# REPETITIVE FINGER JOINT FLEXION WITHOUT EXTERNAL LOAD LEADS TO ARTICULAR CARTILAGE THINNING IN AN *IN VIVO* RABBIT MODEL

*Marecek, GS; **/***Opel, CF; **/****Rempel, DM; +**/****King, KB
*Northwestern University, Chicago, IL; +****University of California, San Francisco, CA
kbking@itsa.ucsf.edu

## INTRODUCTION:

Repetitive hand tasks are a risk factor for the development of musculoskeletal disorders, including degenerative joint disease [1,2]. Additionally, high occupational loads have been described as a risk factor for the development of osteoarthritis [3,4]. However, few studies have identified repetitive use independent of loading as a risk factor for joint injury. Thus, it remains unclear which component, repetition frequency or force, is primarily responsible for joint damage. To examine the difference in the effect of frequency vs. force, we have developed a novel *in vivo* animal model of cyclic joint loading [5]. In this model, the rabbit forepaw digits can be flexed with or without an added external load. This study compares the changes in articular cartilage thickness caused by repetition alone to those changes caused by repetition plus an external load.

## METHODS:

All procedures were conducted with the prior approval and oversight from the University of California's Care and Use of Animals Committee and with institutional approval. The digits of eight adult female New Zealand White rabbits were repetitively flexed over 60 cumulative hours. The loading was performed under anesthesia. A Grass-Telefactor stimulator was used to stimulate the flexor digitorum profundus (FDP) at 1 Hz for 2 hours per day, 3 days per week (about 10 weeks). Since rabbit FDP tendons are fused as they pass through the carpal tunnel area, all digits flexed at the same rate. A resistance load was applied to only the third digit (D3) by means of a lightweight brass cuff attached to a load cell. The mean peak fingertip force was 0.42 N with a loading rate of 4.2 N/s. These parameters were selected to be within the physiologic range of the muscle and frequency of motion experience by workers who perform repeated tasks [6]. The other digits of the stimulated limb remained unloaded, and the contralateral control limb was not stimulated.

After the loading protocol was completed, the rabbits were euthanized and the MCP joints of the second digit (D2, flexed only) and the third digit (D3, flexed and loaded) in the stimulated paw and the matching contralateral control joints were collected with the joint capsules intact. The joints were fixed in formalin, decalcified in EDTA, dehydrated, and embedded in paraffin. Thin sections (7 μm) were taken in the sagittal plane and stained with safranin O, fast green, and iron hematoxylin. Digital images of the sections were obtained using an Axioskop 2 Mot light microscope with an AxioCam digital camera (Zeiss). Six sections from each joint were selected for analysis and a 300 x 500 $\mu m^2$ region of interest was mapped on the proximal joint surface at the area of maximal contact, as determined previously [5]. Axiovision software (Zeiss) was used to calculate the mean thickness of uncalcified and calcified cartilage (cartilage area/width). Mean thickness of calcified cartilage as a percent of total cartilage was also calculated.

The data from the D2 MCP joints were compared to their respective contralateral controls with students' paired t-tests, $\alpha$=0.05. The data from the D2 MCP joints were compared to the data from the D3 MCP joints of the same rabbits using students' paired t-tests, $\alpha$=0.05. Analysis of differences between digits was performed using Repeated-Measures Analysis Of Variance (RMANOVA) with two factors: digit and limb.

## RESULTS:

A summary of the data obtained from D2 and D3 joints is presented in Table 1. Data for the D3 joints have been previously published [3].

The mean thickness of uncalcified cartilage decreased 11.84% in the flexed D2 as compared to its contralateral control. The mean thickness of calcified cartilage was also decreased in the flexed D2 as compared to control, with a mean difference of 6.71%. There was no difference in calcified cartilage as a percent of total thickness. The interaction term for limb and digit was not significant for either uncalcified or calcified cartilage mean thickness.

In these rabbits, the D2 and D3 joints were comparable in size. In the control joints, there was no statistically significant difference in uncalcified [$P$=0.11], calcified [$P$=0.56], or total [$P$=0.17] cartilage thickness between the D2 and D3 joints.

There was no statistically significant difference in the percent change in the D2 joints vs. the D3 joints for either uncalcified cartilage mean thickness [$P$=0.72] or calcified cartilage mean thickness [$P$=0.59].

**Table 1:** Mean thickness of uncalcified and calcified articular cartilage

| | Digit [1] | Control μm | Loaded μm | Change | $P$ [2] |
|---|---|---|---|---|---|
| Uncalcified cartilage | D2 | 115.80 | 102.09 | -11.84% | 0.03 |
| | D3 | 129.42 | 115.64 | -9.70% | 0.03 |
| Calcified cartilage | D2 | 75.21 | 70.03 | -6.71% | 0.03 |
| | D3 | 78.15 | 73.43 | -4.09% | 0.32 |

[1] D2 digit was flexed without loading and D3 digit was flexed with an external load. [2] Significant differences between control and loaded were tested using two-tailed paired t-test

## DISCUSSION:

This is the first study to examine the relative importance of force and frequency of repetition on musculoskeletal tissue changes. We found that repetition alone is sufficient to induce structural changes in articular cartilage *in vivo*. The cartilage thinning may be attributed to a reactivation of endochondral ossification. In normal joint development and likely joint maintenance, a balance between shear and hydrostatic stress is necessary; excessive shear stress leading to more bone and insufficient shear stress leading to less bone [7]. Alterations in the stress profile at the joint surface may thus promote ossification of the calcified cartilage and advancement of the tidemark.

An alternative explanation for cartilage thinning would be enzymatic degradation of the extracellular matrix. However, data from studies in progress using this model do not suggest proteoglycan loss at this level of frequency and force.

The loss of uncalcified and calcified cartilage in the D2 may indicate an early stage of a disease process. Examination of longer loading durations are required to conclude whether the tissue changes observed are pathologic or normal remodeling.

The absence of a significant difference between the loaded D3 and unloaded D2 suggests that under these loading conditions, frequency, rather than force, is the more pertinent physiologic risk factor for the development of cartilage thinning due to repetitive loading.

Future investigations will focus on the effects of varying frequency, and the interaction between force and frequency as a risk factor for occupational overuse injuries. Such information would be useful in identifying at-risk populations and undertaking appropriate preventative measures such as task design and tool design interventions. Furthermore, understanding the mechanobiology of articular joints would help improve the design of functional engineered osteochondral tissues.

This study was supported by the Centers for Disease Control and Prevention (Grant Number: OH007786).

## REFERENCES:

1. Hunter DJ, et al. Arthritis Rheum 2004;50:1495-500
2. Hadler NM, et al. Arthritis Rheum 1978;21:210-20
3. Croft P, et al. British Medical Journal 1992;304:1269-72
4. Manninen P, et al. Scand J Work Environ Health 2002;28:25-32
5. King KB, et al. Osteoarthritis and Cartilage 2005 in press
6. Silverstein BA, et al. Br J Ind Med 1998;43:779-84.
7. Carter DR and Wong M. J Orthop Res 1988;6:804-16

## AFFILIATED INSTITUTIONS FOR CO-AUTHORS:

** University of California, Berkeley, CA
***Presently employed by Genentech Inc., South San Francisco, CA