3.32	2.28 ± 1.03	0.44 ± 0.56	0.12 ± 0.06	1.45 ± 0.74	0.85 ± 0.38
	Impacted[b]	1087 ± 127	3.0 ± 0.5	0.65 ± 0.11+	10.50 ± 3.92	2.42 ± 0.96	0.34 ± 0.31	0.13 ± 0.05	1.72 ± 0.77	1.10 ± 0.73+
2 Year Exercise No Impact	Left[a]	----------	----------	0.55 ± 0.09	9.45 ± 2.15	2.11 ± 0.62	0.77 ± 0.91	0.09 ± 0.05	1.89 ± 1.43	0.86 ± 0.38
	Right[a]	----------	----------	0.57 ± 0.09	9.04 ± 2.74	2.00 ± 0.68	0.66 ± 0.79	0.11 ± 0.05	1.69 ± 0.84	0.81 ± 0.35

* *Significantly different than contralateral non-impacted limb by a two way repeated factor ANOVA with a SNK post-hoc test ($p < 0.05$).*

[x] *Significantly different than impacted limb of the 1 Year Exercise Controls group.*

[+] *Significantly different than 2 yr Exercise No-Impact impacted limb.*

[a]N=10, [b]N=11, [c]N=9, [d]N=12

Non-imp = non-impacted limb

Table 1: Transversely isotropic material properties of non-impacted and impacted patellae (Avg ± stdev).

Histology and Comparison Between Groups and Studies

Gross examination of the histological sections suggested more degradation of the traumatized retro-patellar cartilage in the one year, no exercise group than the one year exercise, one year exercise control, or time zero groups. In the one year no-exercise group 3 of 12 had ossification on the impacted limb (Figure 18), and 4 of 12 had erosion of the cartilage on the impacted limb (Figure 19). Also, 6 out of 12 had a loss of proteoglycans on the non-impacted limb, and 11 out of 12 had a loss of proteoglycans on the impacted limb (Figure 20). In the 1 year exercise impact group from the first study, 0 out of 9 had ossification, only 2 of 9 had severe erosion, 5 of 9 had a loss of PGs in the impacted limb, and 3 of 8 had a loss of proteoglycans in the non-impacted limb. No ossification or erosion was found in any specimens from the one-year control group, and only 4 of 11 animals showed a slight loss of proteoglycans on either limb.

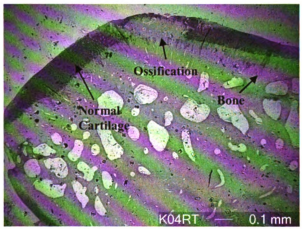


Figure 18. Example of focal ossification in an impacted patella from the 1 year No Exercise group. Photograph was taken at 40 x.

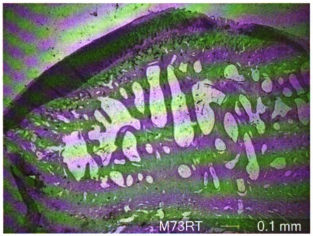


Figure 19. Example of erosion of the cartilage in an impacted patella from the 1 year No Exercise group. Photograph was taken at 40 x.

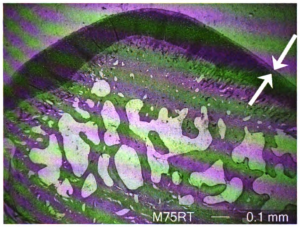


Figure 20. Example of loss of proteoglycans (shown as loss of dark staining). Sample is from an impacted patella from the 1 year No Exercise group. Photograph was taken at 40 x.

Analyses of the histological data revealed no differences between limbs in the one year exercise control group. In the one year exercise impact group the score for pathological cells and clusters in the impacted limb was significantly higher than in the non-impacted limb ($p=0.036$). In the one year no exercise group significant differences between limbs were documented for surface geometry ($p=0.042$), and loss of proteoglycan staining ($p=0.015$). A significant difference was also documented in the

average loss of proteoglycan staining across limbs between the one year exercise impact group and the one year no-exercise group (p=0.005) (Table 2).

	One Year Exercise with Impact		One Year No Exercise		One Year
	Non-impacted	Impacted	Non-impacted	Impacted	Controls
Surface integrity	1.46 (0.00-4.00)	1.79 (0.67-3.00)	0.39 (0.00-1.33)	1.17 (0.00-2.67)*	0.77 (0.00-2.33)
Loss of PG Staining	0.42 (0.00-2.00)	0.58 (0.00-1.67)	0.30 (0.00-0.67)	0.81 (0.00-1.67)*,+	0.30 (0.00-1.33)
Fissures	1.00 (0.00-2.33)	1.46 (0.33-3.00)	0.42 (0.00-1.33)	1.06 (0.00-2.00)	0.64 (0.00-1.33)
Clones/Clusters/ Pathological Cells	1.33 (0.00-2.67)	2.21 (0.67-3.33)*	1.48 (0.00-3.00)	2.17 (0.33-3.00)	1.62 (0.00-4.00)
Ossification	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.22 (0.00-1.33)	0.00 (0.00-0.00)
Exposure of Subchondral Bone	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.11 (0.00-1.33)	0.00 (0.00-0.00)
Erosion	0.00 (0.00-0.00)	0.13 (0.00-1.00)	0.00 (0.00-0.00)	0.22 (0.00-1.00)	0.30 (0.00-0.67)

* *Significantly different from contralateral non-impacted limb by Wilcoxon-Signed Rank Test.*
\+ *Significantly different from one year exercise impact group by Kruskal-Wallis ANOVA on Ranks.*
Table 2: Histology scores for the non-impacted and impacted patellae of the one year exercise impact, one year no exercise, and controls groups (mean (range)).

In examining the thickness of the zone of calcified cartilage (ZCC), a significant difference was found between contralateral limbs in the 1 year no-exercise group at the central location (p=0.008), yet while the trends were the same no statistical differences were recorded at the medial or lateral locations (Figure 21). There were also no significant differences found between contralateral limbs at any of the three locations in the 1 year exercise impact, or the 1 year control groups (Figure 21). Significant differences were found between impacted limbs of the 1 year exercise impact compared to the 1 year no-exercise groups at the central (p=0.023) and lateral (p=0.019) locations of the patella (Figure 21).

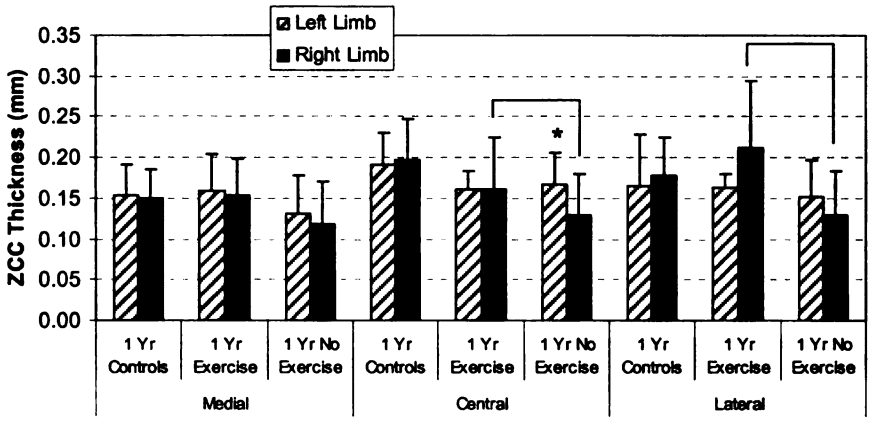


Figure 21. Bar chart of the zone of calcified cartilage thicknesses of the and one year exercise, one year no-exercise, and one year control groups. Thickness measurements were made at 40 x. * Indicates a significant difference compared to the contralateral non-impacted limb.

A thickening of the subchondral bone was found in the 1 year no-exercise group compared to the time zero group, but the difference was only significant at the medial location ($p=0.010$) (Table 3, Figure 22). A thickening of the subchondral bone was also found in the 1 year exercise impact group in comparison with the time zero group, which was significant at all three locations: medial ($p<0.001$), central ($p=0.003$), and lateral ($p<0.001$) (Table 3, Figure 22). A significant thickening was also found in the 1 year exercise group vs. the 1 year no-exercise group at the three locations: medial ($p=0.041$), central ($p<0.001$), and lateral ($p=0.002$) (Table 3, Figure 22). While differences were noted in subchondral bone thickness between groups, interestingly no significant differences were noted between contralateral limbs at any of the three locations in any group.

Group	Limb	Subchondral bone			Cartilage
		Medial	Central	Lateral	
Time Zero	Non-impacted	$0.50 \pm 0.04^{*,+}$	$0.84 \pm 0.19^{*}$	$0.62 \pm 0.15^{*}$	0.51 ± 0.09
	Impacted	$0.52 \pm 0.10^{*,+}$	$0.91 \pm 0.21^{*}$	$0.64 \pm 0.17^{*}$	0.58 ± 0.13
1 Year No Exercise, Impact	Non-impacted	$0.62 \pm 0.24^{*}$	$0.84 \pm 0.32^{*}$	$0.72 \pm 0.16^{*}$	0.57 ± 0.07
	Impacted	$0.59 \pm 0.15^{*}$	$0.84 \pm 0.32^{*}$	$0.68 \pm 0.18^{*}$	0.58 ± 0.08
1 Year Exercise, Impact	Non-impacted	0.64 ± 0.19	1.04 ± 0.27	0.86 ± 0.22	0.65 ± 0.11
	Impacted	0.72 ± 0.12	1.09 ± 0.25	0.76 ± 0.13	0.63 ± 0.09

* *Significantly different from 1 year exercise group.*

\+ *Significantly different from 1 year no-exercise group.*

Table 3: Subchondral bone thickness and articular cartilage thickness measurements (mm) for the non-impacted and impacted patellae of the time zero, one year exercise impact, one year no exercise, and groups. Thickness was measured at 40X with a calibrated eye-piece at the central region of the patella and midline of the lateral and medial facets (average ± S.D.); the cartilage thickness was measured at the sites of biomechanical testing on the lateral facet (average ± S.D.).

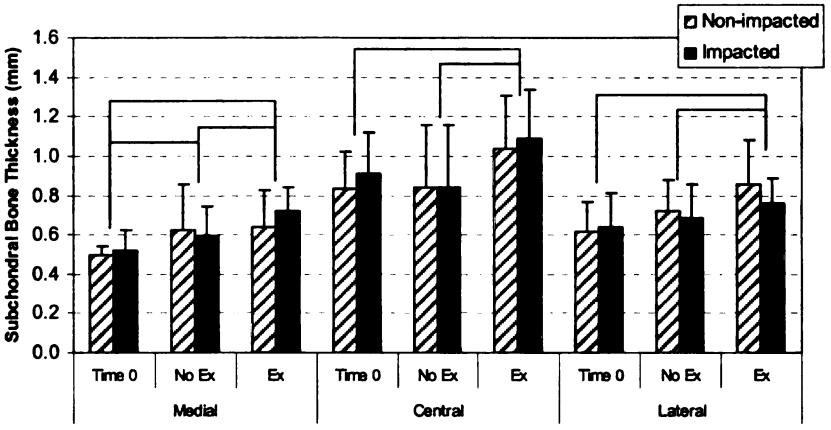


Figure 22. Bar chart of the subchondral bone thicknesses of the time zero and one year exercise and no-exercise groups. Thickness measurements were made at 40 x.

DISCUSSION

The objective of the current study was to follow the cartilage and bone changes in exercise and non-exercise animals out to 24 months following a high-energy 10.0 J impact. It has previously been shown that a high-energy 10.0 J impact results in more severe histological changes than a lower intensity 6.0 J impact in a regularly exercised animal model after 7.5 months (Weaver, 2001). In the current study it was hypothesized that raising the level of impact energy to 10.0 J would again accelerate the degenerative process seen previously with a 6.0 J impact, and that the cartilage in non-exercised animals would degenerate more rapidly than animals exposed to regular exercise after impact trauma.

The results of the current study indicated more histological changes in the no-exercise group compared to the exercise group, in terms of the loss of proteoglycan staining, ossification, erosion, and exposure of subchondral bone. This agrees with the findings from a previous 2 year study with a 6.0 J impact which showed more histological degradation in non-exercised animals (Weaver and Haut, 2005). The results of the current study were consistent with findings from another study, comparing a 6.0 J impact to 10.0 J, which documented severe histological changes in the higher-impact group (Weaver, 2001). It also must be noted that in previous studies with a 6.0 J impact, after only 1 year post-impact no changes in these histological parameters, such as ossification, erosion, and exposure of subchondral bone, were noted (Ewers *et al.*, 2002; Newberry *et al.*, 1997; Newberry *et al.*, 1998). With these changes present at 1 year post-impact in the current study, it is a good indication that the higher energy of impact accelerated the degenerative process, especially in the non-exercising animals.

Previous studies performed by our laboratory have documented significant softening of the retro-patellar cartilage as early as 4.5 months after a 6.0 J blunt trauma in an exercising model, although changes in subchondral bone were not noted until 7.5 months post-trauma (Ewers *et al.*, 2002). However, even after 24 months there was no major histological degradation noted in exercised animals (Weaver and Haut, 2005). This would suggest that at one year post-impact (6.0 J) the cartilage is still in the early stages of degenerative disease (osteoarthritis). Uchio et al. suggest that increases in water content and decreases in collagen integrity in the early stages of disease may reflect lower cartilage stiffness, yet a deterioration of structure and exposure of subchondral bone corresponds with the generation of a higher stiffness (Uchio *et al.*, 2002). The previous 2 year exercise study showed no major histological degradation in retro-patellar cartilage of the exercised animals, yet it did show a significant softening of the cartilage in the impacted limb (Weaver and Haut, 2005). This could be explained by an increase in water content and a decrease in collagen integrity of the tissue (Uchio *et al.*, 2002). The same 2 year study showed major changes in the impacted limb for a no-exercise group, in terms of erosion, ossification, and loss of proteoglycans (Weaver and Haut, 2005). While the exercised group showed a significant softening of the cartilage in the impacted limb, there was no difference in mechanical properties between limbs for the no-exercise group (Weaver and Haut, 2005). The softening effect seen in the exercise group may have been negated in the no-exercise group due to the progressive ossification and calcification of the cartilage. Based on Uchio's theory, this demonstrates that regular exercise may help delay the onset of an end stage of osteoarthritis.

In both the 1st and 2nd studies the impacted limb had a significantly greater amount of fissuring compared to both the non-impacted control groups and the contralateral non-impacted limb. This agrees well with results from a previous study by our laboratory (Ewers *et al.*, 2002), and thus verifies that a blunt impact to the patello-femoral joint causes fissuring of the articular surface.

The current study showed a slight trend for a stiffening of the impacted limb in the exercise groups at both 1 and 2 years post impact, and a trend for a softening of the impacted limb in the 1 year no-exercise group, although these results were not statistical. Also, a significant increase in articular cartilage thickness was found in the impacted limb at time zero compared to the 1 year non-impacted control group, and a non-statistical difference compared to the 1 year exercise group. In Chapter 2 it was discussed that an increase in fluid content leads to a decrease in stiffness. This increase in articular cartilage thickness in the time zero group may be due to impact-induced swelling, which would explain the decrease in mechanical stiffness and increase in permeability in comparison with the exercise groups. In the second study, the 2 year exercise impact group also showed a slight increase in stiffness, and a decrease in the k_1 permeability of the impacted limb, and actually revealed a slight increase in articular cartilage thickness compared to the 1 year no-exercise and the 2 year control groups. In a previous study by another laboratory using a 10.0 J impact model (Mazieres *et al.*, 1987), the investigators documented complete loss of articular cartilage at 6 months post impact. It is important to note that the current study did not show complete loss of articular cartilage after 1 or 2 years post-impact, in either exercised or non-exercised animals.

Mechanical and biological changes in the articular cartilage are induced by the remodeling of both the zone of calcified cartilage and the subchondral bone (Burr and Schaffler, 1997). The current study showed a significant decrease in the zone of calcified cartilage (ZCC) thickness in the impacted limb of the 1 year no-exercise group in comparison to the 1 year exercise group. These findings agree with previous studies which correlate a thickening of the ZCC with regular exercise (Kiviranta *et al.*, 1987; Oettmeier *et al.*, 1992).

Previous studies by our laboratory have consistently shown a thickening of the subchondral plate in the impacted limb (compared to its contralateral non-impacted limb) starting as early as 7.5 months post-trauma, in both exercise and no-exercise animals (Ewers *et al.*, 2002; Ewers and Haut, 2000; Newberry *et al.*, 1997; Newberry *et al.*, 1998). Mazieres *et al.*, (1987) documented a thickening of the subchondral bone at 3 months post impact using a 10.0 J impact. Interestingly, the current study showed no statistical changes between limbs in thickness of the subchondral plate after one year in either the exercise group or no-exercise group. However, it must be noted that the study by Mazieres used White New Zealand rabbits, in a non-exercising model. The combination of this smaller breed of rabbit along with cage-activity may have further helped speed up the degenerative process of osteoarthritis. Although the current study showed no changes in subchondral bone thickness between limbs, the subchondral plate of the exercise group was significantly thicker than that of the no-exercise group. Recalling the more severe histological changes of the non-exercised animals, it is possible that regular exercise caused an increase in the thickness of the subchondral plate

in both impacted and non-impacted limbs, and also helped to delay degradation of the articular cartilage.

There were a number of limitations of the current study which must be mentioned. One major limitation was that two separate studies were involved. Therefore two separate groups of animals were used at different times. Also, the mechanical indentation tests in the first study were performed by a different investigator (D.P.). In the first study a preload of 0.005 N was manually applied. However, it was realized that this preload was within the range of mechanical noise of the system which could have possibly triggered the indentation to begin before the tip was in contact with the specimen. Due to this problem, the indentation procedure for the second study was set for an automated preload of 0.05 N to ensure that the indenter tip was in full contact with the specimen. This change caused an increase in the values of the mechanical properties, making it difficult to directly compare these results between studies. However, regardless of the changes in testing protocols between studies, the differences between limbs were likely not affected, and the trends of changes between limbs in each study were still comparable.

Another limitation of the study was that the histology sectioning of the two year animals has not yet been completed, so the damage beneath the surface of these two year animals is still unknown. One last limitation to mention was that there was no quantitative measurement of proteoglycan content of the cartilage. Other researchers have shown that proteoglycan synthesis, regular exercise, and stiffness may go hand in hand. It may have been beneficial to see if the exercised animals did have an upregulation in proteoglycans compared to the non-exercised animals. Histologically the data did show an increase in the loss of PG staining in the impacted limb of the no-exercise group,

which is a good indication that regular exercise may have caused an alteration in the PG synthesis.

In summary, 10 Joules of blunt impact to the flexed rabbit patello-femoral joint was found to result in more significant histological degradation of the retro-patellar cartilage after one year post-impact than shown previously with a 6 J blunt impact. However, the severe damage that was seen by Mazieres *et al.,* (1987) was still not observed in our animal model. The current study did show that regular exercise helped to delay the degenerative process in comparison to a non-exercising animal model after 1 year post-impact. Mazieres *et al.,* (1987) also showed complete loss of articular cartilage 6 months after trauma, yet there was no indication of an end-stage disease even after 1 year in the non-exercising group. This may indicate that an impact even more severe than 10 J may be necessary to induce end-stage joint degeneration in our larger breed of animal.

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CHAPTER FOUR

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

The previous chapters describe the results of different levels of blunt impact to the patello-femoral joint in *in vivo* exercising animal models, as well as differences between exercise and no exercise, both *in vivo* and *in vitro*. Exercise itself has been suggested to be a therapeutic treatment itself, and certain nutraceuticals were thought to have an increased beneficial effect on joint cartilage when combined with regular exercise.

In Chapter 1 a combination nutraceutical Cosamin®DS (glucosamine and chondroitin sulfate) was shown to have a small but positive effect on the reduction of surface damage after a blunt impact to the patello-femoral joint. A trend for an increase in tissue proteoglycans was found in the impacted limbs of the Cosamin®DS supplemented groups. This corresponded to an increase in mechanical stiffness, and decrease in permeability of the articular cartilage. It was hypothesized that a softening of the cartilage in the impacted limb of the non-supplemented groups would occur, however this effect was not seen. The study was the first for our lab involving regular exercise *pre*-impact. Exercise before the blunt impact may have helped strengthen the cartilage and protect it from severe damage that had been seen in previous studies by our laboratory. Future studies should investigate the effects of pre-impact exercise vs. no exercise, and their effect on tissue stiffness and surface damage following blunt insult to a joint. A higher-energy impact model should also be considered in combination with the nutraceutical. Effects of the glucosamine and chondroitin sulfate may not become evident until severe cartilage degradation can be induced in the test joint.

The animals in Chapter 1 also received a very high dose of the nutraceutical for an extended time period. Subtle but positive effects which were seen in the short-term supplemented group seemed to be negated in the long-term supplemented animals. A future study may want to consider effects of this high dosage of Cosamin®DS over an even more extended time period, to examine the possibility of any long-term negative effects.

Chapter 2 described pilot and experimental studies on exercise vs. no-exercise in chondral explants using an *in vitro* "cartilage exerciser". The results of this study suggested that mechanical loading of cartilage explants allowed for less fluid gain of the tissue, in turn causing a slight stiffening of the cartilage. It is also believed that increased matrix swelling may cause the cartilage to be more susceptible to injury (Morel *et al.*, 2005). Future studies should examine the effects of exercise vs. no-exercise prior to injurious compression. It is possible that pre-impact exercise may limit the degree of damage to the cartilage. Also, previous *in vitro* studies by our laboratory have shown a reduction in cell death around impact-induced fissures in cartilage explants which have been treated with glucosamine (Rundell, 2005). Therefore, future studies may also want to consider what effects glucosamine may have in combination with pre-impact exercise compared to either non-supplemented or non-exercised controls.

In Chapter 3 an increased level of blunt force trauma (10.0 J) was administered to both exercising and non-exercising animals. A previous study by another laboratory has shown complete degradation of articular cartilage 6 months post-impact with the same impact intensity, in a non-exercising animal model (Mazieres *et al.*, 1987). The results of Chapter 3 showed minimal signs of cartilage degeneration in the impacted groups,

although more histological degradation was seen in the non-exercised animals. However, the severity of degradation was no where near the level that Mazieres *et al.* documented, even in the non-exercised animals. This could be due to the larger size of the animals used by our laboratory. Future studies should consider increasing the impact energy to try and initiate more severe degradation in our large animals. This degradation should be examined in both exercising and no-exercising animals after a full 2 years. Also, aside from histological examination, proteoglycan contents should be measured to see if exercise helps prevent a loss of tissue PGs.

A current method of examining cartilage, called delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC), is a non-invasive method which has recently been getting considerable attention. DGEMRIC allows a visual image of the cartilage to be taken, in which cartilage damage can be examined and proteoglycan measurements can be made. This method could be very useful in future studies if more degradation of the cartilage can be induced. Rather than conducting two different studies to examine short-term and long-term effects on the cartilage, dGEMRIC would allow for continual examination of the cartilage over time, using long-term *in vivo* studies.

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Appendix A: Raw Data from Chapter 1

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
B3R2	Left	0.67	10.63	1.74	0.21	0.13	3.35	0.88
B3R2	Right	0.51	9.58	1.66	0.31	0.05	3.40	0.68
BU23	Left	0.75	11.39	2.35	0.23	0.13	4.44	1.13
BU23	Right	0.54	9.80	1.80	0.31	0.12	8.95	0.56
BU24	Left	0.66	6.69	1.28	0.22	0.15	2.69	2.03
BU24	Right	0.59	5.52	1.39	0.18	0.13	1.50	1.55
BU25	Left	0.51	8.00	1.23	0.19	0.10	3.05	0.84
BU25	Right	0.50	9.21	1.63	0.22	0.13	2.47	1.61
BU26	Left	0.52	10.66	1.74	0.34	0.08	2.11	0.88
BU26	Right	0.46	10.00	1.48	0.25	0.08	4.16	0.94
BU27	Left	0.63	5.12	1.20	0.21	0.10	3.91	0.86
BU27	Right	0.57	5.68	1.03	0.19	0.12	8.25	1.19
BU28	Left	0.73	5.52	1.40	0.15	0.19	6.08	3.24
BU28	Right	0.63	5.07	1.28	0.17	0.14	2.62	1.64
GAR2	Left	0.56	9.35	1.76	0.21	0.15	2.79	0.82
GAR2	Right	0.54	10.38	1.78	0.26	0.08	2.34	0.58
KO3	Left	0.70	12.63	2.78	0.22	0.14	2.35	0.45
KO3	Right	0.65	10.81	2.70	0.25	0.17	2.68	0.68
RC121	Left	0.56	9.69	2.30	0.29	0.13	3.10	0.70
RC121	Right	0.60	11.63	2.55	0.26	0.19	2.65	0.73
RC128	Left	0.52	7.77	1.61	0.19	0.12	4.32	0.79
RC128	Right	0.61	9.58	1.59	0.20	0.12	7.20	0.68
V526	Left	0.55	7.25	1.23	0.22	0.08	5.43	1.03
V526	Right	0.57	7.84	1.17	0.17	0.12	2.69	1.00
Average		**0.59**	**8.74**	**1.70**	**0.23**	**0.12**	**3.86**	**1.06**
Standard Dev.		**0.08**	**2.24**	**0.50**	**0.05**	**0.03**	**1.97**	**0.60**

Table 1. Mechanical indentation data for the non-supplemented group in the acute study.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
BB07	Left	0.68	3.90	1.25	0.15	0.17	5.38	2.23
BB07	Right	0.83	6.44	2.02	0.19	0.18	4.24	1.79
BBR1	Left	0.61	8.66	1.80	0.17	0.10	3.52	0.79
BBR1	Right	0.58	8.26	1.55	0.20	0.10	2.49	0.95
BU17	Left	0.53	9.85	2.10	0.27	0.09	1.91	0.95
BU17	Right	0.57	9.07	2.00	0.19	0.13	1.71	1.02
BU18	Left	0.52	8.92	1.66	0.21	0.10	3.25	0.97
BU18	Right	0.58	9.56	1.98	0.30	0.10	1.47	0.73
BU19	Left	0.47	7.70	1.88	0.21	0.15	5.83	0.60
BU19	Right	0.67	8.07	2.14	0.19	0.12	3.06	0.86
BU20	Left	0.60	11.78	2.16	0.26	0.09	1.51	0.57
BU20	Right	0.64	11.61	2.06	0.22	0.12	1.85	0.89
BU21	Left	0.65	8.18	1.39	0.19	0.08	1.48	0.76
BU21	Right	0.48	10.00	1.55	0.22	0.05	1.19	0.57
BU30	Left	0.67	13.80	2.40	0.33	0.12	2.35	0.62
BU30	Right	0.60	11.76	2.24	0.25	0.09	2.79	0.65
BU31	Left	0.41	6.00	0.90	0.15	0.10	4.55	1.38
BU31	Right	0.64	7.82	1.31	0.18	0.08	2.40	1.03
RC120	Left	0.51	8.00	1.43	0.15	0.15	5.35	0.83
RC120	Right	0.59	10.41	1.82	0.24	0.08	4.94	0.61
RC129	Left	0.74	11.03	2.33	0.20	0.14	1.93	1.14
RC129	Right	0.66	6.71	1.64	0.21	0.15	1.56	1.26
RHIF	Left	0.57	4.12	1.10	0.21	0.12		1.31
RHIF	Right	0.56	8.96	1.61	0.17	0.12	3.45	0.91
WHAT	Left	0.65	7.55	1.55	0.18	0.17	2.06	1.11
WHAT	Right	0.63	11.10	2.14	0.20	0.12	2.73	0.76
Average		**0.60**	**8.82**	**1.77**	**0.21**	**0.12**	**2.92**	**0.97**
Standard Dev.		**0.09**	**2.33**	**0.39**	**0.04**	**0.03**	**1.40**	**0.38**

Table 2. Mechanical indentation data for the supplemented group in the acute study.

Acute Non-Supplemented			Acute Supplemented		
Rabbit	**Patella**	**Wet weight (μg/mg)**	**Rabbit**	**Patella**	**Wet weight (μg/mg)**
B3R2	Left	22.6	BB07	Left	22.8
B3R2	Right	24.0	BB07	Right	23.3
BU23	Left	8.1	BBR1	Left	22.1
BU23	Right	5.4	BBR1	Right	23.2
BU24	Left	16.9	BU17	Left	21.1
BU24	Right	17.5	BU17	Right	23.8
BU25	Left	22.5	BU18	Left	13.2
BU25	Right	17.4	BU18	Right	16.2
BU26	Left	22.5	BU19	Left	17.6
BU26	Right	19.7	BU19	Right	21.4
BU27	Left	10.8	BU20	Left	24.5
BU27	Right	19.8	BU20	Right	24.6
BU28	Left	13.2	BU21	Left	7.5
BU28	Right	24.3	BU21	Right	17.0
GAR2	Left	21.6	BU30	Left	22.6
GAR2	Right	23.9	BU30	Right	26.6
KO3	Left	35.9	BU31	Left	20.2
KO3	Right	34.4	BU31	Right	20.4
RC121	Left	27.3	RC120	Left	8.0
RC121	Right	34.5	RC120	Right	7.6
RC128	Left	24.9	RC129	Left	21.4
RC128	Right	21.0	RC129	Right	26.2
V526	Left	24.6	RHIF	Left	21.3
V526	Right	22.2	RHIF	Right	25.2
			WHAT	Left	24.3
			WHAT	Right	27.5
Average		**21.5**			**20.4**
Standard Dev.		**7.5**			**5.7**

Table 3. Proteoglycan data for each group in the acute study.

		ARTICULAR CARTILAGE								
Rabbit	**Limb**	**Geometry**	**Fissures**	**PG Stain**	**Cells**	**Disruptions**	**Max Thickness**	**Focal Ossification**	**Exposure of Subchondral bone**	**Erosion**
V526	Left	1.3	0.0	1.0	1.3	4.0	1.3	0.0	0.0	0.0
V526	Right	0.0	0.7	0.0	0.3	2.7	1.7	0.0	0.0	0.0
GAR2	Left	0.0	0.0	0.0	0.3	1.3	1.7	0.0	0.0	0.0
GAR2	Right	1.7	0.3	0.0	2.7	3.3	0.7	0.0	0.0	0.0
BU27	Left	0.0	0.3	0.0	1.0	2.0	2.3	0.0	0.0	0.0
BU27	Right	1.7	1.7	1.3	1.0	0.0	2.0	0.0	0.0	0.0
B3R2	Left	0.0	0.3	0.0	3.0	0.0	1.7	0.0	0.0	0.0
B3R2	Right	0.0	0.3	0.0	1.0	0.0	1.7	0.0	0.0	0.0
RC121	Left	1.7	2.0	0.0	1.7	0.0	2.3	0.0	0.0	0.0
RC121	Right	0.0	0.3	0.0	0.7	1.3	2.3	0.0	0.0	0.0
BU25	Left	0.3	0.3	0.0	2.0	1.3	1.3	0.0	0.0	0.0
BU25	Right	0.0	0.0	0.0	1.3	1.3	1.7	0.0	0.0	0.0
KO3	Left	0.3	0.3	0.0	1.7	0.0	3.0	0.0	0.0	0.0
KO3	Right	1.0	0.7	0.0	0.3	2.7	2.7	0.0	0.0	0.0
BU23	Left	0.0	0.0	0.0	0.3	1.3	2.0	0.0	0.0	0.0
BU23	Right	0.7	0.7	0.0	1.0	0.0	2.3	0.0	0.0	0.0
RC128	Left	0.7	0.7	0.0	1.0	0.0	1.7	0.0	0.0	0.0
RC128	Right	1.7	0.7	0.7	1.3	0.0	2.7	0.0	0.0	0.0
BU24	Left	0.0	0.0	0.0	0.7	2.7	2.7	0.0	0.0	0.0
BU24	Right	0.0	0.0	0.0	0.0	0.7	2.7	0.0	0.0	0.0
BU28	Left	0.7	2.0	0.0	1.7	1.3	3.0	0.0	0.0	0.0
BU28	Right	0.7	1.0	0.0	1.7	1.3	3.0	0.0	0.0	0.0
BU26	Left	0.3	0.3	0.0	1.0	1.3	1.7	0.0	0.0	0.0
BU26	Right	0.7	0.7	0.0	2.7	1.3	2.3	0.0	0.0	0.0

Table 4. Histology scores for the acute non-supplemented group. Scores were averaged over the medial, central, and lateral locations.

		CALCIFIED CARTILAGE					SUBCHONDRAL BONE				
Rabbit	Limb	Tide Mark	Thickness	Spikes	Stain	Cells	Average Thickness	Morphology	Red Cells	Red Patches	Trabecular Bone
V526	Left	3.0	0.3	3.7	3.0	2.0	1.0	1.0	2.0	2.0	0.0
V526	Right	0.0	0.7	2.0	3.0	3.3	1.7	3.0	2.0	2.0	3.0
GAR2	Left	0.0	0.0	4.0	3.0	2.0	1.3	3.0	4.0	4.0	0.0
GAR2	Right	2.3	0.0	4.0	3.0	2.7	1.0	1.7	2.0	2.0	4.0
BU27	Left	1.0	1.7	2.0	2.7	4.0	2.0	2.0	2.0	2.0	2.0
BU27	Right	3.0	2.0	2.7	2.0	4.0	2.3	2.0	1.3	2.0	3.0
B3R2	Left	2.0	0.3	2.0	3.0	0.7	2.7	3.0	4.0	3.3	3.0
B3R2	Right	3.0	0.0	2.0	2.3	1.3	2.3	2.7	4.0	2.0	2.0
RC121	Left	0.0	0.7	2.0	2.0	3.3	1.7	1.0	4.0	2.0	0.0
RC121	Right	0.0	0.3	2.0	3.0	3.3	2.0	3.0	2.7	2.0	2.0
BU25	Left	0.0	1.3	3.3	2.0	2.0	2.3	2.3	4.0	4.0	1.0
BU25	Right	0.0	1.7	3.7	2.0	2.7	3.0	3.0	4.0	3.3	1.0
KO3	Left	1.0	1.0	1.3	2.0	0.7	2.0	1.0	4.0	2.0	0.0
KO3	Right	1.0	0.7	2.0	2.0	2.0	1.0	1.0	2.7	1.3	0.0
BU23	Left	1.0	1.0	2.3	2.0	2.0	2.7	3.0	4.0	2.0	3.0
BU23	Right	0.0	0.7	2.0	2.0	4.0	3.0	3.0	4.0	2.0	3.0
RC128	Left	0.0	1.7	3.3	2.0	4.0	2.7	2.7	2.0	2.7	2.0
RC128	Right	0.0	0.7	1.7	2.3	3.3	2.7	3.0	2.0	2.0	3.0
BU24	Left	2.0	0.7	2.0	2.0	2.7	1.7	1.3	2.0	2.0	3.0
BU24	Right	3.0	0.7	2.0	2.0	3.3	2.0	2.3	2.0	2.0	3.0
BU28	Left	2.0	0.0	2.0	2.7	4.0	1.0	3.0	3.3	2.0	3.0
BU28	Right	1.7	0.3	1.7	2.0	2.7	2.3	2.0	2.0	2.0	3.0
BU26	Left	0.0	1.3	1.3	2.0	4.0	2.0	3.0	3.3	3.3	2.0
BU26	Right	0.3	0.7	2.0	1.7	4.0	2.7	3.0	4.0	3.3	2.0

Table 5. Histology scores for the acute non-supplemented group. Scores were averaged over the medial, central, and lateral locations.

		ARTICULAR CARTILAGE								
Rabbit	Limb	Geometry	Fissures	PG Stain	Cells	Disruptions	Max Thickness	Focal Ossification	Exposure of Subchondral bone	Erosion
BU21	Left	0.0	0.3	0.0	0.3	1.3	1.7	0.0	0.0	0.0
BU21	Right	0.7	1.0	0.0	0.7	0.0	1.7	0.0	0.0	0.0
BU20	Left	0.0	0.0	0.0	1.7	2.0	2.0	0.0	0.0	0.0
BU20	Right	0.3	0.7	0.0	0.3	3.3	2.3	0.0	0.0	0.0
BBR1	Left	0.0	0.0	0.0	0.0	1.3	2.7	0.0	0.0	0.0
BBR1	Right	1.0	0.7	0.0	0.3	3.3	1.7	0.0	0.0	0.0
BU19	Left	0.0	0.3	0.0	0.0	0.0	1.3	0.0	0.0	0.0
BU19	Right	2.0	2.0	0.3	0.7	0.0	1.3	0.0	0.0	0.0
BU30	Left	0.0	0.3	0.0	1.0	0.0	2.7	0.0	0.0	0.0
BU30	Right	2.3	2.0	0.0	1.7	0.0	3.0	0.0	0.0	0.0
BU31	Left	2.7	2.7	0.0	0.7	1.3	1.3	0.0	0.0	0.0
BU31	Right	0.7	0.7	1.7	2.3	1.3	2.0	0.0	0.0	0.0
BB07	Left	0.7	1.3	0.3	1.3	0.0	3.3	0.0	0.0	0.0
BB07	Right	0.7	1.0	0.0	1.7	0.0	3.0	0.0	0.0	0.0
RHIF	Left	1.7	1.7	0.3	0.7	1.3	2.3	0.0	0.0	0.0
RHIF	Right	0.3	1.3	0.0	0.0	1.3	2.3	0.0	0.0	0.0
BU17	Left	0.7	0.0	0.3	1.3	0.0	2.7	0.0	0.0	0.0
BU17	Right	1.7	1.0	0.3	0.0	1.3	3.0	0.0	0.0	0.0
BU18	Left	0.3	0.3	0.0	2.3	0.0	2.0	0.0	0.0	0.0
BU18	Right	0.0	0.0	0.0	0.3	1.3	2.3	0.0	0.0	0.0
RC120	Left	0.3	0.7	0.0	0.3	1.3	2.0	0.0	0.0	0.0
RC120	Right	1.3	1.0	0.3	1.0	1.3	1.7	0.0	0.0	0.0
WHAT	Left	1.0	1.3	0.0	2.0	0.0	1.3	0.0	0.0	0.0
WHAT	Right	1.7	2.0	0.3	1.0	4.0	1.3	0.0	0.0	0.0

Table 6. Histology scores for the acute supplemented group. Scores were averaged over the medial, central, and lateral locations.

		CALCIFIED CARTILAGE					SUBCHONDRAL BONE				
Rabbit	Limb	Tide Mark	Thickness	Spikes	Stain	Cells	Average Thickness	Morphology	Red Cells	Red Patches	Trabecular Bone
BU21	Left	0.0	0.0	2.3	2.0	0.0	1.7	2.7	3.3	3.3	2.0
BU21	Right	0.7	0.0	2.0	2.0	0.0	1.3	2.0	0.0	2.7	2.0
BU20	Left	2.0	0.3	2.0	3.0	2.0	2.3	2.3	4.0	4.0	1.0
BU20	Right	3.0	0.0	4.0	3.0	2.0	1.7	1.3	0.0	4.0	1.0
BBR1	Left	0.0	0.3	1.3	2.0	1.3	2.3	1.7	3.3	2.0	1.0
BBR1	Right	3.0	1.0	2.3	3.0	4.0	3.0	2.3	2.0	2.0	2.0
BU19	Left	0.0	0.3	2.0	2.0	2.0	2.3	2.3	4.0	4.0	1.0
BU19	Right	0.3	0.0	2.7	2.0	2.7	1.3	1.3	4.0	4.0	1.0
BU30	Left	0.0	0.3	1.7	2.0	0.7	2.7	3.0	4.0	2.7	3.0
BU30	Right	0.0	1.0	3.3	2.0	3.3	3.0	3.0	4.0	3.3	3.0
BU31	Left	1.0	0.3	2.3	2.0	2.0	2.0	2.0	1.3	4.0	2.0
BU31	Right	2.0	2.3	2.7	2.0	3.3	3.7	2.0	0.0	4.0	3.0
BB07	Left	1.0	0.7	2.3	2.0	2.7	3.0	2.7	0.0	2.0	2.0
BB07	Right	0.0	1.0	2.0	2.0	0.7	1.7	1.3	0.0	2.0	1.0
RHIF	Left	0.0	0.7	1.7	2.0	1.3	2.0	2.0	2.0	2.0	0.0
RHIF	Right	2.0	0.7	1.7	2.0	3.3	2.3	2.3	3.3	2.0	3.0
BU17	Left	0.7	0.3	2.0	2.0	1.3	2.3	3.0	0.0	4.0	2.7
BU17	Right	2.0	0.0	1.3	2.3	3.3	3.0	3.0	2.0	2.0	3.0
BU18	Left	0.3	0.3	1.0	2.0	2.0	2.7	3.0	4.0	2.7	3.0
BU18	Right	2.0	0.3	2.0	3.0	4.0	2.7	2.3	2.0	1.3	2.0
RC120	Left	0.0	0.7	2.0	2.3	3.3	1.7	1.0	2.0	2.0	0.0
RC120	Right	0.0	1.0	2.0	2.0	3.3	1.3	3.0	0.0	0.7	0.0
WHAT	Left	0.0	0.3	1.0	2.3	2.7	1.7	3.0	4.0	1.3	1.0
WHAT	Right	1.5	1.5	2.0	2.5	4.0	1.7	3.0	3.3	2.0	1.0

Table 7. Histology scores for the acute supplemented group. Scores were averaged over the medial, central, and lateral locations.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
BU43	Left	0.47	9.07	1.97	0.21	0.17	1.44	0.66
BU43	Right	0.56	7.78	2.20	0.17	0.15	1.57	0.67
BU53	Left	0.65	6.61	2.03	0.22	0.18	2.23	1.89
BU53	Right	0.63	10.58	2.75	0.23	0.16	1.89	0.62
BU54	Left	0.63	9.95	2.31	0.24	0.17	1.47	1.00
BU54	Right	0.50	9.82	2.29	0.14	0.10	2.17	0.65
BU56	Left	0.50	9.04	1.90	0.87	0.05	1.23	0.60
BU56	Right	0.60	9.73	2.02	0.16	0.13	4.20	0.64
BU57	Left	0.59	10.03	2.25	0.23	0.11	1.81	0.67
BU57	Right	0.57	8.39	1.23	0.14	0.12	1.73	0.67
BU58	Left	0.61	12.00	2.76	0.28	0.14	1.62	0.58
BU58	Right	0.57	11.28	2.43	0.29	0.17	0.96	0.73
BU59	Left	0.62	10.42	2.55	0.22	0.17	2.30	0.67
BU59	Right	0.71	7.48	1.93	0.16	0.18	2.25	1.39
K-311	Left	0.60	9.12	2.32	0.23	0.17	1.03	1.04
K-311	Right	0.50	10.07	2.25	0.36	0.12	1.06	0.64
K-611	Left	0.59	7.73	2.31	0.89	0.14	2.60	0.88
K-611	Right	0.53	10.54	2.41	0.21	0.15	1.50	0.67
RSOB	Left	0.67	6.40	1.89	0.18	0.17	3.22	1.29
RSOB	Right	0.64	8.29	2.38	0.19	0.18	1.67	1.01
ZBS9	Left	0.60	6.19	1.64	0.20	0.16	2.63	1.72
ZBS9	Right	0.71	6.60	1.75	0.19	0.17	4.60	1.36
ZBU3	Left	0.62	6.94	1.79	0.21	0.15	4.90	0.79
ZBU3	Right	0.59	6.33	1.41	0.14	0.15	3.53	1.29
Average		0.59	8.77	2.12	0.26	0.15	2.23	0.92
Standard Dev.		0.06	1.72	0.38	0.19	0.03	1.11	0.38

Table 8. Mechanical indentation data for the non-supplemented group in the chronic study.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
BU31	Left	0.51	9.45	2.31	0.26	0.20	1.37	0.85
BU31	Right	0.67	11.39	2.91	0.35	0.17	1.64	0.85
BU37	Left	0.68	7.42	1.96	0.20	0.18	1.36	3.33
BU37	Right	0.56	11.16	2.40	0.27	0.13	2.19	0.50
BU38	Left	0.54	6.48	1.87	0.20	0.13	1.68	1.00
BU38	Right	0.65	12.66	2.94	0.27	0.12	1.48	0.56
BU39	Left	0.51	10.07	1.86	1.19	0.12	1.53	0.51
BU39	Right	0.55	7.52	1.83	0.20	0.17	2.00	0.91
BU40	Left	0.63	6.87	1.44	0.18	0.13	3.20	1.02
BU40	Right	0.60	7.65	1.57	0.16	0.13	1.79	0.79
BU42	Left	0.61	10.55	2.22	0.19	0.13	2.12	1.06
BU42	Right	0.67	9.79	2.12	0.20	0.18	1.70	0.93
BU51	Left	0.58	10.45	2.12	1.20	0.08	1.34	0.84
BU51	Right	0.54	10.64	2.22	0.86	0.07	1.25	0.69
BU52	Left	0.61	4.73	0.82	0.17	0.15	2.27	1.12
BU52	Right	0.70	6.87	1.31	0.17	0.10	2.43	1.16
GAR7	Left	0.62	10.64	1.32	0.29	0.07	1.94	0.63
GAR7	Right	0.52	9.28	1.23	0.77	0.05	1.53	0.78
K-6	Left	0.49	10.22	2.49	1.93	0.07	1.11	0.64
K-6	Right	0.57	13.54	2.91	0.32	0.14	1.55	0.54
RSO415	Left	0.66	8.90	1.88	0.24	0.10	2.45	0.62
RSO415	Right	0.69	8.64	2.28	0.24	0.18	1.95	0.97
ZBU1	Left	0.60	6.04	1.27	0.20	0.14	3.84	0.95
ZBU1	Right	0.62	8.55	1.86	0.14	0.17	3.09	0.62
Average		0.60	9.15	1.97	0.42	0.13	1.95	0.91
Standard Dev.		0.06	2.15	0.56	0.45	0.04	0.67	0.55

Table 9. Mechanical indentation data for the short-term supplemented group in the chronic study.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
BU34	Left	0.59	10.06	1.69	0.23	0.12	1.65	0.89
BU34	Right	0.80	9.64	2.69	0.24	0.16	3.14	0.77
BU35	Left	0.67	3.54	0.71	0.15	0.09	3.84	2.68
BU35	Right	0.69	6.39	1.61	0.19	0.16	5.22	1.65
BU36	Left	0.74	7.35	2.16	0.20	0.18	2.30	1.98
BU36	Right	0.61	8.07	2.42	0.23	0.15	1.24	1.19
BU41	Left	0.57	7.62	1.89	0.82	0.05	1.57	0.66
BU41	Right	0.68	12.98	2.66	0.25	0.17	2.04	0.58
BU45	Left	0.63	9.37	2.36	0.28	0.16	2.18	0.76
BU45	Right	0.59	5.75	1.91	0.17	0.19	3.02	0.91
BU46	Left	0.60	7.62	2.07	0.19	0.12	1.95	1.13
BU46	Right	0.64	8.23	1.86	0.25	0.15	3.27	1.33
BU47	Left	0.63	7.73	1.95	0.21	0.16	2.57	1.60
BU47	Right	0.71	9.47	2.57	0.19	0.18	2.50	0.73
BU48	Left	0.59	9.86	2.31	0.22	0.15	2.31	0.83
BU48	Right	0.56	10.03	2.18	0.21	0.19	1.85	0.78
BU49	Left	0.50	8.94	1.13	1.97	0.00	1.71	0.72
BU49	Right	0.48	8.94	1.82	1.86	0.03	2.24	0.72
BU50	Left	0.67	11.47	2.90	0.20	0.17	1.50	0.58
BU50	Right	0.66	8.77	2.53	0.19	0.15	2.30	0.71
K-1	Left	0.57	10.12	2.10	1.96	0.09	1.50	0.56
K-1	Right	0.55	10.92	2.27	0.33	0.12	3.00	0.62
K-3	Left	0.61	9.12	2.65	1.53	0.05	1.82	0.76
K-3	Right	0.67	9.65	2.00	0.20	0.13	1.45	0.81
Average		0.63	8.82	2.10	0.51	0.13	2.34	1.00
Standard Dev.		0.07	1.94	0.50	0.62	0.05	0.90	0.52

Table 10. Mechanical indentation data for the long-term supplemented group in the chronic study.

Chronic Non-supplemented			Chronic Short-term Supplemented			Chronic Long-term Supplemented		
Rabbit	Limb	Wet weight (μg/mg)	Rabbit	Limb	Wet weight (μg/mg)	Rabbit	Limb	Wet weight (μg/mg)
BU43	Right	40.9	BU31	Right	43.8	BU34	Right	39.9
BU53	Right	44.2	BU37	Right	40.0	BU35	Right	23.5
BU54	Right	36.4	BU38	Right	40.6	BU36	Right	41.1
BU56	Right	39.7	BU39	Right	52.0	BU41	Right	42.1
BU57	Right	33.7	BU40	Right	27.2	BU45	Right	32.2
BU58	Right	41.5	BU42	Right	39.1	BU46	Right	27.0
BU59	Right	49.7	BU51	Right	30.8	BU47	Right	42.0
K-311	Right	43.8	BU52	Right	42.8	BU48	Right	41.1
K-611	Right	32.1	GAR7	Right	29.4	BU49	Right	33.3
RSOB	Right	44.3	K-6	Right	30.6	BU50	Right	37.9
ZBS9	Right	33.5	RSO415	Right	42.5	K-1	Right	33.0
ZBU3	Right	36.7	ZBU1	Right	32.6	K-3	Right	33.0
BU43	Left	42.4	BU31	Left	38.9	BU34	Left	36.8
BU53	Left	43.1	BU37	Left	38.2	BU35	Left	16.7
BU54	Left	31.4	BU38	Left	42.3	BU36	Left	43.5
BU56	Left	41.8	BU39	Left	49.9	BU41	Left	43.7
BU57	Left	34.3	BU40	Left	29.5	BU45	Left	37.2
BU58	Left	38.7	BU42	Left	35.5	BU46	Left	35.2
BU59	Left	51.1	BU51	Left	31.5	BU47	Left	38.2
K-311	Left	43.0	BU52	Left	20.0	BU48	Left	50.2
K-611	Left	41.1	GAR7	Left	33.9	BU49	Left	26.2
RSOB	Left	43.1	K-6	Left	34.9	BU50	Left	38.4
ZBS9	Left	28.2	RSO415	Left	39.3	K-1	Left	30.8
ZBU3	Left	38.9	ZBU1	Left	31.6	K-3	Left	40.5
Average (Right)		39.7			37.6			35.5
Average (Left)		39.8			35.5			36.5
Std Dev. (Right)		5.4			7.4			6.2
Std Dev. (Left)		6.1			7.4			8.8

Table 11. Proteoglycan data for the each group in the chronic study.

		ARTICULAR CARTILAGE								
Rabbit	Limb	Geometry	Fissures	PG Stain	Cells	Disruptions	Max Thickness	Focal Ossification	Exposure of Subchondral bone	Erosion
BU43	Left	0.0	0.0	0.0	0.7	0.0	1.3	0.0	0.0	0.0
BU43	Right	1.3	1.3	0.0	2.0	0.0	1.7	0.0	0.0	0.0
BU53	Left	1.3	1.3	0.7	1.3	0.0	1.7	0.0	0.0	0.0
BU53	Right	1.3	0.7	0.3	1.7	0.0	1.3	0.0	0.0	0.0
BU54	Left	0.3	0.7	0.0	0.3	2.7	2.0	0.0	0.0	0.0
BU54	Right	1.0	1.0	0.3	1.3	2.7	1.3	0.0	0.0	0.0
BU56	Left	2.3	2.7	1.0	1.3	1.3	1.3	0.0	0.0	0.0
BU56	Right	2.3	2.0	0.3	1.3	0.0	1.7	0.0	0.0	0.0
BU57	Left	1.0	1.3	0.3	1.3	1.3	1.3	0.0	0.0	0.0
BU57	Right	0.3	1.0	0.0	0.3	2.0	1.3	0.0	0.0	0.0
BU58	Left	0.0	0.0	0.0	0.3	2.0	1.0	0.0	0.0	0.0
BU58	Right	0.0	0.0	0.0	0.3	0.7	2.0	0.0	0.0	0.0
BU59	Left	0.3	0.3	0.0	0.3	0.0	2.0	0.0	0.0	0.0
BU59	Right	0.0	0.7	0.0	1.3	2.7	2.0	0.0	0.0	0.0
K-311	Left	0.0	0.0	0.0	0.7	0.0	1.3	0.0	0.0	0.0
K-311	Right	0.0	0.0	0.3	2.0	1.3	1.0	0.0	0.0	0.0
K-611	Left	0.3	1.0	0.0	1.0	0.0	1.3	0.0	0.0	0.0
K-611	Right	1.3	2.0	1.3	2.0	4.0	1.3	0.0	0.0	1.3
RSOB	Left	1.7	1.7	0.3	0.7	0.0	2.3	0.0	0.0	0.0
RSOB	Right	1.7	1.7	1.0	1.0	2.7	2.3	0.0	0.0	0.0
ZBS9	Left	1.3	1.0	0.3	1.0	1.3	2.3	0.0	0.0	0.0
ZBS9	Right	0.7	0.3	0.0	0.7	2.7	2.3	0.0	0.0	0.0
ZBU3	Left	0.0	0.0	0.3	0.3	2.7	2.3	0.0	0.0	0.0
ZBU3	Right	0.7	0.0	0.3	0.0	4.0	2.7	0.0	0.0	0.0

Table 12. Histology scores for the chronic non-supplemented group. Scores were averaged over the medial, central, and lateral locations.

		CALCIFIED CARTILAGE					SUBCHONDRAL BONE				
Rabbit	Limb	Tide Mark	Thickness	Spikes	Stain	Cells	Average Thickness	Morphology	Red Cells	Red Patches	Trabecular Bone
BU43	Left	0.0	1.3	3.7	2.0	2.0	1.7	0.7	4.0	4.0	1.0
BU43	Right	0.0	0.3	4.0	2.0	2.0	2.3	1.3	4.0	4.0	2.0
BU53	Left	0.0	0.7	1.7	2.0	1.3	1.7	1.7	4.0	2.0	2.0
BU53	Right	0.0	0.3	1.7	2.0	2.0	0.7	1.3	4.0	2.0	2.0
BU54	Left	0.3	0.7	4.0	2.0	2.0	3.0	3.0	4.0	3.3	3.0
BU54	Right	0.7	0.7	4.0	2.3	1.3	2.0	1.0	4.0	3.3	2.0
BU56	Left	0.0	1.0	2.7	2.7	4.0	1.0	1.0	2.0	2.0	2.0
BU56	Right	0.0	0.3	3.3	1.7	4.0	1.0	0.0	4.0	2.0	0.0
BU57	Left	0.0	0.7	3.3	2.7	3.3	1.0	0.0	2.0	1.3	3.0
BU57	Right	0.0	1.0	2.3	2.0	2.0	1.3	1.0	2.0	1.3	3.0
BU58	Left	1.0	2.0	1.7	2.7	2.7	0.3	0.3	2.0	0.0	0.0
BU58	Right	0.0	1.0	1.7	2.0	2.7	1.0	0.7	4.0	1.3	3.0
BU59	Left	0.0	0.7	2.7	2.0	1.3	0.7	1.3	4.0	3.3	2.0
BU59	Right	0.0	0.7	2.3	2.0	2.7	1.0	0.3	4.0	2.0	1.0
K-311	Left	0.0	1.0	1.7	2.0	0.7	1.7	0.3	2.7	1.3	1.0
K-311	Right	0.0	0.3	1.7	2.7	0.0	1.7	1.7	2.0	2.0	2.0
K-611	Left	0.0	1.3	2.7	2.0	0.7	1.0	2.0	4.0	0.7	0.0
K-611	Right	0.7	0.3	2.0	3.0	2.0	0.7	2.0	2.0	1.3	0.0
RSOB	Left	0.0	0.7	3.7	2.0	3.3	2.0	2.7	2.0	2.0	3.0
RSOB	Right	0.0	0.3	3.3	2.7	2.7	1.7	3.0	4.0	2.7	3.0
ZBS9	Left	0.0	1.0	1.7	2.0	3.3	1.7	1.7	4.0	2.0	1.0
ZBS9	Right	0.0	1.0	1.3	1.3	2.0	0.7	0.7	2.0	2.0	0.0
ZBU3	Left	1.0	1.3	2.7	2.7	3.3	1.7	1.7	2.0	2.0	2.0
ZBU3	Right	1.3	1.0	2.7	2.0	2.0	2.0	2.0	2.0	2.0	2.0

Table 13. Histology scores for the chronic non-supplemented group. Scores were averaged over the medial, central, and lateral locations.

		ARTICULAR CARTILAGE								
Rabbit	Limb	Geometry	Fissures	PG Stain	Cells	Disruptions	Max Thickness	Focal Ossification	Exposure of Subchondral bone	Erosion
BU31	Left	0.0	0.0	0.0	1.0	2.0	1.0	0.0	0.0	0.0
BU31	Right	0.3	0.0	0.0	0.0	3.3	1.3	0.0	0.0	0.0
BU37	Left	1.7	1.7	1.3	1.7	2.0	1.0	0.0	0.0	0.0
BU37	Right	0.3	0.7	0.7	2.0	1.3	1.0	0.0	0.0	0.0
BU38	Left	1.7	0.7	0.3	2.0	3.3	1.7	0.0	0.0	0.0
BU38	Right	1.3	2.3	0.3	1.3	0.7	1.3	0.0	0.0	0.0
BU39	Left	0.7	0.7	0.3	1.7	1.3	0.3	0.0	0.0	0.0
BU39	Right	0.7	1.3	0.0	1.3	4.0	2.0	0.0	0.0	0.0
BU40	Left	0.3	1.7	0.3	0.7	2.7	2.0	0.0	0.0	0.0
BU40	Right	1.3	0.7	0.0	1.0	4.0	1.3	0.0	0.0	0.0
BU42	Left	0.7	1.3	0.0	0.0	0.7	1.7	0.0	0.0	0.0
BU42	Right	0.0	0.0	0.0	0.0	1.3	1.3	0.0	0.0	0.0
BU51	Left	0.3	0.3	0.3	0.0	0.0	1.3	0.0	0.0	0.0
BU51	Right	1.0	1.3	0.3	0.7	1.3	1.3	0.0	0.0	0.0
BU52	Left	0.0	1.3	0.0	4.0	0.0	1.0	0.0	0.0	0.0
BU52	Right	0.0	1.3	0.7	2.0	0.0	1.0	0.0	0.0	0.0
GAR7	Left	0.7	1.0	0.3	2.0	0.7	1.7	0.0	0.0	0.0
GAR7	Right	1.3	1.3	1.0	3.3	2.0	0.7	0.0	0.0	0.0
K-6	Left	0.0	0.0	0.0	1.0	0.0	0.7	0.0	0.0	0.0
K-6	Right	0.0	0.3	0.0	2.0	0.0	1.3	0.0	0.0	0.0
RSO415	Left	0.0	0.0	0.3	0.3	0.7	2.0	0.0	0.0	0.0
RSO415	Right	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0
ZBU1	Left	0.3	0.3	0.3	0.0	0.0	2.3	0.0	0.0	0.0
ZBU1	Right	0.3	0.0	0.0	0.0	0.7	2.0	0.0	0.0	0.0

Table 14. Histology scores for the chronic short-term supplemented group. Scores were averaged over the medial, central, and lateral locations.

		CALCIFIED CARTILAGE					SUBCHONDRAL BONE				
Rabbit	Limb	Tide Mark	Thickness	Spikes	Stain	Cells	Average Thickness	Morphology	Red Cells	Red Patches	Trabecular Bone
BU31	Left	0.0	0.3	3.0	2.0	4.0	1.7	3.0	4.0	2.0	0.0
BU31	Right	0.0	0.3	3.7	2.3	2.7	1.7	1.7	4.0	1.3	0.0
BU37	Left	0.7	2.3	3.0	2.0	2.0	1.0	3.0	2.7	1.3	0.0
BU37	Right	1.0	2.7	2.7	1.7	2.7	1.0	3.0	2.0	2.0	0.0
BU38	Left	0.0	1.0	4.0	2.7	4.0	1.7	3.0	4.0	4.0	2.0
BU38	Right	0.0	0.7	3.7	1.7	2.7	1.3	3.0	4.0	4.0	3.0
BU39	Left	1.3	0.3	2.3	1.0	2.7	1.0	2.0	2.7	0.7	2.7
BU39	Right	0.0	0.7	2.0	2.0	2.7	2.3	3.0	4.0	2.0	3.0
BU40	Left	0.7	1.0	2.3	2.0	3.3	1.3	3.0	4.0	3.3	0.0
BU40	Right	0.0	1.7	2.7	2.0	2.7	1.0	1.7	4.0	2.7	0.0
BU42	Left	0.0	2.0	4.0	2.0	3.3	3.0	1.0	4.0	4.0	2.0
BU42	Right	1.0	0.7	4.0	1.3	3.3	2.0	1.0	4.0	4.0	3.0
BU51	Left	0.0	1.7	2.7	1.7	2.7	1.0	1.7	4.0	1.3	3.0
BU51	Right	0.0	1.3	1.3	2.0	3.3	0.7	1.0	4.0	2.0	2.0
BU52	Left	0.0	0.0	2.0	2.0	2.7	0.0	0.3	2.7	0.0	0.7
BU52	Right	0.0	1.0	2.7	1.3	2.0	0.7	1.0	4.0	1.3	0.0
GAR7	Left	0.0	1.0	4.0	2.3	3.3	1.0	1.0	4.0	2.0	0.0
GAR7	Right	1.0	1.3	4.0	2.0	2.0	1.0	1.0	4.0	2.7	2.0
K-6	Left	0.0	0.7	2.0	1.7	2.0	1.3	1.7	4.0	0.0	1.0
K-6	Right	0.0	0.7	1.3	2.0	2.7	1.7	1.7	4.0	0.7	2.0
RSO415	Left	0.0	0.7	2.0	2.0	3.3	1.0	3.0	4.0	2.0	2.0
RSO415	Right	0.0	1.0	2.0	2.0	2.0	2.3	1.7	2.0	1.3	2.0
ZBU1	Left	0.0	1.0	1.0	2.0	2.7	3.7	3.0	2.0	2.0	3.0
ZBU1	Right	1.0	0.3	1.7	3.0	3.3	1.3	3.0	4.0	2.0	3.0

Table 15. Histology scores for the chronic short-term supplemented group. Scores were averaged over the medial, central, and lateral locations.

		ARTICULAR CARTILAGE								
Rabbit	Limb	Geometry	Fissures	PG Stain	Cells	Disruptions	Max Thickness	Focal Ossification	Exposure of Subchondral bone	Erosion
BU34	Left	2.7	2.3	0.0	1.7	2.0	1.3	0.0	0.0	0.0
BU34	Right	0.7	1.0	0.3	1.0	0.0	1.0	0.0	0.0	0.0
BU35	Left	2.7	2.7	1.0	1.0	3.3	1.0	0.0	0.0	0.0
BU35	Right	1.0	1.3	0.3	0.7	0.0	2.3	0.0	0.0	0.0
BU36	Left	1.7	2.3	0.3	0.7	2.7	1.3	0.0	0.0	0.0
BU36	Right	1.7	1.7	0.3	1.0	0.0	1.3	0.0	0.0	0.0
BU41	Left	1.3	1.3	0.3	0.3	0.0	1.3	0.0	0.0	0.0
BU41	Right	1.7	2.0	0.0	1.0	1.3	1.3	0.0	0.0	0.0
BU45	Left	0.0	0.7	0.0	1.0	0.0	1.3	0.0	0.0	0.0
BU45	Right	0.3	0.3	0.0	2.0	1.3	0.3	0.0	0.0	0.0
BU46	Left	0.0	0.3	0.0	1.3	2.7	1.3	1.3	0.0	0.0
BU46	Right	0.3	0.0	0.0	0.3	0.0	2.0	0.0	0.0	0.0
BU47	Left	0.0	0.0	0.0	1.0	0.0	2.3	0.0	0.0	0.0
BU47	Right	0.7	0.3	0.0	2.3	0.0	2.0	0.0	0.0	0.0
BU48	Left	0.3	0.7	0.0	0.7	1.3	1.3	0.0	0.0	0.0
BU48	Right	0.3	0.0	1.7	0.3	0.0	0.3	0.0	0.0	0.0
BU49	Left	1.7	1.7	0.3	1.0	2.7	1.0	0.0	0.0	0.0
BU49	Right	1.3	1.7	0.0	1.0	2.0	0.3	0.0	0.0	0.0
BU50	Left	0.3	1.3	0.0	0.7	1.3	1.7	0.0	0.0	0.0
BU50	Right	1.0	1.0	0.0	0.3	0.0	2.3	0.0	0.0	0.0
K-1	Left	0.3	1.0	0.0	2.0	0.0	1.3	0.0	0.0	0.0
K-1	Right	0.3	1.7	0.0	0.7	3.3	1.0	0.0	0.0	0.0
K-3	Left	0.0	0.0	0.0	3.0	2.0	1.3	0.0	0.0	0.0
K-3	Right	0.3	0.0	0.0	1.0	4.0	1.7	0.0	0.0	0.0

Table 16. Histology scores for the chronic long-term supplemented group. Scores were averaged over the medial, central, and lateral locations.

		CALCIFIED CARTILAGE					SUBCHONDRAL BONE				
Rabbit	Limb	Tide Mark	Thickness	Spikes	Stain	Cells	Average Thickness	Morphology	Red Cells	Red Patches	Trabecular Bone
BU34	Left	0.0	2.7	3.3	2.0	4.0	1.3	3.0	4.0	2.7	3.0
BU34	Right	0.0	1.7	3.3	2.0	3.3	2.3	1.0	2.0	2.0	3.0
BU35	Left	0.7	0.3	2.7	2.7	2.7	1.0	3.0	4.0	2.7	2.0
BU35	Right	0.0	1.3	3.0	2.0	4.0	1.0	0.0	4.0	3.3	0.0
BU36	Left	0.0	0.7	2.0	2.0	2.7	1.7	3.0	2.0	3.3	3.0
BU36	Right	0.0	0.7	2.0	1.7	2.7	1.7	3.0	4.0	3.3	3.0
BU41	Left	1.0	1.0	1.7	1.7	2.0	2.0	2.0	4.0	2.0	2.0
BU41	Right	0.0	0.3	1.7	2.7	2.0	0.0	3.0	2.0	1.3	1.0
BU45	Left	0.0	0.7	2.3	2.7	2.7	1.3	2.3	4.0	2.0	3.0
BU45	Right	0.0	0.0	2.0	3.0	4.0	0.3	1.0	4.0	1.3	0.0
BU46	Left	0.0	1.3	3.0	2.3	4.0	0.7	3.0	4.0	3.3	0.0
BU46	Right	0.0	1.7	3.3	2.0	4.0	1.7	2.3	4.0	3.3	2.0
BU47	Left	0.3	1.0	1.0	2.0	2.0	1.3	1.0	4.0	0.7	0.0
BU47	Right	0.0	1.7	2.3	2.0	2.7	2.3	1.0	4.0	1.3	2.0
BU48	Left	0.3	0.7	4.0	2.7	2.0	1.0	3.0	4.0	4.0	3.0
BU48	Right	0.0	0.3	4.0	2.3	2.7	2.0	3.0	4.0	4.0	3.0
BU49	Left	0.0	0.7	2.3	2.3	3.3	1.0	1.3	4.0	2.0	2.0
BU49	Right	0.0	0.7	2.7	2.7	4.0	2.3	2.7	4.0	3.3	2.0
BU50	Left	0.0	0.7	2.3	2.0	0.7	1.7	1.7	4.0	2.7	3.0
BU50	Right	0.0	0.7	2.3	2.0	2.7	1.3	2.7	4.0	2.7	3.0
K-1	Left	0.0	2.0	2.0	2.0	4.0	0.7	3.0	4.0	0.7	0.0
K-1	Right	0.0	1.3	1.7	2.3	4.0	0.7	0.0	4.0	0.0	1.3
K-3	Left	0.0	0.0	2.0	2.0	2.0	1.0	1.7	4.0	0.0	0.0
K-3	Right	0.5	1.0	1.7	2.7	2.0	1.3	1.7	4.0	1.3	2.0

Table 17. Histology scores for the chronic long-term supplemented group. Scores were averaged over the medial, central, and lateral locations.

Appendix B: Raw Data from Chapter 2

Group	Sample	P_{max} (N)	P_{min} (N)	Thickness (mm)	G_u	G_r	% Cell Death
2 Day No Ex	1	0.031	0.006	0.535	0.063	0.015	0.0%
2 Day Ex	2	0.177	0.027	0.580	0.348	0.064	22.1%
2 Day No Ex	3	0.149	0.026	0.510	0.306	0.064	0.0%
2 Day Ex	4	0.143	0.030	0.410	0.315	0.079	27.0%
2 Day Ex	5	0.248	0.032	0.515	0.507	0.080	41.4%
2 Day No Ex	6	0.031	0.009	0.495	0.065	0.023	5.6%
7 Day Ex	7	0.035	0.012	0.420	0.077	0.030	48.5%
7 Day No Ex	8	0.101	0.025	0.505	0.209	0.061	33.6%
7 Day No Ex	9	0.029	0.008	0.410	0.063	0.022	7.6%
7 Day Ex	10	0.080	0.016	0.410	0.176	0.042	35.5%
7 Day No Ex	11	0.097	0.022	0.455	0.207	0.055	4.0%
7 Day Ex	12	0.165	0.035	0.395	0.365	0.093	38.9%
2 Day Ex	13	0.161	0.025	0.490	0.336	0.062	23.2%
2 Day No Ex	14	0.029	0.006	0.465	0.062	0.016	20.5%
2 Day Ex	15	0.239	0.035	0.580	0.468	0.081	8.9%
2 Day No Ex	16	0.107	0.023	0.440	0.231	0.058	7.6%
2 Day No Ex	17	0.040	0.008	0.430	0.086	0.021	12.3%
2 Day Ex	18	0.098	0.016	0.445	0.210	0.042	33.7%
7 Day No Ex	19	0.028	0.007	0.410	0.062	0.018	32.9%
7 Day Ex	20	0.073	0.012	0.405	0.161	0.031	59.4%
7 Day Ex	21						
7 Day No Ex	22	0.082	0.023	0.410	0.180	0.060	62.6%
7 Day Ex	23	0.174	0.041	0.370	0.392	0.111	39.7%
7 Day No Ex	24	0.099	0.036	0.450	0.211	0.093	18.9%
Time Zero	25	0.080	0.011	0.410	0.176	0.030	
Time Zero	26	0.240	0.022	0.390	0.534	0.059	
Time Zero	27	0.090	0.024	0.410	0.198	0.062	
Time Zero	28	0.070	0.020	0.420	0.153	0.054	

Table 1. Raw data for the 6/14/05 pilot study.

Group	Sample	P_{max} (N)	P_{min} (N)	Thickness (mm)	G_u	G_r	PG Content (μg/mg Wet Weight)
2 Day No Ex	1	0.391	0.042	0.420	0.852	0.109	28.586
2 Day Ex	2	0.323	0.052	0.620	0.616	0.118	44.584
2 Day No Ex	3	0.176	0.043	0.665	0.327	0.095	46.091
2 Day Ex	4	0.281	0.035	0.560	0.559	0.084	36.431
2 Day Ex	5	0.508	0.070	0.575	1.000	0.165	35.538
2 Day No Ex	6	0.569	0.047	0.625	1.084	0.106	42.463
7 Day Ex	7	0.177	0.048	0.695	0.322	0.104	55.754
7 Day No Ex	8	0.176	0.029	0.495	0.364	0.072	29.200
7 Day No Ex	9	0.523	0.065	0.575	1.029	0.154	42.771
7 Day Ex	10	0.670	0.061	0.730	1.190	0.131	49.722
7 Day No Ex	11	0.548	0.078	0.535	1.109	0.189	46.094
7 Day Ex	12	0.486	0.052	0.560	0.966	0.124	51.537
2 Day Ex	13	0.090	0.019	0.580	0.176	0.044	31.907
2 Day No Ex	14	0.121	0.031	0.610	0.233	0.072	51.230
2 Day Ex	15	0.029	0.009	0.480	0.061	0.022	38.246
2 Day No Ex	16	0.028	0.005	0.635	0.053	0.012	39.169
2 Day No Ex	17	0.092	0.033	0.745	0.162	0.069	38.862
2 Day Ex	18	0.387	0.061	0.705	0.699	0.133	47.599
7 Day No Ex	19	0.240	0.048	0.745	0.422	0.102	49.108
7 Day Ex	20	0.182	0.035	0.510	0.373	0.086	45.475
7 Day Ex	21	0.483	0.071	0.570	0.954	0.168	54.245
7 Day No Ex	22	0.377	0.072	0.500	0.781	0.179	49.415
7 Day Ex	23	0.044	0.011	0.595	0.086	0.026	48.217
7 Day No Ex	24	0.196	0.048	0.630	0.372	0.109	48.800
Time Zero	25	0.261	0.038	0.390	0.580	0.102	44.586
Time Zero	26	0.053	0.014	0.495	0.110	0.035	37.641
Time Zero	27	0.630	0.065	0.495	1.307	0.162	50.307
Time Zero	28	0.801	0.073	0.550	1.602	0.175	52.738
Time Zero	29	0.346	0.037	0.505	0.714	0.092	49.416
Time Zero	30	0.107	0.023	0.485	0.225	0.059	51.817

Table 2. Raw data for the 6/28/05 pilot study.

Group	Sample	P_{max} (N)	P_{min} (N)	Thickness (mm)	G_u	G_r	PG Content (μg/mg Wet Weight)
2 Day No Ex	1	0.034	0.004	0.335	0.077	0.012	47.444
2 Day Ex	2	0.399	0.080	0.440	0.858	0.206	52.552
2 Day No Ex	3	0.031	0.005	0.315	0.072	0.014	45.289
2 Day Ex	4	0.118	0.029	0.560	0.235	0.069	44.862
2 Day Ex	5	0.424	0.100	0.495	0.881	0.249	63.660
2 Day No Ex	6	0.151	0.027	0.405	0.331	0.071	43.170
7 Day Ex	7	0.182	0.046	0.535	0.368	0.111	51.293
7 Day No Ex	8	0.072	0.023	0.445	0.155	0.060	34.614
7 Day No Ex	9	0.063	0.017	0.565	0.125	0.041	43.600
7 Day Ex	10	0.420	0.049	0.460	0.892	0.125	47.443
7 Day No Ex	11	0.027	0.009	0.575	0.054	0.021	38.031
7 Day Ex	12	0.245	0.055	0.555	0.489	0.132	30.770
2 Day Ex	13	0.164	0.033	0.400	0.362	0.087	50.001
2 Day No Ex	14	0.124	0.049	0.430	0.268	0.128	35.047
2 Day Ex	15	0.147	0.032	0.555	0.294	0.076	121.331
2 Day No Ex	16	0.042	0.016	0.490	0.087	0.041	36.338
2 Day No Ex	17	0.030	0.006	0.385	0.067	0.017	36.740
2 Day Ex	18	0.461	0.060	0.515	0.945	0.146	68.799
7 Day No Ex	19	0.262	0.040	0.375	0.588	0.107	39.321
7 Day Ex	20	0.170	0.030	0.395	0.377	0.080	
7 Day Ex	21	0.232	0.055	0.385	0.516	0.146	41.878
7 Day No Ex	22	0.055	0.011	0.455	0.116	0.027	30.769
7 Day Ex	23	0.299	0.052	0.430	0.649	0.134	38.459
7 Day No Ex	24	0.031	0.010	0.500	0.064	0.024	50.861
Time Zero	25	0.094	0.015	0.390	0.210	0.040	50.859
Time Zero	26	0.309	0.070	0.495	0.641	0.174	66.245
Time Zero	27	0.040	0.012	0.440	0.087	0.030	63.660
Time Zero	28	0.119	0.031	0.475	0.250	0.077	49.139
Time Zero	29	0.132	0.031	0.460	0.279	0.078	58.119
Time Zero	30	0.095	0.026	0.675	0.176	0.058	44.432

Table 3. Raw data for the 7/12/05 pilot study.

Group	Sample	P_{max} (N)	P_{min} (N)	Thickness (mm)	G_u	G_r	% Cell Death
2 Day No Ex	1	1.549	0.173	0.785	1.773	0.238	3.6%
2 Day Ex	2	0.568	0.068	0.675	0.697	0.100	1.7%
2 Day No Ex	3	0.645	0.064	0.605	0.830	0.099	1.0%
2 Day Ex	4	0.548	0.110	0.810	0.617	0.148	8.5%
2 Day Ex	5	1.545	0.104	0.725	1.837	0.149	10.5%
2 Day No Ex	6	1.815	0.201	0.880	1.956	0.260	2.0%
2 Day Ex	7	1.059	0.134	0.900	1.128	0.172	9.2%
2 Day No Ex	8	1.123	0.127	0.930	1.174	0.160	5.0%
3 Day No Ex	9	1.466	0.207	0.685	1.789	0.304	1.0%
3 Day Ex	10	1.571	0.143	0.735	1.856	0.203	8.0%
3 Day No Ex	11	2.157	0.221	0.855	2.361	0.290	4.3%
3 Day Ex	12	0.977	0.131	0.595	1.265	0.203	18.9%
3 Day Ex	13	1.896	0.203	0.795	2.155	0.276	20.9%
3 Day No Ex	14	1.271	0.099	0.690	1.546	0.145	1.0%
3 Day Ex	15	0.750	0.050	0.560	0.994	0.080	48.2%
3 Day No Ex	16	0.943	0.088	0.635	1.190	0.133	3.5%
Time Zero	25	0.995	0.066	0.845	1.644	0.131	
Time Zero	26	0.489	0.057	0.695	0.889	0.125	
Time Zero	27	3.087	0.123	0.765	3.577	0.171	
Time Zero	28	0.866	0.055	0.670	1.600	0.122	
Time Zero	29	2.860	0.191	0.780	3.282	0.262	
Time Zero	30	1.660	0.131	0.750	1.942	0.184	
Time Zero	31	1.977	0.103	0.715	2.366	0.148	
Time Zero	32	1.591	0.062	0.765	1.843	0.086	
Time Zero	33	0.840	0.090	0.755	1.469	0.189	

Table 4. Raw data for the 11/1/05 pilot study.

Group	Sample	P_{max} (N)	P_{min} (N)	Thickness (mm)	G_u	G_r	% Cell Death	% Change in Thickness
1 Day No Ex	1	1.455	0.074	0.625	1.848	0.113	0.0%	2%
1 Day Ex	2	3.153	0.140	0.710	3.786	0.202	0.0%	5%
1 Day No Ex	3	2.031	0.313	0.770	2.346	0.434	0.0%	6%
1 Day Ex	4	3.128	0.145	0.790	3.567	0.198	0.0%	4%
1 Day Ex	5	1.843	0.090	0.655	2.293	0.134	0.0%	2%
1 Day No Ex	6	1.824	0.263	0.865	1.984	0.344	0.0%	21%
1 Day Ex	7	1.368	0.101	0.655	1.703	0.150	0.0%	13%
1 Day No Ex	8	2.454	0.108	0.540	3.296	0.174	0.0%	17%
3 Day No Ex	9	2.090	0.121	0.725	2.485	0.173	1.0%	7%
3 Day Ex	10	1.088	0.120	0.810	1.225	0.161	4.2%	13%
3 Day No Ex	11	2.144	0.192	0.675	2.633	0.282	1.0%	-2%
3 Day Ex	12	1.848	0.138	0.625	2.346	0.211	2.8%	-2%
3 Day Ex	13	1.034	0.050	0.415	1.507	0.087	4.3%	12%
3 Day No Ex	14	1.505	0.162	0.670	1.855	0.240	2.4%	24%
3 Day Ex	15	3.393	0.287	0.725	4.034	0.410	2.0%	10%
3 Day No Ex	16	1.347	0.086	0.585	1.757	0.134	1.5%	2%
6 Day No Ex	17	0.868	0.243	0.655	1.081	0.364	5.3%	1%
6 Day Ex	18	1.438	0.151	0.630	1.819	0.230	4.4%	13%
6 Day No Ex	19	2.366	0.146	0.665	2.926	0.217	79.8%	11%
6 Day Ex	20	2.934	0.137	0.825	3.273	0.184	14.3%	9%
6 Day Ex	21	1.270	0.082	0.550	1.694	0.132	15.4%	15%
6 Day No Ex	22	4.107	0.279	0.840	4.539	0.370	14.7%	13%
6 Day Ex	23	0.680	0.091	0.570	0.896	0.144	5.8%	9%
6 Day No Ex	24	0.523	0.075	0.685	0.638	0.110	100.0%	18%
Time Zero	25	2.191	0.175	0.795	2.490	0.238		15%
Time Zero	26	0.775	0.141	0.780	0.889	0.194		17%
Time Zero	27	1.798	0.158	0.760	2.089	0.221		6%
Time Zero	28	1.445	0.104	0.660	1.793	0.155		12%
Time Zero	29	1.224	0.239	0.690	1.489	0.349		15%
Time Zero	30	0.867	0.124	0.625	1.101	0.188		18%
Time Zero	31	1.621	0.125	0.610	2.079	0.193		10%
Time Zero	32	1.300	0.128	0.760	1.511	0.178		22%

Table 5. Raw data for the 11/8/05 study.

Group	Sample	P_{max} (N)	P_{min} (N)	Thickness (mm)	G_u	G_r	% Cell Death	% Change in Thickness	PG Content (μg/mg Wet Weight)
1 Day No Ex	1	0.737	0.029	0.465	0.595	0.038	10.6%	2.5%	33.16
1 Day Ex	2	0.358	0.058	0.455	0.288	0.077	0.0%	2.5%	43.29
1 Day No Ex	3	0.825	0.173	0.495	0.673	0.225	4.2%	4.5%	48.76
1 Day Ex	4	0.420	0.022	0.425	0.331	0.030	4.3%	-2.0%	27.99
1 Day Ex	5	1.186	0.049	0.435	0.942	0.065	1.7%	0.5%	48.03
1 Day No Ex	6	0.353	0.093	0.450	0.283	0.123	1.8%	2.0%	47.35
1 Day Ex	7	1.526	0.095	0.545	1.261	0.120	7.6%	1.0%	46.33
1 Day No Ex	8	0.360	0.037	0.380	0.275	0.050	1.9%	6.0%	42.94
3 Day No Ex	9	0.781	0.086	0.470	0.631	0.112	1.6%	-1.5%	27.06
3 Day Ex	10	1.698	0.126	0.515	1.394	0.163	9.3%	2.0%	47.38
3 Day No Ex	11	0.359	0.092	0.485	0.292	0.120	1.3%	6.0%	45.11
3 Day Ex	12	0.995	0.102	0.430	0.788	0.135	3.0%	0.0%	37.30
3 Day Ex	13	1.236	0.084	0.460	0.995	0.109	3.7%	3.0%	49.22
3 Day No Ex	14	1.636	0.095	0.460	1.316	0.125	2.7%	1.5%	40.55
3 Day Ex	15	0.897	0.124	0.490	0.730	0.160	4.1%	3.5%	47.83
3 Day No Ex	16	0.728	0.057	0.460	0.586	0.075	2.9%	5.5%	34.11
6 Day No Ex	17	0.852	0.043	0.415	0.668	0.057	12.5%	1.5%	35.67
6 Day Ex	18	0.948	0.080	0.405	0.738	0.107	11.1%	0.5%	40.07
6 Day No Ex	19	0.920	0.087	0.380	0.703	0.116	6.9%	0.0%	51.00
6 Day Ex	20	0.945	0.041	0.365	0.712	0.055	12.1%	-0.5%	44.92
6 Day Ex	21	1.234	0.094	0.480	1.001	0.122	7.3%	0.5%	62.20
6 Day No Ex	22	0.348	0.028	0.340	0.256	0.038	7.3%	0.5%	44.84
6 Day Ex	23	0.637	0.075	0.405	0.497	0.099	6.9%	0.5%	28.34
6 Day No Ex	24	0.578	0.043	0.405	0.451	0.057	5.1%	2.0%	30.26
Time Zero	25	0.402	0.025	0.385	0.308	0.034		2.0%	43.82
Time Zero	26	0.745	0.071	0.440	0.593	0.093		0.0%	47.19
Time Zero	27	0.753	0.079	0.485	0.612	0.103		3.0%	48.18
Time Zero	28	0.276	0.039	0.385	0.212	0.052		5.5%	46.88
Time Zero	29	0.319	0.036	0.410	0.250	0.048		2.0%	41.60
Time Zero	30	1.402	0.100	0.520	1.152	0.128		1.5%	50.01
Time Zero	31	0.622	0.042	0.465	0.501	0.055		5.5%	46.36
Time Zero	32	0.848	0.136	0.505	0.694	0.175		2.0%	47.54

Table 6. Raw data for the 11/16/05 study.

Appendix C: Raw Data from Chapter 3

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
KF	Left	0.61	5.23	1.59	0.13	0.18	1.64	0.22
KF	Right	1.08	3.00	0.77	0.13	0.18	2.49	1.48
M29	Left	0.58	4.00	0.53	0.13	0.10	0.68	0.28
M29	Right	0.50	0.51	0.35	0.20	0.15	2.51	1.92
M35	Left	0.50	2.07	0.58	0.15	0.13	0.61	0.69
M35	Right	0.47	1.15	0.49	0.19	0.12	1.90	0.90
M40	Left	0.55	1.47	0.60	0.25	0.10	2.25	0.87
M40	Right	0.79	3.29	0.95	0.14	0.18	0.66	2.71
M41	Left	0.83	2.51	0.95	0.27	0.13	2.01	0.47
M41	Right	0.92	4.70	1.11	0.15	0.11	1.97	0.31
M42	Left	0.71	1.42	0.67	0.31	0.15	1.47	0.92
M42	Right	0.72	4.78	0.97	0.24	0.06	0.70	0.68
M43	Left	0.54	3.26	0.83	0.16	0.12	0.73	0.49
M43	Right	0.80	1.18	0.59	0.19	0.15	1.97	1.57
M44	Left	0.53	1.29	0.53	0.14	0.16	1.59	9.20
M44	Right	0.61	2.92	0.63	0.21	0.10	1.26	1.39
M45	Left	0.46	2.60	0.57	0.15	0.10	1.39	0.40
M45	Right	0.56	3.38	0.66	0.23	0.10	1.11	0.38
R331	Left	0.58	3.12	0.90	0.18	0.13	2.92	0.17
R331	Right	0.58	2.05	0.99	0.50	0.10	0.45	0.40
Average		**0.64**	**2.69**	**0.76**	**0.20**	**0.13**	**1.52**	**1.27**
Standard Dev.		**0.16**	**1.32**	**0.28**	**0.09**	**0.03**	**0.73**	**1.98**

Table 1. Mechanical indentation data for the time zero group in the first study.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
KB2	Left	0.52	1.14	0.59	0.20	0.10	1.19	1.10
KB2	Right	0.58	5.27	1.35	0.18	0.19	0.54	0.27
KB5	Left	0.70	2.92	0.75	0.17	0.13	2.08	0.39
KB5	Right	0.52	2.34	1.02	0.29	0.15	0.66	0.34
M14	Left	0.51	5.41	1.05	0.16	0.12	0.45	0.28
M14	Right	0.58	5.81	1.73	0.17	0.17	0.60	0.19
M15	Left	0.53	8.33	1.03	0.21	0.07	0.46	0.09
M15	Right	0.59	7.76	1.59	0.21	0.10	0.26	0.18
M16	Left	0.40	7.42	1.28	0.22	0.03	0.61	0.13
M16	Right	0.53	5.28	1.04	0.14	0.12	1.61	0.28
M18	Left	0.61	4.14	1.03	0.16	0.15	0.55	0.24
M18	Right	0.70	4.52	1.36	0.17	0.17	1.01	0.25
M20	Left	0.54	7.77	1.62	1.35	0.12	0.31	0.15
M20	Right	0.50	2.06	0.46	0.19	0.05	2.08	0.33
M21	Left	0.37	2.00	0.86	0.33	0.15	0.27	0.82
M21	Right	0.67	2.65	0.88	0.13	0.18	1.67	0.32
M24	Left	0.66	6.89	0.96	0.17	0.12	2.64	0.20
M24	Right	0.57	3.89	0.87	0.14	0.09	0.83	0.24
M31	Left	0.41	6.70	1.33	0.21	0.10	0.65	0.24
M31	Right	0.60	7.64	1.53	0.15	0.10	0.46	0.07
M36	Left	0.58	3.02	1.03	0.21	0.13	1.87	0.41
M36	Right	0.56	3.29	0.87	0.15	0.14	1.30	0.26
Average		**0.55**	**4.83**	**1.10**	**0.24**	**0.12**	**1.00**	**0.31**
Standard Dev.		**0.09**	**2.23**	**0.33**	**0.25**	**0.04**	**0.70**	**0.23**

Table 2. Mechanical indentation data for the 1 year exercise no-impact group in the first study.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
BB15	Left	0.65	3.31	1.03	0.18	0.14	0.66	0.43
BB15	Right	0.80	6.36	1.11	0.18	0.11	1.19	0.19
KB4	Left	0.63	3.01	0.88	0.20	0.11	0.81	0.44
KB4	Right	0.60	3.36	1.05	0.17	0.18	0.98	0.45
M13	Left	0.64	3.31	0.85	0.15	0.09	0.56	0.79
M13	Right	0.68	4.04	0.99	0.22	0.13	1.34	1.31
M17	Left	0.72	3.55	0.79	0.20	0.10	2.22	1.04
M17	Right	0.54	1.67	0.73	0.23	0.10	2.34	0.53
M26	Left	0.52	6.20	1.14	0.19	0.08	1.95	0.11
M26	Right	0.56	3.12	0.85	0.25	0.13	1.35	0.29
M27	Left	0.48	2.06	0.82	0.21	0.15	1.48	0.52
M27	Right	0.70	3.97	1.06	0.16	0.15	1.33	0.34
M34	Left	0.68	3.10	1.06	0.16	0.21	1.04	0.68
M34	Right	0.61	4.33	1.29	0.21	0.16	1.50	0.44
M37	Left	0.65	3.87	0.91	0.22	0.17	1.32	0.29
M37	Right	0.53	7.03	1.33	0.15	0.14	0.79	0.10
M39	Left	0.87	3.50	1.06	0.19	0.16	0.76	0.60
M39	Right	0.63	6.82	1.40	0.15	0.16	2.16	0.14
Average		**0.64**	**4.03**	**1.02**	**0.19**	**0.14**	**1.32**	**0.48**
Standard Dev.		**0.10**	**1.56**	**0.19**	**0.03**	**0.03**	**0.55**	**0.32**

Table 3. Mechanical indentation data for the 1 year exercise impact group in the first study.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
KO4	Left	0.56	12.84	2.43	0.86	0.13	0.82	0.69
KO4	Right	0.57	8.94	2.07	1.00	0.10	2.14	0.70
M73	Left	0.59	9.33	1.94	0.22	0.13	2.93	0.93
M73	Right	0.50	8.06	1.85	0.16	0.15	3.52	0.74
M75	Left	0.49	10.68	2.26	1.47	0.05	1.58	0.79
M75	Right	0.60	8.23	2.43	0.74	0.09	1.88	0.74
M79	Left	0.54	8.14	2.28	1.45	0.05	1.71	0.69
M79	Right	0.55	7.46	1.82	0.77	0.10	2.00	1.06
M81	Left	0.54	12.13	2.96	0.28	0.17	2.11	0.53
M81	Right	0.50	5.50	1.89	0.77	0.05	3.85	1.12
M83	Left	0.64	5.93	1.80	0.20	0.12	2.28	1.53
M83	Right	0.70	12.69	2.52	0.24	0.13	3.55	0.52
M84	Left	0.63	10.62	2.58	0.92	0.08	1.66	0.83
M84	Right	0.68	12.56	2.47	0.24	0.12	1.70	0.81
M85	Left	0.55	10.63	2.77	2.08	0.06	1.37	0.58
M85	Right	0.56	9.62	2.25	0.76	0.10	1.70	1.03
M86	Left	0.70	10.83	2.20	0.94	0.08	1.68	0.82
M86	Right	0.59	8.57	2.06	0.80	0.05	2.39	0.62
M87	Left	0.59	7.62	1.70	0.91	0.06	3.44	0.37
M87	Right	0.57	12.72	2.50	0.36	0.11	2.48	0.51
M89	Left	0.49	9.00	2.33	0.93	0.08	1.86	0.55
M89	Right	0.54	10.69	2.22	1.18	0.12	1.41	0.47
M91	Left	0.55	10.37	2.59	0.89	0.12	2.61	0.60
M91	Right	0.61	10.19	2.41	0.22	0.15	2.27	0.80
Average		**0.58**	**9.72**	**2.26**	**0.77**	**0.10**	**2.20**	**0.75**
Standard Dev.		**0.06**	**2.07**	**0.33**	**0.48**	**0.04**	**0.77**	**0.25**

Table 4. Mechanical indentation data for the 1 year no-exercise group in the second study.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
5ER	Left	0.53	10.22	2.54	0.20	0.10	2.94	0.55
5ER	Right	0.61	9.83	2.25	0.25	0.14	3.15	0.69
A-1	Left	0.52	9.00	1.31	1.36	0.03	1.73	0.66
A-1	Right	0.57	7.28	0.83	0.18	0.05	1.83	0.75
BBA	Left	0.70	12.68	2.82	0.22	0.11	2.07	0.52
BBA	Right	0.63	7.85	1.61	0.15	0.11	2.19	0.74
M42	Left	0.48	10.52	2.49	1.27	0.10	0.87	0.92
M42	Right	0.49	12.33	2.77	2.38	0.09	0.66	0.56
M52	Left	0.56	7.87	2.43	0.80	0.08	1.95	0.72
M52	Right	0.54	10.84	2.58	0.64	0.10	0.97	0.61
M60	Left	0.56	9.52	1.72	0.24	0.10	1.21	0.86
M60	Right	0.59	7.62	2.00	0.14	0.16	1.48	1.03
M63	Left	0.51	8.59	2.20	1.37	0.10	1.39	1.00
M63	Right	0.62	12.18	2.73	0.27	0.14	1.60	0.60
M64	Left	0.60	7.23	1.22	0.23	0.10	4.21	1.56
M64	Right	0.59	6.85	1.05	0.15	0.09	1.85	1.49
M65	Left	0.45	8.50	1.99	1.77	0.05	1.17	0.85
M65	Right	0.49	8.69	1.95	1.73	0.02	1.34	0.75
NB	Left	0.62	10.43	2.36	0.22	0.17	1.42	0.97
NB	Right	0.61	6.88	2.21	0.68	0.16	1.95	0.91
Average		0.56	9.24	2.05	0.71	0.10	1.80	0.84
Standard Dev.		0.06	1.83	0.59	0.69	0.04	0.84	0.28

Table 5. Mechanical indentation data for the 2 year exercise no-impact group in the second study.

Rabbit	Patella	Thickness (mm)	E11 (MPa)	E33 (MPa)	G13 (MPa)	nu31	k_1 ($m^4/(Ns)$ $x10^{-15}$)	k_3 ($m^4/(Ns)$ $x10^{-15}$)
LB283	Left	0.46	8.50	3.14	1.47	0.05	1.02	0.70
LB283	Right	0.70	15.19	3.50	0.77	0.11	0.94	0.56
LB285	Left	0.62	12.37	3.19	0.19	0.16	1.12	0.74
LB285	Right	0.66	11.98	2.75	0.30	0.13	1.26	1.00
M41	Left	0.56	7.20	1.22	1.05	0.12	1.67	1.17
M41	Right	0.62	10.25	1.68	0.23	0.10	1.49	0.66
M43	Left	0.54	10.83	2.43	1.14	0.07	1.19	1.10
M43	Right	0.68	11.24	2.59	0.85	0.10	1.87	0.90
M45	Left	0.63	10.32	2.68	0.22	0.18	1.50	1.01
M45	Right	0.72	14.01	3.32	0.30	0.17	2.14	0.78
M46	Left	0.65	12.70	2.96	0.26	0.18	1.26	0.72
M46	Right	0.60	9.56	2.64	0.26	0.14	2.16	0.89
M47	Left	0.64	12.91	3.38	0.34	0.14	0.98	0.59
M47	Right	0.68	13.32	2.84	0.36	0.14	1.14	1.94
M48	Left	0.68	9.29	1.64	0.20	0.12	1.60	0.93
M48	Right	0.80	8.26	2.38	0.15	0.18	3.31	0.86
M49	Left	0.63	8.21	1.97	0.23	0.15	2.83	1.32
M49	Right	0.58	4.15	1.15	0.12	0.13	2.60	1.58
M51	Left	0.66	11.19	2.93	0.26	0.16	1.36	0.74
M51	Right	0.63	12.10	3.18	0.24	0.21	1.14	1.24
T24B	Left	0.56	10.58	1.13	0.25	0.02	1.97	0.67
T24B	Right	0.45	5.50	0.60	0.18	0.03	1.53	2.79
Average		**0.63**	**10.44**	**2.42**	**0.42**	**0.13**	**1.64**	**1.04**
Standard Dev.		**0.08**	**2.72**	**0.84**	**0.37**	**0.05**	**0.64**	**0.52**

Table 6. Mechanical indentation data for the 2 year exercise impact group in the second study.

		ARTICULAR CARTILAGE								
Rabbit	Limb	Geometry	Fissures	PG Stain	Cells	Disruptions	Max Thickness	Focal Ossification	Exposure of Subchondral bone	Erosion
M16	Left	2.3	1.0	1.3	3.3	2.7	0.3	0.0	0.0	0.0
M16	Right	1.0	1.3	0.3	1.0	0.0	2.3	0.0	0.0	0.0
M15	Left	0.0	0.0	0.3	2.7	1.3	1.0	0.0	0.0	0.0
M15	Right	0.7	1.3	0.0	0.7	0.0	2.7	0.0	0.0	0.0
M14	Left	1.7	1.3	0.7	2.3	0.0	2.3	0.0	0.0	0.0
M14	Right	0.0	0.0	0.0	3.0	0.0	2.0	0.0	0.0	0.0
KB5	Left	0.7	0.7	0.3	0.7	0.0	2.3	0.0	0.0	0.0
KB5	Right	0.0	0.0	0.0	0.3	0.0	2.7	0.0	0.0	0.0
KB2	Left	1.0	1.3	0.0	2.3	0.0	2.7	0.0	0.0	0.0
KB2	Right	1.0	1.3	1.0	4.0	0.0	1.3	0.0	0.0	0.7
M31	Left	0.0	0.0	0.0	1.0	1.3	2.0	0.0	0.0	0.0
M31	Right	1.0	0.7	0.0	2.3	0.0	1.3	0.0	0.0	0.0
M24	Left	0.0	0.0	0.0	3.7	0.0	1.3	0.0	0.0	0.0
M24	Right	1.0	0.7	0.3	3.3	0.0	1.3	0.0	0.0	0.0
M21	Left	1.0	0.7	0.7	0.3	0.0	1.7	0.0	0.0	0.0
M21	Right	0.0	0.3	0.0	1.3	0.0	2.0	0.0	0.0	0.0
M20	Left	0.0	0.0	0.0	0.7	0.0	1.7	0.0	0.0	0.0
M20	Right	0.3	0.3	1.0	0.0	0.0	1.3	0.0	0.0	0.0
M18	Left	1.0	0.7	0.7	0.0	0.0	2.7	0.0	0.0	0.0
M18	Right	1.7	1.0	0.0	1.7	0.0	2.3	0.0	0.0	0.0
M36	Left	2.0	1.0	0.0	0.0	1.3	2.0	0.0	0.0	0.0
M36	Right	0.7	0.3	0.0	1.0	0.0	1.7	0.0	0.0	0.0

Table 7. Histology scores for the 1 year exercise no-impact group in the first study. Scores were averaged over the medial, central, and lateral locations.

		CALCIFIED CARTILAGE					SUBCHONDRAL BONE				
Rabbit	Limb	Tide Mark	Thickness	Spikes	Stain	Cells	Average Thickness	Morphology	Red Cells	Red Patches	Trabecular Bone
M16	Left	0.3	0.3	1.7	2.0	2.0	1.3	1.7	4.0	2.0	0.0
M16	Right	1.0	1.0	1.3	2.0	1.3	2.3	0.7	4.0	2.0	0.0
M15	Left	0.0	1.0	2.0	2.0	2.7	1.3	1.3	4.0	2.7	0.0
M15	Right	0.0	0.0	2.0	2.0	2.0	3.0	1.0	4.0	2.7	1.0
M14	Left	0.0	1.7	2.7	2.0	2.7	2.7	1.7	4.0	2.7	3.0
M14	Right	0.0	1.7	2.7	2.0	2.7	3.3	1.3	4.0	4.0	2.0
KB5	Left	0.0	0.0	2.0	2.0	1.3	3.3	2.0	4.0	3.3	3.0
KB5	Right	0.0	0.0	2.0	2.0	2.0	2.3	2.0	2.0	3.3	3.0
KB2	Left	0.0	0.7	2.3	2.3	3.3	2.7	1.7	4.0	3.3	2.0
KB2	Right	1.0	2.3	2.3	2.0	4.0	1.7	2.0	4.0	2.7	4.0
M31	Left	1.0	1.3	2.7	2.0	1.3	2.3	2.7	4.0	3.3	0.0
M31	Right	0.0	0.3	1.3	2.3	0.7	1.0	1.7	2.7	2.0	4.0
M24	Left	0.0	3.7	0.7	4.0	4.0	4.0	2.0	0.0	0.0	3.0
M24	Right	0.0	2.7	1.3	2.0	2.7	4.0	2.0	0.0	0.0	3.0
M21	Left	0.0	1.3	1.3	2.0	2.7	1.7	2.3	2.7	2.0	0.0
M21	Right	0.0	0.7	1.0	2.0	1.3	2.7	2.0	4.0	2.0	2.0
M20	Left	0.0	0.7	2.7	2.0	4.0	2.3	2.3	2.7	2.7	1.7
M20	Right	0.0	1.0	3.3	2.0	4.0	2.7	2.0	4.0	3.3	2.0
M18	Left	0.0	0.3	2.0	2.0	2.0	3.0	3.0	2.7	2.0	3.0
M18	Right	0.0	1.0	1.0	2.0	2.7	2.3	3.0	4.0	2.0	3.0
M36	Left	0.0	0.3	2.0	2.0	2.0	2.7	1.7	2.7	2.0	1.0
M36	Right	0.0	0.7	1.3	2.0	2.0	1.7	2.0	2.7	2.7	2.3

Table 8. Histology scores for the 1 year exercise no-impact group in the first study. Scores were averaged over the medial, central, and lateral locations.

		ARTICULAR CARTILAGE								
Rabbit	Limb	Geometry	Fissures	PG Stain	Cells	Disruptions	Max Thickness	Focal Ossification	Exposure of Subchondral bone	Erosion
KB4	Left	3.3	2.0	0.0	0.7	0.0	2.0	0.0	0.0	0.0
KB4	Right	1.0	0.7	0.0	0.7	0.0	3.0	0.0	0.0	0.0
M13	Left	1.0	1.3	0.0	2.0	0.0	2.0	0.0	0.0	0.0
M13	Right	1.7	1.7	1.0	3.3	0.0	1.3	0.0	0.0	0.0
M17	Left	0.3	0.3	0.7	2.7	0.0	1.3	0.0	0.0	0.0
M17	Right	0.7	0.3	0.7	3.0	0.0	1.3	0.0	0.0	0.0
M26	Left	2.0	1.0	0.7	1.3	0.0	1.7	0.0	0.0	0.0
M26	Right	2.3	1.7	1.3	2.3	0.0	1.7	0.0	0.0	0.0
M27	Left	4.0	2.3	2.0	0.0	0.0	2.0	0.0	0.0	0.0
M27	Right	3.0	2.3	0.0	2.0	0.0	2.5	0.0	0.0	1.0
M34	Left	1.0	1.0	0.0	1.0	1.3	3.0	0.0	0.0	0.0
M34	Right	1.0	1.7	0.0	1.3	1.3	2.7	0.0	0.0	0.0
M37	Left	0.0	0.0	0.0	2.0	0.0	2.0	0.0	0.0	0.0
M37	Right	2.7	3.0	1.7	1.7	1.3	0.7	0.0	0.0	0.0
M39	Left	0.0	0.0	0.0	1.0	0.0	2.3	0.0	0.0	0.0
M39	Right	2.0	0.3	0.0	3.3	1.3	2.3	0.0	0.0	0.0

Table 9. Histology scores for the 1 year exercise impact group in the first study. Scores were averaged over the medial, central, and lateral locations.

			CALCIFIED CARTILAGE				SUBCHONDRAL BONE				
Rabbit	Limb	Tide Mark	Thickness	Spikes	Stain	Cells	Average Thickness	Morphology	Red Cells	Red Patches	Trabecular Bone
KB4	Left	KB4	0.0	0.3	2.0	2.0	2.3	1.3	4.0	2.0	2.0
KB4	Right	KB4	0.0	0.0	1.7	2.0	3.3	1.3	3.3	0.7	2.0
M13	Left	M13	0.0	1.3	2.7	2.3	2.7	2.0	4.0	2.0	2.0
M13	Right	M13	1.0	2.7	3.3	2.3	2.3	1.7	4.0	3.3	1.3
M17	Left	M17	0.0	2.0	2.3	2.0	1.3	1.7	4.0	2.7	2.0
M17	Right	M17	0.0	1.0	2.0	2.3	3.3	1.7	3.3	3.3	3.0
M26	Left	M26	0.3	0.3	1.3	2.0	3.0	1.7	3.3	2.0	2.7
M26	Right	M26	0.0	1.0	2.0	2.0	2.7	1.7	2.0	2.0	2.0
M27	Left	M27	1.0	0.7	2.0	2.0	3.0	1.7	4.0	2.0	3.0
M27	Right	M27	1.0	2.0	3.0	2.7	3.0	2.7	4.0	0.7	3.0
M34	Left	M34	0.0	1.7	3.7	2.3	2.7	1.7	4.0	2.7	1.0
M34	Right	M34	0.7	1.7	3.0	3.0	2.3	2.3	4.0	2.7	1.0
M37	Left	M37	0.0	1.0	1.7	2.0	2.3	1.3	2.0	2.0	2.0
M37	Right	M37	1.0	2.3	1.7	2.0	2.0	2.0	2.7	2.0	4.0
M39	Left	M39	0.0	0.3	2.0	2.0	3.3	2.7	1.3	2.0	3.0
M39	Right	M39	0.0	1.0	1.5	2.0	3.0	1.7	4.0	2.0	3.0

Table 10. Histology scores for the 1 year exercise impact group in the first study. Scores were averaged over the medial, central, and lateral locations.

		ARTICULAR CARTILAGE								
Rabbit	Limb	Geometry	Fissures	PG Stain	Cells	Disruptions	Max Thickness	Focal Ossification	Exposure of Subchondral bone	Erosion
KO4	Left	0.0	0.3	0.0	3.0	0.0	1.7	0.0	0.0	0.0
KO4	Right	1.3	0.7	1.7	3.0	0.0	1.3	1.3	0.0	0.3
M73	Left	0.0	0.0	0.7	1.3	0.0	1.0	0.0	0.0	0.0
M73	Right	2.0	2.0	1.7	2.0	0.0	1.0	0.0	0.0	1.0
M75	Left	0.7	0.3	0.0	2.7	0.0	1.0	0.0	0.0	0.0
M75	Right	0.0	0.0	0.3	0.3	0.0	2.0	0.0	0.0	0.0
M79	Left	1.3	1.3	0.0	0.7	0.0	1.7	0.0	0.0	0.0
M79	Right	0.7	1.7	0.7	3.0	0.0	1.3	0.0	0.0	0.0
M81	Left	0.3	0.0	0.0	1.7	0.0	1.0	0.0	0.0	0.0
M81	Right	2.0	0.3	1.0	2.0	0.0	1.0	1.3	0.0	0.0
M83	Left	0.3	0.7	0.7	0.3	0.0	1.7	0.0	0.0	0.0
M83	Right	2.7	2.0	0.3	1.0	0.0	2.7	0.0	0.0	0.0
M84	Left									
M84	Right	1.3	1.3	0.0	2.7	0.0	2.3	0.0	0.0	0.0
M85	Left	1.0	1.3	0.3	2.3	0.0	1.3	0.0	0.0	0.0
M85	Right	1.0	0.0	1.0	3.0	0.0	0.3	0.0	0.0	0.3
M86	Left	0.0	0.0	0.3	0.3	0.0	2.3	0.0	0.0	0.0
M86	Right	0.0	0.3	0.7	2.7	0.0	2.3	0.0	0.0	0.0
M87	Left	0.7	0.7	0.7	0.0	0.0	1.7	0.0	0.0	0.0
M87	Right	1.0	1.7	0.7	1.3	0.0	1.3	0.0	0.0	0.0
M89	Left	0.0	0.0	0.7	2.3	0.0	1.0	0.0	0.0	0.0
M89	Right	0.3	0.7	0.3	2.7	0.0	1.0	0.0	0.0	0.0
M91	Left	0.0	0.0	0.0	1.7	0.0	1.3	0.0	0.0	0.0
M91	Right	1.7	2.0	1.3	2.3	0.0	1.7	0.0	1.3	1.0

Table 11. Histology scores for the 1 year no-exercise impact group in the first study. Scores were averaged over the medial, central, and lateral locations.

		CALCIFIED CARTILAGE					SUBCHONDRAL BONE				
Rabbit	Limb	Tide Mark	Thickness	Spikes	Stain	Cells	Average Thickness	Morphology	Red Cells	Red Patches	Trabecular Bone
KO4	Left	0.3	0.3	2.0	2.0	1.3	3.0	3.0	2.0	1.3	3.0
KO4	Right	0.0	0.3	2.3	1.7	1.3	2.3	3.0	2.0	0.7	3.0
M73	Left	0.0	1.3	4.0	2.3	1.3	1.3	2.0	4.0	4.0	0.0
M73	Right	1.0	1.0	4.0	1.7	1.3	1.7	2.0	4.0	4.0	1.3
M75	Left	0.7	2.0	3.3	2.0	3.3	0.3	1.3	4.0	2.0	0.0
M75	Right	0.0	1.0	3.0	2.0	4.0	2.7	2.7	3.3	2.0	2.0
M79	Left	0.0	1.0	2.0	2.0	1.3	1.3	3.0	4.0	2.0	1.0
M79	Right	0.0	0.7	1.5	2.0	1.3	1.3	3.0	4.0	2.0	0.0
M81	Left	0.0	0.7	2.3	1.7	1.3	1.3	3.0	2.0	2.0	0.0
M81	Right	1.7	0.7	2.7	1.3	1.3	1.7	3.0	1.3	1.3	3.0
M83	Left	0.0	0.3	1.0	2.0	1.3	2.7	3.0	2.0	0.7	3.0
M83	Right	1.0	1.0	1.0	2.0	2.0	2.0	3.0	4.0	0.0	3.0
M84	Left										
M84	Right	0.0	0.7	2.3	2.0	2.0	2.3	3.0	1.3	2.0	0.0
M85	Left	0.0	0.7	2.7	1.7	0.7	3.3	3.0	0.0	2.0	3.0
M85	Right	3.0	0.0	1.0	1.3	2.0	1.3	1.3	2.0	2.0	1.3
M86	Left	0.0	1.7	4.0	1.7	2.0	3.3	3.0	2.0	2.0	3.0
M86	Right	0.0	1.3	4.0	1.7	2.0	3.3	3.0	2.0	2.0	3.0
M87	Left	0.0	1.0	2.3	2.0	3.3	3.3	2.3	2.0	2.0	3.0
M87	Right	1.0	0.7	2.7	2.0	0.7	2.7	3.0	2.0	0.7	3.0
M89	Left	1.0	0.3	1.3	1.7	2.7	2.0	2.3	3.3	2.0	0.0
M89	Right	0.3	0.3	2.3	1.7	2.7	1.3	3.0	0.7	0.0	0.0
M91	Left	0.0	0.7	3.3	2.0	2.0	1.7	3.0	4.0	2.0	0.0
M91	Right	0.0	0.0	3.3	2.0	2.0	2.3	2.7	2.0	2.0	2.0

Table 12. Histology scores for the 1 year exercise no-impact group in the first study. Scores were averaged over the medial, central, and lateral locations.

Time Zero					1 Year Exercise Impact					1 Year No-Exercise				
Rabbit	Limb	Medial (mm)	Central (mm)	Lateral (mm)	Rabbit	Limb	Medial (mm)	Central (mm)	Lateral (mm)	Rabbit	Limb	Medial (mm)	Central (mm)	Lateral (mm)
KF	Left	0.552	0.771	0.508	BB15	Left	N/A	N/A	N/A	KO4	Left	0.62	1.08	0.8
KF	Right	0.470	0.563	0.508	BB15	Right	N/A	N/A	N/A	KO4	Right	0.62	0.92	0.6
M29	Left	0.466	1.109	0.499	KB4	Left	0.68	0.96	0.6	M73	Left	0.46	0.5	0.6
M29	Right	0.465	0.918	0.743	KB4	Right	0.9	1.2	0.8	M73	Right	0.46	0.6	0.62
M35	Left	0.530	1.022	0.875	M13	Left	0.64	1.18	0.76	M75	Left	0.34	0.36	0.48
M35	Right	0.518	1.043	0.604	M13	Right	0.7	0.76	0.88	M75	Right	0.44	1.36	0.86
M40	Left	0.453	0.728	0.499	M17	Left	0.38	0.74	0.6	M79	Left	0.48	0.76	0.54
M40	Right	0.563	0.842	0.487	M17	Right	0.8	1.22	0.9	M79	Right	0.5	0.74	0.56
M41	Left	0.563	0.596	0.596	M26	Left	0.46	1.64	1.1	M81	Left	0.58	0.58	0.68
M41	Right	0.508	0.683	0.552	M26	Right	0.7	1.12	0.6	M81	Right	0.44	0.76	0.78
M42	Left	0.530	0.640	0.432	M27	Left	0.9	0.94	0.86	M83	Left	0.5	1.14	0.9
M42	Right	0.525	0.984	0.711	M27	Right	0.6	1.2	0.9	M83	Right	0.54	0.9	0.72
M43	Left	0.492	1.066	0.842	M34	Left	0.58	1	0.84	M84	Left	N/A	N/A	N/A
M43	Right	0.727	1.334	1.050	M34	Right	0.58	1.26	0.72	M84	Right	0.66	0.84	0.74
M44	Left	0.459	0.669	0.634	M37	Left	0.56	0.92	0.84	M85	Left	0.88	1.16	0.88
M44	Right	0.459	0.859	0.564	M37	Right	0.6	0.64	0.6	M85	Right	0.54	0.34	0.24
M45	Left	0.453	0.938	0.604	M39	Left	0.92	0.96	1.24	M86	Left	0.64	1.28	0.98
M45	Right	0.354	0.964	0.518	M39	Right	0.84	1.34	0.64	M86	Right	0.68	1.5	0.94
R331	Left	0.465	0.836	0.689						M87	Left	1.22	1.08	0.7
R331	Right	0.629	0.918	0.672						M87	Right	0.9	0.8	0.78
										M89	Left	0.5	0.7	0.8
										M89	Right	0.46	0.58	0.64
										M91	Left	0.62	0.6	0.56
										M91	Right	0.8	0.76	0.68
Average		**0.509**	**0.874**	**0.629**	**Average**		**0.68**	**1.07**	**0.81**	**Average**		**0.60**	**0.84**	**0.70**
Std. Dev.		**0.077**	**0.195**	**0.155**	**Std. Dev.**		**0.16**	**0.25**	**0.18**	**Std. Dev.**		**0.19**	**0.31**	**0.17**

Table 13. Subchondral bone thickness measurements for the time zero, 1 year exercise impact, and 1 year no-exercise groups.

1 Year Exercise No-Impact					1 Year Exercise Impact					1 Year No-Exercise				
Rabbit	Limb	Medial (mm)	Central (mm)	Lateral (mm)	Rabbit	Limb	Medial (mm)	Central (mm)	Lateral (mm)	Rabbit	Limb	Medial (mm)	Central (mm)	Lateral (mm)
KB2	Left	0.10	0.14	0.12	BB15	Left	N/A	N/A	N/A	KO4	Left	0.09	0.13	0.10
KB2	Right	0.18	0.22	0.14	BB15	Right	N/A	N/A	N/A	KO4	Right	0.08	0.09	0.09
KB5	Left	0.11	0.14	0.11	KB4	Left	0.13	0.16	0.19	M73	Left	0.22	0.24	0.17
KB5	Right	0.11	0.10	0.13	KB4	Right	0.16	0.16	0.17	M73	Right	0.14	0.18	0.11
M14	Left	0.16	0.22	0.28	M13	Left	0.16	0.18	0.18	M75	Left	0.18	0.18	0.11
M14	Right	0.14	0.26	0.19	M13	Right	0.24	0.25	0.32	M75	Right	0.17	0.16	0.22
M15	Left	0.13	0.16	0.08	M17	Left	N/A	N/A	N/A	M79	Left	N/A	N/A	N/A
M15	Right	0.15	0.21	0.15	M17	Right	0.10	0.18	0.17	M79	Right	N/A	N/A	N/A
M16	Left	N/A	N/A	N/A	M26	Left	0.14	0.16	0.15	M81	Left	N/A	N/A	N/A
M16	Right	0.09	0.19	0.19	M26	Right	0.15	0.15	0.16	M81	Right	N/A	N/A	N/A
M18	Left	0.17	0.19	0.18	M27	Left	0.15	0.13	0.15	M83	Left	0.08	0.13	0.14
M18	Right	0.15	0.18	0.12	M27	Right	0.14	0.03	0.34	M83	Right	0.09	0.10	0.12
M20	Left	0.22	0.22	0.15	M34	Left	0.21	0.19	0.16	M84	Left	N/A	N/A	N/A
M20	Right	0.16	0.26	0.27	M34	Right	0.14	0.16	0.16	M84	Right	0.14	0.15	0.19
M21	Left	0.15	0.16	0.13	M37	Left	0.23	0.18	0.18	M85	Left	0.10	0.17	0.14
M21	Right	0.14	0.16	0.21	M37	Right	0.18	0.21	0.25	M85	Right	0.04	0.06	0.06
M24	Left	0.20	0.23	0.20	M39	Left	0.10	0.13	0.14	M86	Left	0.15	0.21	0.24
M24	Right	0.21	0.25	0.24	M39	Right	0.12	0.15	0.13	M86	Right	0.21	0.23	0.20
M31	Left	0.16	0.25	0.26						M87	Left	0.14	0.17	0.12
M31	Right	0.16	0.14	0.15						M87	Right	0.14	0.11	0.13
M36	Left	0.13	0.18	0.12						M89	Left	0.09	0.13	0.16
M36	Right	0.16	0.16	0.16						M89	Right	0.08	0.09	0.08
										M91	Left	0.12	0.15	0.18
										M91	Right	0.10	0.12	0.10
Average		**0.15**	**0.19**	**0.17**	**Average**		**0.16**	**0.16**	**0.19**	**Average**		**0.12**	**0.15**	**0.14**
Std. Dev.		**0.04**	**0.05**	**0.06**	**Std. Dev.**		**0.04**	**0.05**	**0.06**	**Std. Dev.**		**0.05**	**0.05**	**0.05**

Table 14. Zone of calcified cartilage thickness measurements for the 1 year exercise no-impact, 1 year exercise impact, and 1 year no-exercise impact groups.

APPENDIX D: RABBIT PATELLOFEMORAL IMPACT SOP

What to bring with you:
1.) Portable computer w/ A2D board, 2.) LPS lubricant, ethanol, 3.) meter stick, 4.) rabbit data sheets (a copy is attached to the end of this SOP), 5.) blank IBM formatted disks

Pre-test set up: (* Leave portable computer off until all cables are connected)

1. Plug in Valadyne strain gauge amplifier (SGA) to the wall. Turn on the SGA and insure that the trigger release switch (see figure 1) is turned off (down position) to protect from accidental triggering.
2. The SGA will need to be on for 15 minutes in order to allow the electronics to stabilize.
3. Assure that all electronic connections are in place. Figures 2 through 4 carefully detail where all connections should be made.
4. Once you have made all the necessary connections, turn on and start up computer. Computer will give a list of possible configurations, choose "Ethernet configuration".
5. Spray some LPS greaseless lubrication on a rag and wipe down the steel rod and gray rails of the impact cart (see figure 5). Do this very sparingly.
6. Use alchohol to clean the sides of the cart (see figure 5) i.e., the portion of the cart where the brakes act. Keep rabbit hair off the rubber brake pads.
7. After the 15 minutes are up, run the program "rabinsur.vi" located on the portable computers desktop. If the program is running (hit small arrow in the top left of screen in Labview) you will see a readout for the load.
8. Using the small screwdriver, zero the load cell on the SGA. Use the opening to the top left of the black dial (see figure 1). Use the readout in Labview.
9. Calibrate the load cell by depressing and holding the shunt cal switch on the Valadyne strain gauge amplifier. If needed readjust the set point to **3349 N on Labview (or 7.53 Volts on the voltmeter)** by using the Gain knob (See figure 1). **** The values in step 9 are susceptible to change whenever the load cell is changed. Always double check these values with the load cell specifications.
10. Double check to make sure the load cell is still zeroed, adjust if necessary.

Rabbit preparation:

1. On the Data Sheet, record the Rabbit name, weight (kg), and sex.
2. Once the rabbit is fully sedated, pull the left hind foot through the very bottom hole of the leather strap of the holding chair and tuck the rest of the strap under the rabbit so it can be fixated to the underside of the chair.
3. Position the right leg so that the femur is pointing vertical and the impactor is directed to hit the middle of the patella.
4. Place the black strap around the right hind foot and pull tight to insure the foot is fully constrained. It is important that the femur remain vertical, but the tibia can be horizontal in order to flatten the patella. Fix the end of the strap, as well as the leather strap, to the Velcro pad on the underside of the chair.
5. Move the clamping bar into position and attach the free end to the distal clamp.
6. Slowly apply even pressure to both clamps until the clamps lock in place.
7. Slide the chair into position so the patella is directly under the head of the impacting cart.
8. Lower the cart so the head of the impacting cart is just above the patella, checking to insure the patella is centered under the head.
9. Raise the impact cart to the desired height. Measure from top of patella to the bottom of the impactor head using the meter stick (see figure 6)
10. The position of the beam holding the upper brake may need repositioning to accommodate the desired height. Do this by removing the screws of the beam and moving the beam to the desired height above the patella.
11. The height and weight of the sled should be:

Energy	Height	Mass
6 J	0.46meters	1.33kg
10 J	1 meter	1 kg

- figure 5 shows 6 J set-up
- 6 J impacts should see between 500 and 600 N as to where 10 J impacts should see from 900 to 1200 N

Impacting the rabbit:

1. Turn on the trigger enable switch on the Valadyne strain gauge amplifier.
2. Click the arrow at the top left of the Labview interface (the "run" arrow) (see figure 7). This should enable the load readout to be constantly changing (if load readout is already constantly changing then the arrow has already been hit). Then click on the large green START button below the graph area on Labview (button that reads "disabled" in figure 7). WAIT AT LEAST 5 SECONDS BEFORE PROCEEDING.
3. Press the red trigger button on the Valadyne SGA to drop the impact cart.
4. Impact cart will drop and the A/D board will trigger and save the data.
5. Labview will prompt user for a filename and location.
6. Switch the trigger enable switch to disable (down position) before removing the rabbit.
7. Excel will automatically run and a macro will plot the data. Note the peak load and time to peak on the data sheet. Also sketch the graph and note any comments.
8. Choose "save as" from the file menu. Save the file as an Excel Worksheet i.e., ".xls" format. Back up all data files to a floppy disk.
9. ***FOLLOWING IMPACTION OF ALL ANIMALS, REMOVE DISK FROM DRIVE (a:\) AND COPY ALL FILES TO THE (g:\user\bimgrad) DIRECTORY FOR PERMANENT DOCUMENTATION!***
10. When testing is done shut down computer before disconnecting cables.
11. Turn off voltmeter and leave on SGA if more testing is going to be done in the next week.

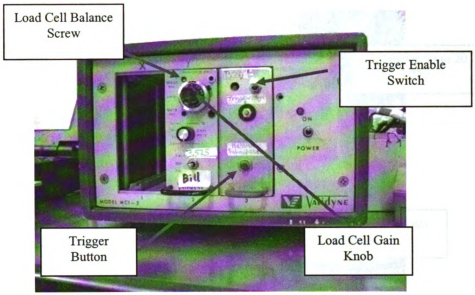

Figure 1. Front view of Validyne Strain Gauge Amplifier (SGA)

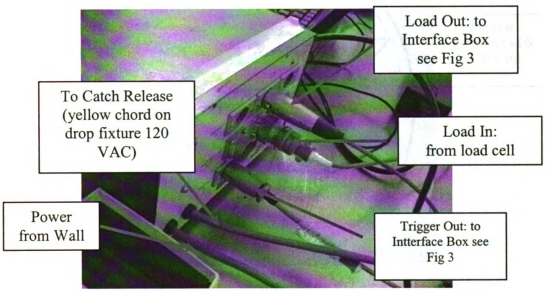

Figure 2. Rear view of Validyne SGA

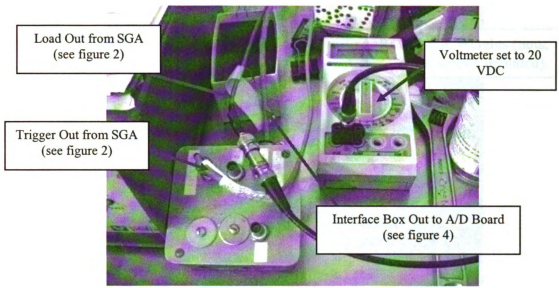

Figure 3. Top view of interface box

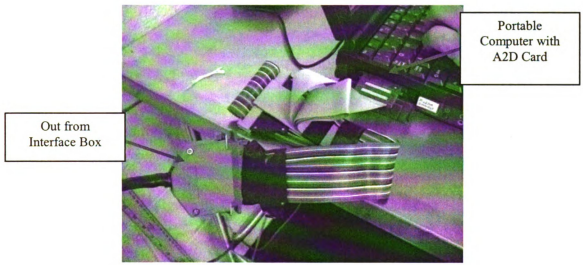

Figure 4. Connection from interface box to A2D card

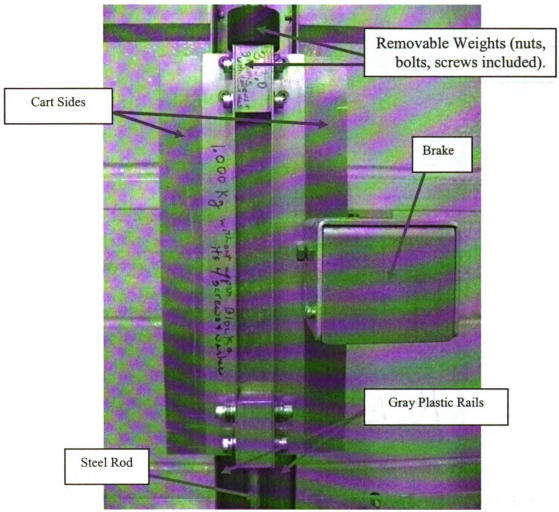

Figure 5. Cart with 1.33 Kg (6 Joule) set up

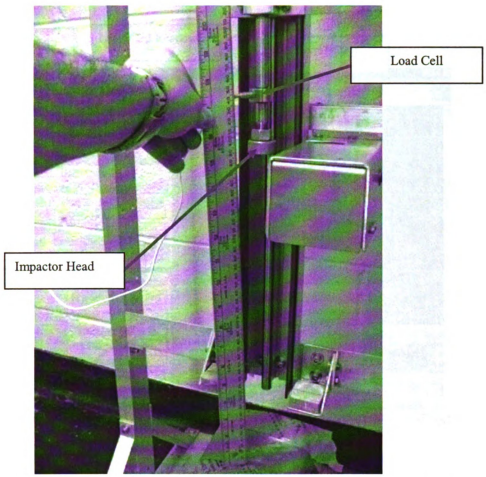

Figure 6. Lower view of impact drop fixture w/ load cell and impactor head

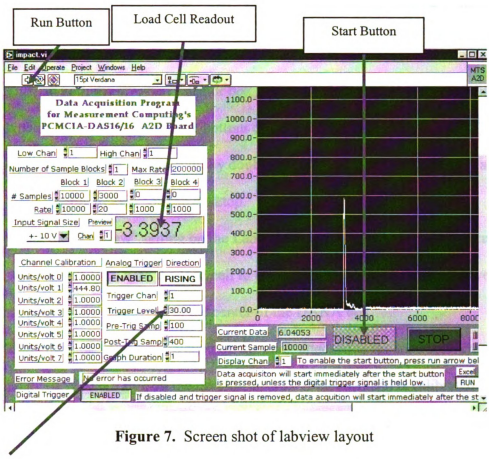

Figure 7. Screen shot of labview layout

**Trigger Value has been changed to 50 in order to prevent accidental triggering

APPENDIX E: RABBIT INDENTATION SOP

Calibration and Program set up:

1. Turn on both the program selector box, and the Validyne strain gage amplifier.
2. Attach the small hook into the bottom of the load cell
3. Open the "preload.vi" program by double clicking on its shortcut, labeled as "Indent with preload" on the desktop.
4. Using the "Preview" setting on the LabVIEW screen, which displays the current force in Newtons, zero the load cell to **0 ± 0.001** using the small screwdriver. The zeroing control is located in the center of the DANA box.
5. Hang the small 100 gram weight on the hook attached to the load cell. This should read **-0.9807 ± 0.002** on the Preview display if the load cell is calibrated correctly. This value corresponds to -0.4000 on the voltmeter. If the value is off more than ± 0.002 then use the gain control (just below the zero control) to adjust the value. Then take off the weight and repeat steps 4 and 5. If the problem persists contact Cliff Becket.
6. Repeat step 5 using the 200 gram weight, the "Preview" read out should be − **1.961 ± 0.002** N, which corresponds to a value of -0.8000 on the voltmeter.
7. In the preload.vi program, verify that the following settings are accurate:

   Block 1: # samples = 1000, rate = 1000

   Block 2: # samples = 6000, rate = 20

   Block 3: # samples = 0

   Preload N = 0.05

   Channel calibration

Units/volt 0 = 1.0000

Units/volt 1 = 2.4517

Units/volt 2 = 1.0000

Analog Trigger = Disabled

These settings control the collection of data. Block one will collect data at 1000 samples a second for one second, block two will then collect 6000 samples for an additional 300 seconds. The data will be displayed in Newtons.

8. Close this program and open the "PreThick.vi" program, which is also located on the desktop of the computer.
9. Verify its setting to be

   Block 1: # samples = 1500, rate = 50

   Block 2: # samples = 0

   Channel calibration

   Units/volt 0 = 1.0000

   Units/volt 1 = 2.4517

   Analog Trigger = Enabled

   Trigger Channel = 1

   Trigger Level = 0.2

   Pre-Trig Samp = 100

   Post-Trig Samp = 1400

11. Run the "Reset MinIndenter" program, located on the desktop, by double clicking. (This must be done any time the program selector box is turned on, before you can reprogram the Mini Indenter.)
12. Open up the "Miniprogram" on the desktop of the computer. This is a HyperTerminal program. The indenter program is set up to be 10, 1 on the program selector box, and the thickness program is set up to be 10, 2.
13. In the Miniprogram, the indenter program is # 500. Type "q500" then hit the carriage return 14 times to display the program. It should read the following:

    500H 1

    502I 2000

    505V 10000

    508K 3

    510O 0

    514R 200

    518W 65535

    521W 65535

    524 W 65535

    527 W 65535

    530 W 65535

    533 W 58725

    536 R -1000

    540

Refer to the mini indent manual for a complete description of these commands. If a line is incorrect, change it by typing "p" then the line number and the appropriate command letter and number. Press Esc to exit edit mode, and type p on the last blank line to specify the end the program.

14. Check the thickness program. Type "q600" followed by 13 carriage returns. It should read the following

    600H 1

    602I 200

    605V 200

    608K 0

    610O 0

    614R 12000

    618H 1

    620I 2000

    623V 10000

    626K 3

    628R -1000

    632

15. Double check these programs by running a simulated test. Place the program selector switches on 10, 1 for the indent program.

16. Rotate the mini indenter jogging knob, located on the top of the indenter, to 0.

17. Push the red start button on the program selector box. Make sure the knob rotates from 0 to 2, which translates to 0.1 mm of downward travel. If this is incorrect, recheck the program in the HyperTerminal and make corrections as necessary.
18. Place the program selector switches on 10, 2 for the thickness program.
19. Push the red start button on the program selector box and make sure the indenter travels downward at a constant rate. Hit the red stop button at the end of the grey cable to stop the test.

Equipment set-up

1. Check to make sure that the black cable labeled "Mini Indenter Trigger Out" (with the BNC connecter) is **NOT** connected to the A2D interface box. The program will not run correctly unless this cable is unattached.

   **However, the trigger signal is still functional and could be used on another A2D interface box hooked up to another computer if so desired.*
2. Loosen all the claps on the mounting plate, 6 total. These are the round knobs located around the edge of the fixture.
3. Attach the small grey base of the camera mount to the center of the horizontal mounting plate.
4. Attach the camera mount to its base. Note that the small reservoir and clamp should be attached to the camera mount, if not do so now.
5. Fill the reservoir with PBS, making sure not over fill. Be very careful not to splash any PBS up onto the load cell, or to spill any PBS near the circuit card located outside of the program selector box.

Test procedure:

1. Remove all soft tissue surrounding the patella with a scalpel, this will ensure a good hold in the clamp.
2. Dab the cartilage surface with India ink to highlight any surface fissures, wipe off excess.
3. On the data sheet, record the rabbit name and all other pertinent information.
4. Sketch all surface lesions and make notes of any other abnormalities such as osteophytes.
5. Place patella into the small clamp on the camera mount.
6. Use a small Allen wrench to tighten the clamp to hold the patella in place. Do not over tighten but make sure the patella is placed firmly against the bottom of the testing fixture and is secured rigidly.
7. Make sure that a lock nut is attached to each of the indenter probes.
8. Verify that the load cell is attached in the correct orientation. The end where the gray cable is attached must be located at the top half of the load cell.
9. Screw in the flat 1mm indenter into the load cell. Find a flat clean location on the lateral facet of the patella for testing. Rotate the camera mount to get this location at horizontal as possible and jog the XY plate so the indenter is located directly above the testing sight. Once the location is roughly where desired, there is a round knob available in order to manually adjust the plate in both the X and Y directions in a much smaller, more accurate amount.
10. Lower the indenter as close as possible with out touching the patella to ensure the testing sight is flat. Adjust the camera mount and plate as necessary.

11. Once in place, tighten all the clamps.
12. Raise the reservoir containing the PBS so the patella is submerged.
13. Zero the load cell with the indenter tip in the PBS. **Note: Make sure the entire indenter tip is not, or will not be submerged. Lowering the top, larger portion of the indenter into the PBS will cause the zero to be off.*
14. Lower the indenter head so that it is very close, but not touching the surface of the patella, (0.5-0.1 mm away).

    **Note: Use the Fast and Slow actuator jog buttons (located on the selector box) to move the indenter up and down while the Z-axis clamps are tightened down.*
15. Verify that the program switches are on 10, 1 and that the "preload.vi" program is in the run mode, (the run arrow in the top left is black). Then click the green start button on the LabVIEW screen and let it run. ***(As soon as the green START button is triggered the indenter will IMMEDIATELY begin running.)*
16. Be sure not to move or bump either the Mini Indenter or the desk it sits on in order to avoid unnecessary jolts or noise in the graph.
17. Once the test is completed, loosen the Z-clamps, raise the indenter and replace the 1mm indenter with the 1.5mm indenter.
18. Save the data file just collected in its appropriate location.
19. Wait 5 min and then repeat steps 13-18.
20. Once the indentation tests are completed, measure the thickness of the indentation sight.

21. Replace the 1.5mm indenter with the needle.
22. Put the program switches on 10, 2.
23. Open the PreThick icon, which opens rabthick.vi.
24. Zero the load cell
25. Lower the load cell until the needle is just above, but not touching the surface.
26. Hit the start button in the PreThick.vi program and then the start button on the program selector box.
27. When the plot on the PreThick.vi program reaches 10-15 Newtons (on the y-axis) stop the test with the shut off switch at the end of the grey cable (labeled "mini indenter stop program button"). This should approximately be the point where the needle hits the bone. ******(Do NOT exceed 20 N! Doing so could cause damage to the load cell.)******

***What happens here is that the needle starts to push on the cartilage, and when it breaks through the graph will show a sudden drop. Then the plot will increase dramatically when the needle hits the bone.*

28. Repeat for three more sights on the patella, so that there is one site tested in each of the four quadrants. Make sure to record each of the sight locations on the data sheet.

APPENDIX F: BOVINE MEDIA STOCK RECIPE

1. Measure 1L of ddH_2O. Pour 600mL of ddH_2O into 2L Erlenmeyer flask with stir bar.
2. Add 1 package of powdered media DMEM:F12 (Gibco #12500-062). 1 package makes 1L of media.
3. Add 20mL AA solution (Gibco #11130-051).
4. Add 3.89g Sodium Bicarbonate ($NaHCO_3$) (for final concentration of 44mM).
5. Add 2mg lactalbumin hydrolysate (for final concentration of 2μg/mL).
6. Add 1μL of diluted sodium selenite stock solution to the media (for final concentration of 1pg/mL).
7. Add 10μL of ascorbic acid stock solution.
8. Add 10μL dexamethasone stock solution (for final concentration of 100μg/mL).
9. Add 10μL of manganese sulfate stock solution to the media.
10. Bring media up to 900mL with ddH_2O and pH to between 7.3 and 7.4, then bring volume to 1L and sterile filter.
11. Before using bovine media for cell culture, add 10mL antibiotics (Biochem stores #15240-062) to the 250mL sterile filtered jar.

Stock Solution Concentrations

Sodium selenite (1mg/mL) - dilute the stock solution 1/1000 for 1μg/mL.
Ascorbic acid (5mg/mL)
Dexamethasone (10mg/mL)
Manganese sulfate (16.9mg/mL)

*media should last for one month

APPENDIX G: BOVINE MEDIA HANDLING SOP

1. Remove FBS and antibiotics (if needed) from freezer and thaw by placing tube in warm water.

2. Set up laminar flow hood by turning on the fan and lights. Sterilize by wiping down the inside surfaces with 70% ethanol. Sterilize 101-1000μL pipetter, marker, 24-well plate, and *anything else placed in hood with 70% ethanol.*

3. Remove two 250mL jars of bovine media from refrigerator and place in hood with thawed FBS and antibiotics.

4. If needed, pour 10mL antibiotic into 250mL of bovine media. Gently mix by swirling. This should protect media from contamination for two weeks.

5. For a standard 24-well plate, 1mL of culture media is needed per well. Pipette 2.5mL FBS into a conical tube. Bring to 25mL by adding antibiotic-treated media (final concentration of 10% FBS by volume media). Vortex to mix.

6. Pipette 1mL of 10% FBS antibiotic-treated bovine media into each well of the 24-well plate. Cover.

7. Set up three petri dishes with bovine media from the untreated jar. Rinse explants in each dish before placing in the fresh 24-well plate.

8. Put remaining FBS in freezer. Sterilize hood again before shutting off.

9. Change media in well plate every 2 days throughout an experiment.