INTRODUCTION:
The development and maintenance of healthy articular cartilage is regulated by both biomechanical and biochemical stimuli [1,2]. Dynamic compression [3] and shear deformation [4] have been shown to stimulate the production of matrix proteoglycans and proteins by chondrocytes. Peptide growth factors such as IGF-I have also been shown to be important regulators of chondrocyte metabolism [2]. Recent studies have indicated that dynamic compression accelerates and augments the biosynthetic response of chondrocytes to IGF-I [5]. These effects of dynamic compression appear to be, at least in part, due to increased fluid flow and augmentation of IGF-I transport into the cartilage matrix [5]. Our objectives in this study were (1) to determine the combined effects of dynamic tissue shear deformation and IGF-I on matrix biosynthesis by chondrocytes in intact cartilage explants, (2) to quantify the dependence of the rate of biosynthesis on shear strain amplitude, and (3) to determine whether shear strain with little or no accompanying fluid flow can augment transport of IGF-I into cartilage and thereby regulate the biosynthetic response of chondrocytes to IGF-I.

METHODS:
Cartilage Explant and Culture: Cartilage disks (3 mm dia., 1 mm thick) were obtained from the femoropatellar groove of 1-2 week old calves and equilibrated free swelling in serum-free DMEM with 10 mM Hepes, 0.1 mM nonessential amino acids, 0.4 mM l-proline, 20 μg/ml ascorbate, 100 U/ml penicillin, and 100 μg/ml streptomycin in a 24 well plate for 2-3 days at 37°C in 5% CO2 atmosphere. Combined Mechanical & IGF-I Stimulation: Cartilage disks were assigned to either static control or shear groups. Static control disks were placed in polysulphone chambers and compressed between impermeable plates to the original cartilage cut thickness of 1 mm (0% offset) for 24 hours [3]. Shear stimulated disks were held at the same 0% offset compression in a similar polysulphone chamber which was added to the culture medium immediately prior to the start of loading. After dynamic shear, the disks were washed, digested with proteinase K, and the digests was added to the culture medium immediately prior to the start of loading. After 24 hr of loading, disks were radiolabeled with 10 μCi/ml [3H]proline and 5 μCi/ml [35S]sulfate as measures of protein and proteoglycan synthesis, respectively. To determine the combined effects of dynamic tissue shear deformation and IGF-I on matrix biosynthesis by chondrocytes in intact cartilage explants, (2) to quantify the dependence of the rate of biosynthesis on shear strain amplitude, and (3) to determine whether shear strain with little or no accompanying fluid flow can augment transport of IGF-I into cartilage and thereby regulate the biosynthetic response of chondrocytes to IGF-I.

RESULTS:
Dose-dependent stimulatory effect of shear strain amplitude on cartilage biosynthesis: Radiolabel incorporation into shear-stimulated disks (pmol/μg-DNA/hr) was normalized to that in control disks (control value = 1 in Fig.1). Dynamic tissue shear at all strain amplitudes above 2% (f = 0.1 Hz) significantly stimulated protein and proteoglycan synthesis by ~30% and ~20%, respectively, in serum free medium with no IGF-I. GAG biosynthesis was not significantly altered in disks subjected to less than 2% shear strain, though protein synthesis appeared lower at 0.5 and 1%. Effects of IGF-I and shear on biosynthesis: Two-way ANOVA indicated that IGF-I and dynamic shear compression each significantly (p<0.01) increased biosynthetic activity, with no significant interaction between the stimuli (p=0.85). Effect of shear on IGF-I transport: The total uptake of 125I-labeled IGF-I into cartilage disks at each time (Fig. 3) was characterized by fitting the data to an exponential (first order kinetics) equation. The characteristic exponential time constant (τ) for 125I-IGF-I transport was calculated for disks in the presence or absence of shear strain. The time constant for static control (11 hr) and shear (10.7 hr) disks were not significantly different from each other (Fig. 3).

DISCUSSION:
Mechanical deformation of chondrocytes within intact cartilage explants caused by applied tissue shear stimulated protein and proteoglycan synthesis at shear strains above 2%. This pattern of stimulation is consistent with a threshold effect of shear strain on matrix biosynthesis by chondrocytes. The anabolic effects of the combination of shear deformation and IGF-I were additive, with no interaction between the stimuli (Fig.2A & 2B). These data, coupled with the observation that each stimulus enhanced the maximal effect of the other, suggest that mechanical shear and IGF-I act on articular chondrocytes via separate biological pathways. This lack of interaction between biomechanical and biochemical stimuli was also found in previous studies of IGF-I with both static [6] and dynamic [5] compression. In the present study, however, there was no temporal acceleration of the effect of IGF-I by tissue shear deformation, as indicated by the similar time constants for the transport of IGF-I into the cartilage in the control (11 hr) and shear (10.7 hr) groups (Fig.3). This is in contrast to studies demonstrating accelerated transport of IGF-I into cartilage explants caused by dynamic mechanical compression [5], and is consistent with the much lower levels of fluid flow induced by simple shear deformation in the present configuration [4,7] compared with that produced by dynamic compression.

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REFERENCES

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