Available online at www.sciencedirect.com
SCIENCE DIRECT®
Archives of Biochemistry and Biophysics 414 (2003) 223–231
ABB
www.elsevier.com/locate/yabbi

Combined effects of dynamic tissue shear deformation and insulin-like growth factor I on chondrocyte biosynthesis in cartilage explants

Moonsoo Jin,[a] Greg R. Emkey,[a] Patrick Siparsky,[a] Stephen B. Trippel,[b] and Alan J. Grodzinsky[a,*]

[a] *Continuum Electromechanics Group, Center for Biomedical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*
[b] *Department of Orthopaedic Surgery, 541 Clinical Dr., Suite 600, Indiana University, Indianapolis, IN 46202, USA*

Received 16 December 2002, and in revised form 1 April 2003

Abstract

Biophysical forces and biochemical factors play crucial roles in the maintenance of the integrity of articular cartilage. In this study, we explored the effect of dynamic tissue shear deformation and insulin-like growth factor I (IGF-I) on matrix synthesis by chondrocytes within native cartilage explants. Dynamic tissue shear in the range of 0.5–6% strain amplitude at 0.1 Hz was applied to cartilage explants cultured in serum-free medium. Dynamic tissue shear above 1.5% strain amplitude significantly stimulated protein and proteoglycan synthesis, by maximum values of 35 and 25%, respectively, over statically held control specimens. In the absence of tissue shear, IGF-I augmented protein and proteoglycan synthesis up to twofold at IGF-I concentrations in the range of 100–300 ng/ml. When tissue shear and IGF-I stimuli were combined, matrix biosynthesis levels were significantly higher than the maximal effect caused by either stimulus alone. However, there was no significant interaction between tissue shear and IGF-I as determined by two-way ANOVA. We then quantified the effect of dynamic tissue shear on the transport of IGF-I into and within cartilage explants. [^{125}I]IGF-I was added to the medium, and the levels of intratissue [^{125}I]IGF-I were directly measured as a function of time over 48 h in the presence and absence of continuous dynamic shear strain. Dynamic shear did not alter the rate of uptake of [^{125}I]IGF-I into the explants, suggesting that convective diffusion of [^{125}I]IGF-I is negligible under the shear strain conditions used. This is in marked contrast to the enhancement of transport reported in response to uniaxial dynamic compression [1]. Taken together, these data suggest that (1) the stimulatory effect of tissue shear is via mechanotransduction pathways and not by facilitated transport of biochemical factors and (2) chondrocytes may possess complementary signal transduction pathways for biophysical and biochemical factors leading to changes in metabolic activity.

Keywords: Cartilage; Tissue shear; IGF-I; Transport; Proteoglycan

Articular cartilage experiences a complex combination of compressive deformation and shear loading during the articulation of synovial joints. Due to the avascular, alymphatic, and aneural nature of cartilage, mechanical loading in vivo is considered to be a potent regulator of cellular processes that regulate cartilage development and homeostasis. In addition to such biophysical forces, numerous studies have demonstrated that soluble factors such as insulin-like growth factor (IGF)[1] I and II and transforming growth factor-beta can stimulate chondrocyte biosynthesis [2,3] while retinoic acid, interleukin 1 (IL-1), and tumor necrosis factor can induce catabolic enzymes and inhibit matrix synthesis [4,5].

The availability of these soluble factors to the chondrocyte is affected by their endogenous concentrations within cartilage and by transport into the tissue from the

* Corresponding author. Fax: 1-617-258-5239.
E-mail address: alg@mit.edu (A.J. Grodzinsky).

[1] *Abbrevations used:* IGF, insulin-like growth factor; IL-1, interleukin 1; IGFBP, IGF-binding protein; PBS, phosphate-buffered saline.

0003-9861/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0003-9861(03)00195-4

synovial fluid [6]. Among the growth factors, IGF-I, which is a 7.6-kDa polypeptide in its free form, has been shown to stimulate the synthesis of matrix components and inhibit catabolic effects induced by IL-1 and retinoic acid in cartilage [7,8]. IGF-I is synthesized in the liver and can act in an endocrine manner. In addition, it has been shown that chondrocytes synthesize IGF-I, IGF-I-receptor proteins that mediate IGF-I signaling, and IGF-binding proteins (IGFBPs) that limit the access of IGF-I to IGF-I receptor [6]. This autocrine mechanism provides a regulatory axis for IGF-I–IGF-I receptor–IGFBP [6] in the stimulatory effect of IGF-I on chondrocytes.

With regard to biophysical factors, dynamic compressive deformation has been shown to stimulate the production of matrix proteoglycans and proteins by chondrocytes while static compression decreases matrix biosynthesis in a dose-dependent manner [9]. These regulatory effects of mechanical compression are related to biophysical changes, including cell and matrix deformation, changes in intratissue pH and charge density, intratissue fluid flow, and pressure gradients [10]. Biophysical and biochemical mediators may also interact. For example, cyclic compression of cartilage has been shown to affect the transport of soluble factors and nutrients in cartilage [11]. While the transport of small molecules (e.g., urea, NaCI) was mostly due to diffusion along concentration gradients, the transport of larger molecules (e.g., serum albumin, 68 kDa) was strongly facilitated by interstitial fluid flow caused by the cyclic loading [11].

Previous studies have indicated that dynamic compression both augments the magnitude and accelerates the time course of the biosynthetic response of chondrocytes to IGF-I [1]. These effects of dynamic compression were due, in part, to the augmentation of IGF-I transport into the cartilage matrix caused by compression-induced intratissue fluid flow [1]. Recently, dynamic tissue shear deformation has also been shown to stimulate the synthesis of matrix proteoglycans and proteins in bovine cartilage [12]. While dynamic tissue shear strain also causes deformation of cells and matrix within cartilage [12], theoretical studies [13] predict that such tissue shear causes relatively little intratissue fluid flow compared to the levels of fluid flow produced by *compressive* strains and strain rates of similar magnitude. Thus, the respective roles of deformation, fluid flow, and growth factor transport in articular chondrocyte biosynthesis remain unclear.

Therefore, the objectives of this study were (1) to quantify the effects of graded levels of dynamic tissue shear strain and, in parallel experiments, graded levels of IGF-I concentration on chondrocyte biosynthesis in bovine cartilage explants, (2) to assess the combined effects of dynamic tissue shear deformation and IGF-I on matrix biosynthesis, and (3) to quantify the effect of tissue shear deformation on the transport of IGF-I into bovine cartilage explants to delineate whether the stimulatory effect of tissue shear is partly due to the facilitated transport of the growth factor into the tissue.

Materials and methods

Cartilage explant and culture

Cartilage explants were obtained from the femoropatellar groove of 1- to 2-week-old bovine calves. After cylindrical specimens of cartilage with underlying bone were drilled, one to three slices (10 mm diameter by 1 ± 0.05 mm thickness) were obtained from the middle zone of the drilled specimens. Then four to six disks (3 mm diameter) were punched from each slice and matched disks were distributed across different conditions described below. These disks were maintained in serum-free, high-glucose (4.5 mg/ml) Dulbecco's modified essential medium supplemented with 10 mM Hepes, 0.1 mM nonessential amino acids, additional 0.4 mM proline, 20 μg/ml ascorbate, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B in a 24-well plate at 37 °C in 5% CO_2 atmosphere for 2 days before they were subjected to mechanical loading or IGF-I (Fig. 1A).

Shear loading and IGF-I stimulation

Cartilage disks were assigned to either static control or shear treatment groups. Control disks were placed in polysulfone chambers (Fig. 2A) and maintained at their original cut thickness of 1 mm (0% offset) between

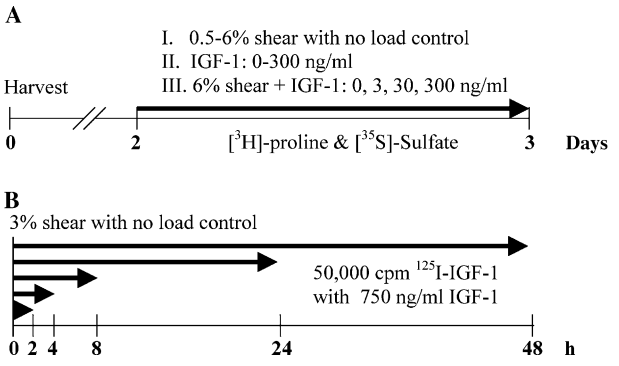

Fig. 1. Experimental design of biosynthesis and transport studies. (A) Cartilage specimens were exposed to (1) dynamic shear deformation in the range of 0.5–6% with no shear control, (2) IGF-I in the range of 0–300 ng/ml or (3) dynamic shear at 6% and IGF-I combined. Culture media was supplemented with [^{3}H]proline and [^{35}S]sulfate as measures of total protein and proteoglycan synthesis. (B) The effect of dynamic tissue shear on IGF-I transport into and within cartilage tissue was assessed by applying shear deformation for 2, 4, 8, 24, and 48 h in the presence of labeled and unlabeled IGF-I in culture media and measuring the radioactivities.

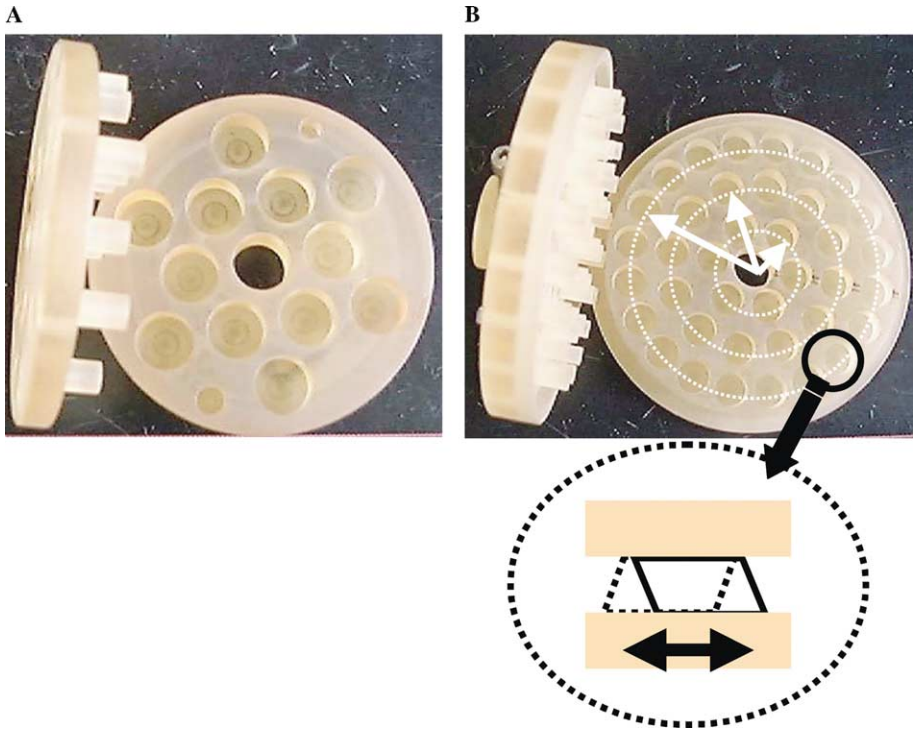

Fig. 2. Chambers that were used for control and shear loading. (A) Control disks were placed in each well of the bottom half and statically compressed between impermeable platens to their original cut thickness of 1 mm. (B) The chamber for shear loading contained three groups of wells located at radii of 0.5, 1, and 1.5 inch from the center of the chamber. This configuration enabled simultaneous application of three different magnitudes of shear strain with a ratio of 1:2:3. The shear chamber was mounted into a tissue loading device, and the bottom half was cyclically rotated with respect to the top half (inset), which applied simple shear to cartilage disks.

impermeable platens for 24 h. Shear-treated disks were held at the same 0% offset compression in a special polysulfone chamber (Fig. 2B) that contained three groups of wells located at radii of 0.5, 1, and 1.5 inch from the center of the chamber. This configuration enabled simultaneous application of three different magnitudes of shear strain with a ratio of 1:2:3, respectively. The shear chamber was mounted into an incubator-housed tissue-loading instrument. Sinusoidal shear strain amplitudes from 0.5 to 6.0% were applied at a frequency of 0.1 Hz for 24 h [13].

To assess the stimulatory effect of IGF-I on matrix synthesis in this explant system, cartilage disks were maintained at 0% compressive offset within the static control chamber, and recombinant human IGF-I (Peprotech, NJ) was added to the media to achieve final concentrations of 0, 3, 30, 100, 150, 200, 250, or 300 ng/ml. To test the combined effects of dynamic shear and IGF-I on biosynthesis, cartilage disks were subjected to either 0% static control or dynamic sinusoidal shear at 6% strain amplitude in the presence of 0, 3, 30, or 300 ng/ml of IGF-I, which was added to the culture medium immediately prior to the start of loading. During the 24-h loading period, disks were radiolabeled with 10 μCi/ml [^{3}H]proline and 5 μCi/ml [^{35}S]sulfate as measures of protein and proteoglycan synthesis, respectively.

Biochemical analysis

After loading, shear and control disks were washed three times over 1 h in phosphate-buffered saline (PBS) supplemented with 0.8 mM sodium sulfate and 0.5 mM L-proline at 4 °C to remove unincorporated radiolabel. All samples were digested in 1.0 ml protease K (100 μg/ml in 50 mM Tris–HCl and 1 mM $CaCl_2$, pH 8.0) for 12–18 h at 60 °C; 100-μl aliquots of the protease K digests were mixed with 2 ml of scintillation fluid for radioactivity counting (RackBeta 1211 counter, Pharmacia LKB Nuclear, Turku, Finland) and the counts were corrected for spillover. For the measurement of DNA, 100 μl of the digests was mixed with 2 ml of the Hoechst 33258 dye in an acrylic cuvette and the fluorescence was measured using a spectrofluorometer (SPF 500C, SLM Instruments, Urbana, IL). Radiolabel incorporation rates were thereby normalized to the DNA content and the duration of loading (Figs. 3–5). The fluid content of each disk was determined from the difference between wet and dry weights, measured before and after loading, respectively.

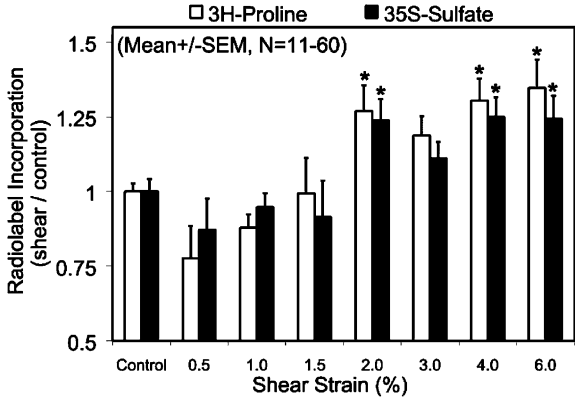

Fig. 3. Stimulatory effect of tissue shear deformation. After 2–3 days in free swelling culture in serum-free media, groups of 11–60 cartilage disks obtained from 10 animals were subjected to shear strains between 0.5 and 6% for 24 h with anatomically matched no-shear control disks. Continuous monitoring of the shear stress waveform ensured that appropriate shear deformation without slippage has been applied to the specimens (see detailed description [12,13]). Using the shear chamber of Fig. 2B with five or six disks placed in each of the three strain amplitude conditions, 2 experiments were performed for 0.5, 1, and 1.5% strain conditions (with matched controls), 3 experiments for 1, 2, and 3% strain (with controls), and 5 experiments for 2, 4, and 6% strain (with controls). The levels of biosynthesis in the matched control groups from these 10 independent experiments were not significantly different from each other ($p > 0.8$; one-way ANOVA). Therefore, radiolabel incorporation in shear-stimulated disks (pmol/μg DNA/h) was normalized to the average of control disks from each individual experiment. Proline and sulfate incorporation were found to depend on shear strain amplitude ($p < 0.001$; one-way ANOVA). Dynamic tissue shear at 2, 4, and 6% strain amplitudes stimulated protein synthesis by 30–35% and proteoglycan synthesis by 20–25% compared to static controls ($p < 0.05$; Tukey's test).

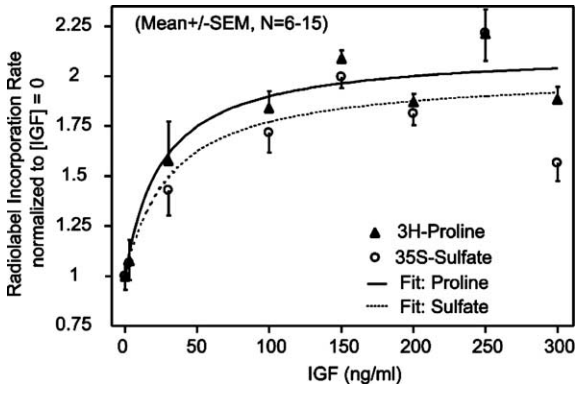

Fig. 4. Stimulatory effect of IGF-I: Cartilage disks were maintained at 0% static compression and IGF-I was added to the culture media at 0, 3, 30, 100, 150, 200, 250, and 300 ng/ml. The levels of matrix biosynthesis (pmol/μg DNA/h) of IGF-I-stimulated disks were normalized to the level of no-IGF-I control disks. A sigmoidal dose–response equation was fit to the measured normalized proline and sulfate incorporation vs IGF-I concentration. IGF-I alone increased proline and sulfate incorporation in a dose-dependent manner up to approx twofold by 100 to 150 ng/ml IGF-I. However, further increases in medium IGF-I concentration did not significantly alter matrix biosynthesis.

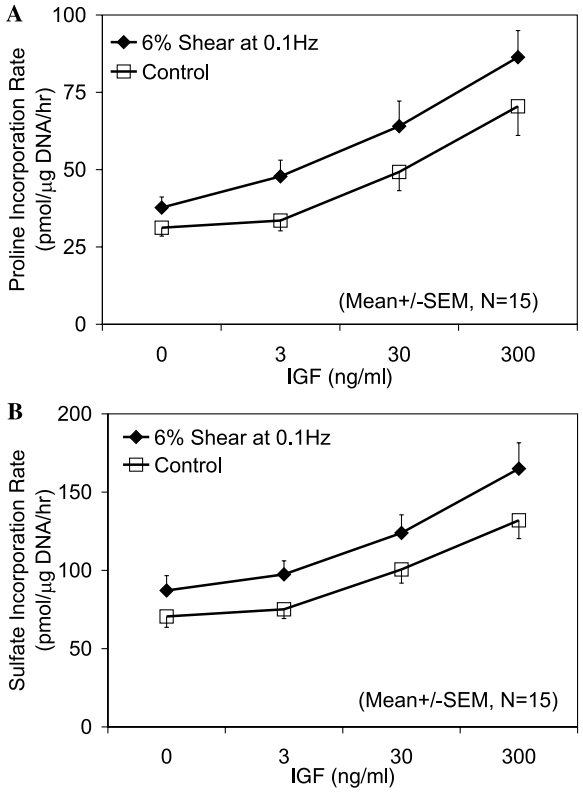

Fig. 5. Combined effects of IGF-I and shear on total protein (A) and proteoglycan (B) synthesis: Cartilage disks were subjected to either 0% static control or dynamic shear deformation at 6% strain in the presence of 0, 3, 30, or 300 ng/ml of IGF-I. IGF-I stimulated total protein and proteoglycan synthesis by around twofold in its maximal effect both for shear-stimulated disks and for no- shear control. Tissue shear further increased the level of biosynthesis by around 25–35%. Two-way ANOVA indicated that IGF-I and dynamic tissue shear each significantly ($p < 0.01$) increased biosynthesis, but with no significant interaction between the stimuli ($p > 0.8$).

IGF-I transport studies

The effect of dynamic tissue shear deformation on IGF-I transport into cartilage was assessed by adding [^{125}I]IGF-I to the medium and measuring uptake of this tracer into cartilage disks at varying time periods compared to uptake into no-shear control disks. Initial equilibration of the [^{125}I]IGF-I (a gift from JJ Van Wyk and LE Underwood, University of North Carolina) with an anion exchange resin (AG 501-X8, Bio-Rad) was used to remove any small-molecular-weight radiolabel from the solution containing macromolecular [^{125}I]IGF-I prior to addition to the explant medium. Disks were subjected to shear (3% shear strain amplitude at 0.1 Hz) or no shear (control) for 2, 4, 8, 24, and 48 h in culture medium containing ~50,000 CPM/ml [^{125}I]IGF-I and 750 ng/ml unlabeled IGF-I that had been added to the medium just before loading (Fig. 1B). The amount of labeled IGF-I added to the medium was less than 1% of unlabeled IGF-I, given the specific activity of approximately 100 μCi/μg. At the

end of each loading period, disks were collected and washed with cold PBS briefly to remove excess media on the surface of disks. Then each disk was digested with 1.0 ml protease K solution as described previously. The amount of [^{125}I]IGF-I within the tissue and in the culture medium was measured by counting 100-μl aliquots of each digest and medium sample mixed with 900 μl of PBS in a gamma counter (Auto Gamma, Packard Instruments, CT). Tissue concentration of [^{125}I]IGF-I was calculated as the total amount of [^{125}I]IGF-I in each digest divided by fluid content of the explant (measured as the difference between wet and dry weight).

Transport models

IGF transport into the tissue was modeled using the first order kinetics equation for the IGF-I concentration [IGF-I] within the tissue at time t ($[\text{IGF-I}]_t$) with respect to the concentration after long-term equilibration $[\text{IGF-I}]_\infty$ and the equilibration time constant τ:

$$[\text{IGF-I}]_t = [\text{IGF-I}]_\infty(1 - \exp(-t/\tau)). \quad (1)$$

This equation is further modified as

$$[\text{IGF-I}]_i/[\text{IGF-I}]_o = c_1(1 - c_2\exp(-t/\tau)), \quad (2)$$

where $[\text{IGF-I}]_i$ represents the intratissue concentration of IGF-I at a given time and $[\text{IGF-I}]_o$ the concentration of IGF-I in the medium (which is maintained constant). In final equilibrium as time t approaches infinity, c_1 represents the ratio of intratissue to medium IGF-I concentration, often called the partition coefficient. During the initial transient phase, the constant c_2 accounts for the slight time difference between the onset of loading and the addition of radiolabel to the medium and factors not directly related to diffusive transport, such as possible adsorption of IGF-I to the surface of cartilage specimen. Eq. (2) was fit to the [^{125}I]IGF-I transport data; best fit values for the three constants, c_1, c_2, and τ, were determined as the set of parameters that yielded a minimum of the sum of squares of the differences between the data and the model, divided by the square of the standard deviation at each time point (see Appendix).

Statistical analyses

Sample quantities are expressed as mean ± SEM. The effect of varying shear strains on matrix synthesis was assessed by one-way ANOVA followed by Tukey's multiple comparison test. Two-way ANOVA was performed to test the significance of the stimulatory effect of shear and IGF-I and the interactions between the two. Power analysis on ANOVA was performed according to Cohen [14]. To assess the effect of shear on the IGF-I transport, the Student t test was performed on the obtained parameter set using the confidence intervals detailed in the Appendix. For all statistical analyses, differences or interactions were considered significant at $p < 0.05$.

Results

Separate effects of tissue shear and IGF-I on chondrocyte matrix synthesis

Proline and sulfate incorporation were found to depend on shear strain amplitude ($p < 0.001$; one-way ANOVA). Dynamic tissue shear at 2, 4, and 6% strain amplitudes stimulated protein synthesis by 30–35% and proteoglycan synthesis by 20–25% compared to static controls ($p < 0.05$; Tukey's test) (Fig. 3). Proteoglycan and protein biosynthesis were not significantly altered in disks subjected to less than 2% shear strain.

Treatment of cartilage disks with IGF-I alone increased both protein and proteoglycan synthesis in a dose-dependent manner up to approximately twofold at a concentration of 100 to 150 ng/ml IGF-I (Fig. 4). Consistent with trends reported previously [1,6,15], the stimulatory effect of IGF-I on chondrocyte biosynthesis plateaued at concentrations above 150 ng/ml (Fig. 4).

Combined Effects of IGF-I and shear on chondrocyte matrix biosynthesis

To explore the combined effects of tissue shear and IGF-I, we used 0, 3, 30, and 300 ng/ml IGF-I in the presence or absence of 6% shear strain amplitude at 0.1 Hz, the shear loading conditions that induced maximal stimulation of matrix biosynthesis (Fig. 3). At all concentrations of IGF-I, tissue shear further stimulated both proteoglycan and total protein synthesis at all levels of IGF-I (Fig. 5). This additional increase in matrix synthesis due to shear deformation was apparent even at 300 ng/ml of IGF-I, a concentration that was maximally stimulatory in the absence of shear. Two-way ANOVA indicated that IGF-I and dynamic tissue shear each significantly ($p < 0.01$) increased biosynthesis of proteins and proteoglycans, but with no significant interaction between the stimuli ($p > 0.8$, power > 0.95), indicating a lack of synergistic interaction between the dynamic tissue shear and the IGF-I under these conditions.

Effect of tissue shear on IGF-I transport

The transport of [^{125}I]IGF-I into cartilage disks was measured after 2, 4, 8, 24, and 48 h of incubation. Disks were either maintained statically at their cut thickness or subjected to 3% dynamic shear strain at 0.1 Hz. The

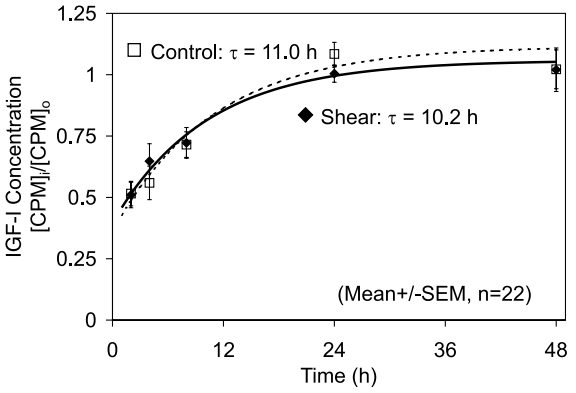

Fig. 6. Effect of tissue shear on IGF-I transport. The transport of [^{125}I]IGF-I into cartilage disks was measured after 2, 4, 8, 24, and 48 h of incubation in medium containing [^{125}I]IGF-I and unlabeled IGF-I; disks were either maintained statically at their cut thickness or subjected to 3% shear strain amplitude at 0.1 Hz. The ratio of IGF concentration in the tissue to that in the medium was plotted, and the best fit for the transport time constant of Eq. (2) was not significantly different between shear and control groups.

ratio of [^{125}I]IGF-I concentration in the tissue to that in the medium at each time point was plotted, and the model of Eq. (2) was fit to the data (Fig. 6) to obtain the set of parameters (c_1, c_2, τ) with a confidence interval of ± 1 standard deviation. The best fit parameters for the control disks were $c_1 = 1.1 \pm 0.05$, $c_2 = 0.7 \pm 0.05$, and $\tau = 11.0 \pm 2.4$, and for sheared disks $c_1 = 1.1 \pm 0.04$, $c_2 = 0.6 \pm 0.05$, and $\tau = 10.2 \pm 2.7$ under the hypothesis (H1) that transport models (Eq. (2)) for the shear and control groups are significantly different (see Appendix; note that from Eq. (2), the constants c_1 and c_2 are dimensionless and τ has the unit of hours). The null hypothesis (H0) is that the two transport models are not different. To test whether the likelihood of the observed outcome by a given model is significantly improved under the hypothesis H1 over H0, we first calculated the set of parameters for the combined group obtained by pooling the shear and control data. This gave $c_1 = 1.1 \pm 0.03$, $c_2 = 0.7 \pm 0.04$, and $\tau = 10.7 \pm 1.83$. Then, from the ratio of the maximum likelihood given H1 to the likelihood given H0, the significance was calculated to be $p = 0.23$. This result suggests that there is no significant improvement in the likelihood of the outcomes by having two different models for shear and control groups (see Appendix for details).

The best fit exponential time constant of $\tau = 11$ h for transport of IGF-I into cartilage disks maintained under static conditions at cut thickness is very close to the $\tau = 12$ h value reported by Bonassar et al. [1] in a previous study using similar calf cartilage plugs having the same geometry. In that study [1], dynamic compression at 3% strain amplitude and 0.1 Hz resulted in a significantly shorter time constant for IGF-I uptake ($\tau = 6.0$). In contrast, 3% dynamic shear strain at 0.1 Hz in the present study did not change the time constant significantly ($\tau = 10.2$; $p > 0.35$).

We also determined whether the ratio of the final concentration of [^{125}I]IGF-I in the tissue to that in the media, c_1, is significantly different between the shear and the control groups. The values of c_1 for the two groups were essentially the same, suggesting that shear deformation did not significantly change the partition coefficient of IGF-I. The fact that the value of the partition coefficient (c_1) is greater than unity may be attributed to Donnan enhancement of the concentration of positively charged IGF-I (p$I \sim 8.3$) within the negatively charged cartilage matrix, consistent with the high fixed charge density of our immature bovine cartilage.

Discussion

The effects on chondrocyte biosynthesis of the soluble growth factor IGF-I and the biophysical stimulus of dynamic tissue shear deformation were investigated separately and in combination using a cartilage explant system. Cyclic tissue shear above 1.5% strain amplitude applied at 0.1 Hz stimulated chondrocyte synthesis of proteins and proteoglycans up to 35 and 25%, respectively, compared to statically maintained control disks (Fig. 3). Previous studies using this same bovine calf cartilage system have shown that ~75–80% of the proline incorporation is into collagen [16]. The stimulation of matrix synthesis by IGF-I reached a plateau at 100–150 ng/ml of IGF-I (Fig. 4), similar to trends observed previously [6]. When these two stimuli were combined, the increase in matrix synthesis was close to the sum of that of each stimulus alone (Fig. 5). Analysis showed significant trends with applied shear or IGF-I but no significant interaction between the two stimuli (ANOVA, $p > 0.8$). This lack of interaction between biomechanical and biochemical stimuli was also found in previous studies of IGF-I using both static [15] and dynamic [1] mechanical compression.

The possibility that tissue shear-induced stimulation of biosynthesis is related to the enhanced transport of soluble factors such as IGF-I into and within cartilage was examined experimentally. Direct measurement of the time constant for uptake of radiolabeled [^{125}I]IGF-I with and without shear showed that dynamic tissue shear had essentially no effect on the intratissue transport of this growth factor (Fig. 6). This finding is consistent with the hypothesis posed previously [12] that tissue shear induces very little intratissue fluid flow within cartilage. Even when using the simple shear configuration of this study (i.e., Fig. 2), any intratissue fluid motion that might occur may be expected to be localized near the leading and trailing peripheral edges of the explant disk. The fluid velocity is estimated to be an order of magnitude lower than the level of fluid flow

induced by dynamic compressive deformation [13]. Therefore, the absence of significant intratissue fluid flow is consistent with our observation that dynamic shear did not enhance convective transport of IGF-I (Fig. 6). In contrast, previous studies have shown that dynamic compressive deformation enhanced the transport of IGF-I into cartilage by nearly twofold [1]. It is interesting to note that neither dynamic shear nor dynamic compression changed the plateau (i.e., the concentration at ~48 h) or equilibrium level ($[\text{IGF-I}]_\infty$ in Eq. (1)) of intratissue IGF-I concentration, while this level was significantly lowered by 50% static compression [15].

The findings that there was no interaction between the stimulatory effects of tissue shear and the IGF and that each stimulus enhanced the maximal effect of the other suggest that chondrocytes can respond simultaneously to physical and biochemical stimuli via complementary signal transduction pathways. This concept has been suggested in previous studies [1,15] of the kinetics of biophysical and biochemical stimulation of chondrocyte biosynthesis. The respective increase and decrease in chondrocyte biosynthesis induced by dynamic and static compression reached a new steady state within 4 h. In contrast, the stimulation of biosynthesis caused by IGF-I reached a new steady state level by 24-48 h. The signal transduction pathways by which these two classes (biophysical and biochemical) of stimuli are transduced into secondary intracellular signals leading to altered cellular activity are still under investigation. Signal transduction by growth factors such as IGF-I has been shown to be initiated by binding to membrane receptors followed by activation of kinase cascades [17,18], altered gene expression [19], and increased matrix synthesis [17]. Compared to the relatively well-studied signal transduction mechanisms for growth factors, the transduction mechanisms associated with biophysical factors are only beginning to be defined. Certain members of the integrin family or stretch-activated ion channels have been proposed as mechano-receptors since integrins are bound by ligands present in extracellular matrix or can be affected by mechanical deformation directly [20–23]. In addition, the effect of mechanical deformation on cell membrane permeability to ions and changes in intracellular ion osmolarity due to the volumetric deformation of cells have been proposed as initial transduction mechanisms [24]. These upstream signaling mechanisms may activate other downstream signals that may be the same as or different from those utilized in growth factor stimulation [25]. Mechanical deformation may also affect translation, posttranslational modification, and intracellular trafficking through morphological changes in organelles [26]. The observations that a mechanical stimulus augments chondrocyte biosynthesis with kinetics very different from those of IGF-I stimulation and without affecting IGF-I transport suggest that these two classes of stimuli are mediated by distinct pathways. The similarity in the downstream effect of these pathways also suggests, however, that, at some point, they may converge.

In our previous study [12], dynamic tissue shear was found to stimulate matrix synthesis by chondrocytes in cartilage explants at an amplitude as low as 1%. Those studies employed low-glucose (5.6 mM or 1 g/L) medium supplemented with 10% serum. In the present study, employing serum-free high-glucose (25 mM or 4.5 g/L) medium, the stimulatory response was found only above 1.5% shear amplitude. The difference may be due to factors other than IGF-I in serum. It is also possible that the presence of high glucose medium may affect the responsiveness of chondrocytes to exogenous stimuli, as observed previously for the case of IGF-I stimulation of chondrocyte biosynthesis [6]. It was found that the augmentation of proteoglycan synthesis due to 100 ng/ml of IGF-I was maximal at 5–10 mM glucose and was marginal outside of this optimal range of glucose concentration.

Although dynamic shear did not alter IGF-I transport into the tissue, the possibility that shear may influence the status of IGF-I within the tissue exists. IGF-I exists in several forms including (1) free IGF-I (7.6 kDa), (2) binary complexes (40–50 kDa) of IGF-I bound to IGF-binding proteins, and (3) ternary complexes (~150 kDa) involving the 85-kDa acid-labile subunit glycoprotein bound to a binary complex [27–29]. The binding of IGFBP to IGF-I modulates the access of IGF-I to cellular receptors and IGF-I action [30–32]. IGF-I that is released from IGFBPs or other complexes in the tissue may increase chondrocyte access to IGF-I above that provided. Whether mechanical deformation regulates IGF-I complex stability is not known. In this study, we used the free form of IGF-I to explore the hypothesis that mechanical and biochemical factors interact to regulate the intratissue presentation of soluble IGF-I to cell receptors relevant to joint motion in vivo.

In summary, we found that the combined stimulatory action of IGF-I and dynamic tissue shear deformation was greater than that of either stimulus alone. This observation is consistent with previous reports on the combined interaction between mechanical compression (dynamic or static) and IGF-I stimulation. Dynamic cartilage compression was previously shown to enhance uptake and intratissue transport of IGF-I associated with intratissue fluid flow [12]. In contrast, dynamic cartilage shear, which causes little or no fluid flow, did not affect IGF-I uptake or transport. Therefore, the finding that the combined effect of IGF-I and dynamic shear further augmented the maximal effect of IGF-I suggests the existence of specific signal transduction machinery sensitive to deformation of chondrocytes by tissue shear.

Acknowledgments

This study was supported by NIH Grants AR33236 and AR45779 (A.J.G.) and AR45749 (S.B.T.).

Appendix

The parameters of the transport Eq. (2) are obtained by minimizing the sum of squares of the difference between the measurement and the prediction divided by the square of the standard deviation of measurements (i.e., χ^2 function), written as

$$\chi^2(c_1, c_2, \tau) = \sum_{i=1}^{N} \frac{\left(y_{\text{measured}}(t_i) - y_{\text{predicted}}(t_i)\right)^2}{\sigma_i^2}.$$

The set of parameters (c_1, c_2, τ) was obtained using the algorithm referred to as Broyden–Fletcher–Goldfarb–Shanno that is a variant of the conjugate gradient method [33]. The confidence interval of each parameter (± 1 standard deviation or 68% confidence interval) is obtained from the square root of the variance–covariance matrix that is computed as the inverse of the second order differential of the χ^2 function evaluated at the given parameter value [34].

With regard to the effect of dynamic tissue shear on IGF-I transport, which was modeled using the first order kinetics Eq. (1), the null hypothesis (H_0) is that the transport models for control and shear are not different. The alternative hypothesis (H_1) is that the two transport models are significantly different. A generalized log-likelihood ratio test can be used to determine whether the two models are significantly different or, equivalently, which hypothesis should be accepted. In addition, the test of significance of the difference between parameters in the two groups may be performed based on the Student t test and the assumption that the difference between the data and the estimation is normally distributed.

A generalized log-likelihood ratio test is a test statistic computed by taking the ratio of the maximum probability under the constraint of the null hypothesis (H_0) to the maximum probability with that constrain relaxed or the alternative hypothesis (H_1), represented as:

$$\lambda = \frac{Max[\Pr(X|\varpi \in H_0)]}{Max[\Pr(X|\varpi \in H_1)]},$$

where $\Pr(X|\varpi \in H_a)$ is the probability of the outcome, X, given the hypothesis, H_a, and the corresponding set of parameters, ϖ. Then, the value $-2\log\lambda$ will be asymptotically χ^2 distributed with degrees of freedom equal to the difference in dimensionality of H_1 and H_0. The parameters of the model under the null hypothesis are obtained by combining the data from two groups into one and finding the best fit of the model to them. Once the sets of parameters, $\varpi \in H_0$ and $\varpi \in H_1$, are computed, the ratio can be calculated under the assumption of the normal distribution of the difference between data and estimations. The degree of freedom equals 3 under theses hypotheses, which is the difference between the six parameters under H_1 ($[c_1, c_2, \tau]_{\text{shear}} + [c_1, c_2, \tau]_{\text{control}}$) and the three parameters under H_0 ($[c_1, c_2, \tau]_{\text{combined}}$).

The significance of the difference between the parameters of the groups is evaluated using the Student t test given as

$$t = \frac{\omega_a - \omega_b}{\sqrt{\frac{SD_a}{n_a - 1} + \frac{SD_b}{n_b - 1}}}$$

where ω_i, SD_i, and n_i represent the value of the parameter, the standard deviation, and the number of samples of group i, respectively. For this test, the degree of freedom is determined as the sum of the number of samples minus two.

References

[1] L.J. Bonassar, A.J. Grodzinsky, E.H. Frank, S.G. Davila, N.R. Bhaktav, S.B. Trippel, J. Orthop. Res. 19 (2001) 11–17.
[2] D.J. McQuillan, C.J. Handley, M.A. Campbell, S. Bolis, V.E. Milway, A.C. Herington, Biochem. J. 240 (1986) 423–430.
[3] K.D. Osborn, S.B. Trippel, H.J. Mankin, J. Orthop. Res. 7 (1989) 35–42.
[4] T.I. Morales, Arch. Biochem. Biophys. 315 (1994) 190–198.
[5] C.R. Flannery, C.B. Little, B. Caterson, C.E. Hughes, Matrix Biol. 18 (1999) 225–237.
[6] K.M. Kelley, T.R. Johnson, J. Ilan, R.W. Moskowitz, Am. J. Physiol. 276 (1999) R1164–R1171.
[7] A.J. Fosang, J.A. Tyler, T.E. Hardingham, Matrix 11 (1991) 17–24.
[8] S. Kondo, S.H. Cha, W.F. Xie, L.J. Sandell, J. Orthop. Res. 19 (2001) 712–719.
[9] R.L. Sah, Y.J. Kim, J.Y. Doong, A.J. Grodzinsky, A.H. Plaas, J.D. Sandy, J. Orthop. Res. 7 (1989) 619–636.
[10] Y.J. Kim, L.J. Bonassar, A.J. Grodzinsky, J. Biomech. 28 (1995) 1055–1066.
[11] B.P. O'Hara, J.P. Urban, A. Maroudas, Ann. Rheum. Dis. 49 (1990) 536–539.
[12] M. Jin, E.H. Frank, T.M. Quinn, E.B. Hunziker, A.J. Grodzinsky, Arch. Biochem. Biophys. 395 (2001) 41–48.
[13] E.H. Frank, M. Jin, A.M. Loening, M.E. Levenston, A.J. Grodzinsky, J. Biomech. 33 (2000) 1523–1527.
[14] J. Cohen, Statistical Power Analysis for the Behavioral Sciences, second ed., Lawrence Erlbaum Associates, Inc, London, 1988.
[15] L.J. Bonassar, A.J. Grodzinsky, A. Srinivasan, S.G. Davila, S.B. Trippel, Arch. Biochem. Biophys. 379 (2000) 57–63.
[16] R.L. Sah, J.Y. Doong, A.J. Grodzinsky, A.H. Plaas, J.D. Sandy, Arch. Biochem. Biophys. 286 (1991) 20–29.
[17] S.B. Trippel, J.J. Van Wyk, H.J. Mankin, J. Bone Joint Surg. Am. 68 (1986) 897–903.
[18] M. Shakibaei, T. John, P. De Souza, R. Rahmanzadeh, H.J. Merker, Biochem. J. 342 (1999) 615–623.
[19] H. Messai, Y. Duchossoy, A.M. Khatib, A. Panasyuk, D.R. Mitrovic, Mech. Ageing Dev. 115 (2000) 21–37.
[20] H.S. Lee, S.J. Millward-Sadler, M.O. Wright, G. Nuki, D.M. Salter, J. Bone Miner. Res. 15 (2000) 1501–1509.
[21] S.J. Millward-Sadler, M.O. Wright, H. Lee, K. Nishida, H. Caldwell, G. Nuki, D.M. Salter, J. Cell Biol. 145 (1999) 183–189.

[22] S.J. Millward-Sadler, M.O. Wright, L.W. Davies, G. Nuki, D.M. Salter, Arthritis Rheum. 43 (2000) 2091–2099.
[23] D.M. Salter, S.J. Millward-Sadler, G. Nuki, M.O. Wright, Clin. Orthop. 391 (2001) S49–S60.
[24] R.J. Wilkins, J.A. Browning, J.P. Urban, Biorheology 37 (2000) 67–74.
[25] W.B. Valhmu, F.J. Raia, Biochem. J. 361 (2002) 689–696.
[26] A.J. Grodzinsky, M.E. Levenston, M. Jin, E.H. Frank, Annu. Rev. Biomed. Eng. 2 (2000) 691–713.
[27] R. Schneiderman, N. Rosenberg, J. Hiss, P. Lee, F. Liu, R.L. Hintz, A. Maroudas, Arch. Biochem. Biophys. 324 (1995) 173–188.
[28] R. Schneiderman, E. Snir, O. Popper, J. Hiss, H. Stein, A. Maroudas, Arch. Biochem. Biophys. 324 (1995) 159–172.
[29] N.R. Bhakta, A.M. Garcia, E.H. Frank, A.J. Grodzinsky, T.I. Morales, J. Biol. Chem. 275 (2000) 5860–5866.
[30] V. Hwa, Y. Oh, R.G. Rosenfeld, Endocr. Rev. 20 (1999) 761–787.
[31] Y.R. Boisclair, R.P. Rhoads, I. Ueki, J. Wang, G.T. Ooi, J. Endocrinol. 170 (2001) 63–70.
[32] Y. Dubaquie, D.L. Mortensen, A. Intintoli, D.A. Hogue, G. Nakamura, P. Rancatore, P. Lester, M.D. Sadick, E. Filvaroff, P.J. Fielder, H.B. Lowman, Endocrinology 142 (2001) 165–173.
[33] W.H. Press, B.P. Flannery, S.A. Teukolsky, W.T. Vetterling, Numerical Recipies: The Art of Scientific Computing, Cambridge University Press, Cambridge, 1990.
[34] M.A. Tanner, Tools for Statistical Inference, third ed., Springer, New York, 1996.