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Muscular loading of joints triggers cellular secretion of PRG4 into the joint fluid

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ABSTRACT

We developed a novel testing system that allows quantification of joint loading and permits analysis of changes in total protein and PRG4 contents in joint fluid of intact knees in live mice. A sequence of 15 repeat, isometric muscular contractions of "low" intensity (less than 50% of the maximal isometric muscular force), and "high" intensity (greater than 55% of maximal) were applied repeatedly (up to five times with a 15 min rest between contractions) to the mouse knee.

Increases in knee joint loading were accompanied with significant increases in total protein ($p < 0.0001$) and PRG4 concentrations in the synovial fluid. Total protein and PRG4 concentrations decreased with repeated "high" intensity loading. However, the addition of cell secretion inhibitors to the knee prior to muscular loading resulted in PRG4 levels that remained below the detection limit for all loading conditions.

These results suggest that changes in synovial fluid proteins and PRG4 concentrations upon joint loading are mediated by cells within the joint, and that these changes may be used as quantitative indicators for the intensity and duration of acute joint loading, and might serve as a powerful clinical tool to assess the effectiveness of rehabilitation and prevention exercise programs.

1. Introduction

Osteoarthritis (OA) is a joint disease, characterized by pain, joint swelling, stiffness and functional impairment. The onset and progression of OA is associated with joint injury, excessive loading, instability, muscle weakness, weight and old age (Spector et al., 1994; Roos, 2005; Reijman et al., 2007). Many of the risk factors for OA are in one way or another related to the magnitude, frequency, and rate of loading of the articular surfaces, and the corresponding biosynthetic responses of the articular cartilage chondrocytes. Cartilage has limited healing capacity and to date, no curative treatment is available. The causes and pathogenesis which lead to clinical OA are insufficiently understood and there is on-going research activity in this field and the results are not always consistent (Helmtrud et al., 2008). OA is typically detected and treated at a relatively late, symptomatic stage, therefore identification of the initiating factors and the rate of progression of early OA, have not been effectively addressed in humans. Animal models of OA have been used to study the onset and early progression of OA by attempting to link joint loading with chondrocyte response (Radin 1984; Thompson et al., 1991; Little and Smith, 2008). However, such attempts have not provided promising markers that might link joint loading with cellular responses in a clinically relevant, *in vivo* system.

Recently, we developed methods to quantify the bone, cartilage, and chondrocyte mechanics in the intact knee joint of live mice loaded by controlled muscular contractions(Abusara et al., 2011). Simultaneously, we can also measure selected generic chondrocyte signalling events, such as intracellular calcium "sparks"(Han et al., 2012). Although this approach allows for unique insight into the mechanics of physiologically loaded joints *in vivo*, it is currently not a feasible approach in human studies.

In response to high magnitude or long term muscular loading, chondrocytes within the intact joint may die (Horisberger et al., 2012). Inappropriate muscular loading has also been associated with gross morphological and histological signs of cartilage degeneration, indicative of the onset of osteoarthritis (Roos et al., 2011). Therefore, it appears that loading conditions produced by muscles surrounding an otherwise healthy joint may trigger the onset or may accelerate the progression of OA. However, the mechanisms underlying these events remain largely unknown (Roos et al., 2011). Since muscular loading of joints through exercise is a frequently used treatment modality in the prevention of joint degeneration or in the rehabilitation from joint injury and disease, it is imperative to understand the dose-effect relationship that

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exists between muscular loading and positive-adaptive and negative-degenerative changes in the intact joint.

In order to examine the relationship between load magnitude and changes in joint fluid (JF), mice knees were loaded with "low" and "high" magnitude forces through knee extensor contractions. Based on pilot results, we set out to test the hypothesis that acute muscular loading of an intact joint changes the composition of JF in a dose-(magnitude and duration) dependent manner. JF can easily be extracted from human joints, and thus might become a powerful marker for guiding exercise interventions aimed at prevention and rehabilitation of joint injuries and diseases.

As an indicator of changes in protein concentration of JF, the relative change in content of proteoglycan 4 (PRG4) in joint fluid samples before and after muscular loading was assessed using western blotting. PRG4 refers to the family of mucin-like, O-linked glycosylated proteins with several names, including lubricin and superficial zone protein, encoded by the PRG4 gene (Jay, 2004). PRG4 proteins, herein referred to as PRG4, are synthesized and secreted by cells that line the joint and are present in JF and at the surface of articular cartilage. PRG4 is critical to the normal lubricating function of JF, as can be seen in the autosomal-recessive disorder camptodactyly-arthropathy-coxa vara-pericarditis (CACP), which is caused by a mutation in the PRG4 gene and leads to non-inflammatory joint failure in juveniles (Marcelino et al., 1999). In addition, PRG4 knockout mice exhibit increased cartilage wear and higher friction in diarthrodial joints than that observed in wild-type animals (Jay et al., 2007).

2. Materials and methods

2.1. Animal preparation

This study was carried out in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the committee for Animal Ethics at the University of Calgary. Twenty C57 adult male mice (10–12 weeks of age) were used in this study. Mice were anesthetized with an isoflurane/oxygen mixture (1–3%). The left knee joints were shaved and secured in a stereo-taxic frame built onto the stage of a dissecting microscope. The medial aspect of the joint was exposed with a 6 mm incision just posterior to the medial collateral ligament (Abusara et al., 2011).

2.2. Fluid sampling from joint space

The articular capsule was carefully released and the joint fluid was collected for analysis. The medial meniscus was excised to provide a direct view of the medial tibio-femoral joint. The exposed medial aspect of the knee was then washed three times and filled with 25 μL phosphate buffered saline (PBS) Fig. 1(a). After 15 min, the joint was passively flexed twice and the PBS sample was collected for analysis. The knee joint ($n=9$, Table 1) was then washed and filled with new PBS, a sequence of 15 repeat muscular loading contractions was applied several times (as described below) to assess the changes in total protein and PRG4 concentrations associated with "low" intensity (less than 50% of maximal muscular force), and "high" intensity (greater than 55% of maximal muscular force) loading. For each set of experimental contractions, the knee was flexed just before the fluid sample was collected and the joint was washed three times and filled with 25 μl PBS prior to the next loading experiment. Approximately 80% of the initially injected fluid was consistently recovered. Therefore, we assumed that the protein concentrations in the recovered samples were excellent indicators of the protein concentrations in the entire joint fluid, and thus a good indicator of total protein content within the joint.

2.3. Muscular loading of the joint

Controlled muscular stimulation of the knee extensors was achieved through two fine wire electrodes inserted into the quadriceps group and electrical stimulation with a Grass (S8800) digital stimulator (Herzog and Leonard, 1997). The free tips of the exposed fine wires were separated by 2 mm, and application of 2–7 V resulted in force of 15–85% of maximal. Maximal stimulation was typically reached at <9 V. Stimulation trains of 0.5 s at 50 Hz were applied every 4 s for 15 repeat contractions (Fig. 1b) and dynamic shear loading was observed (Abusara et al., 2011). Potentiation and fatigue effects of the muscles were offset using small, manual adjustments of the stimulation parameters. Knee extensor torques were measured with a force measuring bar (Entran Sensors & Electronics, USA) attached to the distal part of the tibia.

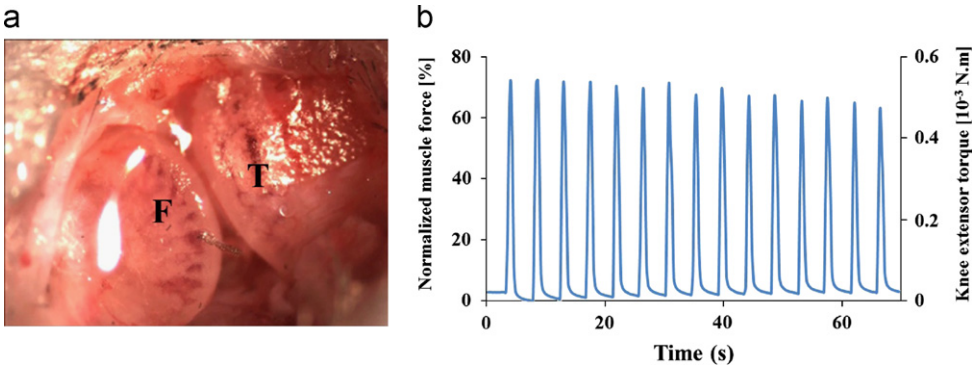

Fig. 1. (a) Exposed mouse knee joint showing the medial tibial plateau (T), and the medial femoral condyle (F) with the meniscus removed. The joint was carefully rinsed with a PBS solution prior to all loading experiments. (b) Normalized (relative to maximum=100%) knee extensor forces as a function of time. Muscles were stimulated 15 times for 0.5 s every 4 s at a voltage and frequency producing approximately 70% of the maximal possible force. The right vertical axis represent the actual knee extensor torque.

Table 1
Illustration of the experimental design showing the number of animals (n) in each treatment. Each row pertains to a single experimental group, and shows the treatment protocols and the analysis that was carried out on joint fluid collected from joints after muscular contraction.

n	TP[a]	WB[b]	Inhibitor	DMSO	**Comments**
9	✓	✓	×	×	Joint fluid was sufficient to analyze both TP and WB.
2	✓	✓	×	✓	Control animals used to show that DMSO concentration used in the experiments has no effect on the results.
4	✓	×	✓	✓	Joint fluid volume was only sufficient for TP analysis after the inhibitor was added.
2	✓	✓	✓	✓	Joint fluid was sufficient to analyze both TP and WB after the inhibitor was added.
3	×	✓	✓	✓	Joint fluid volume was only sufficient for WB analysis after the addition of the inhibitor.

[a] Total protein concentration.
[b] Western Blot.