IN VIVO CYCLICAL JOINT LOADING DECREASES UNMINERALIZED CARTILAGE THICKNESS IN THE RABBIT METACARPOPHALANGEAL

*Opel, C; */**Rempel D; */**King, KB
* Department of Bioengineering, University of California, Berkeley. ** Department of Medicine, University of California, San Francisco
kbking@itsa.ucsf.edu

INTRODUCTION:

Understanding the mechanobiology of joint loading is essential for progress in treating osteoarthritis as well as other degenerative joint diseases and to help engineer cartilage tissue replacements. This study examines articular joint tissue changes resulting from *in vivo* cyclical loading in a rabbit model. This new animal model has the potential to advance our understanding of adult endochondral ossification and degenerative joint disease.

METHODS:

All procedures received prior approval and oversight from the University of California's Care and Use of Animals Committe and with institutional approval. Loading was performed with the rabbits under anesthesia. The digits of adult female New Zealand White rabbits (n = 8) were repetitively loaded, in two-hour increments, three days a week for ten weeks (60h total). This unique model controls both the peak force and rate of loading *in vivo* and does not require surgical intervention, eliminating the various confounding elements of some other animal models. The loading protocol was designed to simulate hand activities associated with the workplace. In this experimental design, the metacarpophalangeal (MCP) joint was expected to receive a greater load than the interphalangeal joints due to the longer moment arm. Therefore, the MCP joints were examined in this study.

A Grass-Telefactor stimulator was used to excite the FDP (*flexor digitorum profundus*) muscle of the experimental limb, causing the digits to flex. A load was applied to the third digit with a light-weight finger cuff attached to a load cell with a resistance of 0.42N. Force analysis (n = 3 rabbits) estimated the force at the MCP joint was 3.7N. The contra-lateral limb (control) was not stimulated nor loaded.

Once the loading was completed, the rabbits were euthanized and the MCP joints of both limbs were removed, fixed in formalin, decalcified, embedded in paraffin, and sectioned in the saggital plane. Thin sections (7 µm) were stained with Iron Hematoxylin, Safranin O and Fast Green. Digital images of the sections were obtained using an Axioskop 2 Mot light microscope with an AxioCam digital camera (Zeiss). Finally, using Zeiss Axiovision software, six sections from the center of each joint were analyzed. Specific outcome measurements were: mean thickness of the unmineralized cartilage layer, mean thickness of the mineralized cartilage layer, and a "complexity ratio" of the osteochondral interface (osteochondral interface length divided by tidemark length). This ratio will thus increase from a minimum value of 1 (where the two lengths are equal) as the complexity of the osteochondral interface increases[1].

To analyze area-specific changes on the articular surface, 3 anatomically distinguishable and equally sized regions of interest (ROIs) were defined. One ROI was on the palmar side of the proximal joint surface, at the point of maximal surface contact with the opposing joint surface during flexion ("Max"). The second ROI was placed at the point of minimum contact on the proximal joint surface ("Min"). The third ROI was placed at the center of the distal joint surface ("Dist").

The experimental and control data sets were compared using two-tailed, paired student's t-tests. The interaction between limb (loaded vs. control) and joint site (Max, Min, or Dist) was analyzed using repeated-measures analysis of variance (Stata v. 8.2, Stata, Corp.).

RESULTS:

We found that the unmineralized cartilage of the loaded joint was significantly thinner than that of the unloaded control joint at the point of maximum contact. The average difference between paired limbs was 10% (p=0.03, n = 8, Figure 1). No difference was observed at the other two sites. The interaction term (limb X site) was not significant ($F_{2,14} = 0.84$, $P = 0.45$).

The mean thickness of the total articular cartilage (unmineralized and mineralized) in the Max site also decreased, but this was not statistically significant ($P = 0.08$). The mean thickness of the mineralized cartilage as a percentage of the mean total thickness did not change. A change in the osteochondral complexity ratio was also found at the Max site. The complexity ratio was 8% less in the loaded joint than the contra-lateral control joint (p=0.046, n =8). This is indicative of either "flattening" of the osteochondral interface or increasing undulation of the tidemark.

DISCUSSION:

The data show that *in vivo* cyclical loading decreases unmineralized cartilage thickness and decreases the osteochondral interface complexity ratio in rabbit MCP joints. Three possibilities may account for the decrease in cartilage thickness: the surface layer degraded, the cartilage compacted, or the mineralized region advanced towards the joint surface at the expense of the unmineralized region.

The third explanation is favored for the following reasons. Since no signs of overt cartilage damage were seen on the joint surface, it is unlikely that the loss of thickness was due to degradation. Second, mechanical load-induced compacting of cartilage has been shown to occur only *in vitro*[2,3] or was temporary during *in vivo* human studies[4]. In contrast, mineralization of cartilage is a documented effect of joint loading[5,6] and is also seen in human joint disease[7]. This may represent reactivated endochondral ossification. In agreement, the decrease in osteochondral interface complexity ratio we observed may suggest the occurrence of bone remodeling[8].

The biomechanical consequences of reactivated ossification are clinically significant: finite element analysis has predicted that increased cartilage mineralization will increase shear stress levels in the deeper cartilage layers[9]. Future analysis of this *in vivo* model will involve biochemical analysis and mechanical testing to elucidate the mechanism of the structural changes observed.

REFERENCES:

1. Norrdin RW, et al. 1998. Bone **22**(2)**:**133-9.
2. Kiraly K, et al. 1998. Anat Rec **251:**28-36.
3. Wong M, et al. 1999. Matrix Biology **18:**391-399.
4. Eckstein F, et al. 1999. Anat Embryol (Berl) **200**(4)**:**419-24.
5. Lane LB and Bullough PG. 1980. J Bone Joint Surg Br **62**(3)**:**372-5.
6. Burr DB and Schaffler MB. 1997. Microsc Res Tech **37**(4)**:**343-57.
7. Patel N and Buckland-Wright C. 1999. Osteoarthritis Cart **7**(6)**:**520-5.
8. Lane LB, et al. 1977. J Bone Joint Surg Br **59**(3)**:**272-8.
9. Anderson DD, et al. 1993. Clin Orthop **286:**298-307.

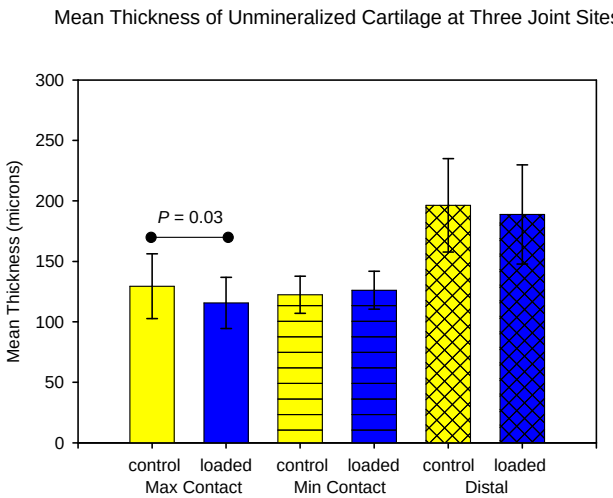

Figure 1. Mean thickness of unmineralized cartilage of *in vivo* cyclically loaded MCP joints. X-axis consists of equally sized regions of interest located at the area of maximum surface contact (Max), at the area of minimum surface contact (Min) and at the midpoint of the distal surface (Distal). N = 8 rabbits.