Osteoarthritis and Cartilage

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Exercise and injury increase chondroitin sulfate chain length and decrease hyaluronan chain length in synovial fluid

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Summary

Objectives: (1) To investigate the effects of exercise and osteochondral (OC) injury on synovial fluid (SF) chondroitin sulfate (CS) and hyaluronan (HA) concentration and chain length, (2) to compare SF and cartilage CS data from joints with OC fragmentation, and (3) to compare SF CS and HA profiles with those seen in serum from the same horses.

Methods: Serum and SF were obtained from (1) normal horses after 8 weeks rest, (2) the same horses after 9 months treadmill training, and (3) horses with OC injury from racing. Articular cartilage was also collected from group 3 horses. Concentrations and chain lengths of CS and HA were determined by gel chromatography and fluorophore-assisted carbohydrate electrophoresis.

Results: SF CS peak chain length in the OC injury group increased significantly (18.7 kDa) when compared to rested horses (11.6 kDa), with exercise producing an intermediate chain length (15.6 kDa). Cartilage and serum from the OC injury group had the abnormally long CS chains seen in SF from these horses. Total SF HA was significantly lower in the OC injury group compared to the rested group. Both the OC injury group and the exercised group had significant decreases in SF HA chain length compared to the rested group.

Conclusions: Chain length of SF CS was increased by exercise and OC injury. Exercise resulted in a modest increase, whereas OC injury caused a marked increase. In contrast to CS, SF HA chain length was decreased by OC injury, and to a lesser extent by exercise. Chain length analysis of SF CS and HA may provide a useful tool for evaluation of joint health.
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Key words: Chondroitin sulfate, Hyaluronan, Synovial fluid, Cartilage, Serum, Exercise, Osteochondral injury, Horse.

Introduction

Synovial fluid (SF) glycosaminoglycan (GAG) composition can vary with age, exercise, and joint injury. The concentration, isomer composition and immunoreactivity of GAGs, such as chondroitin sulfate (CS), keratan sulfate, and hyaluronan (HA) have been widely studied as biomarkers to monitor changes in the metabolism of joints[1–6]. Exercise and injury produce changes in CS sulfation patterns and concentrations in articular cartilage and SF[1,7–13]. Although exercise was reported to have no effect on SF HA concentration[14], there are many reports that injury and osteoarthritis (OA) affect SF HA concentration[15–20] and chain length[21,22].

Accurate assessment of the role of biomarkers in diagnosis has been hampered by the fact that cartilage and SF samples are seldom available from patients with early OA. Validation requirements for biomarkers of OA are many,

including the presence of the biomarker in articular cartilage and knowledge of its function. There should also be an understanding of the relationships between changes in cartilage metabolism and changes in the biomarker in body fluid compartments[23]. As a translational model of OA, the horse presents a number of advantages. Horses are relatively long-lived (over 30 years) and therefore reduce the more rapid aging effect seen in other animal models[24]. Human and horse cartilage thickness are very similar (∼2 mm), with comparable age-related changes[25–27]. In addition, horses are readily adaptable to treadmill exercise, and equine joints can be repetitively sampled without general anesthesia or lavage. SF can be collected concurrently with blood, which provides abundant sample volumes of pertinent tissues for multiple biomarker assessment.

Abnormally long CS chains have been reported for newly synthesized aggrecan in post-injury canine OA cartilage[28]. To date, there is no published study on the effect of exercise and joint injury on GAG chain length in SF. In the current study, we have used horses to evaluate the effects of exercise and injury on the chain lengths of CS and HA by using paired samples of SF, cartilage, and serum. Our objective was to compare the structure of these GAGs in different body compartments to establish potential metabolic relationships that may lead to simplified

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diagnostic evaluation of joint diseases. The specific purposes of this study were (1) to investigate the effects of exercise and osteochondral (OC) injury on SF CS and HA concentration and chain length, (2) to compare SF and cartilage CS data from joints with OC fragmentation, and (3) to compare SF CS and HA profiles with those seen in serum.

Materials and methods

STUDY ANIMALS AND EXPERIMENTAL DESIGN

SF and serum were obtained from three groups of horses: (1) Rested group: SF from the middle carpal joints of nine Thoroughbred horses (3–6 years of age) after they had been given pasture rest for 8 weeks. (2) Exercised group: after 9 months of treadmill exercise, the same joints from group 1 horses were sampled after a period of maximal exercise (stress testing) in which treadmill speed was incrementally increased until the horse could no longer maintain position on the treadmill. Serum samples were obtained immediately after maximal exercise and then again 1 h later. Over the 9-month period, treadmill exercise was performed every Monday–Wednesday–Friday, at 4 m/s (trot) for 0.6 km, then 8 m/s (slow gallop) for 3 km, and then 4 m/s for 0.4 km, for total distance of 4 km. On Wednesdays and Fridays, the slow gallop was performed at a 6° incline. (3) OC injury group: in addition to serum collection, SF and cartilage were collected from 11 middle carpal joints of nine Thoroughbred racehorses (3–6 years of age) undergoing arthroscopic surgery for removal of OC fragments, which were injuries resulting from racing. These fragments were removed from the dorsal articular borders of the third and radiocarpal bones. The protocol was approved by the University of Florida Institutional Animal Care and Use Committee. Blood was collected from the jugular vein *via* needle venipuncture. After being allowed to clot, serum samples were centrifuged and decanted. SF was collected by needle arthrocentesis. If SF samples were contaminated with blood, they were also centrifuged and decanted. All samples were stored at $-80°C$ until assayed.

PROCEDURES

SF (0.25 ml in 0.75 ml of 0.1 M ammonium acetate, pH 7), serum (1 ml), and cartilage samples (5–10 mg in 1 ml of 0.1 M ammonium acetate, pH 7) were digested for 6 h with proteinase K (1 mg proteinase K/sample; GIBCO, Carlsbad, CA), followed by enzyme inactivation at 100°C for 10 min and filtration through a 0.45 μm nylon membrane. SF digests were then fractionated on gel filtration columns (HR 10/30; GE Healthcare, Pittsburg, PA) packed with allyl dextran/bisacrylamide (Sephacryl S-1000, on which 10% of the sample was loaded; GE Healthcare) or agarose (Superose 6, on which 90% of the sample was loaded; GE Healthcare), using 0.1 M ammonium bicarbonate (pH 7) as the mobile phase (0.5 ml/min; FPLC Pump P-500; GE Healthcare). Serum and cartilage digests were fractionated only on Superose 6. Columns were cleaned with 0.1 M sodium hydroxide between samples. Sephacryl S-1000 has a fractionation range (M_r) of 5×10^5 to $>10^8$. Superose 6 has a range up to 10^6. Column effluent was collected in 1 ml fractions by using a fraction collector (GE Healthcare). Fractions 1–6 were discarded and fractions 7–24 were collected and analyzed (S-1000 fractions designated A7–A24 and Superose 6

fractions designated B7–B24). The Superose 6 column was previously calibrated for CS molecular weight[29].

After drying in a vacuum concentrator (Thermo Electron Corp., Waltham, MA), fractions were resuspended in 100 μl of 100 mM ammonium acetate (pH 6.2) and digested with 30 mU each of chondroitinase ABC and chondroitinase ACII (Seikagaku, Associates of Cape Cod, East Falmouth, MA) at 37°C overnight. Disaccharide digests were vacuum dried before labeling for fluorophore-assisted carbohydrate electrophoresis (FACE)[30]. Labeling was performed by addition of 5 μl of 0.1 M 2-aminoacridone HCl (25 mg in 150 μl acetic acid/850 μl dimethyl sulfoxide (DMSO); Molecular Probes, Carlsbad, CA), followed by incubation at room temperature for 10 min. Then 5 μl of 1 M sodium cyanoborohydride (in ultrapure water; Sigma, St. Louis, MO) was added and incubated overnight at 37°C. Galactose-6-sulfate (60, 120, 240, 480, and 960 pmol; Sigma) was labeled simultaneously as an internal standard. On the next day, 30 μl of 25% glycerol was added to each sample, followed by vortex mixing and then centrifugation at $13,000 \times g$ for 2 min.

Components sufficient for pouring two resolving gels consisted of 11.25 ml of 40% acrylamide (Sigma), 1.5 ml of 25% glycerol, 1.5 ml of 0.45 M Tris (hydroxymethyl) aminomethane buffer (Tris base; Fisher, Pittsburg, PA; pH 7.0), and 0.75 ml of ultrapure water. After addition of 75 μl of 20% ammonium persulfate (Sigma) in ultrapure water and 15 μl of tetramethylethylenediamine (TEMED; Fisher), the components were mixed and poured into a 10 cm × 10 cm vertical gel system (Owl, Portsmouth, NH). After the resolving gel was set, components for two stacking gels consisted of 1.5 ml of 40% acrylamide, 1.5 ml of 25% glycerol, 1.5 ml of 0.45 M Tris base buffer, and 3 ml of ultrapure water. After the addition of 25 μl of 20% ammonium persulfate and 5 μl of TEMED, the components were mixed and poured on top of the resolving gel and an 8-well comb was inserted to create loading wells. Gels were rinsed with water and loaded in an electrophoresis tank containing electrophoresis buffer (18.15 g Tris base, 8.37 g boric acid, and 558 mg ethylenediaminetetraacetic acid (EDTA) in 1.5 L of ultrapure water; pH 7) maintained at 4°C. Labeled samples (5 μl) and internal standard (2 μl) were loaded on each of the six center lanes. Internal standard and disaccharide standards (ΔdiHA, Δdi0S, Δdi6S, and Δdi4S), made by chondroitinase digestion of HA polymer (ICN Immunobiologicals, Lisle, IL) and CS (J-Flex, Springtime, Inc., Cockeysville, MD), were loaded in the two outside lanes for band confirmation. Electrophoresis was performed with a constant 500 V power source (Biorad, Hercules, CA) for 2 h. After electrophoresis, gels were removed from their glass plates, illuminated by ultraviolet (UV) light (GE Healthcare), and digitally imaged using the Kodak 1D system (Eastman Kodak, New Haven, CT). Images were analyzed with image analysis software (Kodak 1D) and quantities of GAG in each sample were calculated.

Average molecular weights (kDa) of CS chains in individual fractions from the Superose 6 column were estimated by the relationship between the experimentally determined number of repeating disaccharides and the partition coefficient (K_D) for the column used, as previously described[29]. Partition coefficient (K_D) was estimated from

$$K_D = \frac{V_e - V_0}{V_t - V_0}$$

where V_e is the elution volume of the fraction, V_0 is the void volume of the column and V_t is the total volume of the column[31]. The molecular mass of the typical CS disaccharide unit was assumed to be 446[29].

STATISTICAL ANALYSIS

The Kruskall−Wallis test was performed to determine differences between groups for each gel filtration fraction, as well as for area under the curve (AUC) for total GAG concentration. $P < 0.05$ was considered significant. Sensitivity, specificity, positive predictive value, and negative predictive value of the SF CS chain length to discriminate between OC injured and normal (rested and exercised values combined) joints were determined by Fisher's exact test. Calculations were made for each CS disaccharide and for all three CS disaccharides combined.

Results

Fluorotagged CS and HA digestion products from SF, serum, and cartilage were separated by FACE according to chain length (fraction number) and quantitated in μg/ml of body fluid or μg/mg of cartilage (wet weight). Recovery of HA polymer standard (200 μg) from both the Sephacryl S-1000 and Superose 6 columns, as analyzed by FACE, was >95%.

CHONDROITIN SULFATE

Data from FACE analysis of SF from all horses (rested, exercised, and OC injury groups) are shown as mean CS disaccharide content for each fraction from Superose 6 chromatography [Fig. 1(A)−(C)]. SF from OC injury joints contained CS that had larger peak chain lengths than that from the rested and exercised groups. The peak fraction of CS from rested horses was at B20, which had an average molecular mass of 11.6 kDa. In the exercised group, the peak fraction of CS was at B19 for Δdi0S and Δdi6S, but B20 for Δdi4S, with an average molecular mass of 15.6 kDa. In contrast, the peak fraction of CS from the OC injury group was at B18, with an average molecular mass of 18.7 kDa. Total CS concentration (AUC ± standard deviation (SD)) in SF from OC injury joints was 25.01 ± 14.49 μg/ml, and there were no significant differences when compared to 33.77 ± 18.87 μg/ml for the rested group and 33.19 ± 18.62 μg/ml for the exercised group [Fig. 1(A)−(C)].

Sensitivity and specificity of the SF CS assay ranged from 82% to 100% (Table I). For these calculations, Superose 6 fraction ≤ B18 was used as a cut-off point for peak SF

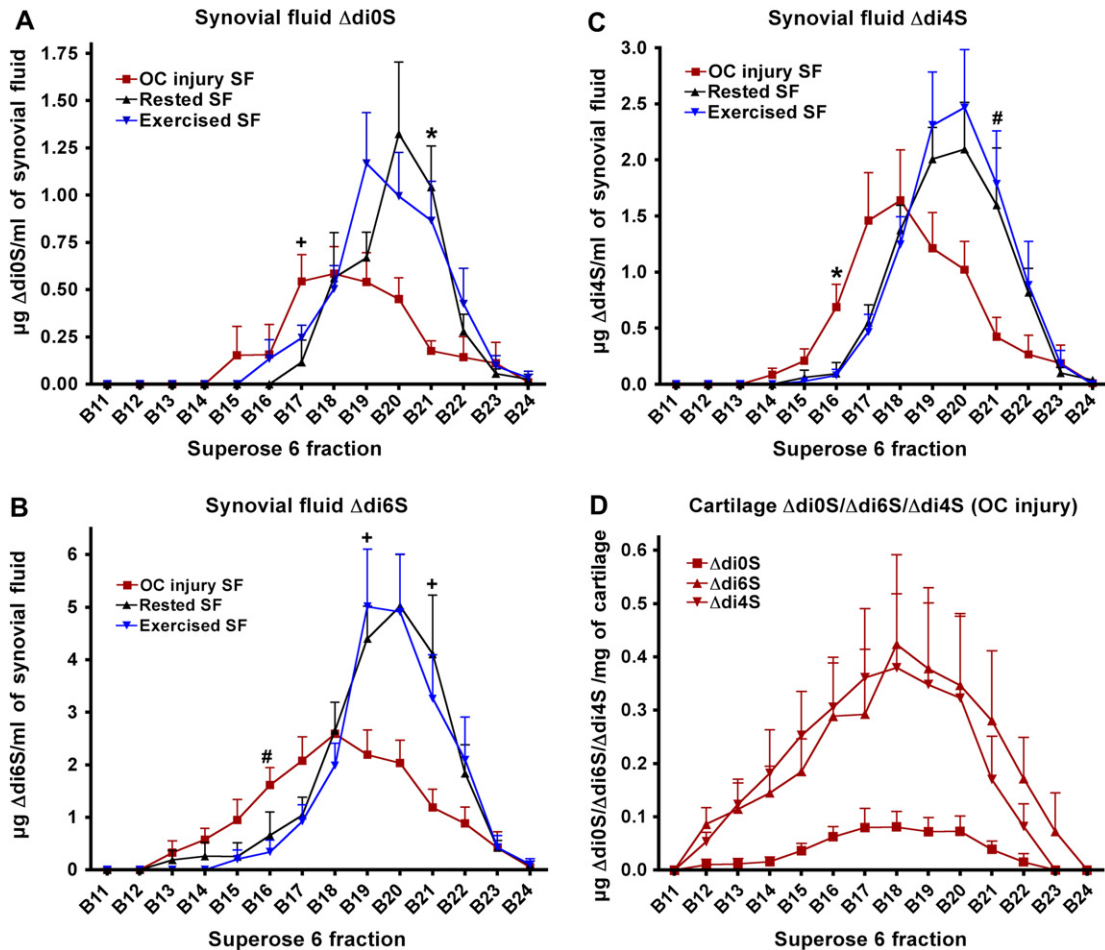Fig. 1. Plot of mean SF and cartilage CS concentrations (μg/ml of SF or μg/mg of cartilage; +SD) vs Superose 6 fraction number. Chain length progressively decreases from fraction B11 to B24. (A)−(C) CS in SF from horses with normal joints after rest and exercise ($n = 9$ each; same joints compared) and from horses undergoing arthroscopic surgery for OC fragment removal ($n = 11$). Note that CS chain length in SF from OC injured joints is longer than that seen in the rested and exercised groups. (D) CS in cartilage from the same horses undergoing arthroscopic surgery for OC fragment removal ($n = 9$). Note that CS chain length in cartilage from OC joints is similar to that seen in the SF from these joints. Differences between groups ($P < 0.05$): + − OC injury vs rested; * − OC injury vs rested and exercised; # − OC injury vs exercised.

Table I
Sensitivity, specificity, positive predictive value, and negative predictive value of SF CS assay to discriminate between OC injured and normal (rested and exercised values combined) joints

Disaccharide	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Δdi0S	100	82	64	100
Δdi6S	100	86	70	100
Δdi4S	91	94	91	94
Δdi0S/Δdi6S/Δdi4S	96	87	76	98

CS values for OC injured joints and fraction $\geq$ B19 was used as a cut-off point for normal (rested and exercised) joints (Fig. 2). Visual examination of the diagnostic performance of the SF CS assay further confirms that the Δdi0S and Δdi6S assays had the highest probability of correctly identifying normal horses (negative predictive value = 100%). In contrast, Δdi4S had the highest probability of correctly identifying OC injured horses (positive predictive value = 91%).

Cartilage was available only from the OC injury group for analysis of CS disaccharide content [Fig. 1(D)]. The peak fraction of CS was at B18, which corresponds with peak fraction in SF from these same joints [Fig. 1(A)−(C)], with a molecular mass of 18.7 kDa. Total CS content of the cartilage (AUC $\pm$ SD) from OC injury joints was highly variable (Δdi0S = 0.49 $\pm$ 0.55 µg/mg; Δdi6S = 2.78 $\pm$ 3.20 µg/mg; Δdi4S = 2.53 $\pm$ 2.76 µg/mg).

Chondroitin-6-sulfate was not detected in the serum. CS peaked at B18 (18.7 kDa) in serum from horses with OC injury. Serum Δdi0S and Δdi4S content in fraction B18 from these horses was significantly different from rested horses [$P < 0.05$; Fig. 3(A) and (B)].

HYALURONAN

Total SF HA content (AUC $\pm$ SD) was significantly decreased for the OC injury group (831 $\pm$ 503 µg/ml) compared with the rested group (1838 $\pm$ 529 µg/ml; $P < 0.05$; Fig. 4). Total SF HA content for the exercised group was 1385 $\pm$ 511 µg/ml, which was not significantly different from the other two groups.

SF from the rested and exercised groups showed prominent HA peaks in fractions A13 and B11 (Fig. 4). Similarly, SF from the OC injury group showed an HA peak at B11, but the Sephacryl S-1000 fractions showed a non-distinct peak at A15, indicating a significant, discriminative loss of the larger HA chains seen in fraction A13 in the rested and exercised groups. Additionally, HA fractions to the right of both peaks for the rested group (A13 and B11) were significantly higher than both the exercised and injured groups, implying effects of both exercise and injury on the SF HA profiles.

Cartilage from the OC injury joints showed an HA peak centered at B10 (Fig. 5). Mean total HA content (AUC $\pm$ SD) in these samples was 0.18 $\pm$ 0.16 µg/mg of cartilage. Total serum HA concentrations ranged from 0.38 $\pm$ 0.23 µg/ml for samples taken 1 h after stress testing (exercised group) to 0.82 $\pm$ 1.07 µg/ml for samples from the rested horses. Serum HA concentrations were quite variable within groups and there were no significant differences in total serum HA between groups. Only in fraction B14 did serum HA show significantly lower values for the OC injury group compared with the exercised group (in samples drawn immediately after and 1 h later; $P < 0.05$).
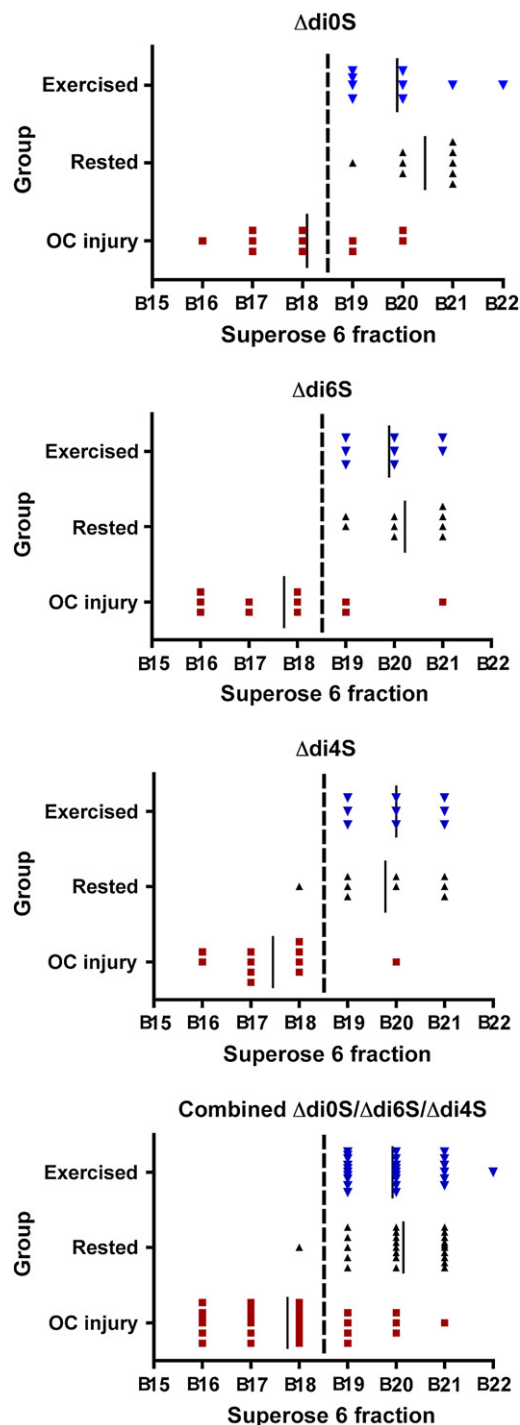

Fig. 2. Horizontal scatter plot of peak SF CS Superose 6 fractions for each joint in the OC injury, rested, and exercised groups. The vertical dashed lines represent the dividing line between fractions B18 (18.7 kDa) and B19 (15.6 kDa). The short vertical solid lines represent the mean value for each group.

Discussion

We report a biochemical analysis of SF and serum CS and HA which discriminates between normal rested and exercised joints and post-injury joints. SF CS and HA both showed significant changes as a result of exercise and OC injury.
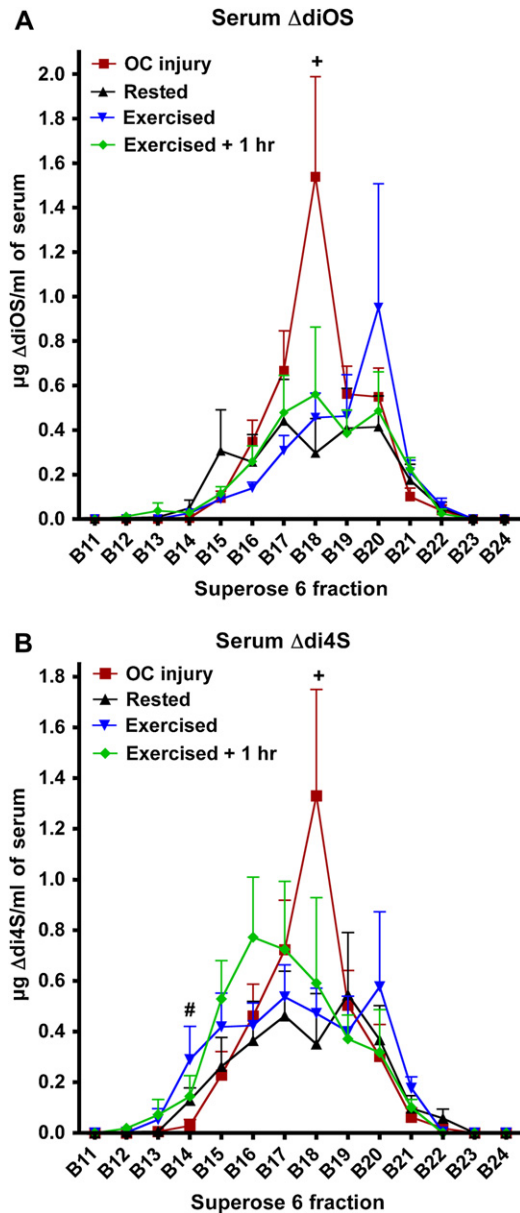
A Serum ΔdiOS

B Serum Δdi4S

Fig. 3. Plot of mean serum CS concentrations (μg/ml + SD) vs Superose 6 fraction number. (A) Δdi0S in serum from horses with normal joints after rest and exercise (collected immediately after and then 1 h after stress test; $n = 9$) and from horses undergoing arthroscopic surgery for OC fragment removal ($n = 9$). (B) Δdi4S in serum from same horses. Note the peaks in OC injury serum occur in fraction B18 (18.7 kDa), similar to those seen in SF and cartilage of this group (see Fig. 1). Differences between groups ($P < 0.05$): + – OC injury vs rested; # – OC injury vs exercised.

Synovial fluid HA

Fig. 4. Plot of mean SF HA concentrations (μg/ml + SD) vs Sephacryl S-1000 fraction (A7–A24) or Superose 6 fraction(B7–B23) from horses with normal joints after rest and exercise ($n = 9$ each; same joints compared) and from horses undergoing arthroscopic surgery for OC fragment removal ($n = 11$). Differences between groups ($P < 0.05$): + – OC injury vs rested; * – OC injury vs rested and exercised; # – OC injury vs exercised; ▽ – rested vs OC injury and exercised; ∞ rested vs exercised. Total HA concentration (μg/ml; AUC) for the OC injury group was significantly lower than for the rested group ($P < 0.05$).

injury joints was similar to that seen in the SF of these joints (Fig. 1), cartilage specimens were not available from the normal horses in this study to make the same comparison. As a result, we were unable to confirm that CS composition in SF is a reflection of that seen in the cartilage of the same joint. Similar comparisons between cartilage and SF CS chain length in normal rested and exercised groups would be needed to determine whether or not SF CS chain length data provided by this assay may provide a direct indicator of the CS composition of the cartilage.

The apparent ability of the assay to distinguish the effects of OC injury on SF CS chain length could lead to diagnostic applications. Negative predictive value is an important parameter of clinical relevance because it helps the clinician know the probability that a joint that is classified as negative by the assay is indeed normal (non-injured). In contrast, positive predictive value helps the clinician to know the probability that a joint that is classified as positive by the assay is indeed injured. The assay performed well in both regards, with Δdi0S and Δdi6S more likely to correctly identify normal joints (100%) and Δdi4S more likely to identify injured joints (91%).

Dimethylmethylene blue (DMMB) assays could be done on Superose 6 fractions instead of FACE analysis for determination of sulfated GAG chain size. This would make the assay much faster and simpler, as well as less expensive

Our results show an increase in the chain length of CS in the SF of OC injury joints. Superose 6 gel chromatography combined with FACE analyses revealed a statistically significant injury-associated increase in CS chain size (18.7 kDa) when compared to rested horses (11.6 kDa), with exercise producing an intermediate chain length (15.6 kDa). The origin of elongated CS chains in SF from injured joints is unknown. Other investigators have suggested that much of the SF CS derives from the articular cartilage[32]. Although CS chain length in cartilage from the OC
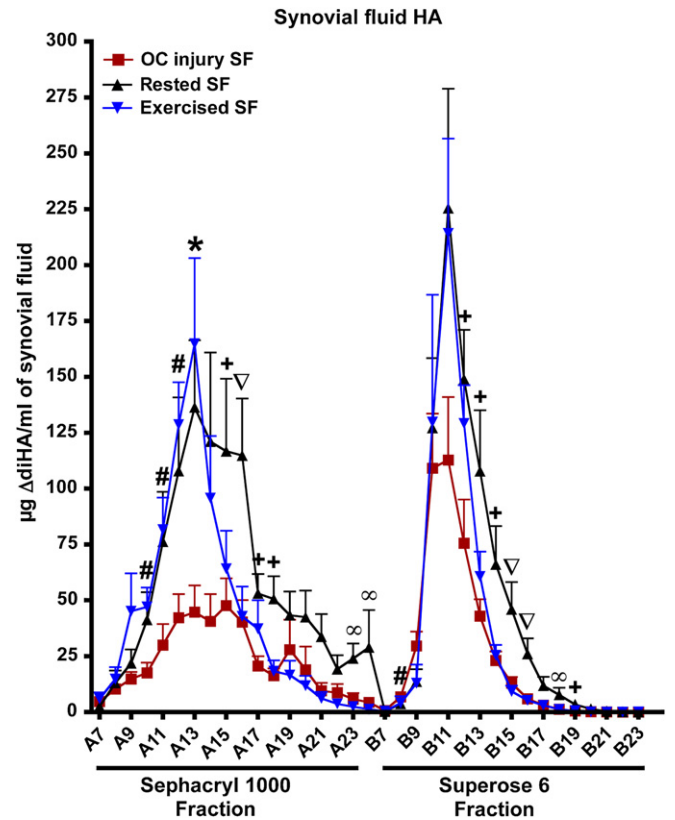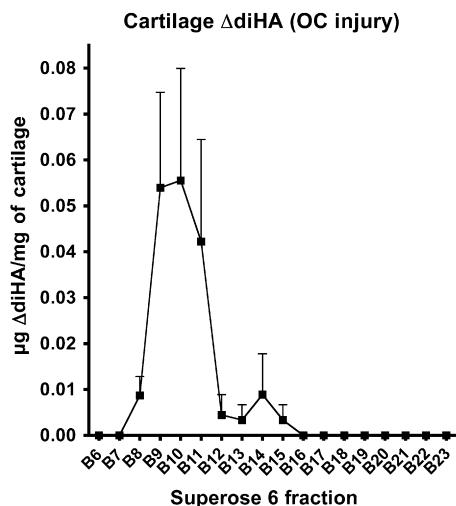
Cartilage ΔdiHA (OC injury)

Fig. 5. Plot of mean cartilage HA concentrations (μg/mg + SD) vs Superose 6 fraction (B7–B23) from horses with OC injury ($n = 9$). Mean total HA content in these samples was 0.18 ± 0.16 μg/mg (AUC $\pm$ SD) of cartilage, with the peak occurring at B10.

to perform[33]. We have determined that the sulfated GAG in these fractions is essentially CS because of Superose 6 purification (data not shown).

Abnormally long CS chains have been reported for newly synthesized aggrecan in post-injury canine OA cartilage[28]. Repeating disaccharides of the CS chain are added by the alternating addition of the monosaccharides, N-acetylgalactosamine (GalNAc) and glucuronic acid (GlcA), to the non-reducing terminal of the elongating chain. Two enzymes, N-acetylgalactosaminyltransferase and glucuronosyltransferase, are required to facilitate this process[34]. Based on in vitro studies using odd- and even-numbered oligosaccharides with a non-reducing terminal, it was reported that non-sulfated GalNAc or 6-sulfated N-acetylgalactosamine (GalNAc6S) were excellent receptors for enzymatic addition of GlcA and thus facilitated elongation of the CS chain. However, in the same model, 4-sulfated GalNAc blocked further elongation of the CS chain. Furthermore, presence of 4-sulfated GalNAc in the penultimate position of the non-reducing terminal also prevented CS chain elongation[35]. In contrast, another study showed oligosaccharides that are 4-sulfated on the penultimate GalNAc at the non-reducing end act as receptors[36]. These latter findings are comparable with our previous work, in which the presence of GalNAc4S and GlcA–GalNAc4S at the non-reducing terminal was more common in articular cartilage from horses with OC fragments than from normal joints[1]. It has been suggested that impaired secretion of proteoglycan from the Golgi cisternae may occur in OA cartilage, thus resulting in abnormally long CS chains[28].

Mean total CS in SF in our study (25.01 μg/ml, injured; 33.77 μg/ml, rested; 33.19 μg/ml, exercised) compared closely with those values reported for rabbits (38 ± 4 μg/ml)[32]. Mean total CS in the cartilage from the OC injury group (5.81 ± 6.44 μg/mg) was highly variable and similar to that reported for mouse cartilage (2.6–4.6 μg/mg)[37], but much lower than that reported for rabbit articular cartilage (27.8 μg/mg)[32].

Our finding that Δdi6S was not detectable in serum is similar to previous reports in which only low or trace amounts were found in serum of rabbits and human patients[6,38]. In the latter study, total serum Δdi0S and Δdi4S data were interpreted by the investigators as being significantly different between human OA patients and controls.

In our study, both serum Δdi0S and Δdi4S showed significant differences between the OC injured and rested groups. This difference was not in total Δdi0S or Δdi4S, but in a single peak at the B18 (18.7 kDa) fraction in the OC injured horses [Fig. 3(A) and (B)]. This peak may be a reflection of the Δdi0S and Δdi4S peaks seen in this fraction in both the articular cartilage and SF. Thus, it appears that the highest concentrations of Δdi0S and Δdi4S in serum, SF, and cartilage of the OC injured horses is in fraction B18, which has an average molecular weight of 18.7 kDa. In contrast, however, this apparent relationship was not seen in the serum and SF Δdi0S and Δdi4S peaks in the rested and exercised horses. It is therefore difficult to draw any conclusions regarding the usefulness of serum CS data as an indicator of events in the joint. It has been shown that approximately one-half of the Δdi4S contained in serum is released from platelets and therefore plasma may be better than serum for monitoring CS[38].

Our findings in OC injured joints agree with previous reports that SF HA is decreased in chain length[21,22] and concentration[3,18,20,39] in OA. Additionally, exercise had significant effects on the SF HA profile (Fig. 4). Regulation of HA synthesis and chain length is not well understood. HA is synthesized by HA synthase, which has three isoforms: HAS1, HAS2, and HAS3[40]. These isoforms have different enzymatic properties, such as rate of synthesis and chain length, and may be stimulated or inhibited by growth factors and cytokines[41]. It has been reported that HAS3 makes shorter chains than HAS1 and HAS2[40]. Induction of HAS3 by interleukin-1β has been shown to be significantly high in OA synovial cells, which may relate to the synovial inflammation in this disease[42,43]. Thus, shorter HA chain length in injured joints may be a result of synthesis of shorter chains by HAS3, as well as degradation of longer chains by oxygen-derived free radicals released by inflammatory cells[44–46].

Synovial cell HAS message expression is higher in vivo than in cell culture, especially in arthritic synovium and has been associated with the effusion that accompanies such joints[41]. Transforming growth factor (TGF-β1) is a major stimulator of HA synthesis in human synovium and might be involved in the mechanisms of joint swelling in inflammatory and degenerative joint diseases[44]. Increased intra-articular pressure results in increased HA synthesis[32,47]. We did not determine HA synthesis rates in these horses. Thus, injury-induced decrease in total SF HA concentration may be dilutional due to synovial effusion, in spite of possible increased synthesis. SFs from normal human knees have yielded HA concentrations from 1.45 to 2.94 mg/ml[21,48]. Values of 3.6 mg/ml have been reported for rabbit SF[32]. Our SF HA concentration values (normal, rested − 1.84 ± 0.53 mg/ml; OC injury − 0.83 ± 0.50 mg/ml) were similar to those previously reported for the horse (normal − 1.26 ± 0.26 mg/ml; arthritic − 0.58 ± 0.16 mg/ml)[39]. By using different assay methods, other investigators have obtained lower values, but have shown similar differences between normal and arthritic joints in horses[3].

Mean total cartilage HA from the OC injury joints (0.18 ± 0.16 μg/mg) was lower than that reported for rabbit cartilage (0.42 μg/mg)[32] and for human cartilage (0.5–2.5 μg/mg)[49]. The HA chains in our cartilage samples were relatively uniform in size, with the majority being in fractions B9–B11 (Fig. 5). This is in contrast to a previous report on HA chain size in human articular cartilage, in which values ranged from 2×10^6 in immature cartilage to 3×10^5 in mature cartilage[49]. We did not fractionate our cartilage digests on the Sephacryl S-1000 column as was done in that study and therefore, we may have missed the larger

chain lengths in our samples, which may also account for our lower total HA content in cartilage.

Exercise has been shown to increase serum HA concentrations in both normal subjects and in patients with rheumatoid arthritis[50,51]. A study involving African American and Caucasian patients supported the use of serum HA concentrations as a biomarker of radiographic OA[52]. In contrast, another study showed no correlation between serum HA concentrations and Western Ontario and MacMaster Universities Osteoarthritis Index (WOMAC) or Kellgren/Lawrence radiological grade, in spite of significant differences in serum HA between normal healthy controls and patients with knee OA[53]. Serum HA concentrations in our study were quite variable and did not discriminate between rested, exercised, and OC injury groups. It is probable that the horses with OC injury in our study were in a much earlier stage of OA than patients seen in human studies. If so, this could account for the difference in results rather than a species difference. The lower limit of detection for our assay appears to be similar to assays used in previous reports and unlikely accounts for the differences. We did not consider the statistically significant difference in serum HA between the OC injured and exercised joints in fraction B14 to be of clinical significance.

Our study had limitations. Although our assay had very high negative and positive predictive values for joint injury, the assay is difficult and time-consuming. As mentioned, adaptation to a DMMB protocol may increase the practicality of measuring SF CS chain length. Another limitation was lack of availability of cartilage samples from the normal rested and exercised horses in the study. As a result, we were unable to confirm the apparent relationship between SF CS and corresponding values in the cartilage.

In conclusion, we have used a combination of gel chromatography and FACE to demonstrate that there was a significant incremental increase in SF CS chain size as a result of exercise and OC injury, compared with the effects of rest. We also demonstrated that OC injury caused a significant decrease in both SF HA chain length and total concentration, when compared to rest. Similarly, exercise resulted in significant decreases in SF HA chain length, with a concurrent, nonsignificant decrease in total concentration, compared to rest. Based on these findings, chain length analysis of SF CS and HA may provide a useful tool for evaluation of joint health.

Acknowledgments

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