

**INJURIOUS COMPRESSION OF BOVINE
ARTICULAR CARTILAGE INDUCES
CHONDROCYTE APOPTOSIS BEFORE
DETECTABLE MECHANICAL DAMAGE**

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BACKGROUND:

Chondrocyte Apoptosis *in vivo*

- Traumatic joint injury is a known risk factor for development of secondary OA
- Apoptotic chondrocytes have been found in human OA cartilage [Kouri, '97; Blanco, '98; Hashimoto, '98] and in an animal model of OA [Hashimoto, '98]
- The number of apoptotic chondrocytes in articular cartilage increases with age in animals [Adams, '98]

BACKGROUND:

In vitro Injury Models

- *In vitro* cartilage injury models show tissue swelling, elevated GAG release, and reduced cell viability and biosynthetic activity [Borelli '97; Jeffrey '97; Quinn '98]
- Injurious compressed explants show non-viable chondrocytes with condensed nuclei [Quinn, '98]
- A cartilage wounding model shows chondrocytes near the wound die via an apoptotic process [Walker, '98; Tew '98]

HYPOTHESIS

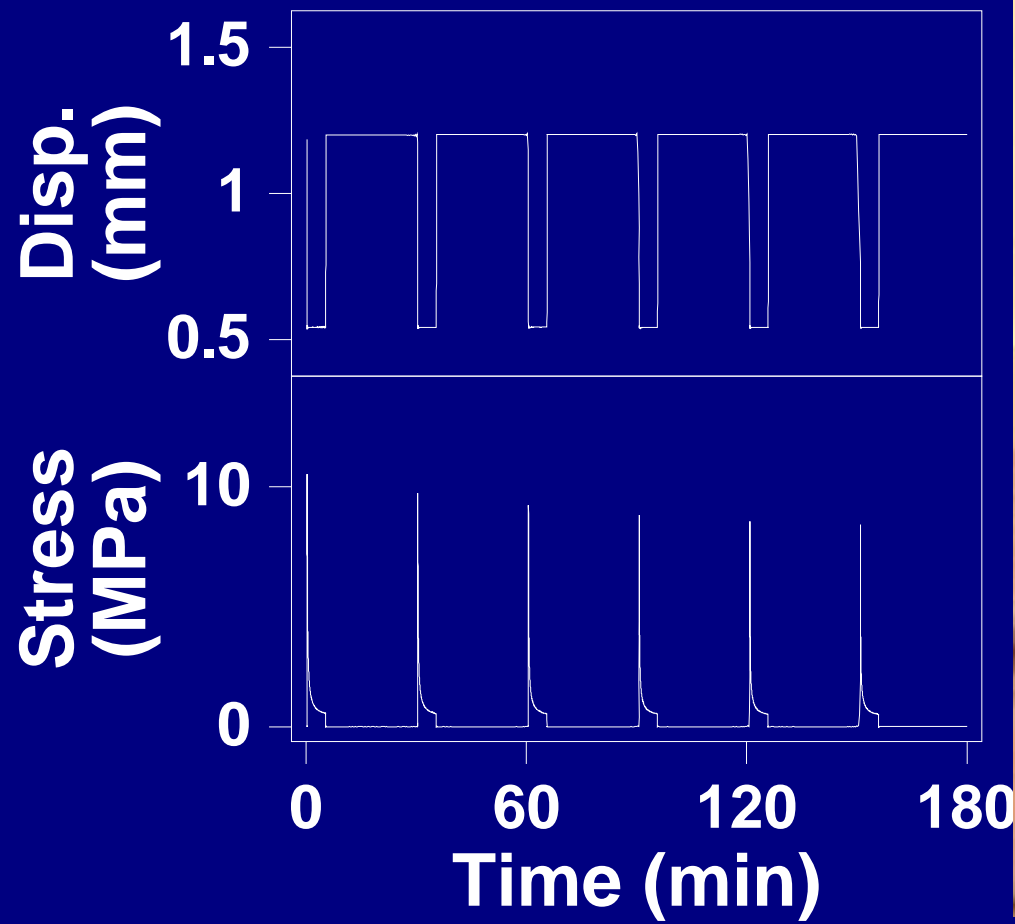
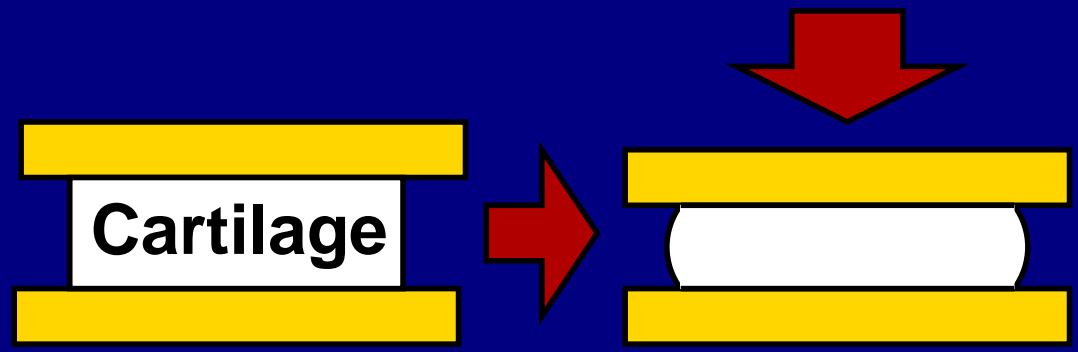
- Injurious compression of normal cartilage may induce chondrocyte apoptosis - another link between traumatic mechanical injury and tissue pathology.

OBJECTIVES

- Determine threshold levels of mechanical compressive stress that may induce chondrocyte apoptosis in cartilage explants
- Compare the effects of these injurious stresses on biomechanical, biochemical, and compositional measures of cartilage damage and degeneration

METHODS

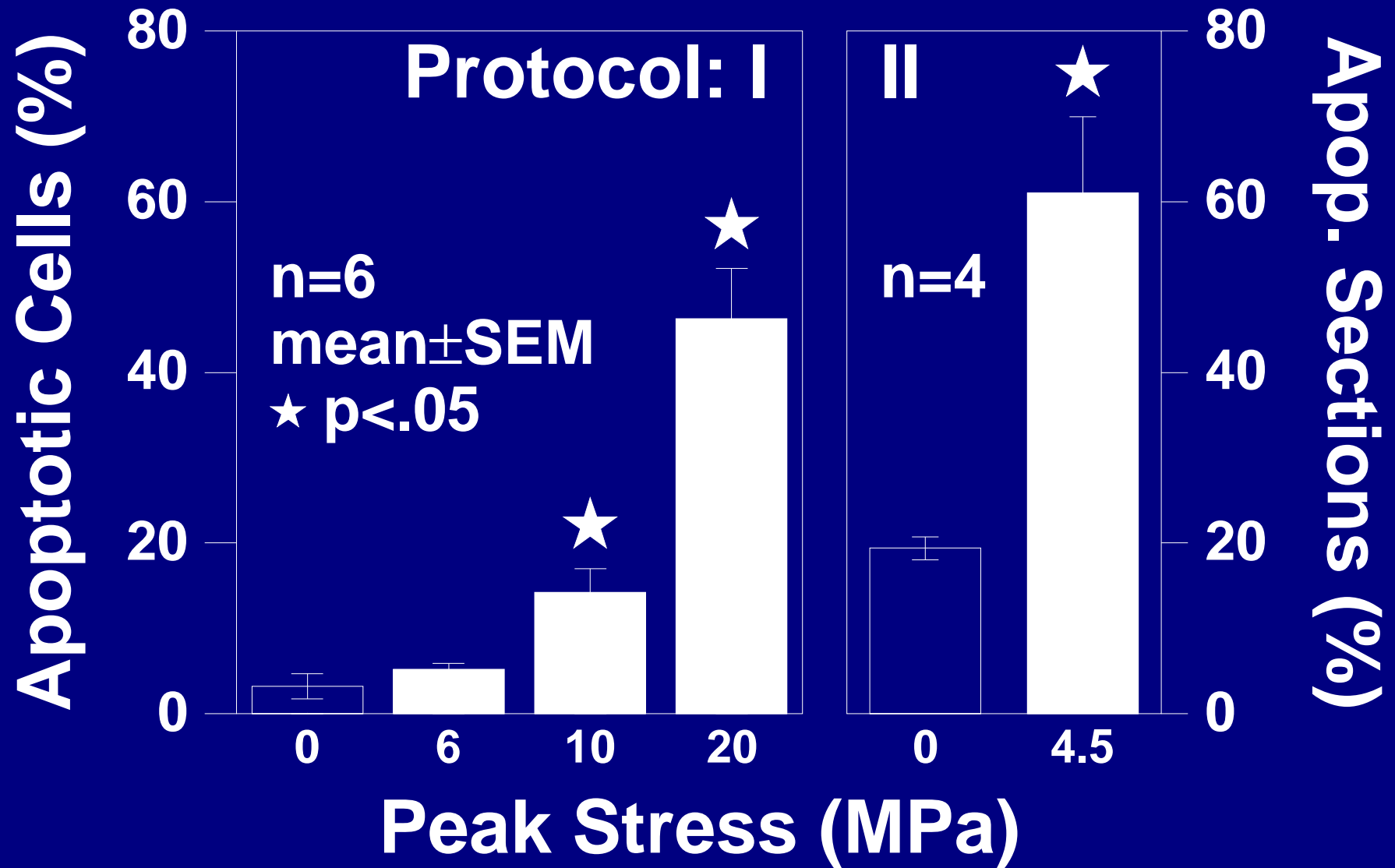
- **Cartilage:** 3 mm diameter by 1 mm thick disks from femoropatellar groove of 1-2 week old calves - maintained in serum supplemented DMEM
- **Injurious Compression:** ramp displacement at 1 mm/s to final strain of 30-50% (held 5 min, released 25 min); 4-25 MPa peak compressive stress; repeated 6x
- **Post-compression:** 0-6 days of free swelling culture; assessment of apoptotic nuclei, biomechanical, and biomechanical changes



METHODS: Detection of Apoptosis

- Disks exhaustively cryostat sectioned (8 μm sections, $\sim 125/\text{disk}$) and stained for apoptotic nuclei using TUNEL
- **Protocol I:** Positive staining nuclei and negative cells were counted in the central regions of 3-5 sections per disk to yield the percentage of apoptotic cells
- **Protocol II:** Number of sections with apoptotic nuclei throughout normalized to the total number of sections
- Artifactual staining along edges was ignored in all cases

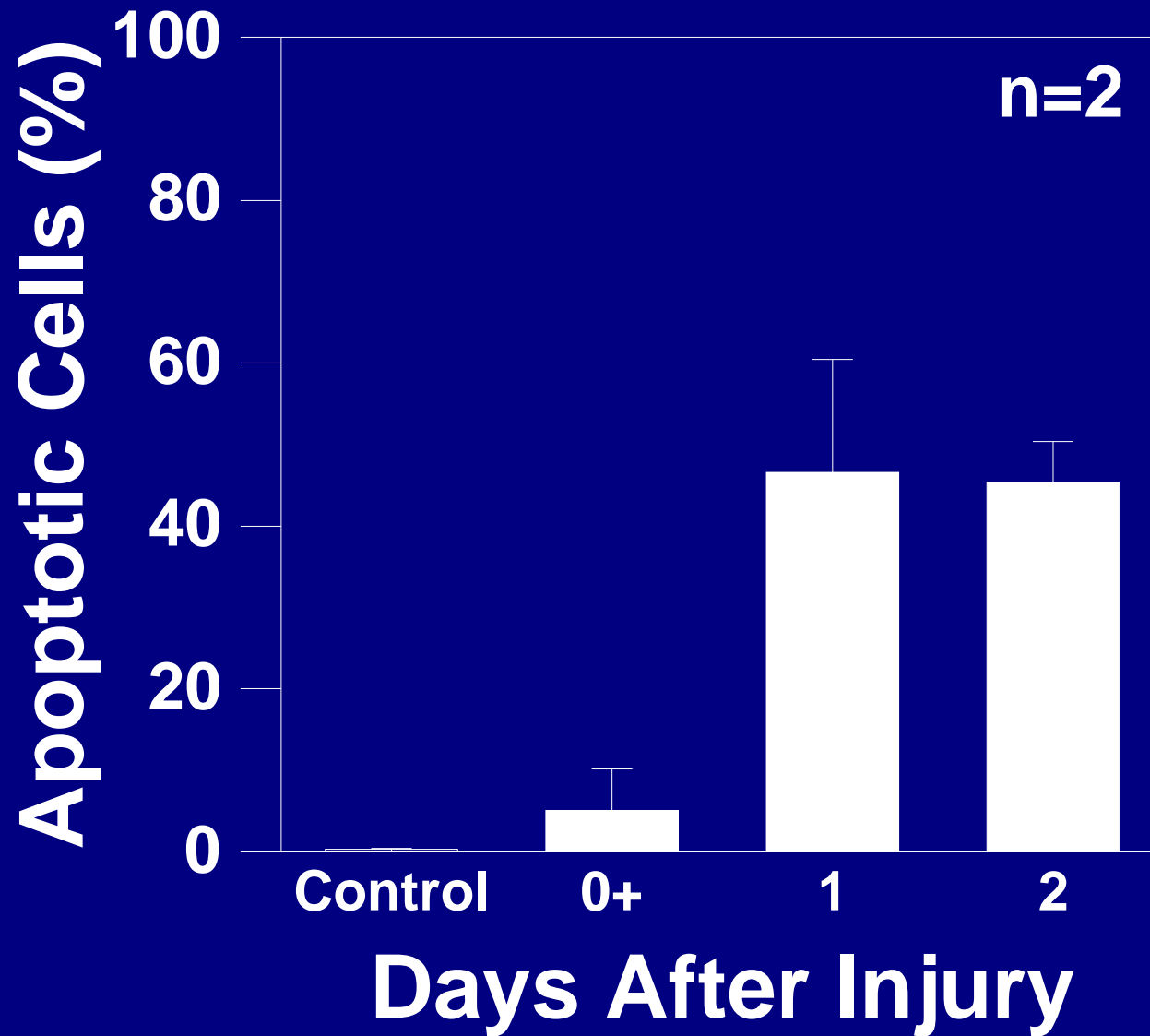
RESULTS: Apoptosis



APOPTOSIS TIME COURSE

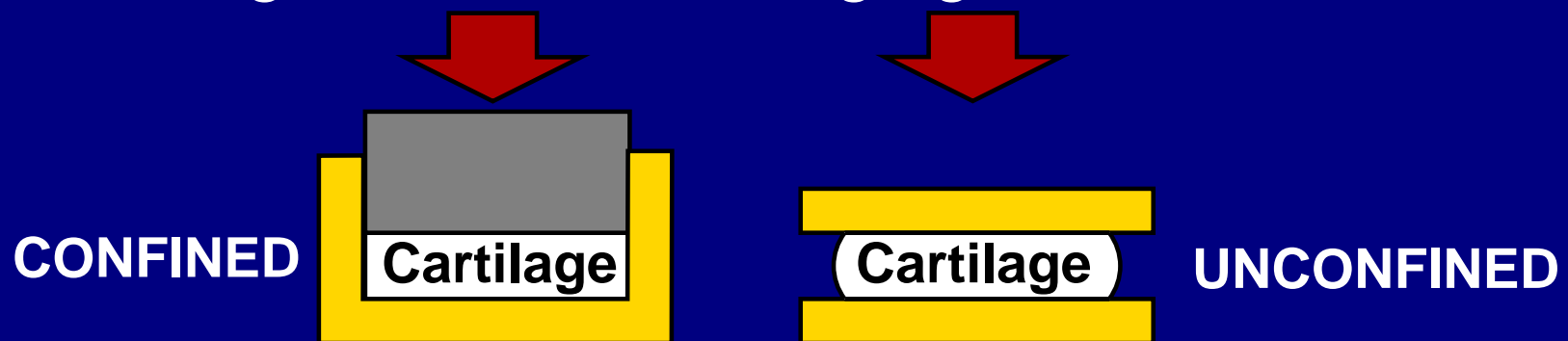
- Measured apoptosis immediately after, 1 day after, and 2 days after a severe (20 MPa) injury
- Initial results show that apoptosis does not occur maximally until 1 day after injurious compression

RESULTS: Time Course

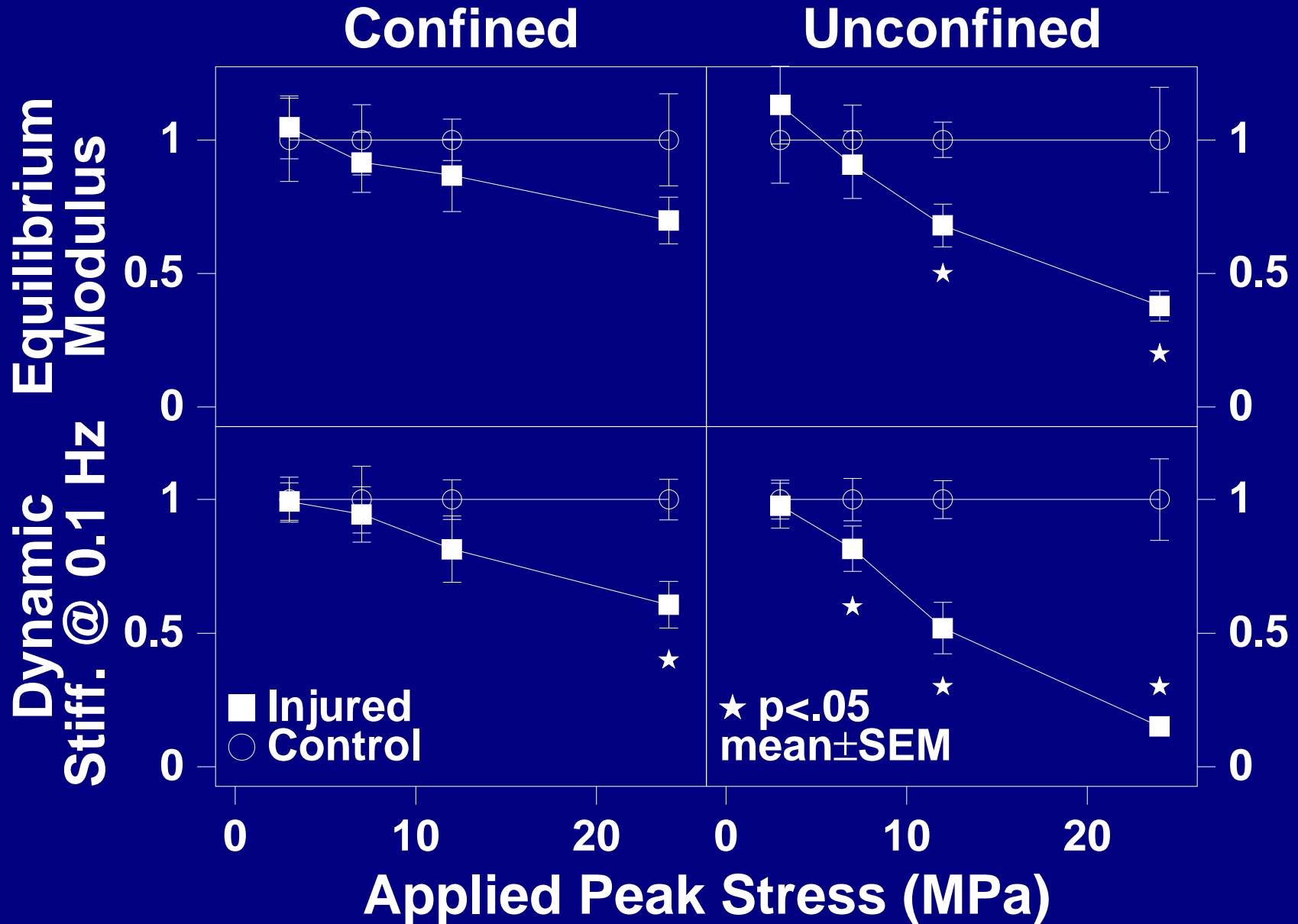


METHODS: Biomechanical Assessment

- Disks were tested in uniaxial confined and unconfined compression post-injury
- **Confined** compression highlights contribution of PG's to compressive stiffness; **Unconfined** compression also emphasizes the contribution of the collagen network tensile strength to restrain “bulging”

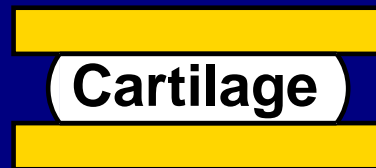
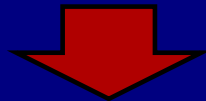


RESULTS: Biomechanical



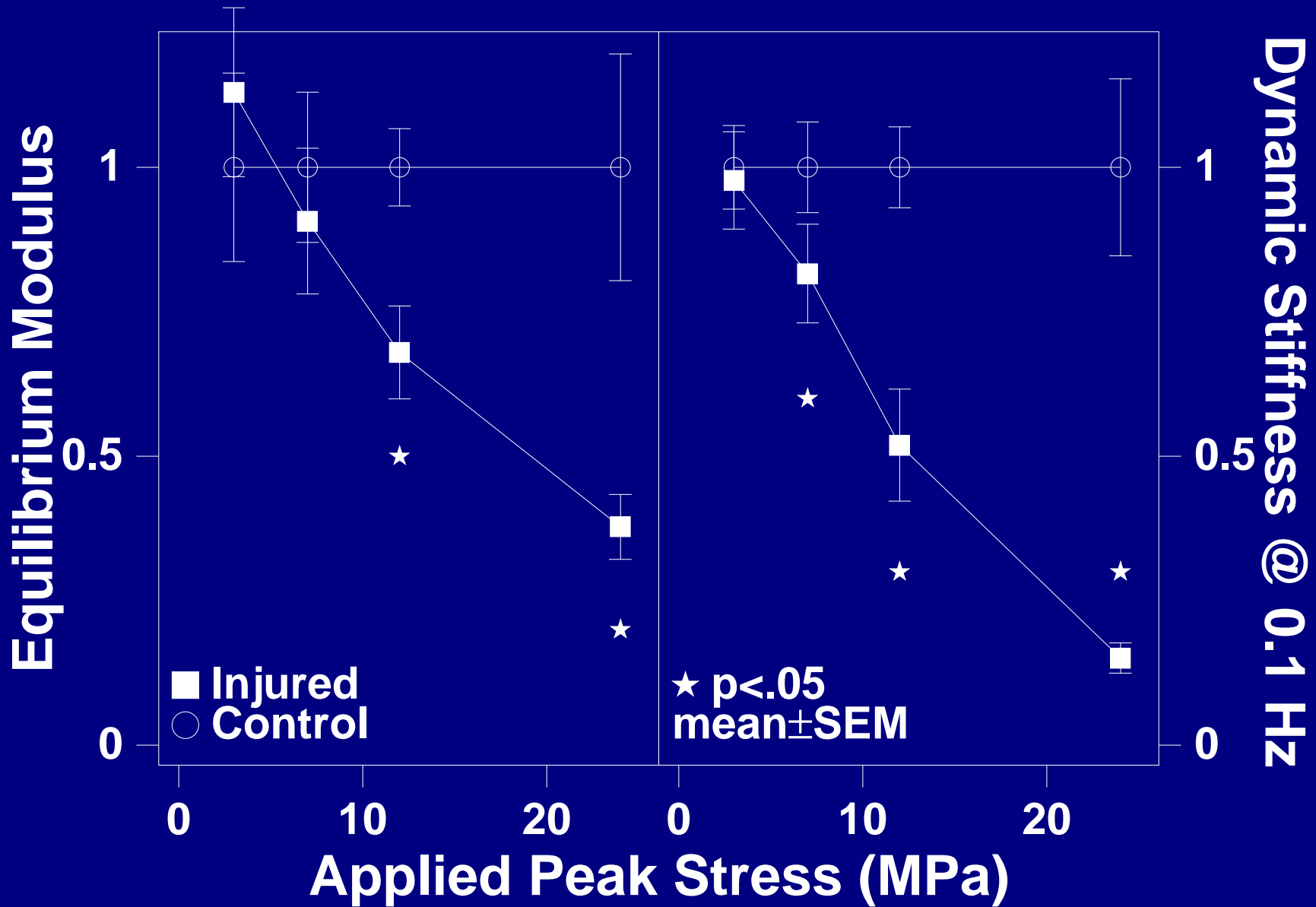
METHODS: Biomechanical Assessment

- Disks were tested in uniaxial unconfined compression post-injury
- Unconfined compression highlights the contribution of the collagen network tensile strength to restrain bulging, and of PGs to compressive stiffness



UNCONFINED

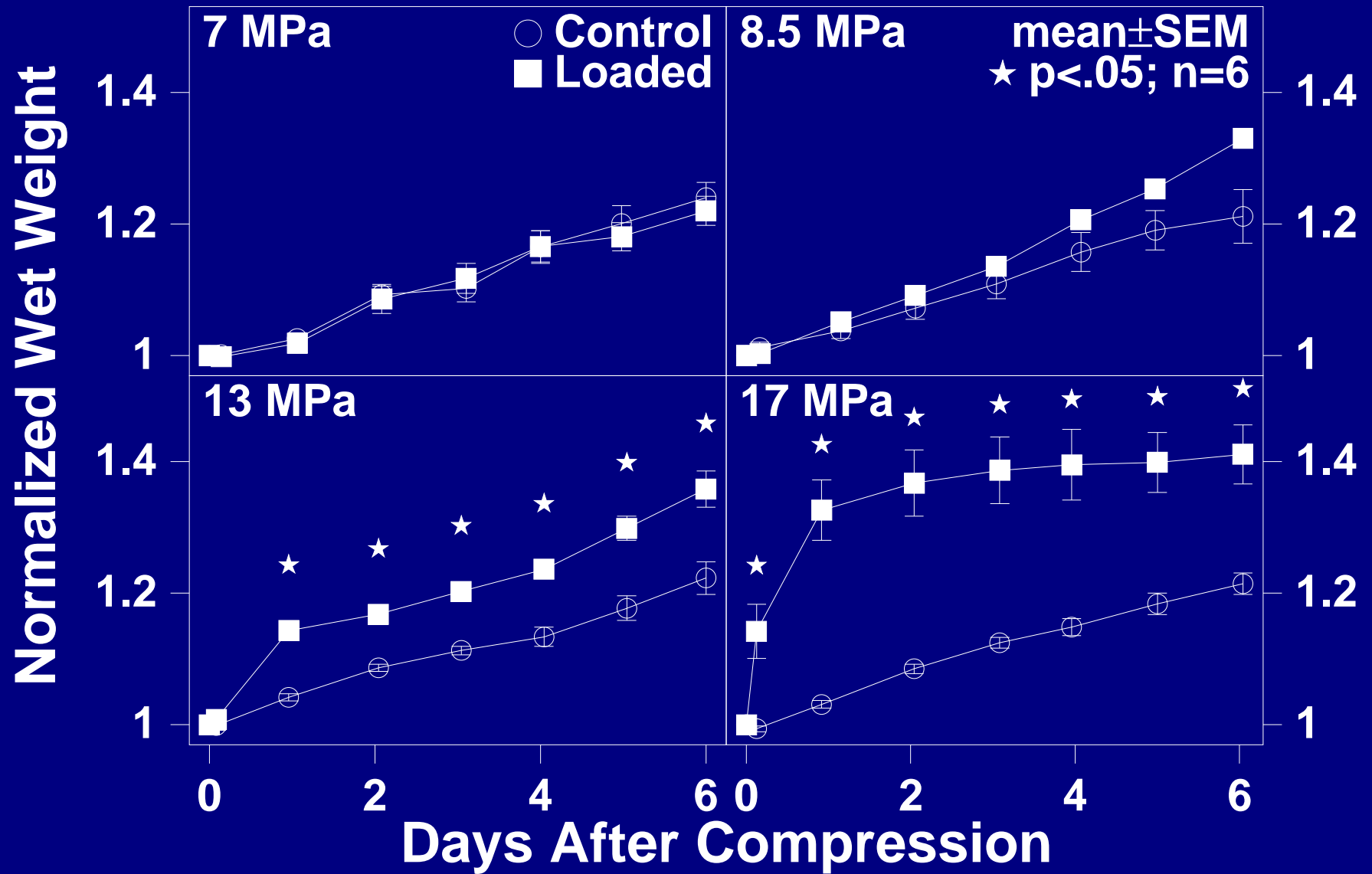
RESULTS: Biomechanical



RESULTS: Tissue Swelling

- Wet weights of injuriously compressed disks were significantly greater than non-injured controls for injurious peak stress of at least 13 MPa
- Wet weight increase occurred within the first day following compression

Tissue Swelling

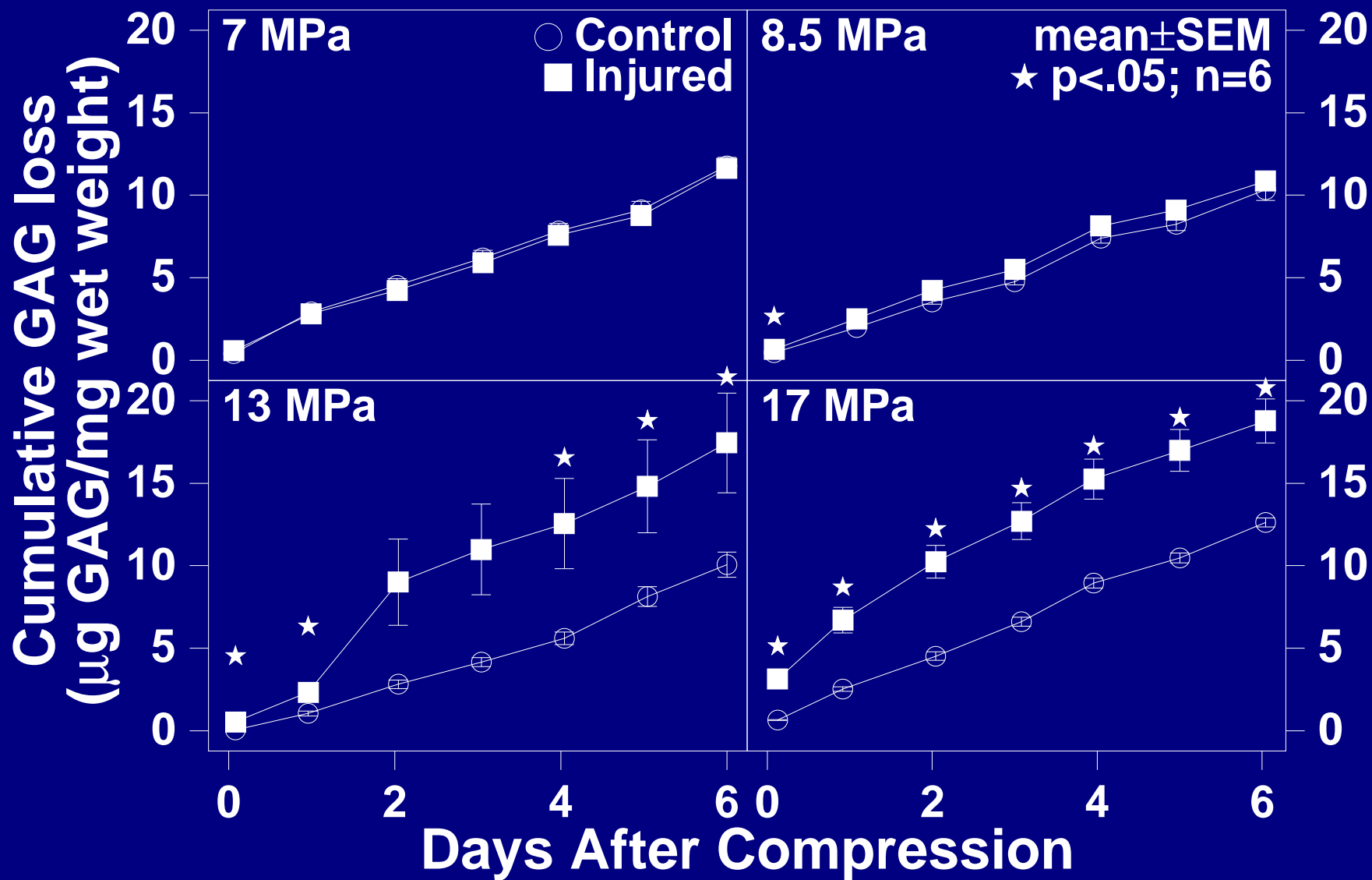


RESULTS: GAG Loss to Media

- sGAG release (by DMMB) increased following injurious compression for peak stresses of at least 13 MPa
- Release rate was elevated for 2-3 days following compression

Nitric Oxide: Increased (50%) only for most severe loading (20 MPa)

GAG Loss to Media



SUMMARY AND CONCLUSIONS

- Injurious mechanical compression caused dose-dependent chondrocyte apoptosis in bovine articular cartilage
- Apoptotic response occurred at peak stresses below threshold needed to induce direct matrix damage, tissue swelling, or increased GAG release

SUMMARY AND CONCLUSIONS

- Apoptosis may therefore be one of the earliest events in response to tissue injury
- Apoptotic chondrocytes have been observed in OA cartilage, and may be related to hypocellularity in OA. Our results are consistent with the possible role of mechanical injury in the induction of an apoptotic response.

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