Osteoarthritis
and Cartilage
Journal of the OsteoArthritis Research Society International

OARSI OSTEOARTHRITIS
RESEARCH SOCIETY
INTERNATIONAL

Intermittent sub-ambient interstitial hydrostatic pressure as a potential mechanical stimulator for chondrocyte metabolism

By Jun-Kyo Suh, Goo H. Baek, Asbjørn Arøen, Christina M. Malin, Christopher Niyibizi, Christopher H. Evans and Andrea Westerhausen-Larson*

Musculoskeletal Research Center, Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA 15213 and *Magee-Womens Research Institute, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA 15213

Summary

Objective: Experimental findings have suggested that the metabolic activities of articular cartilage can be influenced by mechanical stimuli. Our mathematical analysis predicted that cyclic compressive loading may create periods of intermittent sub-ambient hydrostatic pressure within the cartilage extracellular matrix. Based on this mathematical analysis, the present study was aimed to investigate whether the intermittent sub-ambient hydrostatic pressure, created in the cartilage extracellular matrix during cyclic compression, has a stimulative effect on the biosynthesis of chondrocytes.

Method: In order to test this hypothesis, the present study developed a custom-designed sub-ambient pressure generator to subject a monolayer culture of chondrocytes to an intermittent sub-ambient pressure. Using this pressure generator, the monolayer chondrocyte culture system was analyzed for ^{35}S-sulfate and ^{3}H-proline incorporation rates for biosynthesis of proteoglycan and collagenous/noncollagenous protein molecules, respectively. Northern analyses for aggrecan and type II collagen mRNAs were also performed.

Results: It was found that the intermittent sub-ambient pressure produced a 40% increase in proteoglycan and a 17% increase in non-collagenous protein synthesis during the pressurization period ($P<0.05$). The collagenous protein synthesis was not affected by the intermittent sub-ambient pressure regimen used in this study. After the intermittent sub-ambient pressurization, the metabolic activities of the chondrocytes returned to normal (control level). The intermittent sub-ambient pressure also produced an increase in the mRNA signals for aggrecan. Therefore, we conclude that intermittent sub-ambient pressure may be one of the potential mechanical stimulators of chondrocytes in articular cartilage during dynamic compression.

Key words: Hydrostatic pressure, Articular cartilage, Cartilage metabolism, Chondrocyte.

Introduction

Under normal conditions, the structure of articular cartilage is maintained by a balanced coupling of chondrocyte anabolism and catabolism. In the degenerative process, matrix resorption is accelerated causing catabolism (degradation) to outpace the anabolic attempt of the chondrocytes to produce new matrix [1]. Many experimental studies conducted over the past two decades have revealed that cartilage homeostasis can be mechanically regulated. While static compression of articular cartilage suppresses the biosynthetic activity of chondrocytes [2, 3], dynamic compression has been shown to either stimulate or suppress the bio-synthetic activities of articular cartilage. This effect depends on the frequency and magnitude of the applied compressive load [4–8]. However, to date, the exact mechanism through which mechanical loading affects cartilage metabolism remains unclear.

Among several mechanical determinants which articular cartilage is subjected to, the hydrostatic pressure of the interstitial fluid is considered to be a primary component of mechanical stress for resisting the compressive loads applied to the cartilage surface during daily activity [9]. Thus, one would expect this fluid pressure to be a major mechanical signal transducer in regulating cartilage metabolism.

Several in-vitro studies have applied a pure hydrostatic pressure to cartilage explants. Lippiello *et al.* [10] showed that ^{35}S-glycosaminoglycan (GAG) synthesis of bovine

Corresponding Author: Jun-Kyo Suh, Ph.D., Depts. of Orthopaedic Surgery and Mechanical Eng., University of Pittsburgh, 648 Benedum Engineering Hall, 3700 O'Hara Street, Pittsburgh, PA 15261. Tel: 412-624-9790; Fax: 412-624-4846; E-mail: jsuh@pitt.edu

cartilage explants was inhibited significantly (a 53% decrease) by static pressure in the range of 0.5–2 MPa. We also found that young bovine cartilage explants produced a significantly lower amount of GAG (a 60% decrease) under a static pressure of 1.2 MPa [11]. However, neither Kimura *et al*. [12] nor Giori [13] found any significant effect of static pressure on GAG synthesis. Hall *et al*. [14] showed that, in the physiological pressure range (5–10 MPa), both short-term (20 seconds) and long-term (2 hours) exposures to hydrostatic pressure stimulated proteoglycan (PG) and protein syntheses in bovine cartilage explants. In a higher pressure range (>20 MPa), however, this biosynthesis was significantly suppressed by long-term exposure, but not by short-term exposure [14]. In a recent study performed by Parkkinen *et al*. [15], cyclic hydrostatic pressure of 5 MPa for 1.5 hours did not affect the sulfate incorporation of bovine cartilage explants at frequencies lower than 0.5 Hz. However, the same pressure stimulated the incorporation by 17% at higher frequencies.

The effect of hydrostatic pressure on isolated chondrocytes has also been studied. Hydrostatic pressure of 13 kPa applied at 0.3 Hz significantly stimulated the biosynthetic activities, as well as the proliferative mitosis, of chick embryonic chondrocytes [16–18]. Parkkinen *et al*. [15] found that the PG synthesis of bovine chondrocytes was inhibited at frequencies ranging from 0.017 Hz to 0.5 Hz when 5 MPa of cyclic pressure was applied for 1.5 hours, but was stimulated at frequencies ≥ 0.25 Hz when an identical pressure was applied for 20 hours. However, Lammi *et al*. [19] showed that cyclic hydrostatic pressure of 5 MPa did not yield any distinguishable change in the PG mRNA level. Smith *et al*. [20] recently demonstrated that cyclic hydrostatic pressure of 10 MPa at 1 Hz for 4 hours resulted in elevated secretion of mRNA encoding for PG core protein and type II collagen in adult bovine chondrocyte cultures.

Ultrastructural changes in chondrocytes subjected to hydrostatic pressure have also been described [18, 21]. Veldhuijzen [18] reported that repetitive hydrostatic pressure led to hypertrophic changes in the dimensions of chondrocytes and their nuclei. Although the Golgi complexes were not affected by repetitive hydrostatic pressure, they assumed a compact form when subjected to continuous hydrostatic pressure [21]. In a recent study using isolated human and ovine chondrocytes, Wright *et al*. [22] showed that the transmembrane electric potential of the chondrocyte decreased by 28% under a continuous hydrostatic pressure of 16 kPa but increased by 42% under

repetitive hydrostatic pressure of the same magnitude at 0.33 Hz.

In summary, although ample evidence for the stimulative effect of repetitive hydrostatic pressure has been demonstrated, the overall effect of this hydrostatic pressure on cartilage metabolism appears to be somewhat inconsistent. We believe that these inconsistencies, described above, could be attributed to the wide range of loading parameters used in the various studies, reflecting the fact that a physiological normal range of mechanical stress/strain conditions within the cartilage extracellular matrix (ECM) is to be further established.

In our laboratory, we used the biphasic theory for articular cartilage [23] to demonstrate that intermittent sub-ambient (negative with respect to the ambient) hydrostatic pressure exists within the ECM when the tissue is subjected to cyclic compression [24, 25]. Based on this mathematical prediction, we hypothesized that the intermittent sub-ambient pressure created within the cartilage ECM during cyclic compression is a potential mechanical stimulator in regulating the biosynthetic activities of chondrocytes. Using a custom-designed monolayer culture system of bovine chondrocytes under intermittent sub-ambient pressure environment, we demonstrated that a repetitive intermittent sub-ambient pressure has a potent stimulative effect on the biosynthetic activities of the chondrocytes.

Mathematical analysis

In order to estimate the mechanical stress environment within cartilage ECM under cyclic compression, we used the linear biphasic theory for articular cartilage which assumes that articular cartilage is a biphasic mixture of a porous (permeable), elastic solid matrix (collagen and proteoglycan) and an inviscid interstitial fluid [23]. Assuming that both the solid matrix and the fluid are intrinsically incompressible, the linear biphasic theory can be written as follows:

Continuity equation:

$$\nabla \cdot (\varphi^s \mathbf{v}^s + \varphi^f \mathbf{v}^f) = 0. \tag{1}$$

Momentum equations:

$$\nabla \cdot \boldsymbol{\sigma}^\alpha + \boldsymbol{\pi}^\alpha = 0, \ \alpha = s, f. \tag{2}$$

Constitutive equations:

$$\boldsymbol{\sigma}^s = -\varphi^s p \mathbf{I} + \lambda^s tr(\boldsymbol{\varepsilon}^s)\mathbf{I} + 2\mu^s \boldsymbol{\varepsilon}^s, \tag{3}$$

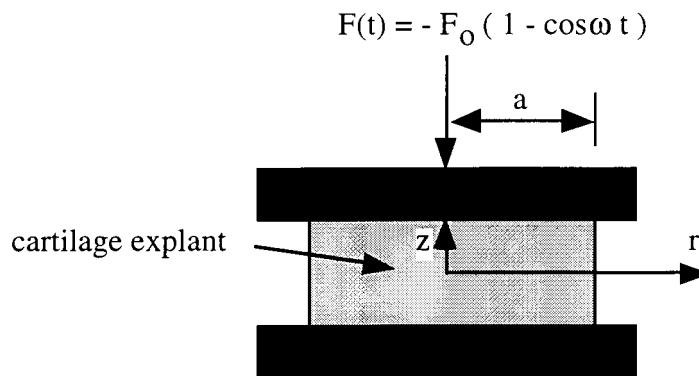$$\boldsymbol{\sigma}^f = -\varphi^f p \mathbf{I}, \tag{4}$$

$$F(t) = - F_O (1 - \cos\omega t)$$

FIG. 1. Schematic of the cyclic unconfined compression of articular cartilage plug with radius=a (Adapted from Suh [25]).

$$\boldsymbol{\pi}^s = -\boldsymbol{\pi}^f = \frac{(\phi^f)^2}{\kappa}(\mathbf{v}^f - \mathbf{v}^s). \tag{5}$$

Here the superscripts s and f refer to the solid and fluid phases, respectively; $\mathbf{v}$ is the velocity; $\boldsymbol{\sigma}$ is the Cauchy stress tensor; $\boldsymbol{\pi}$ is the diffusive momentum exchange; p is the hydrostatic pressure; φ^α is the volume fraction of the α phase; $\boldsymbol{\varepsilon}^s$ is the infinitesimal strain tensor; and λ^s and μ^s are the Lamè constants of the solid phase. This biphasic theory has been widely accepted as the model which best simulates the interstitial fluid flow-dependent viscoelastic behavior of this tissue [9].

We utilized an unconfined compression model which has been widely used to study the mechanical stimulation of cartilage metabolism *in vitro* with either displacement control [2, 3, 5] or force control [26]. In this study, a cyclic compressive force was applied to the cartilage specimen through the impermeable loading platen as shown in Fig. 1 [25]:

$$F(t) = -F_o(1 - \cos \omega t), \tag{6}$$

and the resulting mechanical stress/strain variables, such as volumetric strain and hydrostatic pressure, within the ECM were calculated from Eqs (1)–(6). The results showed that the mechanical behavior of the cartilage ECM strongly depends on the loading frequency, ω (Figs 2 and 3). When the loading frequency is low (e.g., $\omega=0.001$ Hz), the deformational response of the tissue matrix can easily adapt to the applied load. Therefore, the solid matrix undergoes a large deformation (Fig. 2) without a large pressure surge (Fig. 3). In contrast, when the loading frequency is high (e.g., $\omega=0.1$ Hz), the tissue deformation cannot respond as quickly as the loading cycles are being applied, causing a high elevation in pressure within the tissue matrix (Fig. 3).

It was found that an oscillating positive-negative (with respect to the ambient pressure) interstitial pressure is created within the cartilage ECM during repetitive loading-unloading cycles (Fig. 3). When the tissue was subjected to a compressive load, the interstitial fluid exuded from the tissue matrix resulting in a decrease in the apparent volume of the tissue matrix. When the compressive load was removed during the cyclic unloading period, the compressed tissue matrix expanded returning to its original volume. This volume expansion in turn created a sub-ambient pressure within the matrix, causing imbibition of the fluid into the tissue matrix. Furthermore, the peak-to-peak magnitude of the oscillating interstitial hydrostatic pressure was proportional to the peak magnitude of the applied cyclic compressive force. Therefore, this theoretical analysis suggested that the peak magnitude of the intermittent
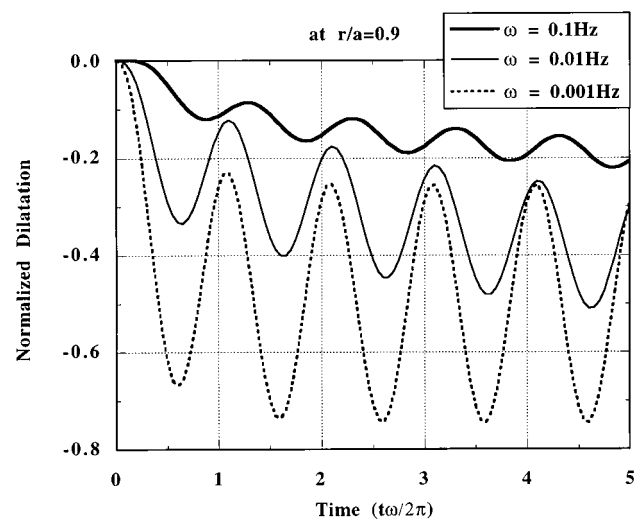
FIG. 2. Normalized volumetric strain ($=\mathrm{tr}(\varepsilon)$/maximum of $\mathrm{tr}(\varepsilon)$) at r/a=0.9 with varying frequencies (0.001 $\sim$ 0.1 Hz) (Adapted from Suh [25]).
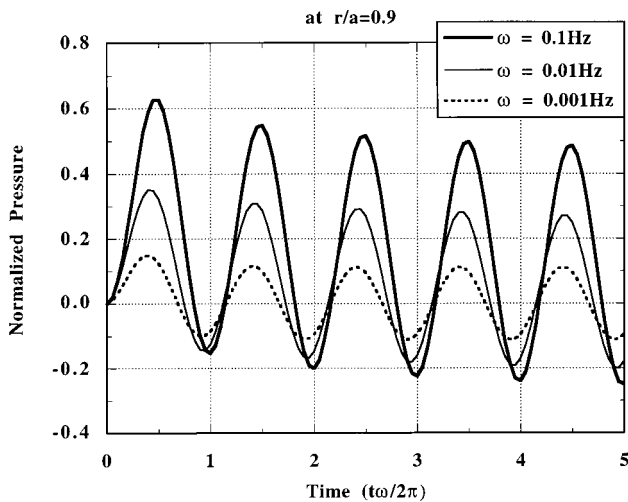
FIG. 3. Normalized pressure ($=p\pi a^2/F_o$) at r/a=0.9 with varying frequencies (0.001 ~ 0.1 Hz) (Adapted from Suh [25]).

FIG. 4. Schematic of intermittent sub-ambient pressure generator.

sub-ambient pressure within the ECM can be very low when the peak magnitude of the applied compressive force is very large.

Experimental analysis

SPECIMEN PREPARATION

Fresh bovine articular cartilage was obtained from the metacarpophalangeal joints of young (1–2 year old) bovine specimens within 4 hours of death. Chondrocytes were isolated from the tissue with 0.2% trypsin digestion for 30 min followed by 0.2% clostridial collagenase digestion for approximately 120 minutes as described by Green [27]. The isolated chondrocytes (5×10^5 cells) were then cultured in tissue culture flasks (Flacon; culture surface area=25 cm^2) with 2 ml of low glucose, HEPES-buffered DMEM supplemented with streptomycin (100 µg/ml)/penicillin (100 U/ml) and 10% fetal bovine serum (FBS) The cells were cultured to a 90% confluent monolayer. The medium was then replaced with 2 ml of fresh medium containing only 1% FBS. The cells were preconditioned in this medium for 24 hours prior to the experiment.

INTERMITTENT SUB-AMBIENT PRESSURE REGIMEN

During the experiment, the cap of the tissue culture flask was replaced with a specially designed air-tight cap connected to a custom-designed low-power vacuum generator. The low-power vacuum system consisted of a 60 cc syringe and a pressure transducer (Micro Switch, Freeport, IL) attached to the tissue culture flask. The vacuum pressure in the culture flask was maintained at 0.8 atmospheric (atm) pressure by using a buttress (Fig. 4).
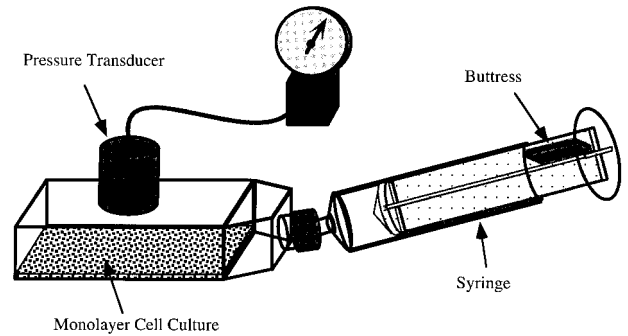
The chondrocytes were subjected to an intermittent sub-ambient pressure of 0.8 atm for 5 minutes, and then released to normal atmospheric pressure (1 atm) for 30 min. This cycle was repeated ten times for a total pressurization period of approximately six hours. The pressurization experiment was performed at 37°C within a 5% CO_2 incubator. The air which had direct contact with the culture medium was regularly replenished during the normal atmospheric pressure period.

During the experiment, either 10 µCi/ml of ^{35}S-sulfate or 5 µCi/ml of L-[2,3-^{3}H]-proline supplemented with 20 µg/ml of L-ascorbic acid was added to the culture medium to deductively measure PG synthesis or total protein and collagen synthesis, respectively. The temporal biosynthetic response of the chondrocytes to the intermittent sub-ambient pressure was determined by using three different time periods (ISP, ATM, and TOT Groups) for the radioactive isotope pulsing (Fig. 5). In the ISP Group, the isotope was added to the medium during the first 6-hour period of intermittent sub-ambient pressure, after which the experiment was concluded. In the ATM Group, the isotope was added to the medium immediately after intermittent sub-ambient pressurization and the chondrocytes were cultured for an additional 18 hours under normal atmospheric pressure at 37°C within the 5% CO_2 incubator. In the TOT Group, the isotope was added to the medium during the intermittent sub-ambient pressurization period and remained in the medium for the 18 hour normal culture period under one atmospheric pressure. Each group consists of seven experimental and seven control specimens.

In order to investigate the degasification phenomenon in the culture system during the intermittent sub-ambient pressure, four additional specimens were used to measure the partial oxygen
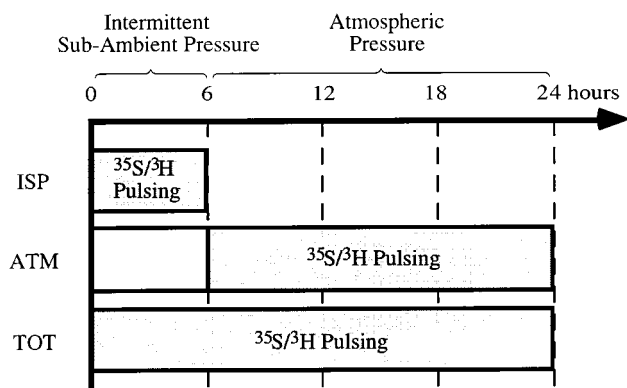
FIG. 5. Design of intermittent negative pressure and ^{35}S-sulfate/^{3}H-proline pulsing. In the ISP Group, the isotope was added to the medium during the first 6 hour period of intermittent sub-ambient pressure, after which the experiment was concluded. In the ATM Group, the isotope was added to the medium immediately after intermittent sub-ambient pressurization and the chondrocytes were cultured for an additional 18 hours under normal atmospheric pressure. In the TOT Group, the isotope was added to the medium during the intermittent sub-ambient pressurization period and remained in the medium for the 18 hour normal culture period under one atmospheric pressure. Each group consists of seven experimental and seven control specimens.

pressure (PO$_2$) of the culture medium after intermittent sub-ambient pressurization for 5 minutes.

^{35}S-SULFATE INCORPORATION ANALYSIS

The medium was retrieved from each flask and eluted on PD-10 columns of Sephadex G-25M (Pharmacia, Piscataway, NJ) [28] using 1 ml of 4 M GuHCl buffer solution (0.05 M Tris, 0.05 M Na$_2$SO$_4$, pH = 7.0) per fraction, yielding a total of 8 fractions for each specimen. The cells in each sample were separately digested with 2 ml of 1% papain solution at 60°C for 48 hours. A 200 µl aliquot from each sample was eluted on the PD-10 columns in a similar manner. When the last fraction was completed, 6 ml of scintillation fluid (Ultra Gold, Packard) were added to each vial and the samples were counted with a scintillation counter. The first peak value of the radioactivity count of the eight fractions represented the newly synthesized PGs. This value was normalized with respect to the total DNA content per sample, which was measured using the fluorometric reaction method with Hoechst 33258 (Hoefer Scientific Inst., San Francisco, CA) [29, 30].

^{3}H-PROLINE INCORPORATION ANALYSIS

The incorporation of L-[2,3-^{3}H]-proline into the extracellular collagen and protein was determined using the collagenase-digestible protein assay of Peterkofsky [31]. Upon the completion of the experiment, both the medium and cells from each sample were completely transferred into a cellulose membrane (Spectra/Por 1, Fisher Scientific, Pittsburgh) and dialyzed against distilled water for 160 hours to remove unincorporated, free [^{3}H]-proline. The total volume of each specimen was approximately 8 ml after dialysis. The specimen was then partitioned into three groups of 2 ml each. Each group was mixed with an additional 2 ml of an enzymatic buffer solution (100 mM Tris-HCl, 300 mM NaCl, 10 mM CaCl$_2$, 0.4 mg/ml NaN$_3$, 0.1% Triton X-100, and 6 mM EDTA) containing either 10 U/ml of highly purified bacterial collagenase (Advance Biofactures, Lynbrook, NY) (Group A), 50 µg/ml of crude collagenase and 50 µg/ml of trypsin (Group B), or no proteases (Group C). After incubation at 37°C for 150 minutes, the undigested protein was separated by centrifugation at 12 000 rpm for 60 minutes in the presence of 5% (w/v) trichloroacetic acid and 1 mg/ml carrier bovine serum albumin. One milliliter of supernatant from each group was mixed with 14 ml of scintillation fluid (Ultra Gold, Packard) and the radioactivity count, representing ^{3}H incorporation, was measured with a scintillation counter. The total collagen synthesis was then calculated as the value of Group A minus the value of Group C and the total non-collagenous protein synthesis as the value of Group B minus the value of Group A. The synthesis rate was finally normalized with respect to the total DNA content of each specimen.

MRNA ANALYSIS FOR AGGRECAN AND TYPE II COLLAGEN

RNA was purified by the acid phenol-guanidinium thiocyanate method [32] and subjected to denaturing gel electrophoresis on 1% agarose gels in the presence of ethidium bromide. Ribosomal RNA bands were photographed under UV light as a control for the integrity and loading of samples. RNA was transferred to nylon membranes (Magna Nylon Membrane, 0.45 µm, MSI) and UV cross-linked (UV-crosslinker, Fisher, Pittsburgh). Specific mRNA was identified by hybridization to DNA probes labeled with [α-^{32}P]dCTP by the random primer method (Multiprime DNA labeling system, Amersham) to specific activities between 1.5 and 2×10^9 dpm/mg. The probes used were a rat cDNA for aggrecan encoding amino acids in the lectin-homologous segment (89% identical to the bovine sequence), a human COL2A1 cDNA encoding amino acids in the C-propeptide, and a human cDNA for the

Table I
^{35}S incorporation into PG synthesis by intermittent sub-ambient pressure

Group	^{35}S Incorp. (mean $\pm$ SD cpm/μg DNA)		Ratio (Exp/Cntrl)
	Control	Experiment	
ISP	119 ± 13.0	167 ± 12.9	1.40*
ATM	488 ± 73.8	464 ± 55.0	0.95
TOT	174 ± 24.6	193 ± 28.1	1.11

(N=7) *P<0.01.

house-keeping gene Glyceraldehyde-3 phosphate Dehydrogenase (GAPDH). Standard hybridization procedures in phosphate buffer with SDS were applied [33] and the filters were exposed to film (XAR-5, Kodak) at $-80°C$. The image of autoradiograms was captured by means of a Cohu 8 bit black and white video camera. Signal intensity was analyzed by the Northern macro of Harmony image analysis system and normalized to GAPDH content.

STATISTICAL ANALYSIS

To determine statistical significance between the various groups (ISP, ATM, and TOT) and their corresponding control groups, ANOVA single factor tests were performed, using Fisher's PLSD post-hoc test.

Experimental results

^{35}S-Sulfate Incorporation

While the ISP and ATM Groups were performed with samples from the same bovine specimen, the TOT Group was performed with a different bovine specimen, therefore the absolute values of the ^{35}S-sulfate incorporation are not directly comparative. It was found that the intermittent sub-ambient pressure (ISP Group) produced a 40%

increase in the PG synthesis of the chondrocytes during the pressurization period (P<0.01) (Table I). After the intermittent sub-ambient pressurization, the metabolic activities of the chondrocytes returned to the control level; the ratio for ATM Group was found to be 0.95 with no statistically significant difference between the experimental and control groups. As a result, the total PG synthesis by the pressurized chondrocytes in a 24-hour time period (TOT Group) increased by 11%.

^{3}H-PROLINE INCORPORATION

All experiments for the total protein/collagen synthesis were performed with chondrocytes harvested from the same bovine specimen. Therefore, the absolute values of the ^{3}H incorporation rate are comparable between groups. It was found that the intermittent sub-ambient pressurization did not cause any differences in the total collagen synthesis in any group. The total non-collagenous protein synthesis increased by 17% under the intermittent sub-ambient pressure (ISP Group); this difference was statistically significant (P<0.05) (Table II). The total non-collagenous protein synthesis was not stimulated after the intermittent sub-ambient pressure (ATM Group). As a result, the overall synthesis of total non-collagenous protein in the TOT Group increased by 5% due to the intermittent sub-ambient pressure.

MRNA ANALYSIS FOR AGGRECAN AND TYPE II COLLAGEN

Application of intermittent sub-ambient pressurization resulted in an 81% increase of mRNA abundance for aggrecan as compared to the control, non-pressurized, specimens (N=2). However, mRNA levels for the type II collagen mRNA (COL2A1) were not affected by intermittent sub-ambient pressurization. Depicted in the upper

Table II
^{3}H incorporation into collagenous (C) and non-collagenous (NC) proteins synthesis by intermittent sub-ambient pressure

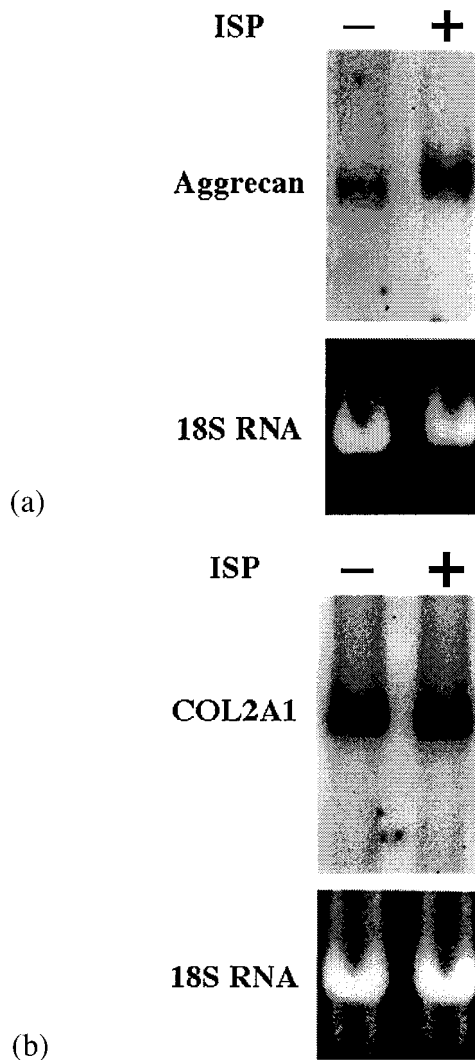| Group	Molecules	^{3}H Incorp. (mean $\pm$ SD cpm/μg DNA)		Ratio (Exp/Cntrl)
		Control	Experiment	
ISP	C	18.8 ± 8.0	18.7 ± 3.9	1.00
	NC	77.5 ± 10.4	91.1 ± 8.5	1.17*
ATM	C	$54.2 \pm 8.4†$	52.1 ± 8.5	0.96
	NC	$281.5 \pm 33.9†$	276.7 ± 36.7	0.98
TOT	C	67.8 ± 21.1	$70.0 \pm 4.8†$	1.03
	NC	320.9 ± 52.3	$337.5 \pm 12.4†$	1.05

(N=7; †N=6) *P<0.05.

(a)

(b)

FIG. 6. Northern Hybridizations for Aggrecan (a) and type II collagen (b).

panel of Fig. 6(a) is the autoradiograph of a Northern hybridization for aggrecan mRNA both without ($-$) and with ($+$) ISP application. The size of the aggrecan mRNA was approximately 8 kb, which is consistent with aggrecan mRNA in other species. As shown in Fig. 6(b), COL2A1 mRNA was not affected by the same treatment. The ethidium bromide stained 18S ribosomal RNA bands can be seen in the lower panels.

PARTIAL OXYGEN PRESSURE DURING INTERMITTENT SUB-AMBIENT PRESSURE

It was found that, after intermittent sub-ambient pressurization for 5 min, the partial oxygen pressure of the culture medium was reduced by 10% (Control Group $= 21.3 \pm 0.4$ kPa; ISP Group $= 19.3 \pm 0.3$ kPa). However, there was no noticeable change in the color of the DMEM culture medium, indicating that the pH level of the culture medium was relatively stable.

Discussion and conclusion

As described earlier, dynamic compression of articular cartilage can stimulate the biosynthetic activities of chondrocytes. Several mechanisms have been proposed for the transduction of dynamic mechanical loading into a biological response of articular cartilage. Under mechanical loading, chondrocytes will experience various types of mechanical signals, such as stress/strain (tensile, compressive, or shear), which can result in the structural deformation of the cells [34–36]. These signals can in turn be selectively transformed into electrical and biological responses through stretch-activated ion channels [37].

In addition to the structural deformation of chondrocytes, compaction of the cartilage ECM due to mechanical compression can also cause various changes in the physicochemical environment around these cells. For example, such loading can alter the diffusive transport of nutrients and other molecules [38]. Compaction can also cause a change in the fixed-charge concentration of GAG molecules with a concomitant osmotic pressure gradient [39, 40], thereby leading to a local change in pH within the tissue matrix [2]. Furthermore, dynamic compression of articular cartilage creates time-dependent mechanical deformation of the ECM, thus producing a hydrostatic pressure gradient and interstitial fluid flow [24, 41]. The interstitial fluid flow within the cartilage ECM can enhance the transportation of large molecules, such as growth factors and newly synthesized matrix macromolecules, through the dense cartilage matrix [42]. It can also create a streaming potential in the tissue matrix [43, 44].

A direct mechanical compression of cartilage explants has been frequently utilized in many in-vitro studies to study the biosynthetic response of articular cartilage under mechanical compression. While direct mechanical compression of cartilage explants closely mimics the physiological loading conditions of articular cartilage, direct mechanical compression of tissue results in a compound phenomenon of matrix deformation, hydrostatic pressure, interstitial fluid flow, and other physicochemical signals within the tissue ECM [45, 46]. As a result, the chondrocytes embedded in the cartilage ECM will receive a variety of mechanical signals during dynamic compressive loading. This will confound the interpretation of the role of each mechanical signal in stimulating cartilage metabolism, and thus make the study

of the mechano-transduction mechanism of chondrocytes very difficult.

The present study proposes another probable pathway of the role of hydrostatic pressure in the mechanical stimulation of articular cartilage. Dynamic compression of the tissue matrix can create an intermittent sub-ambient hydrostatic pressure environment within the cartilage ECM, which can in turn directly stimulate the biosynthetic activities of chondrocytes. This study demonstrates the direct stimulatory effect of intermittent sub-ambient pressure on the metabolism of chondrocytes, specifically, proteoglycan and non-collagenous protein synthesis. However, it does not show any effect on the collagenous protein synthesis. These findings were supported by the Northern analysis which showed an increase of mRNA abundance for aggrecan. While a 10% reduction in the partial oxygen pressure was noticed during the intermittent sub-ambient pressurization period, the decreased oxygen content in the culture medium did not appear to stimulate the biosynthetic activities of chondrocytes. It was previously reported that a deceased oxygen content in the culture medium adversely affected matrix syntheses of chondrocytes [47].

Several limitations of the present study deserve a detailed discussion. While we have mathematically predicted the existence of intermittent sub-ambient pressure within the cartilage ECM, the ultimate proof of the magnitude of the intermittent sub-ambient pressure would be ideally obtained through a direct measurement of the intra-matrix pressure of articular cartilage. Unfortunately, it is not feasible with present technology to experimentally measure this pressure. In the present study, the magnitude (0.8 atm) and the frequency (5 minutes on/30 minutes off) of the intermittent sub-ambient pressure, while far from the physiological relevancy during normal joint loading, was chosen from the technical aspect of the experimental design. Since the chondrocytes were subjected to sub-ambient pressure within a closed tissue culture flask, normal incubation with fresh air (5% CO_2) was allowed for 30 minutes between every sub-ambient pressure application in order to provide sufficient gas exchange within the culture medium. The tissue culture system coupled with the low-power vacuum generator provided an appropriate environment for monolayer cell culture without any contamination or cytotoxicity during the experiment. In this study, the cell viability was confirmed when the activity of the cells returned to control levels after the pressurization period (ATM Group). As the magnitude and frequency of the sub-ambient pressure are changed

in future studies, however, these issues need to be addressed to ensure cell viability.

The underlying hypothesis of the present study was derived from the linear biphasic theory, in which the deformation of the solid matrix was limited to a small deformation (i.e., less than 10%) and the permeability parameter was assumed to remain constant under mechanical loading. In addition, the solid matrix, which is a mixture of collagen fibrils and proteoglycan molecules, was also assumed to be a linearly elastic, isotropic material and the interstitial fluid to be an inviscid material. Including the large deformation of cartilage tissue under physiological conditions and the intrinsic viscoelastic characteristics of the solid matrix would yield a more precise mathematical prediction of the mechanical behavior of articular cartilage. We expect that these model improvements would result in a different overall quantitative magnitude of the stress and strain, but they are unlikely to alter the trends regarding the existence of intermittent sub-ambient pressure. Nonetheless, an improved mathematical model will help us to better predict the magnitude of the intermittent sub-ambient pressure within the cartilage matrix.

In summary, we conclude that the intermittent sub-ambient pressure may be one of the direct mechanical stimulators of chondrocytes in articular cartilage under dynamic loading. Future studies will need to further investigate the effect of the intermittent sub-ambient pressure, especially in the form of a combination of supra-ambient and sub-ambient hydrostatic pressures, on PG synthesis and the specific pathway for the stimulatory role of these hydrostatic pressure signals on the metabolism of chondrocytes. For example, studying the changes in the structural organization of the cytoskeleton and the expression of specific macromolecules in response to these hydrostatic pressure signals will provide further insight to the exact mechanism of this phenomenon.

References

1. Tyler JA, Bolis S, Dingle JT, Middleton JFS. Mediators of matrix catabolism. In: Kuettner KE, Schleyerbach R, Peyron JG, Hascall VC, Eds. Articular Cartilage and Osteoarthritis. New York: Raven Press 1992:251–64.

2. Gray ML, Pizzanelli AM, Grodzinsky AJ, Lee RC. Mechanical and physicochemical determinants of the chondrocyte biosynthetic response. J Orthop Res 1988;6:777–92.

3. Guilak F, Meyer BC, Ratcliffe A, Mow VC. The effect of static loading on proteoglycan biosynthesis and turnover in articular cartilage explants. Trans Orthop Res Soc 1991;16:50.

4. Palmoski MJ, Brandt KD. Effects of static and cyclic compressive loading on articular cartilage plugs in vitro. Arthritis Rheum 1984;27:675–81.

5. Sah RL-Y, Kim YJ, Doong J-YH, Grodzinsky AJ, Plaas AHK, Sandy JD. Biosynthetic response of cartilage explants to dynamic compression. J Orthop Res 1989;7:619–36.

6. Parkkinen JJ, Lammi MJ, Helminen HJ, Tammi M. Local stimulation of proteoglycan synthesis in articular cartilage explants by dynamic compression in vitro. J Orthop Res 1992;10:610–20.

7. Kim YJ, Sah RL, Grodzinsky AJ, Plaas AHK, Sandy JD. Mechanical regulation of cartilage biosynthetic behavior: Physical stimuli. Arch Biochem Biophy 1994;311:1–12.

8. Lee DA, Bader DL. Compressive strains at physiological frequencies influence the metabolism of chondrocytes seeded in agarose. J Orthop Res 1997;15:181–8.

9. Mow VC, Ateshian GA, Spilker RL. Biomechanics of diarthrodial joints: A review of twenty years of progress. J Biomech Eng 1993;115:460–7.

10. Lippiello L, Kaye C, Neumata T, Mankin HJ. In vitro metabolic response of articular cartilage segments to low levels of hydrostatic pressure. Connect Tissue Res 1985;13:99–107.

11. Suh J-K, Marui T, Malin C, Bai S. Dynamic hydrostatic pressure and cartilage biosynthesis: Theoretical and experimental analysis. Trans Orthop Res Soc 1996;21:329.

12. Kimura JH, Schipplein OD, Kuettner KE, Andriacchi TP. Effects of hydrostatic loading on extracellular matrix formation. Trans Orthop Res Soc 1985;10:365.

13. Giori NJ. Mechanical influences on the differentiation and modulation of skeletal and connective tissues, Ph.D. Thesis, Stanford University, 1994.

14. Hall AC, Urban JPG, Gehl KA. The effects of hydrostatic pressure on matrix synthesis in articular cartilage. J Orthop Res 1991;9:1–10.

15. Parkkinen JJ, Ikonen J, Lammi MJ, Laakkonen J, Tammi M, Helminen HJ. Effects of cyclic hydrostatic pressure on proteoglycan synthesis in cultured chondrocytes and articular cartilage explants. Arch Biochem Biophys 1993;300:458–65.

16. Veldhuijzen JP, Bourret LA, Rodan GA. In vitro studies of the effect of intermittent compressive forces on cartilage cell proliferation. J Cell Physiol 1979;98:299–306.

17. van Kampen GPJ, Veldhuijzen JP, Kuijer R, van de Stadt RJ, Schipper CA. Cartilage response to mechanical force in high-density chondrocyte cultures. Arthritis Rheum 1985;28:419–24.

18. Veldhuijzen JP, Huisman AH, Vermeiden JPW, Prahl-Andersen B. The growth of cartilage cells in vitro and the effect of intermittent compressive force. A histological evaluation. Conn Tissue Res 1987;16:187–96.

19. Lammi MJ, Inkinen R, Parkkinen JJ, Hakkinen T, Jortikka M, Nelimarkka LO, Jrverlainen HT, Tammi MI. Expression of reduced amounts of structurally altered aggrecan in articular cartilage chondrocytes exposed to high hydrostatic pressure. Biochem J 1994;304:23–730.

20. Smith RL, Rusk SF, Ellison BE, Wessells P, Tsuchiya K, Carter DR, Caler WE, Sandel LJ, Schurman DJ. In vitro stimulation of articular chondrocyte mRNA and extracellular matrix synthesis by hydrostatic pressure. J Orthop Res 1996;14:53–60.

21. Parkkinen JJ, Lammi MJ, Pelttari A, Helminen HJ, Tammi M, Virtanen I. Altered Golgi apparatus in hydrostatically loaded articular cartilage chondrocytes. Ann Rheum Dis 1993;52:92–198.

22. Wright MO, Stockwell RA, Nuki G. Response of plasma membrane to applied hydrostatic pressure in chondrocytes and fibroblasts. Conn Tissue Res 1992;28:49–70.

23. Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic creep and stress relaxation of articular cartilage in compression: theory and experiments. J Biomech Eng 1980;102:73–83.

24. Suh J-K, Li Z-F, Woo SL-Y. Dynamic behavior of a biphasic cartilage model under cyclic compressive loading. J Biomechanics 1995;28:357–64.

25. Suh JK. Dynamic unconfined compression of articular cartilage under a cyclic compressive load. Biorheology 1995;33:289–304.

26. Torzilli PA, Grigiene R, Huang C, Friedman SM, Doty SB, Boskey AL, Lust G. Characterization of cartilage metabolic response to static and dynamic stress using a mechanical explant test system. J Biomechanics 1997;30(1):1–9.

27. Green WT. Behavior of articular chondrocytes in cell culture. Clin Orthop 1971;75:248–60.

28. Morales TI, Wahl LM, Hascall VC. The effect of bacterial lipopolysaccharides on the biosynthesis and release of proteoglycans from calf articular cartilage cultures. J Bio Chem 1984;259(11):6720–9.

29. Labarca C, Paigen K. A simple, rapid, and sensitive DNA assay procedure. Anal Biochem 1980;102:344–52.

30. Kim YJ, Sah RL, Doong JYH, Grodzinsky AJ. Fluorometric assay of DNA in cartilage explants using Hoechst 33258. Anal Biochem 1988;174:168–76.

31. Peterkofsky KB, Diegelmann R. Use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. Biochemistry 1971;10:988–94.

32. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9.

33. Church GM, Gilbert W. Genomic sequencing. Proc Natl Acad Sci USA 1984;81:1991–5.

34. Broom ND, Myers DB. A study of the structural response of wet hyaline cartilage to various loading situations. Conn Tissue Res 1980;7:227–37.
35. Freeman PM, Natarajan RN, Kimura JH, Andriacchi TP. Chondrocytes cells respond mechanically to compressive loads. J Orthop Res 1994;12:311–20.
36. Guilak F, Ratcliffe A, Mow VC. Chondrocyte deformation and local tissue strain in articular cartilage: A confocal microscopy study. J Orthop Res 1995;13(3):410–21.
37. Sacks F. Mechanical transduction in biological systems. CRC Cr Rev Biomed Eng 1988;16(2):141–69.
38. Maroudas A. Physiochemical properties of articular cartilage. In: Freeman MAR, Eds. Adult Articular Cartilage. Kent, England: Pitman Medical 1979:215–90.
39. Eisenberg SR, Grodzinsky AJ. Swelling of articular cartilage and other connective tissues: Electromechano-chemical forces. J Orthop Res 1985;3:148–59.
40. Lai WM, Hou JS, Mow VC. A triphasic theory for swelling and deformation behaviors of articular cartilage. J Biomech Eng 1991;113:245–58.
41. Mow VC, Hou JS, Owens JM, Ratcliffe A. Biphasic and quasilinear viscoelastic theories for hydrated soft tissues. In: Mow VC, Ratcliffe A, Woo SL-Y, Eds. Biomechanics of Diarthrodial Joints. New York: Springer-Verlag 1990:215–60.
42. O'Hara BP, Urban JPG, Maroudas A. Influence of cyclic loading on the nutrition of articular cartilage. Ann Rheum Dis 1990;49:536–9.
43. Lee RC, Frank EH, Grodzinsky AJ, Roylance DK. Oscillatory compressional behavior of articular cartilage and its associated electromechanical properties. J Biomechanics 1981;103:280–92.
44. Kim YJ, Bonassar LJ, Grodzinsky AJ. The role of cartilage streaming potential, fluid flow and pressure in the stimulation of chondrocyte biosynthesis during dynamic compression. J. Biomechanics 1995;28(9):1055–66.
45. Mow VC, Lai WM. Recent development in synovial joint biomechanics. SIAM Review 1980;22(3):275–317.
46. Grodzinsky AJ. Electromechanical and physico-chemical properties of connective tissue. CRC Cr Rev Biomed Eng 1983;9:133–99.
47. Lee RB, Bartels E, Urban JPG. The role of oxygen in cartilage metabolism. Trans Orthop Res Soc 1995;20:385.