Effects of Equine Joint Injury on Boundary Lubrication of Articular Cartilage by Synovial Fluid

Role of Hyaluronan

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Objective. To compare equine synovial fluid (SF) from injured and control joints for cartilage boundary lubrication function; concentrations of the putative boundary lubricant molecules hyaluronan (HA), proteoglycan 4 (PRG4), and surface-active phospholipids (SAPLs); relationships between lubrication function and composition; and lubrication restoration by addition of HA.

Methods. Equine SF from normal joints, joints with acute injury, and joints with chronic injury were analyzed for boundary lubrication of normal articular cartilage (kinetic friction coefficient $\mu_{\text{kinetic}}$). Equine SF samples were analyzed for HA, PRG4, and SAPL concentrations and HA molecular weight distribution. The effect of the addition of HA, of different concentrations and molecular weight, on the $\mu_{\text{kinetic}}$ of equine SF samples from normal joints and joints with acute injury was determined.

Results. The $\mu_{\text{kinetic}}$ of equine SF from joints with acute injury (0.036) was higher (+39%) than that of equine SF from normal joints (0.026). Compared to normal equine SF, SF from joints with acute injury had a lower HA concentration (−30%) of lower molecular weight forms, higher PRG4 concentration (+83%), and higher SAPL concentration (+144%). Equine SF from joints with chronic injury had $\mu_{\text{kinetic}}$, PRG4, and SAPL characteristics intermediate to those of equine SF from joints with acute injury and normal equine SF. Regression analysis revealed that the $\mu_{\text{kinetic}}$ value decreased with increasing HA concentration in equine SF. The friction-reducing properties of HA alone improved with increasing concentration and molecular weight. The addition of high molecular weight HA (4,000 kd) to equine SF from joints with acute injury reduced the $\mu_{\text{kinetic}}$ to a value near that of normal equine SF.

Conclusion. In the acute postinjury stage, equine SF exhibits poor boundary lubrication properties, as indicated by a high $\mu_{\text{kinetic}}$. HA of diminished concentration and molecular weight may be the basis for this, and adding HA to deficient equine SF restored lubrication function.

In synovial joints, articular cartilage bears load and slides relative to apposing tissue surfaces, with friction and wear reduced through a number of biophysical mechanisms including boundary lubrication (1,2). Boundary lubrication of articular cartilage is mediated by synovial fluid (SF) components that reduce the interaction of articulating surfaces (3–5). Normal SF contains the molecules hyaluronan (HA) (6), proteoglycan 4 (PRG4) (7,8), and surface-active phospholipids (SAPLs) (9), which are implicated in contributing to the boundary lubrication of articular cartilage. Each of these molecules is present at high concentrations in SF (10–
and has been localized at the surface of articular cartilage (9,14,15), as would be expected for a boundary lubricant. In this article, the term PRG4 is used, since it is the name assigned by the Human Genome Organization Committee for the protein known as lubricin, superficial zone protein, and megakaryocyte-stimulating factor (7,8,16).

Previous studies have shown that alteration of the friction-lowering function of SF may contribute to the deterioration of articular cartilage in joint disease and after joint injury (17–21). However, the lubrication function of SF varied substantially in those studies, as did the biomechanical test methods and counterface materials used in the lubrication tests. Lubricant solutions exhibit boundary-mode friction in cartilage-on-cartilage assays that is less than that for glass-on-rubber (17) and cartilage-on-glass (21,22) friction assays, and similar to that for glass-on-latex friction assays (18–20). After acute injury (23,24), such as anterior cruciate ligament rupture, meniscal tear, or intraarticular fracture, synovial joints are predisposed to deterioration and premature osteoarthritis (OA). Such deterioration may involve a reduction in the functional boundary lubrication of articular cartilage due to alterations in the concentrations of SF lubricant molecules (20,25,26).

The diminished lubrication properties of pathologic SF after acute injury have been associated with lower concentrations of PRG4 (20). In OA, the friction coefficient of SF tended to increase from normal when tested at a latex–glass interface (19), and the concentration and molecular weight distribution of HA were shifted to lower levels (27–30). However, the concentration and molecular weight distribution of HA have not been associated directly with decreased lubrication. Additionally, the concentration of phospholipids in SF was lower in acute injury, but higher in OA (10,12), compared to SF from uninjured joints. However, the contribution of SAPL to the boundary lubrication of articular cartilage has been a subject of controversy (31–33). It remains to be established if SF lubricant dysfunction occurs after different types of joint injury and whether such alterations relate to variations in the concentrations and quality of lubricant molecules.

Race horses commonly have osteochondral fractures and OA of the carpal and metacarpophalangeal joints, and thus provide a natural model for the study of joint injury (34). Horses with acute joint injuries are often evaluated for treatment of osteochondral chip fragments or slab fractures, and such joints exhibit signs of acute synovitis. In contrast, some horses are evaluated for more chronic joint damage and secondary OA changes. The SF of such injured joints may be affected with regard to both lubrication function and lubricant composition.

The objectives of this study were to determine, in equine SF from acutely injured, chronically injured, and normal joints, the coefficient of friction at a cartilage–cartilage interface in the boundary lubrication regime; the concentrations and/or molecular weight of HA, PRG4, and SAPL; the relationships between lubrication function and composition; the contribution of HA to cartilage–cartilage lubrication at different molecular weight and concentrations; and whether the addition of the deficient molecules to equine SF restores lubrication function.

**MATERIALS AND METHODS**

**Materials.** Materials for lubrication testing were obtained as described previously (3). (Additional details are available from the author upon request.) HA was obtained in 6.4-kd, 51-kd, and 780-kd forms (all from Lifecore Biomedical), ~800-kd form (molecular weight range 620–1,170 kd; polydispersity index 1.6 [35]) (SupArtz; Smith & Nephew), and 4,000-kd form (Healon; Advanced Medical Optics). Anti-lubricin rabbit polyclonal antibody, raised against amino acids 1356–1374 of human lubricin, was from Abcam (catalog no. ab28484).

**Synovial fluid samples.** Normal bovine SF was prepared as described previously (3) (n = 5 pools, each from different adult animals). Equine SF samples were acquired by
one of the authors (CWM) from adult horses (2–4 years old) undergoing arthroscopic surgery. SF was aspirated from injured cartilaginous joints (n = 14) or metacarpophalangeal joints (n = 6), as well as from contralateral joints as controls (n = 20). Injuries were classified as acute or chronic, based on the estimated duration between joint injury and arthroscopic treatment, as well as arthroscopic observations. Equine SF samples from joints with acute injury (n = 10) were from horses that presented for surgery within 3 weeks of clinical diagnosis, often with signs of moderate to severe synovitis. Equine SF samples from joints with chronic injury (n = 10) were from horses that presented for surgery more than 3 weeks after injury, in which articular cartilage degeneration was often observed and synovitis was generally less severe. Figure 1A shows an arthroscopic view of a normal equine metacarpophalangeal joint; Figure 1B shows a view of the same location in a joint with an acute injury. Figure 1C shows a normal equine proximal intermediate carpal bone articular surface in the medial side of the antebrachio-carpal joint, and Figure 1D shows a joint with chronic injury, with a fragment off the proximal intermediate carpal bone with erosion of articular cartilage on the surface, partial thickness erosion on the distal lateral radius (lower), and thickened synovial villi, indicative of chronic synovitis (secondary OA).

All equine SF samples were clarified of cells and debris by centrifugation (at 3,000g for 30 minutes) immediately after joint aspiration. The supernatants were then collected, stored at −20°C for up to 2 weeks, and then dispensed into aliquots and stored at −80°C until used for the analysis described below.

**Experimental design.** Assessment of variations in equine SF (experiment 1). Biomechanical and biochemical analyses were performed to determine the effect of injury on equine SF lubrication function and composition. Portions of samples of normal bovine SF (n = 5), normal equine SF (n = 20), equine SF from joints with acute injury (n = 10), and equine SF from joints with chronic injury (n = 10) were analyzed by biomechanical lubrication tests for friction-lowering properties as indicated by the kinetic steady-state (equilibrium) and static (start-up) coefficients of friction, \( \mu_{\text{static}} \) and \( \mu_{\text{kinetic}} \), respectively. Other portions were analyzed by biochemical assays for the concentrations and/or molecular weights of the putative lubricant molecules HA, PRG4, and SAPL. Univariate and multivariate regression analyses were performed to assess the relationship between friction coefficient and lubricant composition.

**Assessment of lubrication properties of HA preparations of varying size and concentration (experiment 2).** Portions of HA preparations were analyzed by biomechanical lubrication tests for friction-lowering properties as indicated by \( \mu_{\text{static}} \) and \( \mu_{\text{kinetic}} \). HA preparations with molecular weights of 6.4, 51, 780, and 4,000 kd were analyzed at concentrations of 0.33, 1.1, and 3.3 mg/ml (n = 6 for each molecular weight and concentration combination).

**Restoration and enhancement of dysfunctional equine SF from joints with acute injury (experiment 3).** Based on the deficient friction-lowering properties and low HA values observed in experiment 1, some samples of equine SF from joints with acute injury (n = 7) and normal equine SF samples (n = 9) were analyzed further. HA in the form of SupArtz (800-kd HA; n = 3) or Healon (4,000-kd HA; n = 4) was added to portions of equine SF from joints with acute injury such that the final concentration of exogenous HA in the equine SF was 1.0 mg/ml, in order to restore HA concentrations to levels similar to those found within the normal range reported for equine SF (0.3–1.3 mg/ml) (30,36–38). Friction tests were then performed on equine SF samples from joints with acute injury alone and equine SF samples from joints with acute injury plus HA. Similar experiments were carried out on normal equine SF samples with 800-kd HA (n = 5) or 4,000-kd HA (n = 4). The addition of exogenous HA to all equine SF samples resulted in a slight (10%) dilution of SF.

**Lubrication testing.** Portions of SF and HA samples were analyzed for \( \mu_{\text{static}} \) and \( \mu_{\text{kinetic}} \) in the boundary lubrication mode on articulating cartilage surfaces as described previously (3,31). Intact articular surfaces were in the form of osteochondral cores and annuli harvested from the lateral and medial facets of the patellofemoral groove of adult bovine stifle joints, stored in phosphate buffered saline (PBS) supplemented with protease inhibitors (PIs) (2 mM Na-EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine HCl, and 10 mM N-ethylmaleimide) at −80°C. A total of 120 osteochondral fragments, consisting of 60 pairs of cores and annuli, from 14 stifle joints were used. (Additional details are available from the author upon request.) Test lubricants were also supplemented with PIs, resulting in a 3% dilution of the sample.

Samples were tested by preconditioning, 18% cartilage compression, 30-minute stress relaxation, and rotation at an effective sliding velocity of 0.3 mm/second, with presliding durations (\( T_{\text{ps}} \), the duration the sample is stationary prior to rotation) of 120, 12, and 1.2 seconds. (Additional details are available from the author upon request.) Friction coefficients were calculated from the torque, \( \tau \), and equilibrium axial load was measured immediately after the 30-minute stress relaxation period, with \( \mu_{\text{static}} \) calculated from the peak \( |\tau| \), measured just after (within 10° of) the start of rotation, and \( \mu_{\text{kinetic}} \) calculated from the \( |\tau| \) averaged during steady-state sliding.

Consistent with the results of previous studies (3,31), \( \mu_{\text{kinetic}} \) did not vary substantially with \( T_{\text{ps}} \) so \( \mu_{\text{kinetic}} \) data are presented as the average at all \( T_{\text{ps}} \). Also consistent were \( \mu_{\text{kinetic}} \) and \( \mu_{\text{static}} \) for PBS (>0.20), so these results were not analyzed further.

**Biochemical analysis of boundary lubricants.** Portions of SF samples were analyzed biochemically for the concentrations of HA, PRG4, and SAPL. (Details are available from the author upon request.) The HA concentration in equine SF samples was determined by an enzyme-linked immunosorbent assay–like method using HA binding protein (39). HA molecular weight distribution (0.05–0.25, 0.25–0.5, 0.5–1, 1–2.5, and 2.5–7 MDa) in equine SF samples was determined by horizontal electrophoresis through a 1% agarose gel (40,41) and image processing. The PRG4 concentration in equine SF samples was quantified after Western blotting using antilubricin antibody and purified equine PRG4 standard (40). The SAPL concentration in equine SF samples was measured by an assay that detects phospholipase-sensitive activity (10). To confirm the specificity of the spectrophotometric SAPL assay, absorption profiles of the assay product of SF samples, pooled for each group, were compared to those of SAPL standards.

**Statistical analysis.** Data are presented as the mean ± SEM. The effects of test lubricant on \( \mu_{\text{static}} \) (with \( T_{\text{ps}} \) as a repeated factor), \( \mu_{\text{kinetic}} \) and lubricant concentrations were assessed by analysis of variance, followed by Tukey’s post hoc test to determine which experimental group means differed
from each other. Since an initial analysis assessing the carpal and metacarpophalangeal joints as factors did not show independent or interactive effects of joint location, all analyses were performed without considering joint site as a factor. The dependencies of \( \mu_{\text{kinetic}} \) and \( \mu_{\text{static}} \) on the biochemical constituents (HA, PRG4, and SAPL) were analyzed by univariate regression as well as multivariate regression. Statistical analysis was performed using Systat 10.2 software.

**RESULTS**

**Lubrication function of equine SF.** The boundary mode friction coefficients varied with test lubricant \( (P < 0.05) \) (Figure 2A) and \( T_{ps} \) \( (P < 0.001) \) (Figure 2B). The lubricating abilities of equine and bovine SF on bovine cartilage were similar for normal SF from equine joints \( (\mu_{\text{kinetic}} = 0.026) \) and bovine joints \( (\mu_{\text{kinetic}} = 0.025) \) \( (P = 0.76) \). The mean \( \mu_{\text{kinetic}} \) for equine SF from acutely injured joints was 39% higher than that for normal equine SF \( (P < 0.05) \), and \( \mu_{\text{kinetic}} \) for equine SF from joints with chronic injury (0.031) tended to be higher than that for normal equine SF \( (+20\%; P = 0.15) \).

While \( \mu_{\text{static}} \) varied with \( T_{ps} \) \( (P < 0.001) \), it was not affected by joint injury \( (P = 0.49) \) and did not show an interaction effect \( (P = 0.76) \) (Figure 2B). As \( T_{ps} \) decreased from 120 seconds to 1.2 seconds, \( \mu_{\text{static}} \) values approached \( \mu_{\text{kinetic}} \) values (Figure 2A). Although mean \( \mu_{\text{static}} \) values were not statistically different between groups, those at the shortest \( T_{ps} \) of 1.2 seconds were highest for equine SF from joints with acute injury (0.042) followed by equine SF from joints with chronic injury (0.036) and normal equine SF and normal bovine SF (both 0.030), similar to the ordering of groups for \( \mu_{\text{kinetic}} \).

**Biochemical analysis of equine SF.** Analysis of the concentrations of HA, PRG4, and SAPL in equine SF revealed variations in lubricant molecule concentrations or molecular weight with joint injury.

**HA.** The concentration of HA in equine SF varied with joint injury \( (P < 0.05) \) (Figure 3A). In contrast to the higher friction coefficients obtained for equine SF from joints with acute injury compared to normal equine SF, the mean HA concentration in equine SF samples from joints with acute injury (0.21 mg/ml) was 30% lower than that in normal equine SF samples (0.30 mg/ml) \( (P < 0.05) \) and 40% lower than that in equine SF samples from joints with chronic injury (0.37 mg/ml) \( (P < 0.05) \).

The molecular weight distribution of HA varied between normal equine SF, equine SF from joints with acute injury, and equine SF from joints with chronic injury \( (P < 0.05) \), shifting with injury to lower molecular weight ranges (Figures 3B and C). Relative to normal equine SF, equine SF from joints with acute injury had HA concentrations that were similar in the lower molecular weight ranges of 0.05–2.5 Md \( (P = 0.16–0.59) \), and markedly lower in the highest molecular weight range of 2.5–7 Md \( (−63\%; P < 0.05) \). HA concentrations in equine SF from joints with chronic injury were similar to those in normal equine SF at all HA molecular weight ranges \( (P = 0.48–0.99) \).

![Figure 2. Effect of joint injury on the boundary lubrication of articular cartilage by equine synovial fluid (SF). Kinetic friction coefficients \( (\mu_{\text{kinetic}}) \) (A) and static friction coefficients \( (\mu_{\text{static}}) \) (B) for normal equine SF (NL-eSF; \( n = 20 \)), equine SF from joints with acute injury (AI-eSF; \( n = 10 \)), and equine SF from joints with chronic injury (CI-eSF; \( n = 10 \)) were plotted on a semilog scale. Values are the mean ± SEM. Differing letters indicate significant differences between groups \( (P < 0.05) \). ANOVA = analysis of variance. Color figure can be viewed in the online issue, which is available at http://online library.wiley.com/journal/10.1002/(ISSN)1529-0131.](Figure2)
PRG4. The concentration of PRG4 in equine SF varied with joint injury (P = 0.01). Western blot analysis of individual samples of normal equine SF, equine SF from joints with acute injury, and equine SF from joints with chronic injury identified a specific immunoreactive band (Figure 4A) (Additional results are available from the author upon request.) Mean PRG4 concentrations were highest for equine SF from joints with acute injury (104 μg/ml; P < 0.05 versus normal equine SF), then equine SF from joints with chronic injury (95 μg/ml; P = 0.066 versus normal equine SF), and then normal equine SF (57 μg/ml) (Figure 4B).

SAPL. The SAPL concentration in equine SF also varied with joint injury (P < 0.001) (Figure 4D). Compared to that of normal equine SF, the mean SAPL concentration was markedly higher in equine SF from joints with acute injury (+144%; P < 0.001) (Figure 4D) and was elevated in equine SF from joints with chronic injury (+64%; P < 0.05). Absorption profiles for the reaction products of SF treated with phospholipase C were similar for all pooled SF samples (Figure 4C), supporting the fidelity of the assay.

Figure 3. Effect of joint injury on hyaluronan (HA) concentration and molecular weight distribution in normal equine synovial fluid (NL-eSF; n = 19–20), equine SF from joints with acute injury (AI-eSF; n = 10), and equine SF from joints with chronic injury (CI-eSF; n = 10). A, HA concentration. B, Electrophoretic separation of typical samples. MWM = molecular weight marker. C, Concentration of HA in the indicated molecular weight ranges. Values in A and C are the mean ± SEM. Differing letters indicate significant differences between groups (P < 0.05). ANOVA = analysis of variance. Color figure can be viewed in the online issue, which is available at http://onlineibrary.wiley.com/journal/10.1002/(ISSN)1529-0131.

Figure 4. Effect of joint injury on proteoglycan 4 (PRG4) and surface-active phospholipid (SAPL) concentrations in normal equine synovial fluid (NL-eSF; n = 19–20), equine SF from joints with acute injury (AI-eSF; n = 10), and equine SF from joints with chronic injury (CI-eSF; n = 10). A, Representative PRG4 Western blots probed with an antibody to lubricin/PRG4. B, PRG4 concentration. C, Spectrophotometric absorption profiles for the reaction products of SAPL from pooled normal equine SF, equine SF from joints with chronic injury, and equine SF from joints with acute injury. D, SAPL concentration. Values in B and D are the mean ± SEM. Differing letters indicate significant differences between groups (P < 0.05 or P < 0.001). ANOVA = analysis of variance. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131.
Regression analysis indicated certain correlations between friction coefficients and equine SF lubricant molecule concentrations. Univariate regression showed a significant negative correlation between kinetic and HA concentration (slope $0.031$ ml/mg; $r^2 = 0.195$, $P < 0.01$) as well as positive correlations between kinetic and SAPL concentration (slope $0.045$ ml/mg; $r^2 = 0.277$, $P < 0.005$) and between kinetic and PRG4 concentration (slope $0.003$ ml/mg; $r^2 = 0.124$, $P < 0.05$). Similar correlation trends were also observed for static (data not shown). Multivariate regression revealed the independent relationship of kinetic to HA, PRG4, and SAPL concentrations (all mg/ml) ($\mu_{\text{kinetic}} = -0.019 \times HA + 0.032 \times PRG4 + 0.029 \times SAPL + 0.029; r^2 = 0.342, P = 0.001$) ($P < 0.01$ for HA; $P < 0.05$ for PRG4; and $P < 0.001$ for SAPL).

**Lubrication properties of HA.** The friction-reducing properties of HA solutions for articular cartilage depended on both HA concentration and HA molecular weight (Figure 5). The $\mu_{\text{kinetic}}$ value decreased with increasing HA concentration ($P < 0.001$) and HA...
molecular weight ($P < 0.001$) (Figure 5A). At a concentration of 0.33 mg/ml, the mean $\mu_{\text{kinetic}}$ values for 6.4-kd and 51-kd HA were similar to that for PBS (0.255) ($P = 0.98$ and $P = 0.85$, respectively), while the mean $\mu_{\text{kinetic}}$ value for 4,000-kd HA was significantly reduced compared to that for PBS ($-64\%; P < 0.01$). Similar trends were observed with HA concentrations of 1.1 mg/ml and 3.3 mg/ml. For 4,000-kd HA, concentrations from 0.33 to 3.3 mg/ml had low mean $\mu_{\text{kinetic}}$ values, which did not differ significantly from that of bovine SF ($P = 0.47$) (Figure 5A). The mean $\mu_{\text{static}}$ value increased with $T_{\text{ps}}$ ($P < 0.001$) and decreased with increasing HA concentration ($P < 0.001$) and HA molecular weight ($P < 0.001$), with trends similar to those for $\mu_{\text{kinetic}}$ observed at all $T_{\text{ps}}$. Results for $\mu_{\text{static}}$ at a $T_{\text{ps}}$ of 120 seconds are shown in Figure 5B, and similar trends were found for $T_{\text{ps}}$ of 1.2 seconds and 12 seconds (data not shown).

**Restoration of dysfunctional equine SF from joints with acute injury.** The boundary lubrication function of equine SF from horses with acute joint injury was normalized by the addition of high molecular weight (HMW) HA. For both $\mu_{\text{kinetic}}$ and $\mu_{\text{static}}$ (at a $T_{\text{ps}}$ of 120 seconds), the experimental groups that had similarly low mean friction coefficients (denoted by an “a” in Figure 6A and Figure 6B, respectively) included normal equine SF alone, normal equine SF plus 800-kd HA, normal equine SF plus 4,000-kd HA, as well as equine SF from joints with acute injury plus 4,000-kd HA. In contrast, the highest mean friction coefficients were seen for equine SF from joints with acute injury alone (for $\mu_{\text{kinetic}}$) (Figure 6A) and equine SF from joints with acute injury plus 800-kd HA (for $\mu_{\text{static}}$) (Figure 6B).

**DISCUSSION**

The results of this study indicate that there is a concordance between the changes in SF lubrication function and SF composition after acute joint injury in race horses, and that in vitro supplementation of abnormal SF with HMW HA restores boundary lubrication function. In the acute stage of injury, the boundary lubrication function of SF is reduced, as indicated by a friction coefficient that is higher than normal ($\mu_{\text{kinetic}} = 0.036$ versus 0.026) (Figure 2). This alteration in lubrication function may be due to the diminished concentration and molecular weight of HA in equine SF from joints with acute injury (Figure 3), despite the elevated concentrations of PRG4 and SAPL in equine SF from these joints compared to normal equine SF (Figure 4). The role of HA appears particularly important, since the friction-lowering properties of HA were size- and concentration-dependent (Figure 5). Also, the addition of 4,000-kd HA to equine SF from joints with acute injury restored boundary lubrication function, lowering $\mu_{\text{kinetic}}$ by ~30% to a level indistinguishable from that of normal equine SF (Figure 6). In the later, chronic stage after injury, the boundary lubrication function of SF appears to be partially recovered, possibly due to restoration and normalization of HA, PRG4, and SAPL concentrations (Figures 3 and 4). Articular cartilage may be particularly vulnerable when boundary lubrication is deficient in the acute stage after injury. During this time, addition of lubricant molecules to SF may restore its lubrication function.

A direct comparison of the boundary lubricating ability and lubricant composition of pathologic SF to its normal counterpart is important for understanding the molecular basis for altered lubrication function. Both pathologic and normal contralateral equine SF were obtained fresh in a sufficient quantity and controlled manner for the experiments performed here. The carpal and metacarpophalangeal joints of the race horses from which SF samples were aspirated are weight-bearing and actively loaded, providing a useful model for the study of joint injury. Although equine cartilage was not studied as a substrate for the boundary lubrication tests of equine SF, normal equine SF tested on adult bovine tissue substrates had a lubricating ability similar to that of bovine SF. Finally, although lubricant molecules may differ between species in concentration and quality, understanding the composition–function relationship of putative lubricant molecules in equine SF provides insight into the molecular basis for SF and articular cartilage alteration with injury, and possible sequelae.

The results of the analysis of the boundary lubricating ability of equine SF after injury are consistent with and extend the findings of previous studies of SF from humans and animal injury models that used non-cartilage substrate friction test systems. At a latex–glass interface, boundary lubrication function was also reduced in SF aspirated from humans with knee joint synovitis (19) and from rabbits after anterior cruciate ligament and posterior cruciate ligament transection (20). Thus, such SF from injured joints reduces surface interactions between articulating substrates at both cartilage–cartilage and cartilage–noncartilage interfaces.

The relatively normal lubricating ability of equine SF from joints with chronic injury and OA human SF (19) was also observed for SF from patients with degenerative joint disease (18), where friction coefficients similar to those obtained in the present study for normal equine SF and normal bovine SF (~0.024) were obtained for a latex–glass interface, consistent with the ability of SF from joints with certain chronic pathologies to reduce the surface interaction between articulating surfaces, similar to that of normal SF. The friction
coefficients obtained in the present study for PBS at a cartilage–cartilage interface (~0.26) were ~3 times higher than those previously obtained for saline controls at a latex–glass interface (~0.09) (18–20). The differences between the absolute friction values found in the present study and those found in previous studies may be attributable to differences in test configurations, substrate materials, and test protocols.

Examining the lubricating ability of pathologic SF on physiologic test substrates mimics certain aspects of naturally articulating surfaces and allows for molecular interactions that occur during physiologic articulation (42), expanding on findings for the boundary lubricating ability of SF at a noncartilage interface. The differences in the effect of injury on SF static and kinetic friction properties, as well as the effect of added HA, remain to be clarified, and may involve detailed surface interactions or other modes of lubrication. Deficient lubrication by equine SF from joints with acute injury also leads to an altered mechanobiologic environment for cartilage, with elevated shear strains observed during tibiofemoral human cartilage articulation in the presence of equine SF from joints with acute injury (43), further supporting the phenomenon of impaired boundary lubrication function of SF after joint injury.

The alterations in SF lubricant composition with injury and disease observed in this study are consistent with the findings of previous studies of HA concentration and molecular weight distribution in SF. The concentration of HA in equine SF from clinically normal horses has been reported to average 0.33–1.3 mg/ml (30,36–38), while that in SF from horses with various arthritides is somewhat lower: 0.18–0.3 mg/ml in horses with posttraumatic arthritis (30,44) and 0.18–0.74 mg/ml in horses with more chronic injury conditions (29,30,44). Consistent with the findings of the present study, the HA concentrations in SF from the acutely injured joints of horses (37) and humans (45,46) were lower than those in control SF. The altered HA concentrations observed in the present study indicate local changes afflicting a specific joint, rather than a systemic change. Thus, although lowered HA concentrations are evident with injury, the wide variability of reported HA values suggests possible effects of the sample source, method of analysis, species, particular joint affected, or state of health, injury, or disease.

The present results extend current knowledge regarding the altered molecular weight distribution of HA in SF in joint diseases. Previous studies of SF have generally described the predominant HA molecular weight range rather than quantitative distributions, with ranges of 0.3–5 Md in SF from patients with rheumatoid arthritis and other joints diseases compared to 2–10 Md in normal SF (10,27,28), consistent with the findings for equine SF from joints with acute injury in the present study. Previously, HA molecular weight in normal equine SF (2–3 Md) was not found to differ significantly from that in SF from horses with established arthritis (1.5–3 Md) (30), consistent with the present findings for equine SF from joints with chronic injury. Thus, the present study provides new, quantitative information about the effect of traumatic injury on HA molecular weight distribution in SF.

The in vitro finding that the addition of HMW HA to equine SF from joints with acute injury restores lubricant function suggests that intraarticular supplementation may modulate and restore, to some degree, the boundary lubrication function of SF after injury. Although the concentration of exogenous HA that was added to equine SF from joints with acute injury was higher than the average levels measured in normal equine SF samples, the increase in concentration by the addition of HA (to 1 mg/ml) was within the range of the HA concentration in normal equine SF reported previously and lower than that in normal human SF. The absence of an apparent effect of the addition of 800-kd HA or 4,000-kd HA to normal equine SF on friction coefficients of articulating cartilage may be due to a saturating effect of HA in the presence of PRG4 in normal SF (31) or to a small effect size.

Further analysis of the dose-dependent effects of exogenous HA on the lubricating ability of HA-deficient SF would help to elucidate whether lower concentrations of added HMW HA would also enhance SF lubrication function. HA supplementation is a common clinical treatment for patients with OA, and is postulated to have disease-modifying and chondroprotective effects (47). Previous studies have focused on clinical outcomes of HA supplementation and biologic mediators rather than direct effects of HA, or other putative lubricant molecules, on SF lubricant function. Since HA has a relatively short half-life, administration of a single bolus of HA may not provide long-lasting enhancement of the lubricant function.

The effects of joint injuries and disease on the concentration of PRG4 in SF appear somewhat variable. The elevation in PRG4 concentration determined here is consistent with the initial transient elevation in biosynthesis and secretion as a response to cartilage injury (48) in vitro. The majority of PRG4 secreted by chondrocytes is released into the SF rather than retained within the matrix (8). While some previous studies of injury in animal models (20,25,49) and humans (50) have found a decrease in PRG4 concentration, other studies...
of acute injury and OA in humans have found an increase (21,51). These variable PRG4 concentrations may be due to a number of factors, including the type of injury, the duration of injury before the SF was analyzed, the sample source, and the method of analysis. Previous studies included soft tissue and joint destabilization injuries, while the present study included osteochondral damage. The particular type of injury may affect both the regulated level of PRG4 secretion, as well as pathways of efflux from the joint, both of which affect the PRG4 concentration in SF.

The injury-mediated increase in phospholipid concentration appears consistent, although the consequences for lubrication are a subject of some controversy. With acute and chronic injury, the phospholipid concentration increased, from 0.1 mg/ml in normal human SF to 0.2–0.3 mg/ml in human OA SF and to 0.5 mg/ml in SF from patients undergoing total knee arthroplasties (10). However, previous studies of the contribution of SAPL to the boundary lubrication of articular cartilage have produced conflicting results (31,32,33). The results of the present study, indicating elevation of both SAPL and friction coefficients, provide evidence against the role of phospholipid as a boundary protectant of the articular cartilage surface after acute injury. More detailed analysis of the distribution of phospholipids may clarify their role (12,13). The increased SAPL concentration after injury may be due to the release of cellular debris, in proportion to the degree of synovitis.

The present study provides further evidence that molecular features of lubricant molecules can affect their functional quality in certain states of posttraumatic joint injury, and such features may be important considerations for therapeutic interventions. The approach of analyzing both the concentration and structure of lubricant molecules present in SF after injury, together with testing their role independently and as a supplement in cases of deficiency, can be used to screen putative lubricant therapies. Such analyses may also clarify the interaction of chemical factors. The results of the present study also suggest that the natural time course of altered lubrication is important. At particular stages following injury, biologic, physical, and surgical treatments to modulate the lubrication of HA, as well as PRG4, may help to protect the articular cartilage and joint against damage. Surgical removal or fixation of an osteochondral fragment may be needed to restore the joint environment. In addition, deficiencies in lubrication may occur not only after naturally occurring injury, but also following arthroscopic procedures, including cartilage repair (52).

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REFERENCES

15. Schumacher BL, Hughes CE, Kuettnerr KE, Caterson B, Aydelotte MB. Immunodetection and partial cDNA sequence of the pro-