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### Introduction

Pathological reduction of cartilage volume during disuse is an important finding after fractures and other injuries where immobilization of the limb is necessary. The current report is on a model of knee articular cartilage breakdown caused by overexercise following disuse immobilization, as well as a study of baseline immobilization.

### Materials and Methods

We used a dog model of disuse atrophy to examine in 4 separate study modes the existence and nature of proteolytic action in contributing to disuse atrophy, as well as observing the effect of a rehabilitative exercise program versus an over-exercise program on the same parameters of structure and proteolysis. Age-matched controls were studied at 4 and 6 weeks. Experimental animals were kept in a special sling allowing weight bearing on 3 limbs for 4 weeks and 6 weeks of disuse atrophy. After 4 weeks of disuse atrophy, either conservative (walking, rehabilitative exercise) or treadmill exercise (overexercise) for 2 weeks were studied. After sacrifice, histology and MMP levels in the cartilage were measured.

**mRNA isolation.** For mRNA detection, the cartilage was homogenized in a guanidine isothiocyanate buffer. The RNA pellet was dissolved in 10 mM TRIS pH 8.0, and 1mM EDTA and precipitated with ethanol

**DNA primers:** DNA primers against canine sequences of MMP-1, 3 and 13 were synthesized through the University of Miami DNA core facility (1,2,3). The DNA primers were diluted with distilled H<sub>2</sub>O to a concentration about 5 nmoles/ml and 2-3 µl of each primer pair were used for PCR amplification of specific genes.

**Semi-quantitative RT-PCR:** DNA primers for elongation factor were also used for internal control of PCR reaction in separate tubes. Different PCR cycles were chosen at linear range amplification of each individual primer pair, and 3 or 4 repeated experiments were performed for each assay.

**Zymography:** Samples of conditioned media were separated on a 12.5% SDS-polyacrylamide gel impregnated with transferrin (0.25 mg/ml) to detect MMP-3 or gelatin (0.5 mg/ml) to detect MMP-2 and 9. Zone of MMP activities were indicated by negative staining [4].

### Results

MMP were increased in tibial plateau disuse cartilage. No histologic changes were observed other than a reduced

safranin 0 staining in the tangential zone, and reduction of cartilage diameter. Following vigorous exercise of animals on a treadmill for 3 weeks, there was compared to baseline disuse; 1) cartilage levels, i.e., significant increase by immunobiochemical assay of MMP-1, 2,3 & 9 activities; 2) Safranin 0 staining in the tangential zone; 21) ulceration and cleft formation with a Mankin scale of 10; 4) Heavy intense immunochemical staining for MMP-1 and MMP-3 in the surface zone in the tangential zone, and in more deeply around ulceration was seen; 5) Some MMP- I staining and increased capillary numbers in the adjacent calcified cartilage per unit area were observed.

In contrast, exercise only within the space of large cages, namely, conservative remobilization resulted in no sign of histological abnormalities in the articular cartilage, and chemical changes were virtually restored to normal.

Zymography and RT-PCR analysis generally confirmed the MMP levels in these cartilage as indicated by immunochemical stains.

### Discussion

These observations fit the hypothesis that cast immobilization initiated significant change in the biochemical and biomechanical properties of cartilage and menisci. We postulate that immobilization followed by aggressive rehabilitation accelerates the process of cartilage degeneration by up-regulating the synthesis of degrading metalloproteinases. Too rapid vigorous exercises after cast removal in humans may cause damage to articular cartilage. The present model will provide a unique opportunity to examining molecular mechanisms of biomechanical force transduction leading to articular degeneration is provided by this model system.

### REFERENCES

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Table Relative MMP level in cartilage.

Cartilage	MMP-2	MMP-3	MMP-9
I/C TP	0.8±0.4	1.4 ±0.3	1.3 ± 0.4
I/C C	1.1±0.6	1.5 ±0.5	0.9 ± 0.2
I/O TP	1.5±0.3	4.5 ±1.2	1.8 ± 0.2
I/O C	1.1±0.2	3.0 ±1.3	2.0 ±0.4

Data based on densitometry analysis of zymographys of MMP  
I/C= immobilized/conservative Rehab. I/O= immobilized /overuse

Data were normalized to baseline MMP Levels In cartilage of control dogs.

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