The effects of matrix compression on proteoglycan metabolism in articular cartilage explants

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Summary

The effects of compressive stress on the rate of proteoglycan synthesis and release were determined in bovine articular cartilage from 4-5-month-old animals. Full depth cartilage explants were compressed in an unconfined configuration at various stresses ranging up to 1.0 MPa. At mechanical equilibrium (after 24 h), no significant changes were detected in the rate of $[^{35}S]$-sulfate ($^{35}SO_4$) incorporation at the low level of compressive stresses used (less than 0.057 MPa). At an intermediate level of compressive stress (0.057, 0.1, 0.5 MPa), $^{35}SO_4$ incorporation rates were reduced to ~60% of control values. At the highest level compressive stress (1.0 MPa) studied, $^{35}SO_4$ incorporation rates were further reduced to ~20% that of controls. Recovery experiments at intermediate stress levels showed increased rates of $^{35}SO_4$ incorporation at 24 h after compression. In explants loaded for 24 h at stresses of 0.1 MPa or higher, there was a stress-dose dependent inhibition of proteoglycan release into the media (up to 61% at 1.0 MPa), and proteoglycan release rates did not return to control values following a 24 h recovery period. While cartilage composition and biosynthetic activity were found to vary significantly with depth in control cartilage, the observed suppression (% change) in biosynthetic activity was relatively uniform with depth in both loading and recovery experiments. The study indicates that compression of the tissue to physiological strain magnitudes serves as a signal to modulate chondrocyte biosynthetic and catabolic responses through the depth of cartilage, while prolonged compression at higher strains may be responsible for tissue and cell damage.

Key words: Chondrocyte, Biosynthesis, Biomechanics, Mechanical signal transduction.

Introduction

It is well accepted that mechanical forces and deformations can alter the metabolic activity of chondrocytes in articular cartilage. For example, in vivo studies have found significant changes in the composition, structure and mechanical properties of articular cartilage exposed to disuse, overuse, or altered joint loading conditions [1-5]. To better understand the mechanisms through which mechanical loading regulates the catabolic and anabolic events involved in the maintenance of the extracellular matrix, a number of in vitro studies have attempted to isolate specific factors involved in this transduction process [6-14]. As an advantage over the in vivo conditions, these in vitro explant culture experiments offer a controlled biomechanical and biochemical environment for determining the response of articular cartilage to mechanical forces or deformations. These studies indicate that in general, static compression can suppress proteoglycan synthesis rates in articular cartilage explants [9, 12] and epiphyseal cartilage explants [6, 7], while cyclic compression at specific frequencies can stimulate proteoglycan metabolism [10, 11, 14]. In the present study, we wish to further our understanding of the effects of mechanical loading on cartilage metabolism in an in vitro explant experiment.

Normal chondrocyte metabolic activity is regulated by both genetic and environmental factors which include soluble mediators (cytokines, growth factors and pharmaceutical agents) [15-17] and matrix composition [18], as well as mechanical factors [1-14] which are associated with functional joint loading. Under physiologic loading conditions, peak stresses as high as 18 MPa have been reported during dynamic loading of the joint [19]. However, the in situ equilibrium compressive strain in cartilage has been observed to be no more than 20% [20]. Furthermore, it is likely that under in vivo conditions, interstitial fluid pressurization in cartilage plays a major role in load support in the joint [21, 22]. Thus, in vivo the mechanical environment around a chondrocyte is likely to be very different from that obtained in explant...
studies, except perhaps for the compressive strain. In addition, other mechanical, physicochemical and electrical effects may result from loading of cartilage, including interstitial fluid flow, osmotic pressure, static and dynamic matrix deformation, streaming potentials, changes in the tissue fixed charge density or pH, and deformation of the chondrocytes [8, 9, 12, 21–25]. Currently, there is evidence that any of these phenomena could be involved in the signal transduction process of the chondrocyte. Isolation of the specific regulatory signals derived from the mechanical and-physicochemical environment of the tissue is further complicated by the inhomogeneity of the chondrocytes and extracellular matrix in the different zones of the tissue [26–29].

To better understand the mechanism(s) through which mechanical, physicochemical and electrical signals are translated into biochemical events by the chondrocyte, it is advantageous to examine the response of chondrocytes to each of these events separately, whenever possible. At compressive equilibrium, no complicating effects will appear from interstitial fluid flow, fluid pressurization, deformation rates, streaming potentials or other electrokinetic phenomena [24]. The objective of this study was to quantify zonal variations in the metabolic response of cartilage explants to equilibrium compressive stresses. Rates of proteoglycan synthesis and release were determined for the explants as a function of the equilibrium stress. The recovery response of the biosynthetic activity was also examined upon unloading of the explants. Finally, considering that both tissue composition [18] and mechanical load [1–12, 14] are known regulators of cartilage metabolic activity, the biochemical composition of the tissue and the compression-induced changes in biosynthesis rates were examined in the different zones of the tissue.

Materials and methods

INSTRUMENTATION FOR EXPLANT COMPRESSION AND MECHANICAL TESTING

To prescribe the load or displacement on a single specimen during an explant culture study and for mechanical testing of the specimens, a load and displacement controlled device (LODEC) was designed and constructed in our laboratory [30]. This closed-loop device uses feedback control on a force signal from an in-line load cell (Model #81, Sensotec Inc., Columbus, OH) mounted on the loading shaft (Fig. 1). Motion of the loading shaft is achieved by a computer-controlled motorized micrometer (StepperMike, Oriel Corp., Stratford, CT) mounted rigidly on a translation stage above the explant culture dish. This system, which has a force resolution of 0.01 N and a displacement resolution of 1 pm, was used for both mechanical testing of the explants and for the tissue culture experiments. Once the initial protocol for the explant compression studies was developed, a specially designed rack was built to allow testing of multiple samples simultaneously. With this instrument, six samples were tested simultaneously using dead weights. Compressive loading was applied to samples in individual chambers through steel shafts aligned by linear bearings.

To facilitate diffusion of nutrients to the chondrocytes in the tissue during compression, the sample was loaded in a state of unconfined compression using porous-permeable, sintered stainless steel platens. The platens have an average pore size of 50 μm and a porosity (ratio of void volume to total volume) of 0.4–0.6. Due to the high permeability of these platens (≈ 10⁻¹¹ m⁴/Ns, approximately 1000 x the tissue permeability), the
unconfined configuration permitted all surfaces of the specimen free access to the culture medium. From previous studies on the diffusion rates of solutes, low molecular weight solutes diffuse rapidly into the tissue [7, 12, 25, 31]. Assuming that the porous platens are freely permeable, a sample thickness of 1 mm has a governing distance of 0.5 mm, corresponding to a diffusion time of less than a few minutes [7, 25, 31].

EXPLAN T HARVESTING AND CULTURE

Full-thickness discs of articular cartilage (~1–1.5 mm thick) were harvested from the central region of carpometacarpal joints of 4–5-month-old calves within 3–4 h post mortem. In a sterile environment, samples were punched out using a 5 mm-diameter stainless steel trephine and the cartilage was removed from the underlying bone using a scalpel. Explants were maintained in culture for up to 6 days in Dulbecco’s Modified Eagle Medium supplemented with 0.1% bovine serum albumin, 50 U/ml penicillin/streptomycin, and 2 mM nonessential amino acids and glutamine, at 37°C, 5% CO₂. The media were changed daily and stored for analysis.

MECHANICAL TESTING PROTOCOL

The equilibrium stress–strain behavior of the calf articular cartilage was determined on a set of 12 independent samples using the unconfined loading configuration. Full-thickness cartilage discs were compressed between the permeable loading platens in the LODEC under unconfined compression. Seven different loads were applied sequentially to each specimen, yielding 0.001, 0.01, 0.028, 0.057, 0.1, 0.5 or 1.0 MPa. Strains were recorded once the tissue had reached equilibrium at each level of stress, which occurred rapidly at the lower levels of applied stress. At the higher levels of applied stress, equilibrium was reached within 3600 s. Because large deformations were produced at the 0.5 and 1.0 MPa levels, the equilibrium strain was also calculated using the standard finite deformation formula given by \( e = (\lambda^2 - 1)/2 \) [32], where the stretch \( \lambda = h/h_0 \), \( h \) is the equilibrium compressed thickness and \( h_0 \) is the initial thickness of the specimen. Thickness measurements were made using an apparatus which utilizes the conductivity of the tissue to indicate contact between the tissue and a measuring probe [27].

EFFECT OF STATIC LOAD ON PROTEOGLYCAN SYNTHESIS RATES

In preliminary studies, \(^{35}\)S-sulfate (\(^{35}\)SO₄) incorporation and sulfated glycosaminoglycan (S-GAG) release of the explants were measured for 7 days after harvesting to assess the stability of the culture system. Compression experiments were performed on days 2–6, at which time, the rates of \(^{35}\)SO₄ incorporation and S-GAG release in control cultures did not vary significantly (see Results). Prior to each experiment, the loading platens were ultrasonically cleaned and flame-sterilized. Explants were compressed in individual petri-dishes in 4 ml of media, and control samples, taken from the same joint surface, were cultured unloaded in an otherwise identical experiment.

Step loads were applied to the explants to yield stresses of 0.001, 0.01, 0.028, 0.057, 0.1, 0.5 or 1.0 MPa. To determine changes in the rate of proteoglycan synthesis, the tissue was compressed for 24 h and incubated in media containing 20 μCi/ml of \(^{35}\)SO₄ during the last 4 h of each compression. To determine the rate of proteoglycan synthesis, specimens were digested with papain overnight at 60°C, and the amount of \(^{35}\)SO₄ that eluted in the \( V_c \) of a PD-10 column (Pharmacia) was determined. \(^{35}\)SO₄ incorporation was normalized to the total S-GAG content of the tissue and expressed as a percentage of the rate of \(^{35}\)SO₄ incorporation in the control explants. In separate tests, to determine the ability of the tissue to recover from the compression effects, the samples were compressed for 24 h and then removed from the loading apparatus and cultured unloaded for 20 h before radiolabeling for 4 h in the presence of 20 μCi/ml of \(^{35}\)SO₄.

At compressive stresses which resulted in significant changes in the biosynthetic activity of the whole explant, the zonal variation in the response to compression was determined. Following compression, radiolabeled explants were divided into three layers using a specially designed cutting jig. The top 100–150 μm (surface zone) was first removed, and the remaining piece was then divided into two equal sections (middle and deep zones) with thickness ranging from 400–800 μm. Each zone was analyzed separately and compared to the same zone from control tissue.

EFFECT OF STATIC LOAD ON PROTEOGLYCAN RELEASE RATES

To determine the effect of static load on the rate of release of proteoglycan from articular cartilage,
media were collected for the day before compression (day 1), after the 24 h period of compression (day 2), and after the 24 h following compression (day 3). The porous filters were stirred for 2 min in the medium before collection to release any glycosaminoglycan still remaining in the filter pores. To assess proteoglycan release, the aliquots of the collected media were analyzed for S-GAG content and normalized to the total S-GAG content of the specimen. Similar results were obtained by expressing synthesis and release rates normalized to the hydroxyproline content of the cartilage. The results for S-GAG release from compressed samples on day $x$ ($x = 1, 2, 3$) (denoted by $R_{\text{test}dayx}$) were normalized to the mean release of control samples on the same day (denoted by $R_{\text{control}dayx}$). In order to account for normal variation between samples, these values for each day are expressed as percentage of the release on day 1 of the same sample, using the following equation:

\[
\text{Normalized release} = \frac{R_{\text{test}dayx}}{R_{\text{control}day1}} \times 100
\]

**DETERMINATION OF TISSUE COMPOSITION**

Samples were weighed before and after lyophilization at $-50^\circ\text{C}$ for 48 h to determine the water content (water weight/wet weight of tissue). The tissue was then digested with papain (1.25 mg/100 mg tissue) for 16 h at 60°C, and aliquots of this digest were taken for separate analyses to determine hydroxyproline and S-GAG contents. To determine the hydroxyproline content of this tissue, as a measure of collagen content, the papain digest was hydrolyzed with HCl at 107°C overnight, dried, and then analyzed by a colorimetric procedure [33].

The S-GAG content, a measure of the proteoglycan content, was determined using the dye 1,9-dimethylmethylene blue [34]. The dye was used at a concentration of 16 mg/l in formate buffer, pH 3.5. Forty microliters of sample or standard (diluted in phosphate buffered saline, pH 7.2) was added to wells of a microtiter plate, 250 μl of dye reagent was added, and the absorbance at 530 nm and 600 nm was measured rapidly (within 5 min) using a Bio-Tek EL309 microplate autoreader. A negative absorbance change is obtained at 600 nm and a positive absorbance change is given at 530 nm. The total change in absorbance was determined as the sum of the change in absorbance at 530 nm and the change in absorbance at 600 nm [35]. Chondroitin sulfate from shark cartilage was used as a standard between 5 and 50 μg/ml.

**STATISTICAL ANALYSIS**

Incorporation of $^{35}$SO$_4$ and release of S-GAG of each explant was normalized to the total S-GAG content of the sample. Data are reported as the ratio of the mean of the experimental to the mean of the control results. Statistical comparisons between samples compressed at different stresses were made using one-way analysis of variance (ANOVA). Two-way ANOVA was used to assess differences between release rates on different days vs different stresses. Post hoc comparisons were made using the Tukey Studentized Range Method and the Student–Newman–Keuls Multiple Range test. Statistical analysis was carried out using the BMDP statistical package (BMDP Statistical Software Inc., Los Angeles, CA), which reports statistical significance at the 90%, 95% and 99% confidence levels.

**Results**

**MECHANICAL TESTING**

Samples which were excised from the joint swelled in culture with a mean increase in thickness of 9.4 $\pm$ 2.6%. The equilibrium stress–strain data of the calf carpometacarpal articular cartilage obtained on the LODEC for the applied compressive stresses (0.001–1.0 MPa) in unconfined compression are shown in Fig. 2. For strains
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Surface Zone

Middle Zone

Deep Zone

linear stiffening effect, which is consistent with prior observations [6, 10, 21, 28]. The mean equilibrium strain, \( e_s \) (surface-to-surface strain), of this tissue ranged from less than 0.01 at 0.001 MPa to 0.45 at 1.0 MPa. At lower stresses, mechanical equilibrium was reached within 1200 s while at higher stresses (0.5–1.0 MPa) and with thicker samples, equilibrium was reached within 3600 s. Removal of compressive stress resulted in nearly complete reswelling of the tissue to its original thickness (95% recovery within 3600 s), except at 1.0 MPa, where the tissue reswelled to approximately 75% of its original thickness at 24 h.

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TISSUE COMPOSITION AND BIOSYNTHETIC RATES

Histological analysis of control (unloaded) samples indicated few apparent differences in the ultrastructure of the different zones of the tissue. While cells at or near the surface of the tissue appeared more flattened as has been described in adult cartilage [29], cell density appeared to be fairly uniform with depth (Fig. 3). However, significant differences were noted in the biochemical composition and biosynthetic rates of the surface zone tissue as compared to the middle and deep zones (Fig. 4(a)–(d)). S-GAG content on a

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Fig. 5. $^{35}$SO$_4$ incorporation and S-GAG release rates in control samples. Preliminary tests were performed for 7 days following harvesting of the explants to assess the stability of the culture system. All compression experiments were performed between days 2–6 following explant harvesting, during which proteoglycan synthesis rates (a) and release rates (b) did not vary significantly.

wet weight basis was significantly lower ($P < 0.01$) and water content was significantly higher ($P < 0.01$) in the surface zone. No zonal differences were detected for the hydroxyproline content on a wet or dry weight basis, or for S-GAG content on a dry weight basis. Proteoglycan biosynthetic rates (dpm/µg S-GAG) were significantly higher in the surface zone as compared to the middle and deep zones ($P < 0.01$).

EFFECT OF STATIC COMPRESSION ON PROTEOGLYCAN SYNTHESIS RATES

All experiments were performed on days 2–6 following harvesting, where it was shown that proteoglycan synthesis and release rates of control samples were constant (Fig. 5(a)). In preliminary studies, the effects of presence of the stainless steel filter for 24 h (with a 4 h radiolabeling period) were examined, and no differences were found between synthesis rates in free-swelling control samples and those maintained in contact with the filter on both sides (experimental = 92 ± 37% of free-swelling controls, $P = 0.57$, t-test). Thus all experimental results are shown normalized to the mean synthesis rates of free-swelling control samples. In samples compressed at stresses less than 0.057 MPa, the rate of $^{35}$SO$_4$ incorpora-

Fig. 6. $^{35}$SO$_4$ incorporation rates with compression and recovery. $^{35}$SO$_4$ incorporation rates, normalized to tissue S-GAG content, are presented for cartilage explants compressed at different stresses after 24 h of load and with recovery at 24 h following removal of the load. Compressive stress did not affect $^{35}$SO$_4$ incorporation rates at stresses of 0.001, 0.01 or 0.028 MPa. A significant decrease in $^{35}$SO$_4$ incorporation rates was observed in samples which were compressed at 0.057 MPa and higher. Recovery experiments suggested a 'rebound' phenomenon following loading at stresses of 0.1 and 0.5 MPa, where $^{35}$SO$_4$ incorporation rates were significantly elevated in the 24 h following compression. At 1.0 MPa, incomplete recovery was observed, suggesting tissue damage or cell death. $\square$: compression; $\blacksquare$: recovery. Values shown are mean ± s.d., N = 10–12, *$P < 0.05$ vs control, **$P < 0.01$ vs control.

EFFECT OF STATIC COMPRESSION ON PROTEOGLYCAN RELEASE RATES

In control cultures, there was a slow but steady release of S-GAG into the media at a rate of 3–5%/day of the total S-GAG in the cartilage explants (Fig. 5(b)). As with the synthesis studies, preliminary studies were performed to ensure that the filters used to compress the tissue did not alter the pattern of S-GAG exchange with surrounding media. These studies showed no significant differences in S-GAG release between free-swelling controls and samples in contact with the porous filter on both sides (experimental = 114 ± 43% of free-swelling controls, $P = 0.79$, t-test). Explants
EFFECT OF STATIC COMPRESSION ON RECOVERY OF PROTEOGLYCANS

EFFECT OF STATIC COMPRESSION ON PROTEOGLYCANS

In cartilage that was compressed and then unloaded and allowed to recover, no differences were noted in the rate of \(^{35}\)SO\(_4\) incorporation compared to controls at stresses less than 0.057 MPa (Fig. 6). Mechanical recovery of the tissue occurred in less than 3600 s, although at 1.0 MPa, recovery was not complete. At two intermediate stresses (0.1 and 0.5 MPa), recovery experiments showed a significant marked increase in the rate of \(^{35}\)SO\(_4\) incorporation 24 h following removal of the compression. At the highest stress level of the test (1.0 MPa), incorporation after 24 h of recovery remained inhibited to ~ 40% that of controls.

EFFECT OF COMPRESSION ON PROTEOGLYCANS WITH DEPTH

In control samples, \(^{35}\)SO\(_4\) incorporation rates (normalized to tissue S-GAG content) were highest in the surface layer of the tissue as compared to the middle and deep layers (Fig. 4(d)). In the compression experiments, the decreased \(^{35}\)SO\(_4\) incorporation rates were observed throughout the depth of the tissue (Fig. 8(a)). The suppression in synthesis rates seemed to be generally greater in
FIG. 8. Zonal variations in the biosynthetic response to compression (a) and recovery following compression (b). Values represent $^{35}$SO$_4$ incorporation rates in compressed tissue normalized to $^{35}$SO$_4$ incorporation rates in the same zone of control tissue. While increased suppression was consistently observed in the surface and deep zones, this trend was not statistically significant ($P > 0.05$). With recovery, no zonal patterns were apparent in the alterations of proteoglycan synthesis rates. Values show mean ± s.d., $N = 10-12$, *$P < 0.05$ vs other zones. 

Discussion

Deformation of the extracellular matrix, and the associated changes in tissue hydration, macromolecular concentration, interstitial pH and cell shape have been suggested as mechanisms by which the chondrocytes may perceive changes in their mechanical environment under static compression [6, 9, 12, 13, 23]. In the unconfined compression configuration, the deformation field within the explant is highly nonuniform at the onset of loading, but as creep progresses with time, an equilibrium state is reached as interstitial fluid flow ceases [36]. At mechanical equilibrium, the effects of matrix deformation can be studied without the confounding factors caused by fluid flow, hydraulic fluid pressure, streaming potentials or other kinetic phenomena. At lower stresses, where assumptions of linear elasticity and infinitesimal strain are still valid (< 20% strain), the equilibrium stress–strain distribution is nearly uniform throughout the radius up to a thin boundary layer near the periphery of the specimen and near the tissue-filter interface [36]. At higher stresses which result in finite deformation of the matrix, the strain distribution within the tissue is more inhomogeneous, characterized by higher dilatation and shear strains at the tissue–filter interface due to radial expansion and frictional effects from the porous filters (F Guilak and JK Suh, unpublished results). Because complex stress and strain fields are developed within the explants, interpretation of changes in metabolic activity due to compressive loading is difficult since it is not known which of the mechanically-induced signals the chondrocytes are actually responding to, even in the equilibrium condition.

Our results showed a decrease in proteoglycan synthesis and proteoglycan release rates with increasing compressive stress, similar to previously reported results [6, 9, 11, 12, 14]. These findings are consistent with several possible mechanisms of cellular transduction, including decreased interstitial water content, increased concentrations of tissue macromolecules and alterations in the pericellular pH [6, 9, 12, 13]. This hypothesis is consistent with the observation that a decrease in proteoglycan content of the tissue results in increased proteoglycan synthesis rates [37]. These findings suggest that chondrocytes may have the ability to utilize load-induced changes in the physicochemical environment of the tissue as a regulatory signal for control of their metabolic activity, and ultimately, the composition of their surrounding extracellular matrix [18].

Mechanically-induced alterations in chondrocyte shape have also been proposed as a possible regulator of cartilage activity [23, 38–40]. In cell culture studies, chondrocyte shape has been shown to play a role in gene expression, and cell size as well as sphericity have been associated with chondrogenesis [41]. Cellular deformation has been hypothesized to regulate metabolic activity through several possible mechanisms.
[42, 43], such as stretch-activated ion channels [40, 44] and cytoskeletonally-mediated deformation of intracellular organelles [45]. While previous studies have shown that chondrocytes undergo both shape and volume changes during compression of the extracellular matrix [39], the pathway through which these phenomena are transduced to a biochemical signal which regulates specific gene transcription is yet to be determined.

Cartilage explant recovery following removal of load after a period of compression also showed a dose dependency effect for both synthesis and proteoglycan release. In previous studies, suppression of proteoglycan synthesis rates was noted in the first 4–5 h following removal of compression [7, 10], followed by increased synthesis rates from 8–48 h following removal of compression [9, 10]. The findings of the present study are consistent with this 'rebound' phenomenon, which was observed 20–24 h following removal of compression at stresses of 0.1–0.5 MPa. Although previous studies have noted an increase in proteoglycan release rates during recovery from static compression [9, 11], in this study continued suppression of proteoglycan release was shown. One potentially important difference is that previous investigations have studied the release of newly synthesized proteoglycan, whereas our study examined the effect on the total proteoglycan population.

At 1.0 MPa compression, proteoglycan synthesis rates were greatly inhibited, and removal of this stress for 24 h did not result in complete recovery. Incomplete reswelling of the tissue following this level of stress implies that ultrastructural damage may have occurred to the extracellular matrix and/or to the chondrocytes, and may indicate a sustained decrease in tissue pore size after removal of compression. While chondrocytes have been shown to recover their morphology following 20–30% compressive strain [23, 38, 39], preliminary studies in our laboratory suggest that at this higher level of strain, a decrease in cell viability may have been responsible for the continued suppression of proteoglycan synthesis rates.

One suggested mechanism for these observed effects is that compression of cartilage will impede solute and proteoglycan diffusion in cartilage, as the diffusion coefficient of small solutes in the cartilage matrix has been shown to depend on both interstitial ion concentration and matrix porosity [24, 25]. For uncompressed explants, the diffusion time constant for small solutes is related to the square of the characteristic distance (tissue thickness) [25, 31]. For explants which are ~ 1 mm thick, this time constant will be of the order of several minutes, which will have little effect on the labeling procedure [7, 25], even in compressed tissue [12]. As regards proteoglycan degradation, the continued suppression of S-GAG release on removal of the compressive load at the high end of our loading range suggests that altered diffusion rates were not responsible for the observed effects. Rather, this finding suggests that an alternative, chondrocyte-mediated mechanism may have been involved at 0.1–0.5 MPa, where nearly complete reswelling of the tissue occurred. The mechanism used for the breakdown of aggregating proteoglycans in cartilage is likely to be, at least in part, caused by the activities of matrix metalloproteinases that are secreted by the cells [46]. A molecular mechanism that results in proteoglycan loss from cartilage is a cleavage of the protein core of aggregating proteoglycan between the G1 domain (involved in aggregation) and the glycosaminoglycan containing region [16, 17, 47, 48]. Thus it seems likely that compression of the tissue at intermediate stress levels (0.1–0.5 MPa) results in a cellular response which reduces the rate of this mechanism, possibly by reducing the synthesis or activation of the metalloproteinases. At higher stresses (1.0 MPa), there is potential for physical damage of the cells, which would result in the release of proteases from the cells into the extracellular matrix and thus lead to accelerated proteoglycan release. Although a suppression of proteoglycan release was still detected at 1.0 MPa, the true effect of reduced specific proteoglycan breakdown may be somewhat masked by the potentially increased release of proteases from lysed cells.

Interestingly, we found that the changes in the rate of proteoglycan synthesis as caused by equilibrium compression did not vary significantly with depth through the tissue. Yet the structure and composition of the extracellular matrix, as well as the biosynthetic rates, morphology and density of the chondrocytes, do vary at different depths in the cartilage, especially in adult tissue [25, 26, 29]. Cartilage permeability [25] and tensile properties [27] also vary with depth, and there is evidence that, in adult tissue, the compressive modulus is lower in the surface and deep zones as compared to the middle zone [28]. Further, at higher applied stresses, frictional effects will result in larger strains at the interface between the tissue and the loading platen. In nearly all samples compressed at 0.1 and 0.5 MPa, a weak but consistent trend of several minutes, which will have little effect on the labeling procedure [7, 25], even in compressed tissue [12]. As regards proteoglycan degradation, the continued suppression of S-GAG release on removal of the compressive load at the high end of our loading range suggests that altered diffusion rates were not responsible for the observed effects. Rather, this finding suggests that an alternative, chondrocyte-mediated mechanism may have been involved at 0.1–0.5 MPa, where nearly complete reswelling of the tissue occurred. The mechanism used for the breakdown of aggregating proteoglycans in cartilage is likely to be, at least in part, caused by the activities of matrix metalloproteinases that are secreted by the cells [46]. A molecular mechanism that results in proteoglycan loss from cartilage is a cleavage of the protein core of aggregating proteoglycan between the G1 domain (involved in aggregation) and the glycosaminoglycan containing region [16, 17, 47, 48]. Thus it seems likely that compression of the tissue at intermediate stress levels (0.1–0.5 MPa) results in a cellular response which reduces the rate of this mechanism, possibly by reducing the synthesis or activation of the metalloproteinases. At higher stresses (1.0 MPa), there is potential for physical damage of the cells, which would result in the release of proteases from the cells into the extracellular matrix and thus lead to accelerated proteoglycan release. Although a suppression of proteoglycan release was still detected at 1.0 MPa, the true effect of reduced specific proteoglycan breakdown may be somewhat masked by the potentially increased release of proteases from lysed cells.
observed effects. At 1.0 MPa, the significant suppression of $^{35}$S$^{4}$ incorporation rates of the surface zone seems to be due to cell necrosis and tissue damage at the surfaces in contact with the porous loading platen.

While specific zonal variations in the composition and biosynthetic rates of the control tissue were observed with depth, these patterns did not correlate with the observed patterns of suppression of proteoglycan synthesis rates in the compressed tissue. These findings suggest that, in young cartilage, tissue composition is not a strong determinant of chondrocyte response to load. Alternatively, the zonal variations in the properties of the extracellular matrix and the chondrocyte mechanical environment may be such that, under static compression, the mechanical environment of the chondrocyte remains relatively uniform in the different zones. In previous studies, it has been shown that chondrocyte response to compression is significantly greater in the middle-zone of adult articular cartilage, where tissue fixed charge density is the highest [12]. These differences may be due to the more pronounced heterogeneity of adult cartilage [29] or chondrocytes [26], and thus an important consideration is that the present study was performed on articular cartilage from relatively young animals (4–5 months old). Extrapolation of the data to mature articular cartilage should be done with caution, since the activities of chondrocytes from cartilage of different ages can vary.

At present, it is unclear how physiological loading of articular cartilage will affect the stress, strain, pressure (hydraulic and osmotic), fluid flow and electrokinetic environment around the individual chondrocyte, or how individual chondrocytes metabolically respond to changes of these physical quantities. One possibility is to examine changes in the chondrocyte mechanical environment and response to mechanical stimulation on a single cell basis [23, 40]. With this knowledge one may hypothesize that physiological changes in quantities such as intracellular strain, membrane stretch, fluid shear stresses or changes in the pericellular fixed charge density may serve as signals to modulate chondrocyte metabolic response. These precisely defined physical quantities (and others) offer new avenues in search of the mechanical and physicochemical transduction signals responsible for modulating chondrocyte activity.

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