Biochemical and biomechanical alterations in equine articular cartilage following an experimentally-induced synovitis

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Summary

The effects of inflammation on the biochemical and biomechanical properties of articular cartilage at two sites (dorsal and palmar) from the radial facet of the equine third carpal bone were examined in response to a synovitis induced with Escherichia coli lipopolysaccharide (LPS). Four groups were studied. In group 1 synovitis was induced at time zero and evaluated at week 6. Group 2 was the sham-treated control for group 1. In group 3 synovitis was induced at time zero and evaluated at week 2. Group 4 was the sham-treated control for group 3.

There was a significant increase (P < 0.05) in newly synthesized proteoglycan PG from both sites in group 3 as compared to the sham-treated groups and group 1. No significant difference in the endogenous PG concentration between groups or sites was detected. Sepharose CL-2B revealed two peaks of newly synthesized PG in all groups; an early peak (Kav 0.11–0.13) and a late peak (Kav 0.48–0.64). Newly synthesized PG profiles from sham-treated groups and group 3 were similar, but the group 3 PG profile exhibited a more pronounced early peak. Conversely, the PG profile from group 1 demonstrated a more prominent late peak. Electrophoresis and Western blot analysis of the pooled late PG peak fractions from the sham-treated and group 1 showed a single toluidine blue stained band from both sites which reacted with monoclonal antibody (MAb) 1C6. By contrast, the late peak from the palmar site in group 3 showed an additional faster moving component on composite gels which did not react with MAb 1C6.

There was a significant decrease in Poisson's ratio and a significant increase in cartilage thickness in groups 1 and 3 which had received synovitis. The increase in cartilage thickness of groups 1 and 3 was also significantly affected by site (dorsal > palmar). There was no significant difference in aggregate modulus or permeability constant among groups.

Primary joint inflammation induced by LPS alters the biochemical and biomechanical properties of the articular cartilage as a function of time and site. An increase in chondrocyte PG synthesis in the early period following synovitis may be a reparative response to the inflammatory insult. Continued alterations in the qualitative PG composition in the later period following synovitis may represent a shift in chondrocyte metabolism to repopulate the existing cartilage matrix.

Key words: Cartilage, Proteoglycans, Synovitis, Biomechanics.

Introduction

The clinical diagnosis of joint inflammation is predicated upon the hallmark signs of effusion, heat and pain. The gross changes of inflammation detectable on the exterior joint surfaces are linked with macroscopic and microscopic alterations occurring in the interior of the joint [1–3]. In short, joint effusion, heat and pain are represented by increased synovial membrane hyperemia and thickening and decreased synovial fluid viscosity and clarity. These macroscopic changes are related to well-defined microscopic events of the inflammatory process including alterations in cell surface adhesion molecule expression, inflammatory cell influx and the release of inflammatory mediators and enzymes. The release of neutrophil or macrophage-derived mediators and/or enzymes, in turn, have been associated with alterations in chondrocyte metabolism and cartilage extracellular matrix destruction [4]. The alterations in tissue homeostasis associated with the inflammatory process are generally assumed to occur uniformly throughout the joint. Conversely, the predisposition of articular cartilage lesions in anatomically specific locations in primary or post-traumatic osteoarthritis has been strongly associated with...
alterations in joint, mechanics [5–7]. While location-dependent changes in the articular cartilage in response to mechanical alterations is clear, the nature of the joint hard tissue response to the inflammatory process has been incompletely investigated. Further investigation of the overall and topographical response of the articular cartilage to inflammation is required in order to determine if a selective capacity for destruction and recovery exists within the joint.

The relationship and significance of topographical variations of articular cartilage biomechanical and biochemical properties has been described for selected joints [8, 9]. Site-specific changes in these properties have also been described in a canine model following an increase in the load applied to the articular tissues via exercise [10–12]. Recent studies also support a link between the location of naturally occurring osteochondral lesions associated with post-traumatic osteoarthritis in the equine midcarpal joint and the innate biomechanical/biochemical characteristics of these sites [13–15]. From these studies it is hypothesized that the response of the articular cartilage to an inflammatory insult may be dependent upon its innate topographical diversity. For example, if an area of articular cartilage within a joint possesses less compressive resistance associated with reduced proteoglycan content or a more permeable matrix organization, the effects of the inflammatory process would be expected to be more pronounced than when compared to an area with greater compressive stiffness, greater proteoglycan content or a less permeable matrix. In addition, the capability of the chondrocytes in a given site to respond to the inflammatory event may be inherently different than that of another site, and therefore the potential to repair even a minor insult may be variable.

The induction of a mild to moderate synovitis which does not create grossly detectable cartilaginous damage provides a method to test this hypothesis [16]. The horse serves as an excellent model for several reasons. Namely, acute synovitis is a common clinical condition seen in the midcarpal joint of young race horses during training and racing. A large percentage of these horses will eventually be treated for post-traumatic osteoarthritis [17]. The frequency, severity and histopathological changes seen with in equine osteoarthritis are similar to that seen in man [18]. In addition, the relative size of the equine joint as compared to the dog or rabbit allows for the collection of adequate sample sizes to permit both biochemical and biomechanical analyses from the same joint reducing within and between subject variability.

Materials and methods

GROUP SELECTION

Ten horses were selected for study (mean age 6.6 years, mean body weight 440.3 kg) on the basis of normal clinical orthopedic examination, radiographic examination and synovial fluid analysis. All procedures were approved by the Institutional Laboratory Animal Care and Use Committee. Horses were exercised on a high-speed treadmill (Swedish Sato, Uppsala, Sweden) for 20 min once weekly at 4 m/s for 4 weeks prior to the start of the study to acclimate horses to the treadmill and to standardize the level of activity on the joint between animals. Between exercise intervals, horses were individually housed in 12×12 ft boxstalls. Horses were exercised at the same time period weekly throughout the course of the study.

SYNOVITIS MODEL

The use of Escherichia coli lipopolysaccharide (LPS) to create a mild to moderate synovitis in the equine joint has been previously reported [16]. Briefly, carpi of groups 1 and 3 (see below) were prepared for aseptic intra-articular injection of 0.125 ng LPS (serotype B:055) in 0.5 ml sterile phosphate-buffered saline (PBS; pH 7.2). Intra-articular injections of LPS were repeated every 48 h for a total of four treatments to maintain the inflammation for a 1-week duration. The midcarpal joints of groups 2 and 4 (see below) received sham-treatment. Sham-treatment consisted of aseptic arthrocentesis without concurrent injection at similar time periods as groups 1 and 3.

EXPERIMENTAL DESIGN

Ten horses with 20 normal carpi were divided randomly into the following groups (Table I): group (1): synovitis induced at time zero and were evaluated at week 6; group (2): no synovitis induced at time zero but received sham-treatment and were evaluated at week 6; group (3): synovitis induced at time zero and were evaluated at week 2; group (4): no synovitis induced but received sham-treatment and were evaluated at week 2. Tissue was harvested from carpi at the end of each study period from groups with synovitis (groups 1 and 3) or without synovitis (groups 2 and 4) at either 2 weeks (groups 3 and 4) or at 6 weeks (groups 1 and 2) after intervention. Carpi were
assigned to groups to avoid comparison of carpi from an individual horse for either the evaluation of time effects (week 2 to 6) or inflammation effects (synovitis or sham-treated).

At the end of the study, comparisons were made between groups 1 and 2 to evaluate the effect of synovitis induced 6 weeks earlier; between groups 3 and 4 to evaluate the effect of synovitis induced 2 weeks earlier; groups 1 and 3 to evaluate the effect of time on carpi with synovitis; and groups 2 and 4 to evaluate the effect of time on carpi without synovitis.

Tissue Sampling

Horses were killed humanely with an overdose of sodium pentobarbital solution. Both midcarpal joints were aseptically opened and the articular cartilage visually evaluated for gross fibrillation or erosion. Articular cartilage specimens were collected from the central aspect of the radial facet divided into dorsal and palmar sections (Fig. 1). Specimens were collected using a power drill and core biopsy instrument designed to provide full-thickness plugs of approximately 2 mm in diameter [19]. Each core biopsy site was continuously irrigated with Ringer’s lactate solution (Baxter Healthcare Corp., Deerfield, IL, U.S.A.) during the harvesting procedure to retard thermal necrosis of the cartilage plugs. Cartilage plugs were individually examined during collection to determine the presence of subchondral bone, which if found was dissected. Cartilage plugs from dorsal and palmar sites were immediately placed into separate transfer vials containing chilled Dulbecco’s modified Eagle’s medium (DMEM; Gibco, BRL, Gaithersburg, MD, U.S.A.).

Proteoglycan Biosynthesis

Articular cartilage from each site and animal were removed from the transfer vials and cultured in individual culture wells (35 mm in diameter) containing 3 ml DMEM supplemented with 10% (v/v) fetal bovine serum. To each culture, 40 μCi of $^{35}$SNa$_2$SO$_4$ (New England Nuclear Research Products, Boston, MA, U.S.A.) in 0.5 ml DMEM media was added to radiolabel newly synthesized proteoglycans. Explant cultures were incubated at 37°C for 18 h in an atmosphere of 5% CO$_2$/95% air. Following incubation, the cartilage plugs were removed and rinsed four times with sterile PBS. Subsequent biochemical analyses was

Table I.
Assignment of limbs to experimental procedures

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Fig. 1. Carpal joint anatomy and collection sites (inset) from the radial facet of the equine third carpal bone. Sites A (dorsal) and B (palmar) represent the central aspect of the radial facet which were used for proteoglycan analysis. Site C represents the medial aspect of the radial facet which was used for biomechanical analysis. Two sites on site C were evaluated by indentation testing and corresponded to the middle of the dorsal portion and the middle of the palmar portion of the radial facet. RF, radial facet; IF, intermediate facet.
performed on articular cartilage explants from eight horses.

Explants from each site and animal were extracted for 48 h at 4°C with 5.0 ml 4.0 M guanidine hydrochloride (GuHCl; Sigma Chemical Co., St Louis, MO, U.S.A.)/0.1 M sodium acetate (pH 5.8) containing the protease inhibitors; 6-aminohexanoic acid (100 mM), disodium-EDTA (10 mM) and benzamidine (5 mM). Extracts were exhaustively dialyzed overnight at 4°C against distilled water (molecular cutoff, 6–8000; Spectrum Medical Industries, Inc., Houston, TX, U.S.A.) to remove unincorporated ³⁵SO₄, and to promote aggregation of the proteoglycan with hyaluronic acid in the GuHCl extract. The volume of the non-dialyzable extract was recorded and radioactivity counted by the liquid-scintillation method in a Tri-carb Parkard model 3255 liquid-scintillation spectrometer (Beckman, Model 3255, Palo Alto, CA, U.S.A.) using a 100 μl aliquot of the total non-dialyzed volume (ICN Biochemicals, Costa Mesa, CA, U.S.A.).

**PROTEOGLYCAN CHARACTERIZATION**

The uronic acid content from each site and animal was determined on a 200-μl aliquot of the total non-dialyzed volume using a modified carbazole method with D-glucuronolactone as standard [20]. Protein content was measured on a 100-μl aliquot of the total non-dialyzed volume using the bicinchoninic acid assay with bovine serum albumin as standard [21]. The remaining non-dialyzable extract was pooled according to site and group, lyophilized and stored at -80°C for further analysis.

Lyophilized samples from each site and group were suspended in 2 ml of 50% glycerol in 20 mM Tris-hydroxy buffer (pH 7.5) containing 100 mM sodium chloride. To one-half of the 2-ml sample, 10 μg of hyaluronic acid (human umbilical cord HA; Sigma Chemical Co., St Louis, MO, U.S.A.) was added and incubated at room temperature for 5 h. Both samples were chromatographed over a Sepharose CL-2B column (0.9 cm x 100 cm) (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) with 0.5 mM sodium acetate (pH 7.0) associative buffer. Void volume (V₀) and included volume (Vₜ) of the column were determined by blue dextran and ³⁵SO₄, respectively. The chromatographic profile of newly synthesized ³⁵SO₄ labeled-proteoglycans was determined by counting 100 μl of each fraction [22, 23]. Fractions eluted from the column which corresponded to the late PG peak (see below) were pooled, dialyzed overnight and lyophilized.

Lyophilized ³⁵SO₄-labeled material was reduced at 37°C for 4 h with 10 mM dithiothreitol dissolved in 4 M GuHCl/1 mM Tris; pH 8.0 followed by alkylation with an equal volume of 80 mM iodoacetic acid in 4 M GuHCl/1 mM Tris; pH 8.0. This procedure derives the proteoglycan monomer hyaluronic acid binding region (HABR) for recognition of monoclonal antibody (MAb) 1C6 (Hybriodoma Bank, Baltimore, MD, U.S.A.). Following reduction/alkylation, samples were dialyzed at 4°C overnight (molecular cutoff 2000) and lyophilized to reduce volume prior to electrophoresis on composite agarose/polyacrylamide gels [24]. Reduced/alkylated samples were electrophoresed in duplicate on two slab gels; one gel was used for toluidine blue staining and the other for Western blotting with MAb 1C6. Slab gels were prerun for 1 h at 50 V prior to loading samples. Samples were dissolved in running buffer (10 mM Tris acetate buffer, pH 6.3/0.25 M sodium sulfate) followed by an equal volume of sample buffer (60% (w/v) sucrose with 0.01% (w/v) bromophenol blue and 0.2% (v/v) Triton X-100). The entire sample from each site was loaded onto the gel in order to maximize detection. Electrophoresis was carried out for 18 h at 4°C at 50 V.

Some gels were fixed (50% methanol and 10% acetic acid) for 1 h and subsequently stained with 0.02% toluidine blue in 0.1 M acetic acid. Toluidine blue-stained gels were destained with 3% acetic acid. Each toluidine-stained gel was transferred to a Gelbond film (FMC; Rockland, ME, U.S.A.) for 20 min and films were dried in a 60°C incubator overnight.

Some gels were used for Western blotting. These were placed on top of two layers of filter paper (Whatman No. 3 MM) and soaked in transfer buffer (0.125 M Trizma base, 0.96 M glycine and 12.5% methanol). Immobilon-P membrane (0.45 μm; Millipore, Boston, MA, U.S.A.) was pre-treated briefly with methanol and distilled water followed by equilibration in transfer buffer and placed on top of the gel followed by two additional pieces of soaked filter paper. Each filter paper/gel sandwich was placed into an electrotransfer apparatus (E-C Electrobolt, St. Petersburg, FL, U.S.A.) containing cold transfer buffer. Gel transfer to Immobilon membrane was performed overnight at 4°C for 19 h at 0.4 amps.

After transfer, Immobilon-P membranes were soaked in 3% gelatin in Tris-buffered saline (TBS; 20 mM Tris and 500 mM sodium chloride, pH 7.5) for 1 h and then washed for 10 min in 0.05% Tween-20-TBS (TTBS). Each membrane was incubated overnight with a 1:1000 dilution of MAb 1C6 in buffer (1% gelatin in TTBS) at room
temperature. Western blots were developed by washing each gel in TTBS for 10 min to remove MAb 1C6 and then incubated with a 1:12 200 dilution of an alkaline-phosphatase anti-mouse IgG antibody in antibody buffer for 2 h at room temperature. Membranes were washed twice more with TTBS (10 min) followed by one wash in TBS (10 min). Membranes were subsequently incubated for 30 min with a mixture of 3 ml alkaline phosphate substrate (100 mM Tris, 100 mM NaCl, 5 mM magnesium chloride, pH 9.5) plus 20 μl nitro-blue tetrazolium and 10 μl 5-bromo-4-chloro-3-indolyl phosphate. Membranes were rinsed with distilled water and air-dried.

The cartilage residue after GuHCl extraction from dorsal and palmar sites in one horse from each group was completely dissolved in 2 N sodium hydroxide for 2 days at 37°C, and then neutralized by an equal volume of 2 N hydrochloric acid for solubilization of all remaining glycosaminoglycan. Aliquots were removed from the final digest to determine uronic acid content. This value was then employed to determine the extraction efficiency of the GuHCl method for horse cartilage PG.

BIOMECHANICAL ANALYSIS

Immediately after collection of samples for biochemical analysis, osteochondral specimens were sectioned from the medial aspect of the radial facet immediately adjacent to the area selected for PG analysis (Fig. 1). Specimens were sectioned in the transverse plane at approximately 2 cm below the level of the proximal articular cartilage surface. Each specimen was briefly rinsed in PBS (pH 7.2) and then stored at -80°C until indentation testing. At no time was any sample thawed prior to testing. Therefore, each sample underwent only one freeze–thaw cycle prior to testing. Prior to indentation testing, each specimen was mounted in the testing machine holder with cyanoacrylate cement and thawed for 1 h at room temperature in a normal (0.15 m) saline solution containing protease inhibitors as previously described [25]. Specimens were maintained in the ‘enzyme inhibitor bath’ throughout the testing procedure. Two sites on each specimen were chosen for indentation testing, one located in the center of the dorsal aspect of the radial facet and one located in the center of the palmar aspect of the radial facet. At each site, the articular surface of the specimen was visually aligned perpendicular to the porous indenter tip. Biphasic creep indentation and recovery testing was performed using a plane-ended circular porous microindentor (1.5 mm) in a creep indentator testing apparatus [26]. A 0.02-N tare load was first applied at each site to ensure full contact between the articular cartilage surface and the indenter tip. The tare load was maintained until cartilage displacement reached an equilibrium, approximately 1200 s. Following this, an instantaneous 0.2-N test load was applied and cartilage displacement recorded until equilibrium was reached [25]. Cartilage creep recovery was performed at each site to confirm appropriate indentation testing apparatus function and tissue health. Creep recovery displacement versus time data was collected over an 1800-s time period after removing the 0.2-N test load, but without altering the tare load. The per cent recovery at each site was calculated by dividing the absolute change in creep recovery displacement by the total creep indentation displacement. The total time required for alignment, testing and recovery of each site was approximately 2–3 h per specimen.

Cartilage thickness at each site was determined after creep recovery of the cartilage following indentation testing. Cartilage thickness was measured by driving a steel needle probe through the articular surface into the underlying subchondral bone and recording the force versus displacement data. Cartilage thickness was calculated by measuring the displacement between the force change as the needle probe contacted the articular cartilage surface to when the needle probe contacted the subchondral bone plate. Cartilage displacement at equilibrium, cartilage thickness and indentator tip radius were used to calculate the intrinsic material properties of each site and specimen in a PC-adapted version of the linear KLM biphasic model of articular cartilage [27]. Included in this model are the aggregate modulus (H), the permeability constant (k), and Poisson’s ratio (ν).

STATISTICAL ANALYSIS

Statistical analysis was performed using a repeated measures mixed ANOVA for site and group corrected for variance among horses (Statistical Analysis Systems, Cary, NC, U.S.A.). Contrasts were evaluated with Bonferroni’s correction applied to the number of comparisons performed. P values <0.05 were considered significant.

Since there was no statistically significant differences in biochemical or biomechanical properties between groups 2 and 4 (sham-treated), values for these groups were combined in the presentation of data.
Results

CLINICAL AND PATHOLOGICAL OBSERVATIONS

Joints injected with LPS exhibited mild to moderate synovitis with a mean ± s.e.m. total nucleated cell count of 194.0 × 10⁶ cells/l and total protein of 47.75 g/l ± 3.3 g/l which began 12 h after the initial injection. No changes in weight, 'attitude', appetite or systemic white blood cell count during the course of the study were noted. At death, the synovial membrane, synovial fluid and articular cartilage in each joint appeared grossly normal.

PROTEOGLYCAN RESULTS

There was a significant increase in newly-synthesized PG in group 3 when compared to groups 2 and 4 (sham-treated) or to group 1 [Fig. 2(a)]. The site of collection had no effect on this difference (i.e. dorsal versus palmar) (data not shown). The significant increase in the newly-synthesized PG concentration in group 3 was not reflected in the endogenous PG concentration when compared to groups 2 and 4 (sham-treated) or group 1 [Fig. 2(b)]. There was no significant difference in either the newly synthesized or endogenous PGs of the articular cartilage between group 1 and groups 2 and 4 (sham-treated). PG extraction efficiency was comparable among groups with an average PG extraction efficiency of 49.6%.

Chromatographic profiles of newly synthesized PG differed considerably among the groups (Fig. 3) and between sites. All groups exhibited at least 2 PG peaks on Sepharose CL-2B; an early peak (Kₑₐ 0.11-0.13) and a later peak (Kₑₐ 0.48-0.64). Newly synthesized PG profiles from groups 2 and 4 (sham-treated) [Fig. 3(a)] and group 3 [Fig. 3(b)] largely overlapped, with the exception of a more pronounced early peak in group 3 [Fig. 3(b)]. By comparison, the newly synthesized PG profile from group 1 [Fig. 3(b)] was very different. In addition, to a large decrease in ³⁵SO₄ associated with the early PG peak, the size of the PG in the later peak

![Fig. 2](image-url)

![Fig. 3](image-url)

tended to be hydrodynamically smaller [Fig. 3(c)]. Dorsal and palmar sites from groups 2 and 4 (sham-treated) and group 1 had similar PG chromatographic profiles, but the early peak was more pronounced in the sham-treated groups than in group 1. In group 3, the dorsal site cartilage showed an early PG peak which was more pronounced than the late PG peak, while PG from the palmar site demonstrated a broad PG peak which eluted from the column between the early and late PG peaks. Addition of HA to the samples did not alter the chromatographic profile in any group or in cartilage from either dorsal or palmar sites.

The $K_m$ of the early PG peak was consistent with PG of large hydrodynamic size. The $K_m$ of the late PG peak was less well defined and therefore further examined to determine the nature of this PG subpopulation. The migration patterns from groups 2 and 4 (sham-treated) and group 1 at dorsal or palmar sites did not differ. These samples exhibited a single band of PG (PG$_1$) after staining with toluidine blue [Fig. 4(a), lane 1]. The migration rate was similar to human cartilage PG monomer A1D1 which was electrophoresed simultaneously with the experimental samples [Fig. 4(a), lane 3]. These samples also reacted positively with MAb 1C6 on Western blots [Fig. 4(b), lanes 1 and 3]. A slab gel analysis of group 3 demonstrated a difference in the migration pattern for specimens collected from the palmar aspect of the facet only. In this area, there appeared to be 2 PG bands, PG$_1$ and PG$_{II}$ [Fig. 4(a), lane 2]. PG$_{II}$ migrated faster than either PG$_1$ or human cartilage PG monomer A1D1 [Fig. 4(a), lane 2]. Western blotting showed that this gel zone did not react with MAb 1C6 [Fig. 4(b), lane 2]. Specimens from the dorsal aspect of the radial facet from group 3 showed a migration pattern similar to both dorsal and palmar sites of group 1, groups 2 and 4 (sham-treated) and human cartilage PG monomer A1D1 samples. Cartilage PG of both sites from group 1 also reacted with MAb 1C6.

Fig. 4. Toluidine blue stained composite agarose/polyacrylamide gels (a) and Western blots with monoclonal antibody (MAb) 1C6 (b) from cartilage GuHCl extracts of the palmar aspect of the radial facet. Lane 1, sham-treated (groups 2 and 4); lane 2, 2 weeks after synovitis (synovitis, week 2 group 3); lane 3, and human cartilage PG monomer A1D1. All samples were reduced/alkylated. Samples from sham-treated groups contained the proteoglycan population PG$_1$, which migrated with human cartilage PG monomer A1D1 and reacted with MAb 1C6. Samples from cartilage specimens 2 week after synovitis (synovitis, week 2 group 3) contained a similar migrating PG$_1$ subunit but also contained a PG$_{II}$ subunit which did not react with MAb 1C6 (arrow).
BIOMECHANICAL RESULTS

Poisson’s ratio was significantly decreased in group 1 and group 3 as compared to groups 2 and 4 (sham-treated), however, there was no effect of anatomical site on Poisson’s ratio [Fig. 5(a)]. Articular cartilage thickness in group 1 and group 3 was significantly greater than groups 2 and 4 (sham-treated) [Fig. 5(b)]. Specimens collected from the dorsal aspect of group 1 and group 3 were significantly thicker as compared to the palmar aspect [Fig. 5(b)]. There was trend \( P=0.07 \) for a significant increase in the aggregate modulus between group 1 and group 3 as compared to groups 2 and 4 (sham-treated) [Fig. 5(c)]. While, there was no significant difference in the permeability constant between groups, there was a significant increase in the permeability constant for palmar sites as compared to dorsal sites in all groups [Fig. 5(d)].

Discussion

The results of the present study demonstrated an increase in chondrocyte newly synthesized PG, an alteration in the hydrodynamic size of the predominant synthesized PG, and an alteration in the biomechanical properties of the articular cartilage in response to an inflammatory process produced by LPS as both a function of time and site. A companion study, demonstrated alterations in the synovial fluid and synovial membrane up to 6 weeks following intra-articular LPS injection [28]. While the classic inflammatory changes (i.e. increased synovial fluid total nucleated cell and...

![Fig. 5](image-url)
total protein concentrations) were short-lived (resolving within 1 week), increased levels of synovial fluid eicosanoids, prostaglandin \( E_2 \) and thromboxane \( B_2 \) persisted. These findings correlated with synovioctye hyperplasia 6 weeks following induced synovitis in the histopathological analysis of the synovial membrane. Therefore while the classic parameters associated with inflammation had subsided by 6 weeks, residual repair mechanisms might still be active in the joint. These results may suggest a link between the chondrocyte response to the presence of inflammatory mediators which presumably act to resolve and repair the cartilage matrix.

The qualitative changes in the newly synthesized and endogenous PG were evident in the Sepharose CL-2B and the electrophoretic and Western blot profiles, respectively. In the groups 2 and 4 (sham-treated), the newly-synthesized PG from dorsal and palmar sites consisted of approximately equal proportions of an early PG peak which in all likelihood is hydrodynamically large aggregan (K\text{av} 0.11–1.13) and a late peak (K\text{av} 0.48–0.64) which is likely to contain aggregan fragments but may contain other hydrodynamically small PGs as well. All profiles represented the typical chromatographic profile of unpurified GuHCl cartilage extracts and demonstrated the polydisperse nature of the newly-synthesized PG of equine articular cartilage. The fact that exogenous HA did not cause PG aggregate formation suggested that newly synthesized equine cartilage PG had a low affinity for HA as has been shown previously for newly synthesized human cartilage PG [29]. However, additional studies to determine the optimal proportions of HA to monomer must be conducted to prove this theory. Further analysis of pooled reduced/alkylated late peak fractions demonstrated a single PG monomer in the endogenous population (PG\text{I}) that exhibited a migration rate identical to human cartilage PG monomer (fraction A1D1). Human cartilage PG monomer A1D1 and equine cartilage PG\text{I} reacted with MAb 1C6 after reduction/alkylation, but PG\text{II} (a significant component of group 3) did not. MAb 1C6 has been shown to react with the HABR (G1 domain) of aggregan after reduction/alkylation and with the G2 domain only after treatment of the latter with keratanase [30]. Since equine cartilage PG was transferred to Immobilon-P from composite gels directly without prior keratanase digestion, these results suggested that MAb 1C6 was reacting with equine HABR [31].

The chromatographic profile of cartilage exposed to synovitis 1 week earlier (group 3) differed between sites and when compared to the sham-treated groups. The dorsal cartilage site of group 3 had a greater proportion of newly synthesized large PG, while the palmar site had a broad profile of both newly synthesized large and small PG. The reasons for this differential response are unknown, but reflect differences in the selective ability of chondrocytes from each site to respond to the synovitis or reflect difference in the relative location of each site to the synovial membrane. Qualitative analysis of the endogenous PG population demonstrated two populations of PG monomer subunits (PG\text{I} and PG\text{II}) in the palmar site of group 3. Since PG\text{I} migrated similarly in all groups and reacted with MAb 1C6, PG\text{I} probably represented a normal PG monomer constituent of the cartilage. However, PG\text{II} from the palmar site of group 3 migrated faster in the gel in comparison to both the PG\text{I} and the human cartilage PG monomer A1D1 and did not react with MAb 1C6. Therefore PG\text{II} may represent a degradation product in the matrix. The large increase in the newly synthesized PG monomer from this site seen on chromatography may reflect an attempt by the chondrocytes to replace PG monomer. Since the PG\text{II} band was not present in cartilage from group 1 it was possible that it undergoes further degradation eventually being released from the matrix during the 4-week period.

The chromatographic profiles from dorsal and palmar sites were essentially identical in cartilage from joints exposed to synovitis 6 weeks earlier (group 1). However, these profiles still remained qualitatively different from the sham-treated groups in the relatively low proportion of large PG and the large contribution of small PG to the total newly synthesized PG population. Comparing these results with those obtained from group 3 suggested that this may represent a gradual shift of chondrocyte metabolism towards that which would normalize and replenish the endogenous population of PG in the articular cartilage matrix. Conversely, since large PG is the principle component of the articular cartilage matrix, the continued production of smaller PG species may also represent an alteration in chondrocyte activity as a result of the synovitis insult. However, the composite profiles and Western blots of the endogenous PG monomer from group 1 cartilage were indistinguishable from that of the sham-treated groups. This result further supported a reversion of the matrix to that seen without synovitis.

Previous \textit{in vitro} studies employing LPS stimulated articular cartilage explants demonstrated that an 0.001 \( \mu \text{g/ml} \) LPS concentration did not stimulate glycosaminoglycan (GAG) release over
control values [32]. In addition, the hydrodynamic size or GAG concentration of PG has been shown to be reversible up to 8 days of continuous culture with LPS [33]. A recent report demonstrated that the response to equine cartilage explants to LPS is age-dependant, with cartilage harvested from a young horse (4-year-old) exhibiting a greater suppression of PG synthesis and a greater release of GAG into the culture media as compared to an older horse (20-year-old) following a 3-day exposure to various concentrations of Salmonella typhosa-derived LPS [34]. In the present study, an increase in PG synthesis was detected in the early time period following in vivo injection of LPS. The differences in these studies may be associated with the presence of in vivo factors which are released in the inflammatory process either by the synovial membrane, cellular infiltrate or articular cartilage which may boost chondrocyte reparative mechanism to the inflammatory insult.

The average PG extraction efficiency of equine full-thickness cartilage plugs harvested in this study was lower than that previously reported for freezer-milled human and rabbit cartilage under similar extraction conditions [23]. However, since there was no statistical difference among samples in PG extraction efficiency, the extraction method was consistent and supports group comparisons.

The results of indentation testing indicated that the biochemical alterations seen in response to inflammation may be associated with changes in the biomechanical material properties which reflect the organizational structure of the articular cartilage extracellular matrix. In the present study, Poisson's ratio of cartilage exposed to synovitis (group 1 and group 3) was significantly lower than in the sham-treated groups. A low Poisson's ratio is indicative of a tissue with greater compressibility. The decreased Poisson's ratio in group 1 and group 3 suggests a change in the matrix organization which could allow for this expansion. The chromatographic profiles of group 1 and group 3 demonstrated alterations in the newly synthesized PG as compared to the sham-treated groups and support this viewpoint. Group 1 and group 3 also had a significantly greater thickness than the sham-treated groups and a significantly greater thickness in the palmar site when compared to the dorsal site. This may suggest location-dependent swelling of the tissue in response to both degradative and synthetic events or matrix re-organizational events [35]. Since increased articular cartilage thickness and chondrocyte metabolism accompany early events in osteoarthritis pathology, the large increase in articular cartilage thickness in the dorsal site in cartilage exposed to synovitis (group 1 and group 3) is of particular interest since this is the predominant site of osteochondral lesions in racehorses [36, 37]. In addition, a trend toward an increase in aggregate modulus of cartilage exposed to synovitis (group 1 and group 3) was noted. Since the aggregate modulus is an indicator of the overall PG content, the increased synthesis of PG in group 3 and the large contribution of newly-synthesized PG monomer in group 1 may contribute to this result.

The results of this study extend previous contentions and provides new evidence that global joint inflammation may change the biochemical and biomechanical properties of the articular cartilage. We demonstrated that a mild short-lived synovitis resulted in early changes in the articular cartilage matrix. This study also supported the view that cartilage alterations in response to a joint injury may be location-dependent. Therefore, inflammation may play a primary role in cartilage response to initial injury. Repeated bouts of acute synovitis may weaken the articular cartilage structure and alter chondrocyte reparative mechanisms. The salient novel finding of the present study was that quantitative and qualitative changes in the articular cartilage continue to progress after the "inflammatory" response has largely subsided.

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