The effect of frequency and force of in vivo loading on proteoglycan content of rabbit articular cartilage

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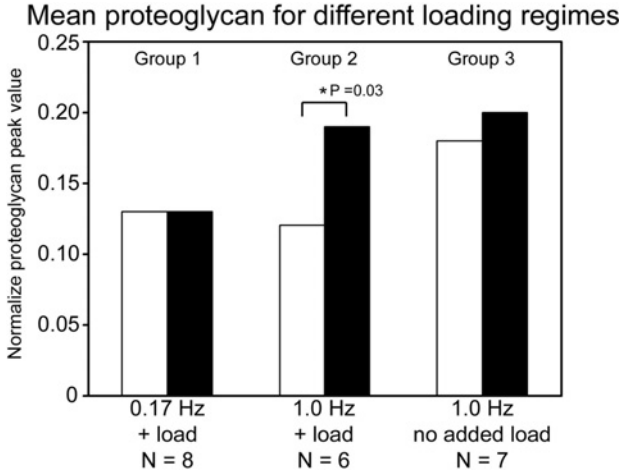
Introduction: Mechanical loading is an important regulator of the metabolic activity of chondrocytes and is essential for maintaining extracellular matrix composition and functional tissue properties. Furthermore, the chondrocyte is very sensitive to frequency and force levels[1-3]. Understanding the effects of frequency and force on chondrocyte metabolism is essential for understanding and treating joint disorders including the development of engineered tissues and of post-traumatic or post-operative rehabilitation protocols. Previous studies have examined these effects on biosynthetic rates of articular cartilage *in vitro*, but no data is available on the dose response of frequency or force on cartilage metabolism *in vivo*. We have developed a novel rabbit model of repetitive joint flexion and loading to examine frequency and force *in vivo* [4]. Using this model, we previously demonstrated an increase in cartilage proteoglycan content with cyclical joint loading of a fixed frequency and force [5]. This study examines the effect of differing levels of frequency and force on the amounts of proteoglycan and collagen in articular cartilage using Fourier Transform Infrared (FTIR) spectroscopic imaging.

Materials and Methods: All procedures received prior approval and oversight from the University of California's Care and Use of Animals Committee. Using a loading protocol that was designed to simulate hand activities associated with the workplace[4], the digits of adult female New Zealand White rabbits were repetitively flexed for 80 cumulative hours (in two-hour increments, three days a week for 14 weeks). The rabbits were divided into three groups, each receiving a specific frequency and force level: Group 1 = 0.17 Hz with added load on the digits; Group 2 = 1 Hz with added load, and Group 3 = 1 Hz without added load. For digits receiving added load, a load was applied to the digit tip with a lightweight finger cuff attached to a load cell with a resistance of 0.42 N. For all of the loaded digits, total amount of work done was the same. The contra-lateral limb (control) was neither stimulated nor loaded. Once the loading was completed, the rabbits were euthanized and the metacarpophalangeal joints of both limbs were removed, fixed in formalin, decalcified, embedded in paraffin, and sectioned in the saggital plane[4]. FTIR spectroscopic imaging was performed on 7 μm thick sections as previously described[5]. All FTIR data processing was done using the ISYS software package. The data sets from the loaded joint and the contra-lateral control joint were compared for each group using the two-tailed, paired student's T-test.

Results: The mean proteoglycan peak values, normalized to collagen peak values for all rabbits tested are reported in Figure 1 for each loading group. There was no change in proteoglycan content in joints flexed and loaded at 0.17 Hz (Group 1). There was also no change in proteoglycan content of joints flexed at 1Hz with no added load (Group 3).



Units are integrated area of sugar peak (1185 - 950 cm^{-1}) normalized to integrated area of amide I peak (1710 - 1595 cm^{-1}). Open bars are control. Filled bars are experimental.

As published previously [5], the amount of proteoglycans increased in the experimental joint compared to the control joint for all rabbits in the 1 Hz group with added load (Group 2; P = 0.03). The mean of the percent increase in the amount of proteoglycan between control and loaded joints in this group was 64%; the median was 29.5%. No change in the collagen content was observed for any of the groups compared to their contralateral control joint. This was expected given the long half-life of cartilage collagen.

Discussion: The data indicate that chondrocytes react selectively and specifically to a set of mechanical stimuli by altering the cartilage tissue biochemistry. Furthermore, the mechanical stimulation of proteoglycan synthesis is dependent on the frequency as well as the magnitude of applied force. Cyclical loading *in vivo* creates oscillating hydrostatic pressure in cartilage and the resulting mechanical tension on the chondrocytes is likely responsible for activation of stretch-activated ion channels [6,7]. A subsequent rapid rise of intracellular calcium levels up-regulates anabolic pathways leading to a detectable increase in proteoglycan content of the tissue. Another metabolic effect of mechanical loading is the activation of integrins which play a role in the response of chondrocytes to mechanical loading by activating the mitogen-activated protein kinase pathway [8,9]. In our model for finger joint flexion, cyclical *in vivo* loading of cartilage at 1 Hz with added load leads to a measurable increase in matrix proteoglycan content[5]. However, we observe no change at the lower frequency (0.17 Hz). This contrasts *in vitro* data in which cyclical load as low as 0.01 Hz increased proteoglycan [1]. In addition, we observe no change at the lower force. Our free body analysis estimates the joint contact pressure is ~1 MPa for groups 1 and 2 and at least 10 times less (<0.01MPa) for group 3. Our results confirm *in vitro* data in which 1 MPa of hydrostatic pressure had a small but not statistically significant effect on proteoglycan [2]. This preferential change in chondrocyte biosynthesis points to a threshold, both in frequency and force of *in vivo* loading, that sustains intracellular signaling pathways long enough for their effects to be manifested in tissue biochemistry. The frequency threshold lies somewhere between 1 and 0.2 Hz and the pressure threshold lies at or around 1 MPa. These data can be used in developing interventions to prevent joint overuse injuries or in the development of therapies to improve joint health.

References: [1]R Sah, J Orthop. Res 7:619, 1989 [2]T Ikenoue, J Orthop Res. 21:110, 2003 [3]K Sauerland, OA & Cart 11:343, 2003 [4]K King, OA & Cart 13:971, 2005 [5]E Saadat, Arth Res Ther 8:R147, 2006 [6]M Wright, Clin Sci 90:61, 1996 [7]F Guilak, J Orthop Res 17:421, 1999 [8]A Mobasheri, Cell Biol Int 26:1, 2002 [9]C Forsyth, Arth Rheum 46:2368,2002