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Changes in protein concentration in murine knee joints with muscular contraction

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SUMMARY

We developed a novel *in vivo* testing system that allows for quantification of joint, cartilage and chondrocyte loading and for analysis of changes in total protein and PRG4 content in the synovial fluid of intact knees in live mice. A sequence of 15 repeat, isometric muscular contractions of "low" intensity (less than 50% of the maximal muscular force), and "high" intensity (greater than 55% of maximal force) were applied repeatedly (up to five times with a 15 minute rest between contractions) to the mouse knee. Increases in knee joint loading were accompanied with significant increases in total protein and PRG4 content in the synovial fluid. Total protein and PRG4 content decreased with repeat sets of "high" intensity loading. The addition of cell secretion inhibitors to the knee prior to muscular loading abolished the protein increase and PRG4 response.
These results suggest that changes in synovial fluid protein and PRG4 content 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.

INTRODUCTION

Recently, we developed methods to quantify joint, bone, cartilage and chondrocyte mechanics in the intact knee of live mice loaded by controlled muscular contractions [1]. Depending on the magnitude and duration of muscular loading, we showed that chondrocytes can die in the fully intact joint [2], and that joints show histological signs of onset of osteoarthritis (OA) [3]. Therefore, it appears that loading provided exclusively by muscles surrounding a completely healthy and intact joint, can trigger the onset of OA and possibly accelerate the rate of progression of OA, but the mechanisms of these events remain unknown [3]. Since muscular loading of joints is a frequently used treatment modality in the prevention of joint degeneration or in the rehabilitation from joint injury and disease, it is imperative that we understand the dose relationship that exists between muscular exercise treatment and positive-adaptive and negative-degenerative events in human joints.
While loading mice knees with "low" and "intense" knee extensor contractions, we observed a discoloration of the synovial fluid that suggested that synovial fluid composition might be changing based on the intensity of knee loading. The purpose of this study was to test this hypothesis and determine whether synovial fluid might be used to quantify joint loading and possibly serve as an indicator of positive-adaptive and negative-degenerative knee joint responses. Synovial fluid can easily be extracted from human joints, and thus might become a powerful marker for guiding muscular prevention and rehabilitation programs.

METHODS

Twenty adult male mice (10-12 weeks of age) were used in this study. Mice were fixed in a custom-built jig onto the stage of a dissecting microscope. The medial aspect of the knee was exposed with a 6mm incision just posterior to the medial collateral ligament (Figure 1a). A sequence of 15 repeat, isometric muscular contractions of "low" intensity (less than 50% of the maximal muscular force), and "high" intensity (greater than 55% of maximal) were applied repeatedly (up to five times with a fifteen minute break) to the mouse knee (Figure 1b).
Changes in total synovial fluid borne protein and proteoglycan 4 (PRG4, also known as lubricin) content for the different loading conditions were measured and compared to resting values. Knee fluid samples were collected after each loading bout and the knee was washed carefully between loading experiment. Knee extensor forces were controlled by adjusting the magnitude (current) and the frequency of electrical stimulation of the muscles using a Grass (S88) stimulator.

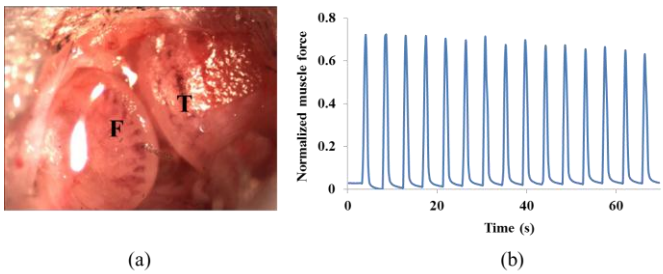

Figure 1: (a) Exposed mouse knee joint showing the medial tibia(T) and femur(F) with the meniscus removed. The joint was rinsed with a PBS solution prior to and in between each loading bout. (b) Normalized (to maximal force = 1.0) muscle force as a function of time. Muscles were stimulated for 0.5s every 4s using a current and frequency producing approximately 70% of the maximal force.

RESULTS

Increases in muscular loading of the mouse knee caused a significant increase in total protein content (Figure 2) and an increase in PRG4 in the joint fluid (Figure 3). When "high" intensity load conditions were repeated, the total protein (Figure 2) and PRG4 (Figure 3) response decreased and, given sufficient repeat sets, was abolished completely.

Figure 4 shows a significant increase in total protein content in the synovial fluid with intense loading, but this increase was abolished after the application of cell secretion inhibitors. Two successive sets of 15 intense loading contractions produced PRG4 release into the joint space (mouse "1" Figure 5). However, PRG4 release was absent for the same loading conditions after application of the cell secretion inhibitors (mouse "2" Figure 5).

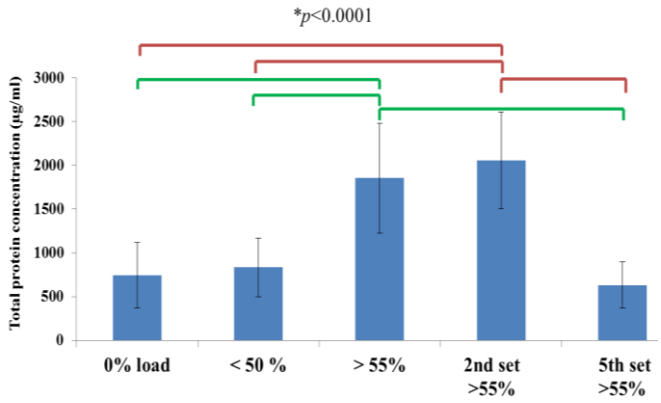

Figure 2: Increases in loading of the mouse knee caused an increase in total protein content of the synovial fluid (means±1SD; n=9). The percentages on the x-axis give the force (max=100%) and 0% load is the initial resting protein content. The last bar shows the 5th set for the "intense" loading conditions. The horizontal bars on top show significant differences between groups.

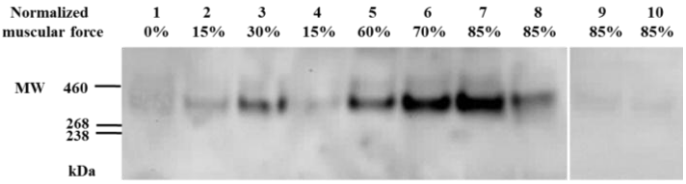

Figure 3: Western blot analysis (for a single mouse) showing PRG4 (with a MW of ~ 460 kDa) for the initial resting condition (column 1) and following "low" intensity (columns 2,3,4, with column 4 being a repeat of trial 2) and "high" intensity loading conditions (columns 5,6,7,8). Columns 9 and 10 represent the 3rd and 4th sets of 15 contractions at 85% of maximal force, respectively.

DISCUSSION AND CONCLUSIONS

We developed an *in vivo* experimental model of knee joint loading that allows for quantifying changes in protein content of synovial fluid following controlled bouts of muscular loading. Our results indicate that increased loading results in increased total protein and increased PRG4 content. Total protein content and PRG4 content decrease with multiple "intense" loading conditions.

We also observed that blocking cell secretion, total protein and PRG4 content in synovial fluid did not change even with intense muscular loading which suggests that changes in synovial fluid content occur through protein release from articular cartilage chondrocytes.

These results suggest that synovial fluid might be used as a biomarker for assessing the intensity and duration of exercise bouts. We will now try to link these (and other synovial fluid borne markers like cytokines and stem cells) biomarkers to positive-adaptive and negative-degenerative processes in the mouse knee. Ultimately we anticipate the use of synovial fluid from human joints to assess and regulate muscular intervention programs for the prevention and rehabilitation of joint injures and diseases.

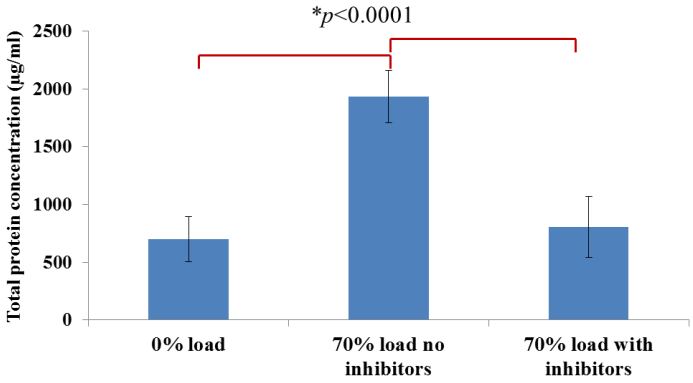

Figure 4: Total protein content significantly increases with increasing knee loading (70% of maximal muscular force in this example) and significantly decreased in the presence of cell secretion inhibitors (mean±SD; n=6). The horizontal bars indicate significant differences between groups.

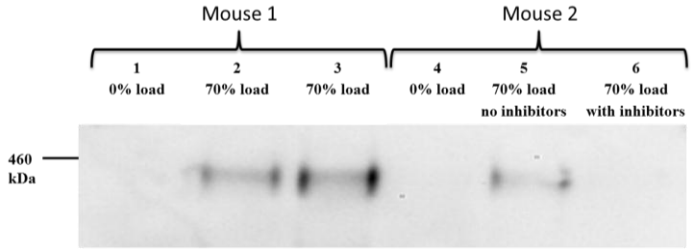

Figure 5: Western Blot for PRG4 with a MW of ~ 460 kDa (n=5). PRG4 appeared after intense (70%) muscular loading (2, 3, 5), but was absent in the presence of cell secretion inhibitors (6). PRG4 was absent prior to joint loading (1, 4).

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[2] Horisberger M, et al. Clin Biomech 2012; 27, 292-298.
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