CYCLICAL IN VIVO LOADING INCREASES CARTILAGE PROTEOGLYCAN CONTENT IN THE RABBIT METACARPOPHALANGEAL JOINT

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INTRODUCTION:

The composition of the extracellular matrix of cartilage dictates its mechanical properties. Proteoglycans (PG) and collagen are two important structural components of the cartilage extracellular matrix [1]. The ability to measure changes both in the amount and distribution of cartilage matrix constituents is essential in understanding early pathological changes of joint diseases. Previous studies have examined the biosynthetic response of articular cartilage with cyclical loading of osteochondral explants *in vitro* [2]. We have developed a novel method of repetitive joint flexion and loading to examine the biosynthetic response of cyclical loading *in vivo* [3]. This study examines the change in the amounts of proteoglycans and collagen in articular cartilage resulting from *in vivo* cyclical loading using Fourier transform infrared (FTIR) imaging spectroscopy.

METHODS:

All procedures received prior approval and oversight from the University of California's Care and Use of Animals Committee and institutional approval. Using a loading protocol that was designed to simulate hand activities associated with the workplace, the digits of adult female New Zealand White rabbits (n = 6) were repetitively loaded for 80 cumulative hours (in two-hour increments, three days a week for 14 weeks). Loading was performed with the rabbits under anesthesia. A Grass-Telefactor stimulator was used to excite the FDP (*flexor digitorum profundus*) muscle of the experimental limb, causing its 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 0.42 N. This unique *in vivo* model applies a controlled peak force equivalent to 17.5% of the maximum muscle force at a frequency of 1 Hz. The contra-lateral limb (control) was neither stimulated nor loaded. Metacarpophalangeal (MCP) joints were examined in this study [3].

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 thin-sectioned (7 µm) in the saggital plane. One section from each joint was selected for analysis and placed onto IR reflecting microscope slides (MirrIR low-e microscope slides, Kevley Technologies, Chesterland, OH).

The FTIR data were collected at 16cm^{-1} resolution using a mid-infrared Michelson-type step-scan interferometer (ThermoNicolet 870; Thermo-Electron Corporation, Madison, WI) coupled to an Imagemax infrared microscope with a 64*64-pixel mercury cadmium telluride focal plane array detector under N_2 purge. A region of interest 200 µm wide was mapped on the proximal palmar surface as determined previously [3]. This region extended 100 µm from the joint surface and included most of the uncalcified cartilage without calcified cartilage or bone.

The FTIR images were baseline-subtracted and background corrected. The amount of proteoglycans was measured as the integrated area of the sugar peak (1185 – 960 cm^{-1}) for each of the FTIR images [4]. The amount of collagen was measured as the integrated area of the amide I peak (1710 – 1595 cm^{-1}) [4]. All FTIR data processing was done using the ISYS software package (Spectral Dimensions, Olney, MD).

The data sets from the loaded joint and the contra-lateral control joint were compared using the non-parametric Wilcoxon signed-rank test.

RESULTS:

An increase in the amount of proteoglycans was observed for the loaded joint compared to the control joint for all rabbits tested (Figure 1). The mean (±SD) of integrated PG peak values of the control joints was 4.46(±1.96). The PG increased to 6.56(±2.42) for the loaded joints. This change was statistically significant (P = 0.03) using the Wilcoxon signed-rank test. The difference was also significant using the parametric two-tailed paired student's t-test. The mean of the percent increase in the amount of PG between control and loaded joints was 64%; the median was 29.5%.

In a cage control rabbit (i.e., identical cage conditions, but no FDP stimulation nor anesthesia), no difference in the amount of PG was observed between right and left joints (data not shown).

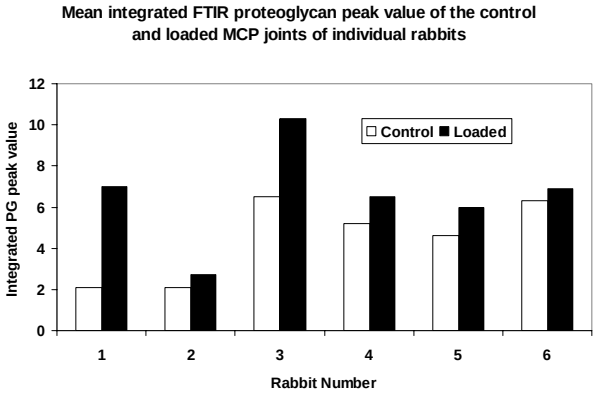

Figure 1: Results of the Fourier Transform Infrared (FTIR) microspectroscopic analysis. Mean integrated proteoglycan peak (1185 – 960 cm^{-1}) value of cartilage from the control and *in vivo* loaded joints of 6 individual rabbits are shown. The mean peak value increased with loading in all rabbits.

The mean (±SD) of integrated collagen peak values of the control joints was 34.18(±6.90) and for the loaded joints was 33.48(±3.80). No significant difference in the amount of collagen between the two joints was observed (P = 0.74).

DISCUSSION:

This is the first study to examine the changes in the cartilage matrix constituents due to *in vivo* loading while controlling the levels of both force and frequency. The results indicate that 80 cumulative hours of physiological loading causes detectable metabolic changes in cartilage indicated by an increase in amount of proteoglycans. We have shown that the increase in amount of proteoglycans demonstrated *in vitro* [2] does occur *in vivo.* This is clinically significant since proteoglycans – particularly aggrecan – provide the osmotic resistance necessary for cartilage to resist compressive loads.

That no significant changes in the amount of collagen were found after 80 hours of cumulative loading is expected given the extremely high turnover time for collagen. The turnover rate of collagen is much slower than that of aggrecan [5].

This study has examined one time point of chronic loading. Therefore, we can not conclude when the PG increase becomes detectable or is sustained. Furthermore, only one loading pattern has been tested. Other mean peak force levels and loading frequencies may result in different amounts of proteoglycans. This new animal model is suitable for testing different frequencies, peak forces and durations of *in vivo* joint loading.

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