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Collagen Damage Is not the Cause of Early Softening in Articular Cartilage
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Introduction
Cartilage damage may start in young adults as a result of abnormal joint loading. Early damage may progress into severe osteoarthritis if poor mechanical conditions persist. Unfortunately, our knowledge on early development of cartilage damage is limited. We have recently shown using col2-3/4M antibody staining that collagen damage is the earliest histological sign of tissue damage following indentation loading [1]. In this study we follow the general hypotheses that cartilage softening, another early sign of osteoarthritis, would occur as a result of this initial collagen damage.

Aim
To evaluate if this hypothesis holds, we explore whether damage in the cartilage collagen network induced by indentation loading, always precedes cartilage softening.

Materials and Methods
Indentation loading using a spherical indenter (Ø2 mm) was applied to calf osteochondral plugs (Ø7.5 mm, 6 mm high). The experiment contained one control group (n=6) whose loading included a baseline of 5% indentation, followed by 600 s periods of 10% indentation during which relaxation was monitored (11 cycles) (Fig. 1a, blue lines). In another group (n=6) a 17.5 s damaging peak load of 15 N indentation was included after each 10% indentation step of the control loading protocol (Fig. 1a, red and blue lines).

![Fig. 1 a: Indenter displacement vs time: control (blue lines) and 15 N compression group (red and blue lines). b: 10% indentation relaxation measurement: peak force (red arrow) and equilibrium force (green line).](image)

To assess cartilage softening, peak and equilibrium forces were monitored during each 10% indentation (Fig. 1b). To determine mechanically induced collagen damage, col2-3/4M (cumulative collagen damage) and col2-3/4Cshort (only enzymatic damage) staining were compared.

Results
The peak and equilibrium forces of the control experiment did not decrease significantly after repeated loading. Samples receiving 15 N showed considerable softening, as peak and equilibrium forces decreased between subsequent 10% indentation loading cycles (Fig. 2).

![Fig. 2: Peak (left) and equilibrium forces (right) during the 10% indentation step, normalized to the first cycle, for controls (top) and samples loaded by 15 N indentation (bottom).](image)

Col2-3/4M and col2-3/4Cshort staining were similar, indicating no mechanically induced collagen damage occurred (Fig. 3).

![Fig. 3: col2-3/4M (left) and col2-3/4Cshort (right) stainings. Positive control shows intense brown staining at the extreme impact site.](image)

Discussion
By applying 15 N indentation, we were able to induce cartilage softening in all samples. Surprisingly, however, we did not detect collagen damage. Therefore, this study falsifies the general hypothesis that collagen damage causes the earliest tissue softening that occurs in mechanically challenged cartilage. Future work will explore alternative explanations for early cartilage softening.

References