Introduction: Traumatic joint injury is a known risk factor for development of secondary osteoarthritis (OA). In vitro arthritic cartilage (AC) injury models have produced OA-like degenerative changes, including tissue swelling [2,3,5], elevated sulfated glycosaminoglycan (sGAG) release [5], reduced cell viability [6,4] and reduced biosynthetic activity [2,4]. These models exhibit high-toxic injuries, with loading sufficient to induce macroscopic tissue damage. Relatively little is known about the effects of less severe loading, which may induce more subtle mechanical and biological changes. Quinn et al [5] observed condensed nuclei in severely injured AC explants, and increased numbers of apoptotic chondrocytes have been found in human OA tissue compared to normal cartilage[1], suggesting that apoptotic cell death may be an important event in OA pathogenesis. To investigate whether compressive injury could induce apoptosis in healthy tissue, we assayed for apoptosis and other biological and mechanical measures of tissue damage for graded levels of injurious compression.

Methods: Cartilage disks (3x1mm) were obtained from the femoro-patellar groove of 1-2 week old calves and maintained in DMEM with 10% FBS, 25 µg/ml gentamicin, and 0.1 mM ascorbic acid at 37°C, 5% CO2. Anatomically matched disks were assigned to free-swelling control or experimental groups. Compression protocol: Graded levels of injury were applied to experimental disks in an unconfined compression mode by ramping at 1 mm/s (100%/s) to a final strain level of 30-50% (peak stresses from 2-30 MPa) using an incubator housed compression apparatus. Six compression-release cycles (5 mm/s, on, 25 min, off) were applied, except for the most severely loaded group for mechanical testing (1 cycle). Disks were then cultured free-swelling for an additional 0-6 days.

ApopTag: Disks from a relatively mild loading condition (30%; 4 MPa) were flash-frozen in liquid N2 four days after compression and serially sectioned into 8 µm sections (~125 sections/disk). The sections were then immobilized onto glass slides, air-dried, fixed, and stained via TUNEL for the presence of apoptotic nuclei (ApopTag peroxidase in situ apoptosis detection kit, Oncor, Gaithersburg, MD). All sections were scored blindly (0-3) for stained nuclei, with staining only at the periphery considered negative (cutting artifact, 0 or 1), and staining in the bulk of the tissue considered positive (2 or 3). The number of positive sections (a score of 2-3) was expressed as a percentage of the total number of sections from each cartilage plug. Vital dye staining: Multiple ~200 µm slices from experimental and control disks were immersed 2-6 days after compression in an 18 µM ethidium bromide, 250 µM fluorescein diacetate PBS solution and then viewed under a fluorescence microscope with a fluorescent and a bright-field microscope.

Biochemistry/Metabolism: Changes in wet weight and release of sGAG and nitric oxide (NO) into the media were measured for relatively mild (35%; 2-4 MPa) to relatively mild (30%; 4 MPa) to severe (55%; 16 MPa) to severe (50%; 16 MPa). Matched disks were assigned to free-swelling control or experimental groups. Mechanical Testing: A compression modulus for any loading condition, although a nonsignificant trend toward degraded stiffness with increasing loading severity was observed (Fig. 3). In contrast, significant reductions were noted in the equilibrium unconfined compression modulus for peak injurious compression stresses at and above 12 MPa and in the dynamic unconfined compression modulus for peak compression stresses at and above 7 MPa. No significant differences from control disks were noted at the mild stress levels comparable to that for which the apoptosis assay was done (n=20, p>0.5)

Discussion: The key finding of this study was a marked increase in the number of apoptotic cells in injuriously compressed cartilage. In particular, we found apoptotic cells death at loading levels that produced no changes in sGAG release, NO release, vital dye staining, wet weight or mechanical properties. The observation that apoptosis precedes these changes suggests that this could be one of the earliest events in response to injury. It has been proposed that reduced cellulurity in OA tissue is achieved through apoptotic cell death [1]. Our findings are consistent with this concept, and suggest a possible paradigm for the development of traumatically induced cartilage degradation. Even low levels of cartilage injury may induce chondrocyte apoptosis without producing any immediate extracellular matrix damage or functional impairment. Ongoing studies are examining apoptosis under a wider range of injurious loading conditions.

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DETECTABLE MECHANICAL DAMAGE

INJURY, DEGRADATION, AND APOTOTIC INDUCTION ARE INDUCED IN CARCILAGE APOTOSIS BEFORE

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