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Molecular basis of osteoarthritis: biomechanical aspects

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Abstract. The unique biomechanical properties of healthy cartilage ensure that articular cartilage is able to transmit force between the joints while maintaining almost friction-free limb movement. In osteoarthritis, the biomechanical properties are compromised, but we still do not understood whether this precedes the onset of the disease or is a result of it. This review focuses on the physical changes to cartilage with age, disease, and mechanical loading, with specific reference to the increased collagen cross-linking that occurs with age (nonenzymatic glycation), and the response of chondrocytes to physiological and pathological loads. In addition, the biomechanical properties and matrix biosynthesis of cartilage from various joint surfaces of the knee and ankle are compared to elucidate reasons why the ankle is less affected by progressive osteoarthritis than the knee.

Key words. Biomechanics; Osteoarthritis; mechanical loading; age; glycation; biosynthesis.

Ranges of physiological forces on joint cartilage

Human articular cartilage experiences wide ranges of stress and strain during normal joint loading. Studies using cadavaric limbs loaded in simulated gait have shown that stresses in the range of 5–10 MPa are normal in the hip, corresponding to loads that are 300–800% body weight [1–4]. In vivo measurements with an instrumented hip endoprosthesis have indicated that higher stresses are possible (up to 18 MPa) during other physiological movements, especially when muscle forces are high [5].

Accompanying strains during certain regimes of joint loading can also be high. Herberhold et al. [6] reported decreases of approximately 40% in patella cartilage thickness after 30 min of static loading of cadaver joints at 3.6 MPa [6]. In loaded areas of knee joints, cartilage thickness recorded at the end of the day was decreased by up to 0.6 mm compared to the start [7]. This phenomena was attributed to accumulated fluid loss from the matrix of loaded areas.

Supranormal stress and strain can lead to injury

In contrast to the above normal ranges of joint forces, stress and strain above the physiological range have the potential to damage the matrix and chondrocytes. Acute trauma to the joint is known to increase the risk of osteoarthritis (OA) [8, 9], while destabilization of the knee joint due to anterior cruciate ligament rupture or meniscal damage causes radiographic signs of OA in many patients [10, 11]. Other mechanical influences that cause abnormal forces, such as joint laxity, obesity, and muscle weakness, are also linked to the progression of OA [12].

In vivo studies have shown that impact trauma can cause osteoarthritic changes. Radin et al. [13–15] impacted patellofemoral joints of rabbits, causing damage to the bone and cartilage and subsequently leading to OA-like degradation. Even impacts that do not appear to fracture the bone can result in cartilage degradation [16].

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Biomechanical properties of normal and osteoarthritic cartilage

Numerous studies have focused on the methods and challenges of measuring the equilibrium properties of the extracellular matrix (ECM) (e.g., compressive, shear, and tensile moduli and Poisson's ratio) and the non-equilibrium poroelastic and poro-viscoelastic properties of normal and OA tissue [see refs 17–19 for reviews]. Since joints experience a wide range of loading conditions, it is not surprising that the biomechanical properties of cartilages vary between joints [20–22] and with position [23–26] and depth [27] on the same normal joint surface (e.g., fig. 1). Higher stiffness is sometimes associated with sites of higher loading [28]. Even cartilage from surfaces directly opposing each other have been found to have different mechanical properties (fig. 1) [1, 21].

Studies have also related the biomechanical properties of cartilage to the molecular composition of tissue ultrastructure [21, 29–31]. For example, Treppo et al. [21] recently reported that the top 1 mm of distal femoral cartilage had a lower confined compression equilibrium modulus, lower glycosaminoglycan (GAG) content per wet weight, and lower dynamic stiffness (fig. 1A, GAG and dynamic stiffness data not shown) compared to the remaining cartilage down to the bone. When the confined compression modulus values of all areas tested from each joint were pooled, the modulus of talar cartilage was found to be higher than that of the distal femoral and tibial cartilage, and the distal femoral cartilage stiffer than the tibial (fig. 1B). The tibial plateau also had the highest water content, and lowest GAG and hydroxyproline content per wet weight (indicative of lower proteoglycan and collagen levels). Distinct sites on joint surfaces were also tested and compared. Talar was split into anterior and posterior on the lateral (white bars, fig, 1C) and medial (black bars) sides. Femoral was split into femorapatella groove, and anterior and posterior areas of the condyles (as well as medial and lateral). Tibial sites tested were anterior and posterior. No significant differences were found within a joint surface, but the medial aspect of the anterior femoral condyle showed a trend toward an increased equilibrium modulus compared to the opposing site on the tibia (fig. 1C). These areas were also the only areas where there was a significant difference in GAG content per wet weight. Multiple linear regression showed that more than 80% of the variation in the equilibrium modulus between joint surfaces could be accounted for by variations in the biochemical properties (water content, sulfated GAGs/wet weight, and hydroxyproline/wet weight).

A characteristic feature of OA is focal damage to the cartilage, including fibrillation and thinning. The mechanical properties of cartilage adjacent to fibrillated cartilage have been extensively studied in an attempt to understand disease progression (fibrillated tissue itself is difficult to assess, as sample preparation can be problematic, if possible at all). Akizuki et al. [32] showed that osteoarthritic

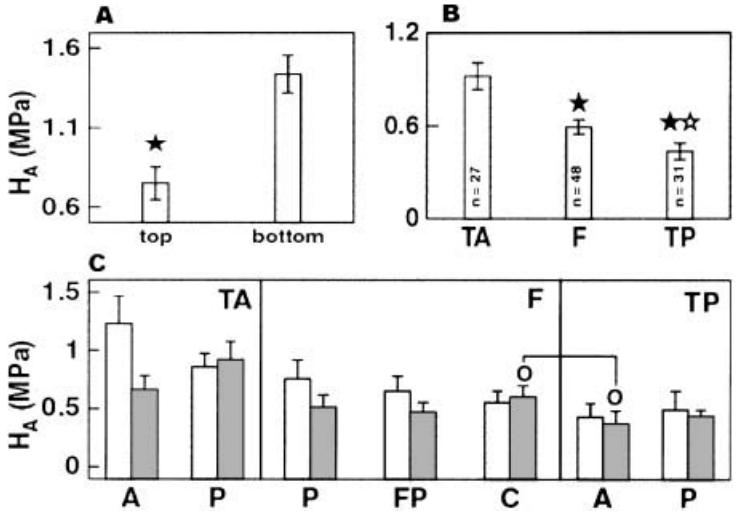

Figure 1. Differences in the confined compression equilibrium modulus (H_A) of human cartilage from the talar dome (TA), distal femur (F), and proximal tibia (TP). (*A*) Distal femoral cartilage: the top 1 mm had a lower equilibrium modulus compared to the remaining cartilage down to the bone. (*B*) When all areas tested of each joint were pooled, the modulus of talar cartilage was higher than that of the distal femoral and tibial cartilage ($p < 0.01$, closed star), and the distal femoral stiffer than the tibial ($p < 0.01$, open star). (*C*) Distinct sites on joint surfaces were also tested. Talar was split into anterior (A) and posterior (P) on the lateral (white bars) and medial (black bars) sides. Femoral was split into femorapatella groove (FP), and anterior (C) and posterior (P) areas of the condyles (as well as medial and lateral). Tibial sites tested were anterior (A) and posterior (P). The medial aspect of the anterior femoral condyle showed a trend toward increased stiffness compared to the opposing site on the tibia (open circle, $p = 0.062$). All bars are means $\pm$ SE, eight knees, five donors. Adapted from Treppo et al. [21] with permission.

cartilage from knee replacement donors had very low tensile moduli compared to controls. Even visually normal cartilage from the apparently unaffected compartment of unilateral knee replacements can be thinner and softer than control cartilage [33]. Animal models are often used because they enable the researcher to assess the effects of early stage degradation on the mechanical properties, before gross damage has occurred. In canine articular cartilage transection and menisectomy models, significant changes in mechanical properties were noted in the cartilage from the affected limbs as compared to contralateral controls [34–36]. Even chondrocytes in osteoarthritic cartilage were found to have altered viscoelastic properties, potentially changing their response to load [37].

Changes in biomechanical properties with age

As age is the most significant risk factor for OA, many studies have examined how the material properties of cartilage change with age. In individuals without cartilage lesions, cartilage thickness does not decrease significantly with age in men (–6%, n.s.), but does decrease in women by approximately 12% ($p < 0.05$), possibly as a result of the more rapid decrease in muscle forces with age in females [38]. Femoral head cartilage shows a large decrease in tensile stiffness and fracture stress with age, whereas talar cartilage (ankle) shows significantly less degradation in properties [39]. The superficial zone of human condyle cartilage shows a steady increase in tensile stiffness, peaking in the third decade of life, then decreasing thereafter [40]. Deep-zone stiffness decreases continuously with age.

As the collagen network has a very low turnover [41], alterations to it could cause changes in its mechanical stiffness and fatigue properties, possibly leading to premature breakdown. The presence of various sugars in the body cause cross-linking of proteins via a process called nonenzymatic glycation [42]. These cross-links are only significant in tissues with low turnover where they can build up. In cartilage, the collagen network is affected, leading to the browning of tissue associated with old age [42]. To study the effect of collagen cross-linking on the mechanical properties of cartilage, without the other changes that occur with age in vivo, researchers have incubated young, unglycated cartilage explants in high concentrations of sugars to create a level of cross-linking otherwise seen only in much older cartilage. For example, threose is a sugar formed by the degradation of ascorbic acid (vitamin C). A 5-day incubation of calf explants in a 200 mM threose solution caused an increase in the uniaxial confined compression equilibrium modulus and dynamic stiffness, as in well as in the equilibrium and dynamic shear stiffness of cartilage explants compared to cartilage incubated without threose (fig. 2) [43]. Other research has also indicated that glycated tissue becomes more brittle [44]. In people with subnormal levels of collagen turnover, or high levels of glycating sugars, the collagen could become highly cross-linked. The altered mechanotransduction and increased brittleness of the cartilage could lead to either cell-mediated or mechanical failure of the cartilage, and predispose the cartilage to OA. Current studies are investigating how glycation of the cartilage affects the chondrocyte response to static and dynamic loads.

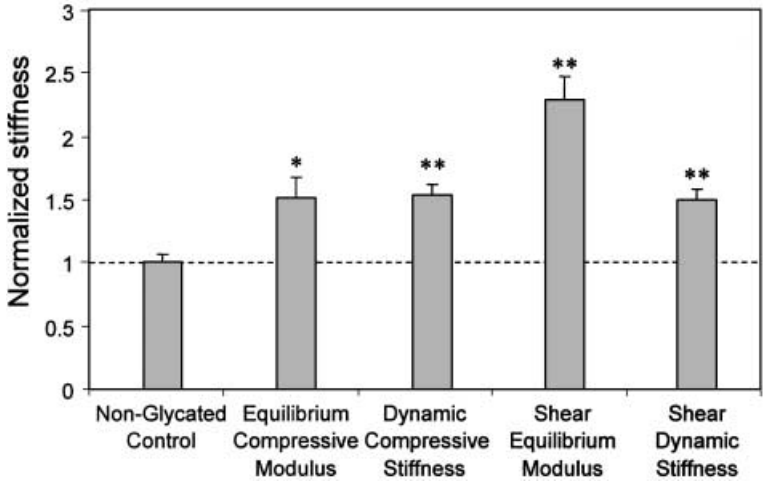

Figure 2. Uniaxial compression equilibrium modulus and dynamic stiffness, and shear equilibrium and dynamic moduli of calf cartilage explants treated with 200 mM threose solution for 7 days to form nonenzymatic glycation cross-links, similar to those that occur with age, normalized to tissue incubated without threose (control). Glycation caused significant increases in all properties tested (mean + SE, * $p < 0.05$, ** $p < 0.01$). Dynamic measurements were made at 0.1, 0.5, and 1 Hz, with a 2% amplitude for uniaxial compression and 1% for shear. The uniaxial equilibrium modulus was calculated from the final stress (after stress relaxation) at 12, 14, and 16% strain. Shear modulus was calculated from the final shear stress at 1, 2, and 3% shear strain.

Chondrocyte response to mechanical loading

Compression of cartilage causes deformation of cells and matrix, gradients in hydrostatic pressure, intratissue fluid flow, and associated electrokinetic effects (e.g., flow-induced streaming potentials). Since the compressive stiffness of chondrocytes is about three orders of magnitude less stiff than of the surrounding ECM, the cells will deform with the matrix [45]. The deformation of the charged ECM will change ionic concentrations, osmolarity, and pH of the cellular environment according to Donnan equilibrium theory [46, 47]. Tissue fluid flow during loading can also dramatically enhance transport of nutrients and macromolecules (e.g., growth factors and cytokines [48]). Therefore, mechanical and chemical changes during loading can alter chondrocyte behavior, and hence matrix synthesis and turnover.

Areas on joints that are more highly loaded during locomotion generally have a higher proteoglycan content compared to adjacent cartilage experiencing lower stress [49–51]. However, these highly loaded chondrocytes synthesize less total proteoglycan (although the synthesis of certain small proteoglycans, especially decorin, is elevated) [47, 52, 53]. Together, these findings suggest that less proteoglycans are degraded and lost from the cartilage in these regions. In young and neonatal cartilages, these trends between areas have not been observed, suggesting that the loading itself is responsible for the change in cartilage matrix composition and also the zonal variation in chondrocyte phenotype [51, 54]. Repeated loading can also increase the proteoglycan content of highly loaded regions of cartilage, as shown in dogs that had been strenuously exercised each day for a year compared to adjacent lower loaded areas and to control dogs [55]. In humans, the level of physical activity does not appear to explain the variation in thickness of knee cartilage observed between subjects, although the surface area of the knee cartilage is 7–9% greater in triathletes than in a less active population. This suggests that a possible mechanism for reducing the stress on cartilage is to increase joint surface area [38].

Magnetic resonance imaging studies of humans show that while there are only weak correlations between body height or weight and the volume and surface area of knee cartilage, the thigh muscle cross-section is a much better predictor, emphasizing the role of muscle forces in ontogenesis and/or cartilage maintenance [38].

To elucidate the effects of load on biosynthesis and gene expression by chondrocytes, explant culture is frequently used. This in vitro model system enables the chondrocytes to remain in their native ECM environment. In addition, well-defined and controlled loading conditions can be applied to explants and the resulting effects on cell-mediated synthesis and degradation of ECM can be quantified. Cartilage explants can be subjected to axial compression, resulting in fluid flow and the accompanying flow-induced ECM changes, or placed under hydrostatic pressure or pure tissue shear, which can result in essentially no volume change or intratissue fluid flow. Such mechanical stimuli can be applied to simulate the ranges of matrix deformation, fluid flow, and pressure that is caused by joint loading.

In animal tissues, static compression generally inhibits the biosynthesis of ECM molecules [56–58], while cyclic compression above a threshold frequency can stimulate biosynthesis [56, 58–60]. Even mechanical deformation due to dynamic tissue shear strain, which causes negligible volume change, fluid flow, or fluid pressurization, has also been shown to stimulate matrix production [61].

To further understand how loading and biosynthesis may be linked to OA, current research is focusing on the response of normal human cartilage explants from joints that rarely become osteoarthritic (e.g., talar dome of the ankle) compared to joint cartilages that are commonly affected by OA (e.g., cartilage from the proximal tibia, distal femur, and patellofemoral surface). Cartilage plugs were prepared from intact, non-osteoarthritic, donor joints (Collins grade 0 and 1) less than 48 h after death. After an initial period of 24 h in DMEM + 10% fetal bovine serum, the plugs were left to freely swell, kept at their cut thickness (0% compression), or statically compressed by 15, 25 or 50% for 16 h in the presence of radiolabeled sulfate and proline precursors. The sulfate is predominately used by the cell to manufacture chondroitin and keratan sulfate for synthesis of aggrecan molecules (and other proteoglycans) [56]. Proline is incorporated in various amounts into most proteins synthesized by chondrocytes, while hydroxyproline is found uniquely in collagens. After multiple washes to remove free radiolabel, the plugs were digested with proteinase K and the remaining radioactivity measured using a scintillation counter.

Static compression of normal talar dome cartilage exhibited similar trends to those of bovine and other animal cartilages. GAG synthesis (as sulfate incorporation) was inhibited when the talar cartilage was subjected to greater than 25% compressive strain (fig. 3). Similarly, collagen synthesis (^{3}H-hydroxyproline incorporation) decreased with increasing compression, and was significantly lower than controls by 15% compression. Data from the normal human knee surfaces are limited, but the results to date from the patellofemoral surfaces show that the chondrocytes were synthesizing similar amounts of proteoglycans compared to talar cartilage. However, the knee cartilages were less affected by static compression. Total protein biosynthesis in talar cartilage was almost double that of the knee tissues at no compression. Uniquely, the knee cartilage increased total protein synthesis with increasing static compression (not shown), so that at 50% compression, the ankle and knee cartilages were synthesizing similar amounts of protein [62].

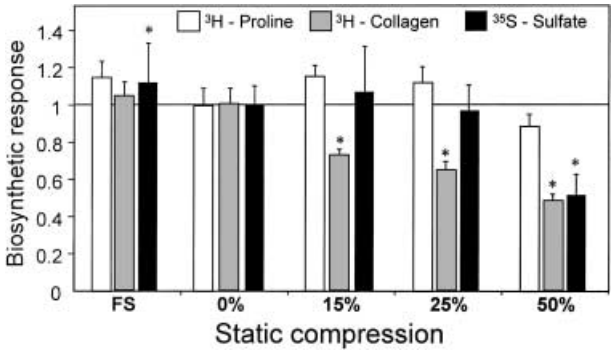

Figure 3. Response of normal talar (ankle) cartilage (Collins grade 0, 1) to varying degrees of static compression. Proteoglycan biosynthesis (^{35}S-sulfate incorporation, black bars) was inhibited by static compression greater than 25%, while total protein synthesis, (^{3}H-proline incorporation, white bars) was unaffected. Collagen synthesis, however (as determined by the amount of ^{3}H-proline converted to ^{3}H-hydroxyproline, quantified by column chromatography [72], gray bars), decreased significantly with compression (mean + SE, n = 30–95, 14 donors, * $p < 0.01$ vs 0%).

The differences in the biosynthetic responses of normal knee and ankle cartilage to static compression could be due to different pools of chondrocytes, or differences in the matrix surrounding them, and thus the stimuli they receive. To study these differences, chondrocytes from the ankle and knee surfaces were isolated and cast into agarose gels. The chondrocytes were allowed to synthesize new matrix (newly seeded chondrocytes may not respond to load due to the absence of matrix [63]) and their response to static load assessed. Preliminary results suggest that the chondrocytes from the different surfaces behave the same once removed from their native matrix, thus emphasizing the potential role of a defective matrix in OA.

As a wide range of human donors were used in this study, biosynthetic trends with age could be studied. Surprisingly, biosynthesis of proteins and proteoglycans was largely unaffected across the age range tested (20–71 years) when the cartilage was statically compressed, but when left in a free-swelling state, total protein synthesis decreased by a factor of two over the 50-year age range (fig. 4). We note that these data are from cartilage specimens obtained from healthy joints. It is therefore difficult to form conclusions from these data regarding the relationship between age and OA. However, these data do emphasize that some age differences are present at the cellular level.

Dynamic stimulation of ankle tissue at 0.1 and 0.01 Hz increased total protein synthesis across a 33- to 73-year-old age range, with no variation with age so far apparent [62] (data not shown). No significant stimulation of proteoglycan synthesis was noted, even in younger tissue. Whether other cartilages behave differently has yet to be examined.

Chondrocyte response to pathological forces

As mentioned previously, traumatic joint injury has been linked to an increased risk of developing OA in later life. Until recently, little was known about the state of the chondrocytes or ECM macromolecules in the time between injury to human joints and the development of disease. Lohmander et al. [64] removed synovial fluid from patients immediately after an articular cartilage or meniscal tear

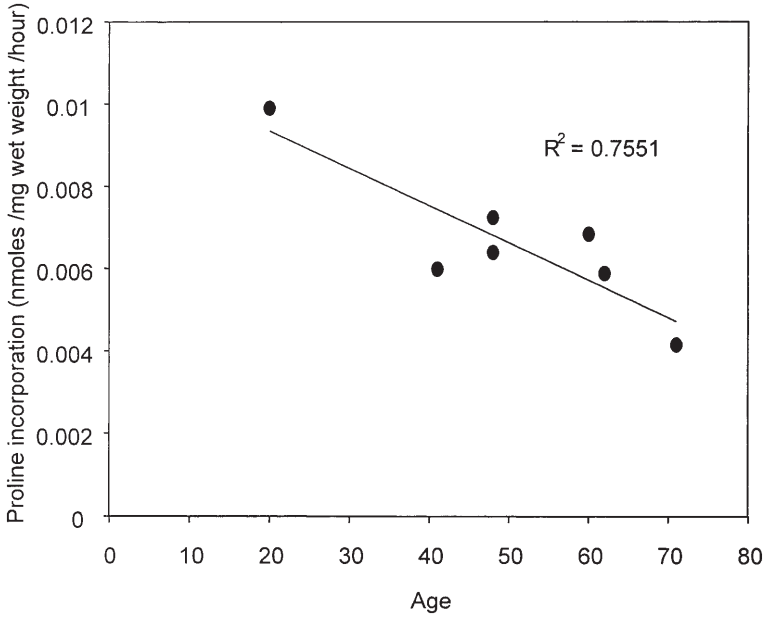

Figure 4. Total protein synthesis (^{3}H-proline incorporation) in free-swelling talar (ankle) cartilage versus donor age. Linear regression analysis indicates a significant decrease in protein synthesis with age ($p < 0.05$, $R^2 = 0.76$).

and up to 15 years postinjury. The synovial fluid samples were analyzed for the presence of degradative enzymes and fragments of enzymatically cleaved or intact matrix molecules. The matrix metalloprotease stromelysin-1 (MMP-3) is thought to be one of the major proteolytic enzymes responsible for the normal turnover of ECM molecules and the enhanced turnover during disease. In the days following injury, the level of MMP-3 in the synovial fluid (measured as the latent or proenzyme form) was elevated by 50–100 times the level in healthy athletes. These levels decreased with time after injury, but remained almost tenfold higher than in uninjured controls even by 10–15 years after injury. Interestingly, the levels of tissue inhibitor of metalloprotease were also elevated after injury.

Collagen degradation also occurred soon after injury, as indicated by a 15-fold increase in the amount of MMP up to 15 years after injury, leaved collagen molecules in the synovial fluid [64]. Cleavage at this site in the C-telopeptide cross-linking domain indicated that mature, rather than newly synthesized, collagen molecules were being cleaved and leaving the tissue. Again, these levels remained higher.

In an animal model [65], cytokines such as interleukin-1 β, tumor necrosis factor-α), and stromelysin (MMP-3) were upregulated in the proximity of cells adjacent to macroscopic damage caused by impact to closed patella joints, although only for 2 weeks following injury. After an extended period, attempts at cellular repair were evident, suggesting that the impact was below the threshold needed for the long-term osteoarthritic changes seen by other authors. Excess mechanical load can also cause tissue injury in vitro, providing a useful model for in vivo trauma. In vitro cartilage impact studies have demonstrated a loss in cell viability above a threshold stress or strain, as well as increases in matrix damage and turnover, tissue swelling, and the presence of degradative factors [65–69]. In one such study, injurious compression gave rise to the emergence of a population of apoptotic cells within bovine cartilage explants in a dose-dependent fashion with increases in the peak stress of the applied compressive load (fig. 5) [70]. Under the same loading conditions, there was a dose-dependent increase in tissue swelling (measured as increased tissue water content) and GAG loss [48], the former suggestive of collagen damage, similar to that observed in other related studies [47, 68, 71].

After traumatic injury to cartilage, stimulation of reparative processes using mechanical as well as biological factors may be possible. To explore this, we examined the possibility that low-amplitude dynamic compression might stimulate anabolic matrix synthesis in injured tissue, just as in normal bovine tissue [56]. However, this stimulatory response was lost for injured tissue subjected to the most rapid compression (1 s^{-1}) to 50% strain even when cell death resulting from the impact is taken into account (fig. 6) [69]. These results emphasize the potential

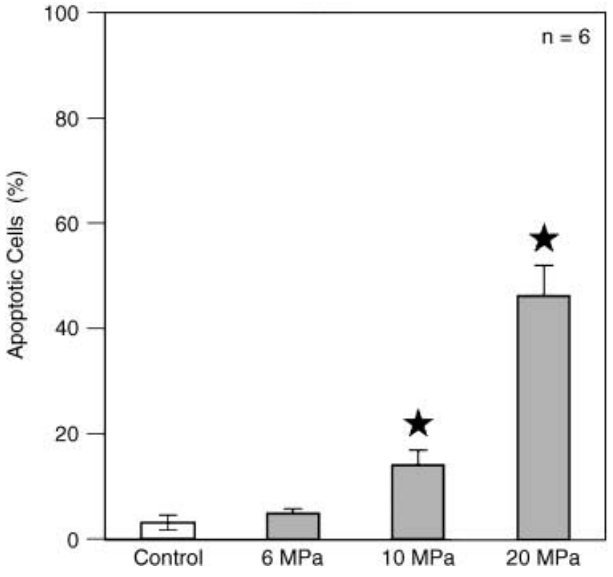

Figure 5. Percentage of apoptotic cells after rapid compression (strain rate = 1 mm/s) of cartilage explant disks to nominal peak compressive stress of 0 (control), 6, 10, and 20 MPa. Disks were subsequently incubated for 4 days and then analyzed for apoptotic nuclei using the TUNEL method on frozen sections of the tissue. Data are means + SE; star indicates $p < 0.05$ by paired t test. Reproduced with permission from Loening et al. [70].

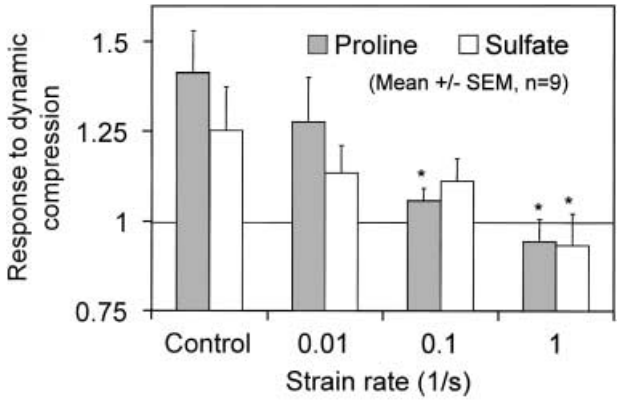

Figure 6. Response to dynamic compression after injurious loading. Calf cartilage explant disks were loaded to 50% strain at three strain rates. Three days after injury, radiolabel incorporation of ^{3}H-proline and ^{35}S-sulfate was measured in response to low amplitude dynamic loading, which is known to stimulate biosynthesis in uninjured calf explants (3% dynamic strain, 0.1 Hz, 10% static offset), or to static offset alone. Control disks received dynamic stimulation but no injury. The dynamic data are normalized to the plugs held at the static offset (line). This protocol accounts for any cell death during injury; that is, the response to dynamic compression is normalized to synthesis of equally injured plugs maintained at the same static offset compression. Significant decreases in proline and sulfate were seen at the higher strain rates versus uninjured controls ($*p < 0.05$) to the extent that at 0.01 and 0.1 s^{-1} strain rate, no significant stimulation was seen (significance not shown). Reproduced from Kurz et al. [69].

for long-term degradation after traumatic injury in vivo, and the inability of chondrocytes to respond to physical stimuli when subjected to forces above the threshold of mechanical damage.

It is clear that a prophylactic response may be required immediately after a biomechanical insult, rather than when OA symptoms present themselves. Levels of catabolic cytokines and enzmyes are upregulated almost immediately after injury and stay increased for many subsequent years. Chondrocytes lose their ability to increase biosynthesis of matrix components in response to dynamic compression and die through necrosis and apoptosis. Even in the absence of injury, glycation of the collagen network could increase susceptibility to fatigue failure. This could be further enhanced by the decrease in free-swelling protein synthesis seen with age. One of the pathways for decreasing the incidence of OA may be ensuring that chondrocyte response to both physiological and pathological mechanical loads is optimized for long-term survival of the cartilage.

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