Concentrations of stromal cell-derived factor-1 in serum, plasma, and synovial fluid of horses with osteochondral injury

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Objective—To determine whether stromal cell-derived factor-1 (SDF-1) concentrations in serum, plasma, and synovial fluid differed among untrained, race-trained, and osteochondral-injured Thoroughbred racehorses.

Animals—22 racehorses without osteochondral injury and 37 racehorses with osteochondral injury.

Procedures—Horses without osteochondral injury were examined before and after 5 to 6 months of race training. Horses with osteochondral injury were undergoing arthroscopic surgery for removal of osteochondral fragments from carpal or metacarpophalangeal or metatarsophalangeal joints (fetlock joints). Serum, plasma, and fetlock or carpal synovial fluid samples were obtained and analyzed for SDF-1 concentration by use of an ELISA.

Results—In horses with fetlock or carpal joint injury, mean synovial fluid SDF-1 concentrations were significantly higher, serum SDF-1 concentrations were significantly lower, and synovial fluid-to-serum SDF-1 ratios were significantly higher than in untrained and trained horses. Synovial fluid SDF-1 concentrations were not significantly different between trained and untrained horses. Plasma SDF-1 concentrations were not different among the 3 groups. Results obtained with serum, compared with synovial fluid and plasma, had better sensitivity for differentiating between osteochondral-injured horses and uninjured horses. In horses with fetlock joint osteochondral injury, serum SDF-1 concentrations were correlated with radiographic and arthroscopic inflammation scores, but not arthroscopic cartilage scores.

Conclusions and Clinical Relevance—Results suggested that serum SDF-1 concentrations were more sensitive than plasma and synovial fluid concentrations for detection of osteochondral injury in the fetlock or carpal joint of racehorses. Analysis of serum and synovial SDF-1 concentrations in horses with experimentally induced joint injury may help define the onset and progression of post-traumatic osteoarthritis and aid in the evaluation of anti-inflammatory treatments. (*Am J Vet Res* 2014;75:722–730)

When joint trauma occurs, the resulting biomechanical and biochemical abnormalities can lead to breakdown of articular cartilage, which is the hallmark of osteoarthritis. Pain associated with osteoarthritis often causes lameness in horses.[1] Because there is no cure, the goal of osteoarthritis management is to prevent or slow its progression.[2] Current diagnostic methods fail to detect early PTOA and have limited ability to monitor progression.[3]

Biomarkers are biological markers that reflect quantitative and dynamic variations in joint tissue remodeling that may allow earlier diagnosis of PTOA.[3] Although some biomarkers are associated with specific cartilage metabolic processes involving collagen or aggrecan, others are less specific, such as the inflammatory markers.[4] One inflammatory biomarker group gaining interest in joint disease research is the chemokines. Chemokines are chemoattractive cytokines that mediate inflammation and immunity in tissues. Like cytokines, their effect is exerted on cells when bound to a specific cell membrane receptor.[5] Stromal cell-derived factor-1 α and β (ie, SDF-1) are in the CXC subfamily CXCL12 that binds to the CXCR receptor type 4 and are thought to be housekeeping chemokines.[5] Thus, their role in cartilage homeostasis is multifaceted.

Stromal cell-derived factor-1 activates and enhances release of MMPs, such as MMP-1, -3, -9, and especially MMP-13, from chondrocytes.[6–9] These MMPs have an important role in cartilage degradation.

Abbreviations

CV	Coefficient of variation
MMP	Matrix metalloproteinase
PTOA	Post-traumatic osteoarthritis
ROC	Receiver operating characteristic
SDF-1	Stromal cell-derived factor-1

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In addition, SDF-1 increases release of *N*-acetyl-β-D-glucosaminidase from chondrocytes,[9] which degrades glycosaminoglycans. However, SDF-1 also induces chondrocytes to proliferate and synthesize DNA, as measured by increased expression of cathespin B.[9] Unfortunately, cathespin B may prevent regenerating chondrocytes from expressing cartilage-specific anabolic genes, despite their high synthetic activity.[5] At high concentrations, SDF-1 can cause chondrocyte necrosis that results in release of high mobility group box chromosomal protein,[1,10] another potent inflammatory mediator. Therefore, it appears that SDF-1 has the potential to shift metabolism to a predominantly catabolic state. Because there is potential to prevent this cartilage degradation by blocking the interaction of SDF-1 with its receptor, SDF-1 may be a good biomarker to measure in osteoarthritis. An SDF-1 blockade effect has been detected in guinea pigs with primary osteoarthritis; in these guinea pigs, catabolic breakdown products released from chondrocytes decreased, compared with untreated controls.[11]

Stromal cell-derived factor-1 has been measured in synovial fluid and serum in humans and guinea pigs with osteoarthritis,[7,8,11–13] but to our knowledge, has not been evaluated in horses. A guinea pig study revealed large increases in synovial fluid concentrations in animals with primary osteoarthritis and PTOA.[13] Similarly, synovial fluid from human osteoarthritis patients has higher SDF-1 concentrations, compared with controls.[7] Synovial fluid SDF-1 concentrations, but not serum concentrations, in human patients with knee osteoarthritis are closely associated with radiographic severity of osteoarthritis.[12] However, serum concentrations are greater in osteoarthritis patients, compared with controls. Combined, these studies indicate that SDF-1 concentrations in serum or synovial fluid may be used to distinguish joints with osteoarthritis from controls.

The purpose of the study reported here was to evaluate whether SDF-1 concentrations in serum, plasma, and synovial fluid differed among untrained, race-trained, and osteochondral-injured racehorses. We hypothesized that training and osteochondral injury would increase serum, plasma, and synovial fluid SDF-1 concentrations and that osteochondral injury would have a greater effect than training.

Materials and Methods

Animals—Serum, plasma, and fetlock joint (metacarpophalangeal or metatarsophalangeal joint) synovial fluid or carpal (antebrachiocarpal or middle carpal joint) synovial fluid were obtained from 2 groups of Thoroughbred racehorses. One group consisted of 22 untrained horses purchased as yearlings (age range, 14 to 21 months). Fetlock and carpal joint radiographs revealed no abnormalities, and horses were free from lameness. Samples were obtained from metacarpophalangeal, antebrachiocarpal, and middle carpal joints of horses prior to undergoing training (designated as untrained horses) and after 5 to 6 months of race training (designated as trained horses). Fetlock and carpal joint radiographs obtained at the end of the training period revealed no abnormalities. A second group consisted of 37 racehorses (3 to 10 years old) undergoing arthroscopic surgery for removal of osteochondral fragments from the fetlock joint (18 horses; 16 metacarpophalangeal and 4 metatarsophalangeal joints) or carpal joints (19 horses; 7 antebrachiocarpal joints and 14 middle carpal joints). Joints were chosen for analysis on the basis of the presence of unilateral or bilateral lameness detected by physical examination, and in most cases, on the basis of results of intra-articular injection of an analgesic. Sixteen of these 37 horses had osteochondral injury in > 1 joint (7 horses with bilateral carpal joint injury; 9 horses with bilateral fetlock [metacarpophalangeal] joint injury), but the joint in the limb with the most severe lameness was chosen for analysis. In 4 of these bilaterally affected horses, synovial fluid from both joints was analyzed because it was difficult to determine which joint affected the horse more (2 middle carpal joints and 2 metacarpophalangeal joints). All known historical data regarding training and medications were recorded; however, little information was available.

Blood was collected by jugular venipuncture into plain tubes and EDTA-containing tubes, and the resulting serum and plasma samples were decanted. Synovial fluid was obtained aseptically via arthrocentesis with a 20-gauge, 1.5-inch needle without lavage, centrifuged (12,000 × *g* for 10 minutes), and decanted. Samples were stored at –80°C until assayed. All procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

Radiographic and arthroscopic scores of osteochondral-injured horses—As records were available, affected joints were assigned a radiographic and arthroscopic score, as described.[14] Briefly, radiographic scores were a summation of subjective scores (range, 0 to 3) for 10 radiographic categories (soft tissue swelling or effusion, subchondral bone sclerosis, subchondral bone lucency, joint space narrowing, number and size of osteophytes, number and size of enthesophytes, and number and size of osteochondral fragments). Therefore, total radiographic score could range from 0 to 30, with higher scores having more radiographic changes. Similarly, total arthroscopic scores were a summation of subjective scores for 5 inflammation categories (range, 0 to 3), 4 cartilage damage categories (0 to 4), and 2 osteochondral fragment categories (0 to 3). Therefore, arthroscopic scores for inflammation could range from 0 to 15, cartilage damage scores could range from 0 to 16, osteochondral fragment scores could range from 0 to 6, and total arthroscopic score could range from 0 to 37; higher scores indicated more arthroscopic changes. Arthroscopic inflammation categories assessed synovial hyperemia, petechiation, villi density or thickening, new villi (more villi present than normal in a given location), and villous atrophy. Arthroscopic cartilage categories were assigned to determine the worst lesion present with regard to localization, extent, and depth of cartilage lesions related to the osteochondral fragment, and depth related to the lesion on the opposing cartilage surface (the so-called kissing lesion). Arthroscopic osteochondral fragment categories were assigned to determine the number of fragments and size of the largest fragment.[14] Arthroscopic and radiographic scores were determined under consensus of authors (MPB and TNT

or MPB and DCD), who were unaware of radiographic appearance of a joint when evaluating the arthroscopic images and vice versa. Scores were assigned collectively after all cases were evaluated and without knowledge of SDF-1 concentration.

Analysis of SDF-1—Concentrations of SDF-1 in serum, plasma, and synovial fluid were measured with a commercially available human ELISA for SDF-1[a] according to manufacturer's guidelines, with appropriate dilutions. A validation of the immunoassay was performed to confirm validity of the assay for equine serum, plasma, and synovial fluid on the basis of recommendations provided for bioanalytical method validation.[15] Plates from the same lot number were used to minimize variation. Fresh serum, plasma, and synovial fluid were collected aseptically from 6 clinically normal Thoroughbreds (6 geldings; 11 to 12 years of age). Blood and synovial fluid were collected as described from 6 middle carpal and 6 antebrachiocarpal joints, and samples for each fluid were pooled together for further analysis. Results of this validation study were used to calculate the power needed to identify potential changes related to exercise and osteochondral injury.

Pooled serum, plasma, and synovial fluid samples were spiked with a known amount of standard to create high (3,000 pg/mL), medium (1,000 pg/mL), and low (200 pg/mL) concentration quality control samples. All quality control samples for each fluid were also pooled together to create a mixed quality control sample. Fresh pooled, quality control, and mixed quality control samples were analyzed at 0, 1:2, 1:4, and 1:8 dilutions to determine precision, accuracy, linearity of dilution, parallelism, percentage recovery, and stability of SDF-1 in each fluid. Samples were analyzed in duplicate 3 times in each plate (intra-assay variability) and across 3 plates (interassay variability). To analyze stability of SDF-1, fresh pooled samples were analyzed immediately af-

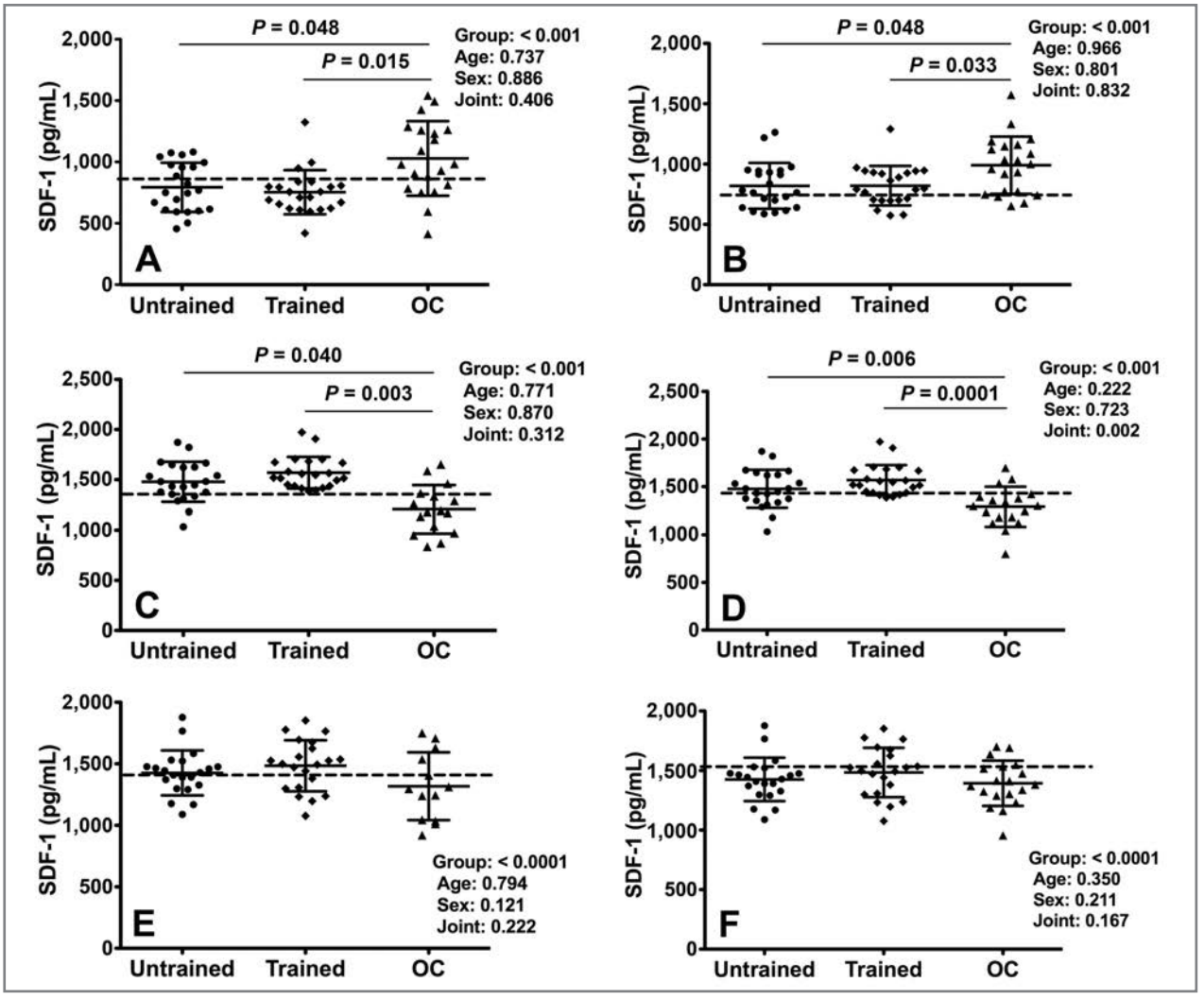

Figure 1—Scatterplots of SDF-1 concentrations in synovial fluid (A and B), serum (C and D), and plasma (E and F) from untrained and trained Thoroughbred racehorces and racehorses with metacarpophalangeal or metatarsophalangeal joint (fetlock joint) osteochondral injuries (OC; A, C, and E) or carpal osteochondral injuries (B, D, and F). Solid lines indicate mean ± SD values. Dashed lines represent the ROC curve threshold values for which there was predictive value for distinguishing horses in the osteochondral-injured group from combined untrained and trained clinically normal horses. Effects of the overall mixed model for the fixed effects of group (untrained, trained, and osteochondral injured), age, sex, and joint examined (middle carpal, antebrachiocarpal, metacarpophalangeal, and metatarsophalangeal) are provided to the right of each scatterplot. *P* values (Tukey post hoc comparisons) are listed in each scatterplot when significant ($P \leq 0.05$) differences were detected among groups.

ter collection as well as after 24 hours at 22°C, after 24 hours at 4°C, and after 1 to 4 freeze-thaw cycles at –80°C.

Statistical analysis—Statistical analysis was performed with statistical software programs.[b–d] All test assumptions were met. Data were assessed for normality by Shapiro-Wilk tests. A mixed model ANOVA was used for analysis of SDF-1 concentration in each fluid to determine differences among groups (untrained, trained, and osteochondral injured) with fixed effects of age (years), sex (sexually intact male, gelding, or female), and joint affected (antebrachiocarpal, middle carpal, metacarpophalangeal, or metatarsophalangeal). The Tukey test for multiple comparisons was used to determine differences among the untrained, trained, and osteochondral-injured horses. Receiver operating characteristic curve analyses were performed to determine the ability of SDF-1 concentrations in synovial fluid, serum, and plasma to distinguish between joints with osteochondral injury and those without injury (untrained and trained combined). Threshold values of SDF-1 that could be used to distinguish joints of healthy versus osteochondral-injured horses were determined by means of the ROC curves by choosing a threshold that resulted in approximately equal sensitivity and specificity. Sensitivity, specificity, and likelihood ratios were recorded for these threshold values and positive predictive values and negative predictive values were computed on the basis of the population data. The Spearman rank test was used to determine correlations of SDF-1 concentrations in all fluids from joints of osteochondral-injured horses and their respective arthroscopic and radiographic scores and number of joints affected. A value of $P \leq 0.05$ was considered significant.

Results

SDF-1 assay validation—Serum samples had a mean intra-assay CV of 7.5% and interassay CV of 10.1%. Plasma samples had a mean intra-assay CV of 7.8% and interassay CV of 13.5%. Synovial fluid samples had a mean intra-assay CV of 7.6% and interassay CV of 10.3%. The limit of detection for serum was 157.0 pg/mL, for plasma was 161.2 pg/mL, and for synovial fluid was 128.5 pg/mL. All diluted samples in all fluids had parallelism to the standard curve. Linearity was determined for serum and plasma at all dilutions, whereas synovial fluid had linearity for 1:2 and 1:4 dilutions but not for 1:8 dilutions (recovery between 56% and 90% of undiluted samples). Recovery of spiked samples in the region of the standard curve where all equine samples congregated was 87% for serum, 95% for plasma, and 71% for synovial fluid. When stored at 22°C (or at 4°C for 24 hours), all fluids had minimal loss of stability. For serum and plasma, loss in stability was < 10% of the first to fourth freeze thaw cycles, and for synovial fluid, loss in stability was < 10% of the first to third freeze thaw cycles. On the basis of data from this validation, a power calculation was performed with a 2-tailed unpaired *t* test ($\alpha = 0.05$) to determine that 22 samples were needed in each group to have an 80% power to detect a difference of 200 pg/mL (25% of the reference synovial fluid concentration) among the 3 groups.

SDF-1 concentrations in synovial fluid—Mean synovial fluid SDF-1 concentrations were significantly higher in horses with fetlock and carpal joint osteochondral injury, compared with untrained and trained horses, but differences between trained and untrained horses were not significant (**Figure 1**). Differences in age, sex, or joint affected were not associated with significant differences in fetlock and carpal joint SDF-1 concentrations. Fetlock and carpal joints had greater likelihood of having osteochondral injury when synovial fluid SDF-1 concentrations were greater than approximately 900 pg/mL (**Table 1**).

SDF-1 concentrations in serum—Mean serum SDF-1 concentrations were significantly lower in horses

Table 1—Results of ROC curve analyses performed to determine whether SDF-1 concentrations in synovial fluid, serum, and plasma could be used to distinguish between Thoroughbreds with osteochondral injury and those without injury (untrained and trained combined) in the metacarpophalangeal or metatarsophalangeal joint (fetlock joint) or carpal joint.

Fluid	No. of joints examined		AUC	*P* value	Threshold (pg/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Likelihood ratio
	OC	No OC								
Synovial fluid										
Fetlock joint	20	44	0.75	0.001	> 860.8	70	73	54	84	3
Carpal joint	21	44	0.72	0.004	> 916.1	67	66	48	81	2
Serum										
Fetlock joint	16	44	0.86	< 0.001	< 1,369	81	86	68	93	6
Carpal joint	18	44	0.81	< 0.001	< 1,402	78	77	58	90	3
Plasma										
Fetlock joint	13	42	0.65	0.1	< 1,320	NS	NS	NS	NS	NS
Carpal joint	19	42	0.58	0.3	< 1,413	NS	NS	NS	NS	NS
Synovial fluid-to-serum ratio										
Fetlock joint	18	44	0.86	< 0.001	> 0.605	89	77	62	94	4
Carpal joint	20	44	0.91	< 0.001	> 0.625	85	84	71	93	5
Synovial fluid-to-plasma ratio										
Fetlock joint	14	42	0.81	< 0.001	> 0.595	71	69	44	88	2
Carpal joint	21	42	0.80	< 0.001	> 0.625	71	76	60	84	3

OC = Osteochondral injury. NS = Not significant.

Threshold values of SDF-1 that could be used to distinguish between horses with and without osteochondral injury were determined from the ROC curves. Sensitivity, specificity, and likelihood ratios were determined for the threshold values, and positive predictive values (PPV) and negative predictive values (NPV) were computed from the population data.

with fetlock or carpal joint osteochondral injury than in untrained and trained horses, but differences between trained and untrained horses were not significant (Figure 1). There was no significant association between age or sex and serum SDF-1 concentrations. However, there was a significant ($P = 0.002$) association between the joint affected (middle carpal vs antebrachiocarpal) and serum SDF-1 concentrations in horses with carpal osteochondral injury, in that horses with osteochondral injury in antebrachiocarpal joints (n = 7) had higher serum concentrations than horses with osteochondral injury in middle carpal joints (14). Serum SDF-1 concentrations in horses with fetlock joint osteochondral injury were correlated with the number of joints affected in the horse ($R = 0.748$; $P = 0.02$), but this was not so for horses with carpal osteochondral injury. Horses had greater likelihood of having osteochondral injury when serum SDF-1 concentrations were less than approximately 1,400 pg/mL (Table 1).

SDF-1 concentrations in plasma—Mean plasma SDF-1 concentrations were not significantly different in horses with fetlock or carpal joint osteochondral injury, compared with untrained and trained horses, and training resulted in no significant difference from untrained horses (Figure 1). There was no association

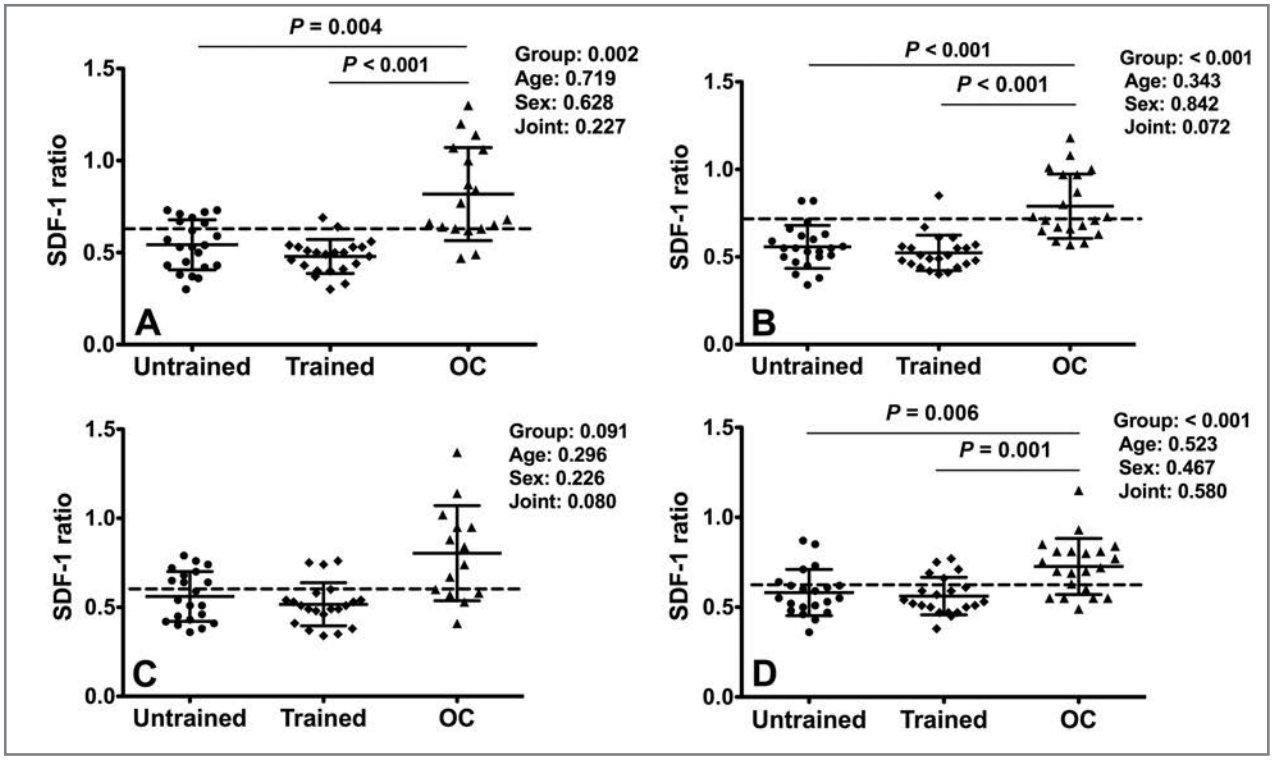

Figure 2—Scatterplots of synovial fluid-to-serum SDF-1 ratios (A and B) and synovial fluid-to-plasma SDF-1 ratios (C and D) for untrained and trained Thoroughbred racehorses and racehorses with fetlock joint osteochondral injuries (A and C) or carpal joint osteochondral injuries (B and D). *See* Figure 1 for remainder of key.

Table 2—Spearman rank correlations of SDF-1 concentrations in synovial fluid, serum, and plasma (and synovial fluid-to-serum and synovial fluid-to-plasma ratios) with radiographic and arthroscopic scores for horses with osteochondral injuries of the carpal and fetlock joints.

	Synovial fluid		Serum		Plasma		Synovial fluid-to-serum ratio		Synovial fluid-to-plasma ratio	
Score	***R***	***P* value**	***R***	***P* value**	***R***	***P* value**	***R***	***P* value**	***R***	***P* value**
Total radiographic										
Carpal joint (n = 20)	0.437	0.07	0.353	0.151	0.422	0.081	0.203	0.419	0.195	0.438
Fetlock joint (n = 17)	0.763	0.017	0.919	< 0.001	0.902	0.001	0.661	0.053	0.458	0.215
Total arthroscopic										
Carpal joint (n = 19)	0.220	0.381	0.088	0.729	0.145	0.567	0.093	0.713	0.122	0.629
Fetlock joint (n = 14)	0.617	0.077	0.828	0.006	0.762	0.017	0.500	0.17	0.300	0.433
Arthroscopic inflammation										
Carpal joint (n = 19)	0.216	0.389	0.100	0.692	0.168	0.504	0.115	0.650	0.155	0.540
Fetlock joint (n = 14)	0.849	0.004	0.852	0.004	0.886	0.001	0.748	0.02	0.529	0.143
Arthroscopic cartilage										
Carpal joint (n = 19)	–0.010	0.967	–0.164	0.516	–0.164	0.516	–0.001	0.997	0.044	0.864
Fetlock joint (n = 14)	0.034	0.931	0.573	0.107	0.282	0.462	0.000	1.000	–0.051	0.896

between age, sex, or joint affected (metacarpophalangeal vs metatarsophalangeal or middle carpal vs antebrachiocarpal) and plasma SDF-1 concentrations. Plasma SDF-1 concentrations were not significantly different between osteochondral-injured horses and clinically normal horses (Table 1). Plasma concentrations were positively correlated with serum SDF-1 concentrations in horses with affected carpal joints ($R = 0.789$; $P < 0.001$) and horses with affected fetlock joints ($R = 0.899$; $P = 0.001$).

Synovial fluid-to-serum ratio—Synovial fluid-to-serum SDF-1 ratios in horses with fetlock or carpal joint osteochondral injury were significantly higher than those of untrained and trained horses, but values in trained versus untrained horses were not significantly different (**Figure 2**). There were no significant associations between age, sex, or joint affected and synovial fluid-to-serum SDF-1 ratios. Horses had greater likelihood of having osteochondral injury when synovial fluid-to-serum SDF-1 ratios were greater than approximately 0.6 (Table 1).

Synovial fluid-to-plasma ratio—Synovial fluid-to-plasma SDF-1 ratios in horses with fetlock joint osteochondral injury were not significantly different from those of untrained and trained horses, and training resulted in no significant difference from untrained horses (Figure 2). Synovial fluid-to-plasma SDF-1 ratios in horses with carpal osteochondral injury were significantly higher than those of untrained and trained horses, but training resulted in no significant difference from untrained horses. There were no associations between age, sex, or joint affected (metacarpophalangeal vs metatarsophalangeal or middle carpal vs antebrachiocarpal) and fetlock or carpal joint synovial fluid-to-plasma SDF-1 ratios. Horses had greater likelihood of having osteochondral injury when synovial fluid-to-plasma SDF-1 ratios were greater than approximately 0.6 (Table 1).

Radiographic and arthroscopic scores in osteochondral-injured horses—No significant correlations were detected between SDF-1 concentrations and radiographic and arthroscopic scores from horses with carpal osteochondral injury. For horses with fetlock joint osteochondral injury, multiple correlations existed between SDF-1 concentrations and radiographic and arthroscopic scores (**Table 2**).

Discussion

The SDF-1 gene has a high degree of cross-species conservation between humans and multiple other species,[16] which allowed us to presume that this would be similar for horses. The validation data revealed parallelism to the standard curve, indicating that SDF-1 in equine serum, plasma, and synovial fluid can potentially be measured with equal effectiveness. The precision of analysis of the unknown samples was acceptable for intra-assay (< 10%) and interassay variability (< 15%) for all fluids, and the SDF-1 analyte was stable through at least 3 freeze-thaw cycles. However, it is important to note that equine synovial fluid appeared to be the least reliable of the 3 fluids because it had matrix effects, especially with increased dilution (> 1:4). Matrix effects are the combined effects of all sample components, such as endogenous proteins other than SDF-1, or influences from viscosity, pH, or salt concentrations that can affect the ability of the assay to accurately determine SDF-1 concentration. Validation results for serum and plasma were comparable and appeared to be less affected by dilution or freeze-thaw cycles than synovial fluid. Despite successful validation of the SDF-1 assay for equine plasma, the data suggested that plasma may be less useful than serum for SDF-1 biomarker studies of joint disease, even though there was a high correlation between serum and plasma SDF-1 concentrations. Platelets bind SDF-1 with high affinity.[17] It is possible that the plasma samples may have contained platelet concentrations sufficient to affect the results of the SDF-1 assay, but no data to support this were obtained.

In a pattern similar to other equine biomarker studies, such as bone alkaline phosphatase and carboxy-terminal telopeptide fragments of type II collagen (CTX-II),[14,18] an increase in synovial fluid concentration, a decrease in serum concentration, and an increase in synovial fluid to serum ratios were observed in osteochondral-injured horses, compared with clinically normal horses. The increase in synovial fluid SDF-1 concentrations, compared with clinically normal horses, was similar to the response reported in guinea pigs with primary osteoarthritis and PTOA[13] as well as human osteoarthritis patients.[7,12] One of the main sources of SDF-1 in joints is synovial fibroblasts, not chondrocytes.[7,8] Stromal cell-derived factor-1 synthesis from synovium is also greater in human osteoarthritis patients than in controls,[7] and guinea pigs with PTOA have higher synovial fluid SDF-1 concentrations with greater synovial membrane change than do guinea pigs with primary osteoarthritis.[13] The main receptor for SDF-1 (CXCR4) is expressed in chondrocytes in the superficial and deep zones of healthy and arthritic cartilage samples, and the level of expression is similar between healthy and diseased cartilage.[7,8] Combined, these reports suggest that as inflammation increases with osteoarthritis, so does SDF-1 production, making it more likely to bind to its receptor on chondrocytes. Once bound, several catabolic events can occur that cause degradation of articular cartilage.[5–10] Our results partially support this theory because synovial fluid SDF-1 concentrations in the fetlock joints of osteochondral-injured horses correlated with arthroscopic inflammation, but there was no correlation with arthroscopic cartilage damage. This was in contrast to a study[13] comparing primary osteoarthritis with end-stage PTOA in guinea pigs in which animals with PTOA had higher synovial fluid SDF-1 concentrations with greater cartilage destruction than did animals with primary osteoarthritis. The differences in cartilage damage in the present study, compared with studies in humans and guinea pigs, may have been present because the fetlock joint osteochondral injuries in the present study had not advanced to end-stage joint disease. Therefore, our results suggested that synovial fluid SDF-1 concentrations increase when inflammation is present, but that these changes do not necessarily reflect the severity of cartilage damage. Thus, it should be emphasized that

SDF-1 does not appear to be a biomarker of joint tissue degradation but is an inflammatory biomarker.

Serum SDF-1 concentrations were lower in osteochondral-injured horses, compared with clinically normal horses, which was contrary to findings in a human study in which serum concentrations were greater in osteoarthritis patients, compared with controls.[12] However, it is interesting that in horses with fetlock joint osteochondral injury, there were positive correlations between serum SDF-1 concentrations and radiographic and arthroscopic scores. This suggests an increase in serum concentration with increasing severity of disease. This enigma regarding serum biomarker results has been reported in a human study[19] and has prompted debate regarding the apparent unpredictability of increased or decreased serum biomarker concentrations in response to degenerative changes in joints.[20] It should be noted that changes in serum are reflective of systemic turnover and do not specifically reflect changes that occur in a single joint. Because of hepatic metabolism, the half-lives of biomarkers are much shorter in blood than in synovial fluid, and the magnitude of the difference depends on the biomarker.[21] Therefore, measured concentrations in serum can be altered by continued degradation of the analyte in synovial fluid or by metabolism in the bloodstream that may result in a change in structure of analyte fragments so that they are no longer detectable by the assay.[22,23] It has been suggested that there may be a fundamental difference in skeletal metabolism of osteoarthritis patients, compared with healthy individuals, that could affect biomarker concentrations.[24] Although unproven in the present study, this may be similar in horses because the present study found decreased serum concentrations similar to some other equine biomarker studies[14,18,25,26] of naturally occurring joint disease.

Another factor that could have led to lower serum SDF-1 concentrations in the osteochondral-injured horses was age. Untrained horses used in this study were < 2 years of age, underwent 5 to 6 months of training, and were compared with mature adult horses (3 to 10 years of age). It is not uncommon for younger horses to have higher biomarker concentrations than mature horses.[25] In the present study, no association between age and serum SDF-1 concentrations was found, but the association between age and biomarker concentrations may not be straightforward.[25]

Ideally, osteoarthritis biomarkers should be investigated in multiple body fluids (serum, urine, and synovial fluid).[20,21,27] Collection of urine and blood from patients is associated with less complications, compared with synovial fluid. However, synovial fluid should provide the most direct information about changes in the joint.[20,21,27] Multiple fluid analyses allow for comparison of concentrations in each fluid, including the interrelationships among fluids as measured with ratios or correlation data, and determination of which fluid best reflects the severity of disease as estimated with clinical measurements such as radiographic and arthroscopic scores.[20,21,27] Synovial fluid usually provides the most useful biomarker data with regard to the joint, but on the basis of the validation data, serum had less matrix effects on the assay than did synovial fluid in the present study. In addition, even though we cannot explain the decrease in serum SDF-1 concentrations with osteochondral injury, serum was more useful than plasma and synovial fluid in distinguishing horses with fetlock or carpal joint osteochondral injury from untrained and trained clinically normal horses. Although the synovial fluid-to-serum SDF-1 ratio was more useful for detection of injury than synovial fluid SDF-1 concentration alone, the difference was not large. This was different from 2 previous equine biomarker studies[14,18] in which synovial fluid-to-serum ratios were far superior to either fluid alone for use in identification of osteochondral-injured horses. Because the present study is the first to examine SDF-1 in equine fluids, further investigation is required to support or refute the conclusions.

Previous biomarker studies in horses and humans have found that exercise can affect biomarker concentrations in serum and synovial fluid and the effect likely varies with each biomarker.[28–30] Results of the present study suggested that SDF-1 concentrations in equine serum and synovial fluid did not change with exercise. It is possible that an exercise response was not detected because the joints had time to adapt to race training over 5 to 6 months. If so, fluids should be analyzed shortly after commencement of exercise to determine whether there is a more acute response to exercise, as has been detected in a human study.[30]

Correlation of clinical scores of osteoarthritis severity with biomarker values is important in assessment and validation of the objectivity of biomarkers for clinical applications. It was recently proposed that diagnosis of early human knee osteoarthritis can be based on presence of pain, minimal radiographic findings (Kellgren-Lawrence grade 0 to 2), and evidence of cartilage lesions on either arthroscopic or MRI examination.[31] On the basis of these criteria and radiographic and arthroscopic scores in the osteochondral injury group in the present study, it could be argued that these horses had evidence of PTOA. Interestingly, correlations of serum, plasma, and synovial fluid SDF-1 concentrations with radiographic and arthroscopic scores provided evidence of differences between the fetlock and carpal joints in response to osteochondral injury. When considered alone, SDF-1 concentrations in injured carpal joints were not correlated with radiographic or arthroscopic scores, whereas SDF-1 concentrations in injured fetlock joints were highly correlated with radiographic and arthroscopic scores; correlation was also found with multiple joint injury. We cannot explain this difference, but it may be related to low numbers of samples analyzed or differences among joints in cartilage metabolism and thickness, joint size, load, and mechanical properties.[32]

The major limitation to this study was inherent variability in the clinical cases of naturally occurring osteochondral injury. Factors such as chronicity, exercise, and previous medical treatments were mostly unknown. Although this is not an uncommon problem when examining clinical cases of naturally occurring joint disease for biomarker studies,[14,18,25,26,33–39] the potential effect on biomarker concentrations can be quite profound. For example, administration of phenylbutazone,[40] lidocaine,[41] methylprednisolone acetate,[42] and

triamcinolone acetonide[43] results in changes in biomarker concentrations in equine joints. In humans, administration of dexamethasone and a COX-2 inhibitor decreases SDF-1 production associated with subacromial bursitis.[44] Therefore, it is likely that if any of the horses in the present study received intra-articular injections of corticosteroids prior to surgery, SDF-1 production in the affected joint would decrease. However, in another equine study[45] in which medication history was known, it was concluded that medication effects on biomarkers were not straightforward because the duration of effects of commonly used medications administered intra-articularly are not well documented. Therefore, even though undisclosed treatments may have had an effect on results of the present study, SDF-1 concentrations still could be used to distinguish osteochondral-injured horses from clinically normal horses.

Results of this study support further investigation of SDF-1 for detection of joint disease in horses. Stromal-derived factor-1 concentrations in serum appeared to be the most sensitive for detection of PTOA injury of the fetlock joint and carpus, compared with plasma and synovial fluid. Further analysis of serum and synovial fluid SDF-1 in experimental equine joint injury studies may be of use in defining the onset and progression of PTOA and evaluation of anti-inflammatory treatments.

a. Quantikine Human SDF-1α Immunoassay, R&D Systems, Minneapolis, Minn.
b. SAS, version 9.2 for Windows, SAS Institute Inc, Cary, NC.
c. SPSS, version 20.0 for Windows, SPSS Inc, Chicago, Ill.
d. GraphPad Prism, version 6.01, GraphPad Software, Inc, La Jolla, Calif.

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