Manuscript Details

Manuscript number	CLBI_2018_521
Title	The Influence of Maximal and Submaximal Cyclic Concentric and Eccentric Exercise on Chondrocyte Death and Synovial Fluid Proteins in the Rabbit Knee
Article type	Research Paper

Abstract

Background: Mechanical stimulation of joints regulates the biosynthetic activity of chondrocytes. It has been argued that excessive loading might cause chondrocyte death, leading to degeneration of cartilage and cause osteoarthritis. The aims of this study were to apply a high, short-term loading, and a low intensity, long-term loading protocol to intact joints in life animals and determine changes in synovial fluid and the percentage of dead cells in rabbit knee cartilage. Method: Nine rabbits were subjected to unilateral exercise loading consisting of five sets of 10 maximal eccentric contractions. Another 6 rabbits were subjected to submaximal concentric contractions for 30 min at 20% of the maximum isometric knee extensor force. Contralateral joints served as unloaded controls. Cell viability was assessed using confocal microscopy. Synovial fluid was analyzed for total protein concentration and total number of identifiable proteins and was compared to protein content of control rabbits (n=4). Findings: Neither the high-intensity, short-term nor the low-intensity, long-term loading protocol caused increased chondrocyte death compared to the unloaded control joints. Total synovial fluid protein concentration was the same before and after exercise. Following the high-intensity exercise protocol, the number of identifiable proteins was decreased, while following the low-intensity exercise protocol, the number of identifiable proteins was increased compared to control. Interpretation: Chondrocytes are well protected in the intact joint and withstood maximal eccentric muscular loading, and maximal endurance loading. Synovial fluid protein content was changed after exercise, and these changes depended crucially on the type of loading.

Keywords	Chondrocytes, Cell viability, Muscular loading, Synovial fluid, Protein content
Taxonomy	Molecular Biology, Biochemistry
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December 29, 2019

Dear Editor Dr. Gefen,

I am resubmitting a manuscript for consideration of publication in Clinical biomechanics. The manuscript is entitled “The Influence of Maximal and Submaximal Cyclic Concentric and Eccentric Exercise on Chondrocyte Death and Synovial Fluid Proteins in the Rabbit Knee” by Nada Abughazaleh, Ziad Abusara, Roman Krawetz, and Walter Herzog.

It has not been published elsewhere and that it has not been submitted simultaneously for publication elsewhere.

Thank you very much for your consideration.

Yours Sincerely,

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Highlights:

- High intensity exercise in intact joint in vivo didn't cause significant chondrocyte death
- Low intensity, long duration exercise in joint in vivo didn't cause significant chondrocyte death
- High intensity, short duration exercise had no significant effect on total synovial fluid proteins
- Low intensity, long duration exercise had no effect on total synovial fluid protein concentration

The Influence of Maximal and Submaximal Cyclic Concentric and Eccentric Exercise on
Chondrocyte Death and Synovial Fluid Proteins in the Rabbit Knee

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Words count: Abstract =328 now 246, Main body =4696 now = 3914

Abstract

Background: Mechanical stimulation of joints regulates the biosynthetic activity of chondrocytes. It has been argued that excessive loading might cause chondrocyte death, leading to degeneration of cartilage and cause osteoarthritis. The aims of this study were to apply a high, short-term loading, and a low intensity, long-term loading protocol to intact joints in life animals and determine changes in synovial fluid and the percentage of dead cells in rabbit knee cartilage.

Method: Nine rabbits were subjected to unilateral exercise loading consisting of five sets of 10 maximal eccentric knee contractions. Another 6 rabbits were subjected to submaximal concentric contractions for 30 min at 20% of the maximum isometric knee extensor force. Contralateral joints served as unloaded controls. Cell viability was assessed using confocal microscopy. Synovial fluid was analyzed for total protein concentration and total number of identifiable proteins and was compared to protein content of control rabbits (n=4).

Findings: Neither the high-intensity, short-term nor the low-intensity, long-term loading protocol caused increased chondrocyte death compared to the unloaded control joints. Total synovial fluid protein concentration was the same before and after exercise. Following the high-intensity exercise

protocol, the number of identifiable proteins was decreased, while following the low-intensity exercise protocol, the number of identifiable proteins was increased compared to control.

Interpretation: Chondrocytes are well protected in the intact joint and withstood maximal eccentric muscular loading, and maximal endurance loading. Synovial fluid protein content was changed after exercise, and these changes depended crucially on the type of loading.

Key words: Chondrocytes, Cell viability, Muscular loading, Synovial fluid, Protein content

Introduction

Articular cartilage (AC) is a specialized fibrous connective tissue that covers the ends of bones in joints. Healthy cartilage can maintain its function for a lifetime, and chondrocytes are active in maintaining the tissue matrix, yet they have limited capability for tissue repair (Temenoff and Mikos, 2000). Cartilage damage often occurs due to aging, sport injuries or diseases (Sophia Fox et al., 2009; Temenoff and Mikos, 2000). Osteoarthritis (OA) is a joint disease that is characterized by a progressive break down of articular cartilage.

Chondrocytes have been shown to die when mechanical loading of the tissue is high (Horisberger et al., 2013a). It would be important to know how much mechanical loading a joint can take, and identify a stress threshold below which cells can repair matrix damage, and above which they cannot (Clements et al., 2001; Horisberger et al., 2011; Milentijevic et al., 2005; Urban, 1994). The differences in “stress thresholds” reported in the literature may be related to methodological differences. Clements et al. (2001) observed that repeat stresses below 6 MPa did not result in chondrocyte death in bovine femoral groove specimens. For pressures above 6 MPa, chondrocyte

death rates were reported to increase linearly with increasing loads (Clements et al., 2001). Stresses of 1.2 MPa applied for 18 hours on canine articular cartilage explants resulted in chondrocyte death in the superficial, but not the deep zones (Burton-Wurster et al., 1993). Unconfined compression of 40 MPa at different loading rates in bovine chondral explants resulted in 47 to 66% cell death.

There has been little systematic research on the effects of loading on chondrocyte viability in vivo. The most physiological loading patterns would be observed when an animal is allowed to walk or run freely and chondrocyte mechano-biology would be measured simultaneously. However, this seems impossible with today's experimental techniques. A next best physiological scenario is loading of joints by controlled muscular contraction. (Bourne, 2011) found that submaximal, cyclic isometric knee loading for 50 min resulted in an increase in chondrocyte death in all areas of the rabbit knee. Cyclic, sub-maximal, eccentric contractions of 20% of the maximal isometric force applied for 50 minutes caused a significant increase in cell death in loaded compared to unloaded knees (Horisberger et al., 2011). Quantification of cell viability after four weeks of applying different type of mechanical loading (eccentric, concentric, and isometric) revealed that cyclic loading applied 3 times a week resulted in an increase in cell death in experimental compared to control joints (Horisberger et al., 2013b).

These results suggest that low levels of muscular force application may cause chondrocyte death, if loads are applied over long periods of time (Horisberger et al., 2011). In vitro studies have provided important information on the mechanical properties of articular cartilage and the mechanobiology of chondrocytes. However, in vitro studies may not represent in vivo responses of chondrocytes appropriately because of altered boundary conditions and loading geometries. (Hall et al., 1991; Jeffrey et al., 1995).

There is selected research of the effects of joint injuries and diseases on the composition of synovial fluid (SF) (Catterall et al., 2010; Sardari et al., 2011). However, the effects of exercise on SF composition are not well characterized. Preliminary studies suggest that exercise may affect SF composition and might have beneficial effects for joint health (Abusara et al., 2013). Expression of PRG4 has been shown to coincide with increased joint motion (Ogawa et al., 2014), while abnormal mechanical stimulation seems to result in a decrease in PRG4 expression (Cleary et al., 2010). Based on results of *in vitro* studies, it has been suggested that compression and cyclic tensile strains regulate the cellular PRG4 metabolism (Grad et al., 2005; Wong et al., 2003). However, systematic studies on acute changes of synovial fluid composition following joint loading have never been performed in intact joints using physiologically meaningful loading parameters.

The purpose of this study was to quantify changes in SF composition and chondrocyte death for physiologically relevant loading conditions. Measurements were made in the rabbit knee. Physiologically relevant loading conditions were applied by controlled stimulation of the knee extensor muscles. We hypothesized that high intensity, short-term and low intensity, long-term loading kills chondrocytes and increases SF protein content, specifically PRG4.

Methods

Testing was performed in healthy, skeletally mature female New Zealand White rabbits. Animals were housed in accordance with the Canadian Council on Animal Care. Rabbits were anesthetized and maintained using an isoflurane and oxygen mixture. A nerve cuff electrode was implanted around the femoral nerve of the right leg and connected to a Grass S8800 stimulator for controlled quadriceps contractions. The left (unloaded) leg was used as a control. Rabbits were placed on a

stereotaxic frame and the pelvis and distal femur were fixed by bone pins. Muscle forces were measured using a strain-gauged lever that was attached to the distal part of the tibia and was connected to a motor driving the leg. Eighteen knee joints were assigned to an experimentally loaded (n=9), and an unloaded control group (n=9). Eccentric knee extensor contractions were performed by flexing the knee against the resistance of the activated quadriceps muscles from a knee angle of 150° to 80° at a speed of 70°/s. Maximal contractions were achieved by stimulating the knee extensors supra-maximally at a frequency of 100Hz. Exercise loading consisted of five sets of 10 eccentric maximal contractions (1s on, 1s off) with a two-minute rest between sets.

A physiologically relevant exercise protocol was applied to another six rabbit knee joints with the contralateral legs (n=6) serving as the unloaded controls. The maximum isometric force was determined at a 100° of knee flexion angle. Then, activation of the muscle was decreased until a force of 20% of the maximum isometric force was obtained. Once the 20% activation level had been determined, the knee joint was subjected to cyclic loading from 70 to 130° of flexion at an angular speed of 120°/s, and then was returned to its initial flexed position at an angular speed of 40°/s. Loading was maintained for 30 minutes, with one loading cycle every 2s for a total of 900 cycles. Following the exercise protocols, rabbits were sacrificed by a Euthanyl overdose. Both hind limbs were removed and placed in a phosphate buffered saline solution.

The femoral groove, lateral and medial femoral condyles, patella and medial and lateral tibial plateaus of control and experimental knees were harvested from each joint (Fig. 1). Each specimen was stained with Syto 13 (a live cell stain) and SytoX orange (a dead cell stain) [Invitrogen, Carlsbad, California, U.S.A.] (Horisberger et al., 2011) which were mixed with dimethyl sulfoxide for quick penetration of the stains into the cartilage (Lewis, 2007). Specimens were stored at 4° C

overnight. After 24 hours, each specimen was washed 3 times for 5 minutes with PBS. The specimens were then analyzed for cell viability using confocal microscopy (Jones et al., 2005).

Bone-cartilage specimens were fixed in a petri dish and immersed in PBS. Confocal laser scanning microscopy (LSM 510, Zeiss Inc., Germany) was used to scan 17 predefined areas on the articular surfaces of the knee: 6 locations on the tibial plateau, 4 locations on the femoral condyles, 4 on the patella, and 3 on the femoral groove. Two lasers, 488nm and 543nm wavelength, were used to scan the surface of the cartilage for live and dead cells with a 10X objective. Images were taken every 1 μ m up to a depth of 75 μ m into the cartilage tissue. The field of view was 512 $\times$ 512 μ m². Dead and live cells were counted using a custom-written Matlab program (Mathworks Inc., Natick, MA) that identified live (green) and dead (red) cells, and the percentage of live and dead cells was determined for each location in experimental and control joints.

Protein analysis

Total protein concentration for SF extracted from the exercised and contralateral joints was determined in the high and low intensity exercise group rabbits using a Bicinchoninic acid assay (BCA assay) with bovine serum albumin standards per manufacturer's instructions (Thermo Scientific, NY, USA). Samples were analyzed for number of proteins using liquid chromatography (LC; Agilent 1260 Infinity chip cube interface) tandem mass spectrometry (MS/MS) on an Agilent 6550 iFunnel quadrupole (Q)- time-of-flight (TOF) mass spectrometer. One μ l of tryptic peptides was loaded onto a C18 trap column. Mascot algorithm (Matrix Sciences; version 2.4) software was used to search the national center for biotechnology information NCBI database and identify the peptides

Statistical analysis

Comparisons of cell viability between corresponding locations of unloaded control and loaded exercised joints were performed using the Wilcoxon Signed Rank Test (SPSS 19, SPSSInc., Chicago, IL, USA). Results are presented as means $\pm$ 1 standard error. Comparison between the average percentages of dead cells between the four locations in the exercised limb was done using a one way repeated measures ANOVA (SPSS 19, SPSSInc., Chicago, IL, USA). Comparison between average protein concentrations and the number of identifiable proteins in the low intensity exercise, the high intensity exercise, and the untreated control group rabbits was done using a 3x2 repeated measures ANOVA (SPSS 19, SPSSInc., Chicago, IL, USA). The level of significance was set at 0.05 for all tests.

Results

The mean quadriceps forces produced in the first cycle of the high intensity exercise were $566 (\pm 53)$ N. In the last cycle, mean forces had dropped by about 55% of the initial forces to a mean of $314 (\pm 26)$ N (Fig. 2). This drop in force indicated that the muscles were driven at maximal effort and were fatigued at the end of the maximal intensity loading protocol. During the low intensity exercise, peak quadriceps forces were maintained at a relatively constant level of 20% of the maximal isometric force. The maximum isometric force at 100° was 450N and the average quadriceps forces were $95 (\pm 8)$ N (Fig. 3).

Knowing that the average patello-femoral and medial and lateral tibio-femoral contact areas in rabbit knees in eccentric contraction are approximately 30.5 mm^2 , 21.7 mm^2 , and 18.1 mm^2 , respectively, and for concentric contractions average approximately 38.1 mm^2 , 23.5 mm^2 , and 21.2 mm^2 , respectively (Leumann et al., 2013), we estimated that the average peak contact pressures in the high intensity and low intensity exercise were approximately 33 and 4.4 MPa, respectively.

There were no significant differences in the percentage of cell death between control and exercised joints ($p > 0.05$) in the high and low intensity exercise protocols (Figs 4 and 5). The mean percentage of dead cells at the four joint locations was similar.

For the high intensity, short duration exercise protocol, the average number of uniquely identifiable proteins was 36 and 32 for the exercised and the contralateral joints, respectively, compared with the low intensity, long duration exercise which had 133 and 119, in the exercised and contralateral joints, respectively. For the non-exercised control group rabbits, the average number of uniquely identifiable proteins was 72 and 65 for the right and left leg, respectively (Fig. 6).

The mean protein concentration in the experimental limbs following the high intensity exercise (19.3 ($\pm$ 6.6) mg/ml) was the same as that observed in the corresponding contralateral limbs (15 ($\pm$ 5.3) mg/ml); (Fig. 7). The mean protein concentration in the experimental limbs following the low intensity exercise (22.7 ($\pm$ 10.0) mg/ml) was the same as in the corresponding contralateral limbs (19 ($\pm$ 5.5) mg/ml). There was no significant difference in mean total protein concentration between the two exercise protocols for the exercised or the contralateral knees, nor was there any difference in total protein concentration between any of the limbs in the two exercise protocol groups compared to the non-exercised control animals.

Discussion

Chondrocyte death has been observed for surface pressures of as little as 0.5MPa for long term static loading (Lucchinetti et al., 2002) and above 6MPa for impact type loading (Clements et al., 2001). It is well accepted, and has been shown experimentally and theoretically, that articular cartilage surface pressures in a joint loaded with muscular contraction can reach in excess of 20MPa easily (Leumann et al., 2013; Ronsky et al., 1995; Sawatsky et al., 2012; Urban, 1994). These findings suggest that joint loading through muscles might be sufficient to kill chondrocytes as pressures in joints can easily exceed those shown to produce chondrocyte death in situ. Impact, static, and cyclic loading have been used to determine chondrocyte viability in cartilage explants in vitro or intact cartilage in vivo (Hall et al., 1991; Lewis et al., 2003; Lucchinetti et al., 2002; Sah et al., 1989; van Kampen et al., 1985). Cyclic indentation impacts resulting in cartilage surface pressures of 5 MPa resulted in significant increases in chondrocyte death compared to unloaded controls (Chen et al., 2001). Similarly, a single impact load applied to intact bovine patellae

resulted in a decrease in cell viability compared to unloaded control specimens (Lewis et al., 2003). However, findings on joint loading using concentric and eccentric muscular loading *in vivo* are rare, and it is hard to compare chondrocyte death across different loading conditions as cell responses depend on the type of loading and the boundary conditions (Horisberger et al., 2013c).

The aim of this study was to determine chondrocyte deaths following high intensity, short-term, cyclic muscle loading and low intensity, long-term cyclic muscle loading at different locations on the articular cartilage in rabbit knees and detect changes in SF composition following each exercise. These two muscular loading protocols were chosen as they represent the maximal loading intensity and the near maximal endurance capacity of the rabbit knee extensor muscles, thereby, they form the limits of muscular capacity.

The forces produced by the high intensity exercise reached peak values of approximately 600N with the corresponding pressures exceeding 27MPa. By the end of the fifth set of the ten maximal eccentric contractions, forces had decreased to about half of the peak value, indicating that this exercise was demanding and resulted in substantial fatigue of the muscles. However, no increase in dead cells compared to control tissues was detected. Our finding supports the idea that chondrocytes can withstand maximal muscular loading for a short period of time, and that chondrocytes are well protected by the ECM and the natural loading conditions in a joint (Alexopoulos et al., 2005; Sophia Fox et al., 2009).

Compressive surface stresses above 25 MPa have been found to kill most chondrocytes under *in vitro* conditions (Clements et al., 2001). In our study, cartilage surface pressures exceeding 25 MPa did not cause increased cell death. Combined these results indicate that *in vitro* studies do not represent the behavior of chondrocytes loaded in the intact joint, and thus, loads that kill

chondrocytes *in vitro* may not cause damage *in vivo*. Joints and cartilage often function properly for the duration of a life. Therefore, one might assume that the functioning of joints and the loading they experience is reflected in the structures and properties of cartilages so that they can fulfill this role. The congruency of joints, the cartilage to cartilage contacts, the cartilage material properties, and the different structures of cartilages and chondrocytes across joints and for different locations within joints, might provide some insight into the durability of this amazing tissue.

According to Lucchinetti et al. (2002), chondrocyte viability depends on the characteristics of the load and the duration of the load. A six hour, 1 MPa uniaxial compressive cyclic load to cartilage explants taken from the occipital joint of adult cows resulted in dramatically increased superficial zone chondrocyte death. Similarly, a 0.5 MPa static load applied for 3 hours to the same preparation resulted in vastly increased cell death compared to control (Lucchinetti et al., 2002). In our study, with the load applied through cyclic muscular contraction, no increase in chondrocyte death was detected when cartilage was subjected to peak pressures of 4.5 MPa for 30 minutes corresponding to 900 loading cycles. Cartilage surface pressures that were four times smaller than those applied by us have been shown to induce cell death in explant tissues (Steinmeyer et al., 1997). Differences in the duration of loading, the frequency of loading, the rate of load application, and differences in boundary conditions between explant tissues loaded with a flat metal plate rather than a curved cartilage surface likely account for the different results.

Average peak pressures in the human hip during walking have been estimated at 5–8 MPa (Park et al., 1999). Since it is safe to assume that normal human walking does not cause chondrocyte death, the results of our concentric exercise protocol are consistent with the idea that normal walking is not associated with cartilage damage in a healthy knee. Our findings also agree with

those by Clements et al. (2001) who found that pressures below 6 MPa do not cause cell death in cartilage/bone specimens of bovine knees (Clements et al., 2001).

The high intensity, short duration and the low intensity, long duration exercise protocols had no effect on total synovial fluid protein concentrations. This result agrees with those obtained by (Frisbie et al., 2008). They exercised horses on a treadmill for five days each week for 91 days, and synovial fluid samples were aspirated once per week from the mid-carpal joints. Total protein concentration remained virtually constant (22mg/ml) in the first 21 days of exercising, then increased, and then returned to baseline levels, suggesting that extensive exercise loading might need more time than three weeks to cause changes in total SF protein concentration (Frisbie et al., 2008). The increase in total SF protein concentration after 3 weeks of exercising may have been caused by the mid-carpal arthroscopic surgery performed on day 21. Another potentially important difference in the Frisbie study and our approach is that we collected the SF “immediately” following the exercise protocol, whereas Frisbie et al. (2008) collected their samples once per week after 5 days of training.

Our results also agree with findings by (Abusara et al., 2013) who reported that total protein concentration in the mouse knee did not change with low intensity exercise in which muscle forces were kept below 50% of the maximal isometric force. However, with high intensity exercise (muscle forces greater than 55% of the maximal isometric force) they detected a significant increase in total SF protein, including a significant increase in PRG4 concentration. Based on their result, we would have expected an increase in protein concentration in our high intensity loading protocol. However, in contrast to (Abusara et al., 2013), our joints were left completely intact, while they breached the medial joint capsule and removed the medial meniscus. Interestingly, when (Abusara et al., 2013) continued their high intensity loading protocol to five sets of 15

consecutive contractions, SF protein and PRG 4 concentrations did not further increase, indicating an “exhaustion” of protein supply with repeated high intensity loading. Our high intensity protocol involved 5 sets of 10 maximal muscular contractions which might have exhausted the protein reservoir system in the joint, but still, we would have expected to see an increase in total protein concentration.

Although total protein concentration did not change following the high and the low intensity exercise protocol, the average number of uniquely identifiable proteins significantly increased following the low intensity exercise protocol, and significantly decreased following the high intensity exercise protocol compared to control group rabbit values. However, the number of uniquely identifiable proteins was the same for knees exposed to the exercise loading and the corresponding non-loaded contralateral knees, suggesting that the acute changes in SF composition with exercise are systemic rather than local. This finding suggests that the protein release mechanisms may not be triggered by mechanical loading, but that mechanical loading produces a biological signal that causes systemic protein release into the synovial fluid. This theory could be tested by using protein release blockers, as done by (Abusara et al., 2013), and block protein release into the SF in the loaded joint, and then check SF protein concentrations in the non-loaded contralateral joint. (Abusara et al., 2013) demonstrated that following application of cell secretion blockers, loading protocols did not produce the previously observed changes in SF composition.

The increase in the number of uniquely identifiable proteins following the low intensity exercise protocol may be associated with the release of anti-inflammatory cytokines or regulatory molecules, which were detected following low intensity exercise and have been reported to inhibit the effect of pro-apoptotic proteins and prevent cell death (Abusara et al., 2013).

284 Other proteins may be removed from the SF by exercise to keep the total protein concentration
285 approximately constant.

286 The high intensity protocol appears to have been associated with an increase in the total number
287 of proteins, specifically lubricating proteins that might help cartilage withstand high loading
288 conditions. A greater number of total proteins tends to decrease the number of uniquely identifiable
289 proteins (Snider, 2014), which is what we observed. This thinking is supported by observations on
290 cartilage explants that were obtained from exercised and non-exercised ponies and cultured in SF
291 for one month. There was no difference in total SF protein concentrations obtained from the
292 exercised and non-exercised explant cultures. However, the levels of specific components of SF
293 changed significantly. For example, the concentration of insulin-like growth factors was greater
294 and glucosaminoglycan was smaller in the exercised compared to the non-exercised explant
295 cultures (Van den Hoogen et al., 1998). (Frisbie et al., 2008) also observed increased
296 concentrations of selected proteins following exercise, while total protein concentration remained
297 the same.

298 Some SF proteins, such as creatine kinase, were increased following the low and high intensity
299 exercise protocols. Creatine kinase catalyzes the conversion of ADP and phosphoryl creatine into
300 ATP and creatine, and is associated with muscle damage (Baird et al., 2012). This finding indicates
301 that the increase in uniquely identifiable SF proteins may not only result from product release from
302 intra-articular cells, but may be transported into the joint space through the blood and synovium
303 which is known to have increased permeability when inflamed and allows for large size molecules
304 to enter the joint (Kushner and Somerville, 1971; Strøm et al., 1989).

A limitation of this work is that we were unable to compare concentrations of specific proteins between exercised and control rabbits, since mass spectroscopy gives a score for highly abundant proteins but not their concentration, and Bicinchoninic acid assay (BCA assay) gives the total protein, but not individual protein concentrations. In future work, it would be important to quantify changes in specific SF protein concentrations with exercise. It would also be important to identify the function of proteins secreted into the SF with exercise, and to determine the long-term effects of these changes. A fundamental understanding of changes in the intra-articular environment caused by exercise might help determine the beneficial effects of exercise on joint health and help quantify the amount of exercise that is healthy compared to what might lead to overloading and degeneration of joint surfaces. Changes in synovial fluid might also serve as indicators of joint health, injury and disease.

Conclusions

We conclude from the results of this study that maximal intensity and near maximal endurance muscular exercise does not cause damage to chondrocytes in the intact joint of life animals, while much lower loading conditions than those used here result in chondrocyte death in *in vitro/in situ* cartilage preparations. Therefore, one needs to be careful to extrapolate results obtained under non-physiological conditions to loading conditions in the intact joint. Furthermore, we confirm that *in vivo* joint loading substantially and instantaneously changes the synovial fluid protein content composition. The detailed effects of these changes on joint lubrication, joint mechanics, and long-term joint health, need careful exploration.

327 **Acknowledgements**

328 Alberta Innovates Health Solutions Fellowship and Team Grant on Osteoarthritis. Canadian
329 Arthritis Network. The Canada Research Chair Program, the Canadian Institutes of Health
330 Research, and the Killam Foundation.

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332 **Conflict of interest**

333 The authors have no conflicts of interest

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Fig. (1): A, Tibial plateaus. B, Femoral condyles. C, Patella. D, Femoral groove. Red spots indicate the areas that were scanned for live/dead cells using confocal microscopy.

Fig. (2): Knee extensor force-time graph of an exemplar set of ten maximal contractions. A) first cycle, B) fifth cycle. Note that the maximal force reached about 600 N at the beginning of the maximal intensity exercise protocol, and that force decreased for the ten repeat contractions and for the five sets of contraction because of intense fatigue.

Fig. (3): Knee extensor force-time graph for a 30-minute exercise at approximately 20% of the maximum isometric force.

Fig. (4): Percentage of chondrocyte death in the control and exercised joints for the low intensity exercise for the: A) femoral condyle, B) femoral groove, C) patella, D) tibial plateau. Bars represent mean + 1 SE.

Fig. (5): Percentage of chondrocyte deaths in control and exercised joints for the high intensity exercise for the: A) femoral condyle, B) patella, C) femoral groove, D) tibial plateau. Bars represent mean + 1 SE.

Fig. (6): Comparison of the number of identifiable proteins in high intensity, low intensity and control group rabbits. (A) low intensity exercise with control rabbits. (B) high intensity exercise with control rabbits.

Fig. (7): Mean (± 1 SE) total protein concentration in the SF of the low and high intensity exercise group animals and the non-exercised left and right knees of the control group rabbits.

There is no conflict of interest in the submitted work