

# Correlation of prostaglandin E<sub>2</sub> concentrations in synovial fluid with ground reaction forces and clinical variables for pain or inflammation in dogs with osteoarthritis induced by transection of the cranial cruciate ligament

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**Objective**—To evaluate the temporal pattern of prostaglandin (PG) E<sub>2</sub> concentrations in synovial fluid after transection of the cranial cruciate ligament (CCL) in dogs and to correlate PGE<sub>2</sub> concentrations with ground reaction forces and subjective clinical variables for lameness or pain.

**Animals**—19 purpose-bred adult male Walker Hounds.

**Procedure**—Force plate measurements, subjective clinical analysis of pain or lameness, and samples of synovial fluid were obtained before (baseline) and at various time points after arthroscopic transection of the right CCL. Concentrations of PGE<sub>2</sub> were measured in synovial fluid samples, and the PGE<sub>2</sub> concentrations were correlated with ground reaction forces and clinical variables.

**Results**—The PGE<sub>2</sub> concentration increased significantly above the baseline value throughout the entire study, peaking 14 days after transection. Peak vertical force and vertical impulse significantly decreased by day 14 after transection, followed by an increase over time without returning to baseline values. All clinical variables (eg, lameness, degree of weight bearing, joint extension, cumulative pain score, effusion score, and total protein content of synovial fluid, except for WBC count in synovial fluid) increased significantly above baseline values. Significant negative correlations were detected between PGE<sub>2</sub> concentrations and peak vertical force ( $r$ , -0.5720) and vertical impulse ( $r$ , -0.4618), and significant positive correlations were detected between PGE<sub>2</sub> concentrations and the subjective lameness score ( $r$ , 0.5016) and effusion score ( $r$ , 0.6817).

**Conclusions and Clinical Relevance**—Assessment of the acute inflammatory process by measurement of PGE<sub>2</sub> concentrations in synovial fluid may be correlated with the amount of pain or lameness in dogs. (*Am J Vet Res* 2004;65:1269-1275)

**T**ransection of the cranial cruciate ligament (CCL) in dogs is an accepted method for experimentally inducing osteoarthritis (OA) that results in changes typical of the early stages of OA in humans and dogs.<sup>1</sup> Transection of the CCL in dogs ultimately results in damage to all of the intra-articular structures, including the synovium, menisci, articular cartilage, and subchondral bone.<sup>1-3</sup> Damage is presumably attributable to the combination of events that are primarily related to joint instability and inflammation.<sup>2</sup> Traditionally, it has been believed that instability leads to an increase in inflammation; however, with surgical transection of the CCL, inflammation is usually initiated during surgery, and the combination will cause a dog to be lame.<sup>4</sup>

With the onset of inflammation, cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)- $\alpha$  are upregulated, and they in turn activate the arachidonic acid cascade. Induction of the arachidonic acid cascade stimulates the synovium to produce various inflammatory mediators, such as prostaglandins (PGs), thromboxanes, leukotrienes, and kinins.<sup>5-7</sup> The most important inflammatory mediator in experimentally induced inflammatory exudates is PGE<sub>2</sub>.<sup>8</sup> It is an eicosanoid capable of mediating a number of features characteristic of inflammation, including vasodilation and increased permeability of small blood vessels, that lead to erythema.<sup>9</sup> In addition, PGE<sub>2</sub> sensitizes peripheral nociceptors,<sup>10,11</sup> stimulates the formation of new blood vessels,<sup>12</sup> and stimulates expression of matrix metalloproteinases (MMPs) via promotion of plasminogen activators.<sup>13</sup> The diversity of these reactions is the result of the multiple receptor subtypes and isoforms that are responsible for regulation of the PG cycle.<sup>14</sup> Prostaglandin E<sub>2</sub> has anabolic and catabolic effects via its regulation of chondrocyte proliferation and the synthesis of extracellular matrix components.<sup>15-17</sup> There are greater amounts of PGE<sub>2</sub> in synovial fluid obtained from joints with varying degrees of OA, compared with amounts in synovial fluid obtained from normal joints of horses.<sup>8,18</sup> Because of the high concentrations of PGE<sub>2</sub> in the synovial fluid early in the disease process, it has been suggested that it could be a useful indicator of the existing inflammatory status.<sup>18</sup>

Force plate analysis has been used as a research tool to objectively measure normal and abnormal forces in dogs.<sup>19-29</sup> It allows for description of the peak forces and impulses (total force applied over time)

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applied in vertical, craniocaudal (braking and propulsion), and mediolateral directions. Many orthopedic studies<sup>19-24</sup> in dogs have included analysis of these ground reaction forces to objectively determine the typical forces applied for various gait cycles at several velocities. In addition, force plate analysis has been used to measure abnormal forces that result from insult to the appendicular skeleton as well as the response of the limbs to various surgical techniques or medical options used for treatment of joint impairment.<sup>25-29</sup> Force plate analysis has been used to document changes in the ground reaction forces that are a result of rupture or transection of the CCL, compared with values for an intact CCL.<sup>25-29</sup> Certain surgical techniques can improve these kinetic data during the early period, suggesting that the force plate is a sensitive tool for identification of subtle differences over time.<sup>29</sup> To the authors' knowledge, PGE<sub>2</sub> concentrations in the synovial fluid of dogs have not been evaluated to determine their correlation with ground reaction forces that result after injury of the CCL.

The purpose of the study reported here was to evaluate the temporal pattern of PGE<sub>2</sub> concentrations in synovial fluid after experimental transection of the CCL in dogs and to correlate those concentrations with subjective and objective measures of pain or lameness. Our hypothesis was that PGE<sub>2</sub> concentrations in the synovial fluid would increase significantly above baseline values by 2 weeks after CCL transection and correlate with objective analysis of lameness measured by use of force plate analysis.

## Materials and Methods

**Animals**—Nineteen purpose-bred adult male Walker Hounds that ranged in weight from 19.0 to 28.5 kg (mean  $\pm$  SD, 22.42  $\pm$  2.39 kg) were used in the study. These dogs were used as the control group in another study of a novel therapeutic agent. All dogs were obtained from approved sources by the Colorado State University Laboratory Animal Resources. Throughout the study, all dogs were housed separately in runs. The study was conducted in accordance with a protocol approved by the Colorado State University Animal Care and Use Committee.

Inclusion criteria required that the dogs did not have evidence of preexisting orthopedic disease of the stifles on the basis of results of routine physical, orthopedic, lameness, radiographic, and force plate examinations. Orthopedic examination included separate lameness and physical examinations of both hind limbs as well as radiographic examination of both stifles. For gait analyses, each dog was acclimated for several weeks to the force platform analysis laboratory and was led by a leash at a trot across the force plate prior to inclusion in the study. The dogs had to be able to adequately trot across the force plate with minimal effort or coaxing from the handler.

**Experimental procedures**—All dogs were anesthetized, and the right hind limb was prepared for aseptic surgery. A lateral parapatellar arthroscopic approach to the right stifle was used to examine the joint for evidence of any intra-articular pathologic changes in the articular cartilage, cruciate ligaments, and menisci. The CCL was then transected arthroscopically<sup>a</sup> by use of a meniscectomy blade. Day of CCL transection was designated as day 0. Analgesia was accomplished by use of a 5-mg fentanyl patch (2.0  $\mu$ g/kg/h) that was

applied the day before surgery and was maintained for 72 hours after surgery. Morphine (1 mg/kg, SC) was also administered after surgery, as needed. Cefazolin (22 mg/kg) was administered IV during surgery, and treatment was continued for 3 days after surgery with cephalexin (10 mg/kg, PO, q 12 h). Dogs were closely monitored during the first 48 hours after surgery for signs of pain, infection, anorexia, or intestinal complications. To encourage development of OA, dogs were exercised in accordance with a protocol that required leash walking for 30 min/d (5 d/wk) from days 2 through 126 after surgery.

**Collection of synovial fluid**—To obtain samples of synovial fluid, each dog was sedated by IM injection of a combination of acepromazine maleate (0.05 mg/kg), butorphanol tartrate (0.2 mg/kg), and glycopyrrolate (0.005 mg/kg). When additional restraint was required, dogs were anesthetized by administration of isoflurane via a face mask; anesthesia was maintained by use of isoflurane. Because the study reported here was an adjunct of a larger study in which multiple biomarkers were analyzed, synovial fluid samples were collected from the right stifle joint (ie, stifle with the transected CCL) by use of a lavage method in which 5 mL of sterile saline (0.9% NaCl) solution was injected into the joint. The limb was then manipulated for 1 to 2 minutes to ensure adequate mixing of saline solution and synovial fluid prior to aspiration of as much fluid as possible. Samples were collected before (baseline) and 14, 70, and 126 days after surgery. An aliquot (0.5 to 1.0 mL) of the total volume collected was added to a tube containing EDTA, and the remainder of the fluid was placed in serum separator tubes. Samples in the EDTA tubes were analyzed to determine total protein content and WBC count. Immediately after collection, serum separator tubes were placed on ice until centrifuged (500  $\times$  g for 10 minutes at 4°C). The samples were then divided into multiple aliquots and frozen at -80°C until assayed. Interval between collection and freezing of samples was < 3 hours.

**PGE<sub>2</sub> analysis**—Most samples used for measurement of PGE<sub>2</sub> content were subjected to 2 to 4 freeze-thaw cycles during sample analysis because of the volume of synovial fluid required for analysis. Synovial fluid samples were subjected to solid-phase extraction procedures. Synovial fluid (500  $\mu$ L) was mixed with 500  $\mu$ L of 80% ethanol and 10  $\mu$ L of glacial acetic acid; this mixture was incubated at 23°C for 5 minutes and then centrifuged (2,500  $\times$  g for 8 minutes). The supernatant was harvested and applied to 1-mL, C2 minicolumns<sup>b</sup> that had been washed twice with 1 mL of 10% ethanol while under vacuum. Columns were then washed with 1 mL of deionized water and 1 mL of hexane, and PGE<sub>2</sub> was eluted (under vacuum) with 1.5 mL of ethyl acetate. The eluted sample was evaporated to dryness under a continuous flow of nitrogen by use of a multiple-port gas evaporation system.<sup>c</sup> Dried samples were stored at -20°C until assayed.

Concentrations of PGE<sub>2</sub> were estimated by use of a commercially available enzyme immunoassay kit<sup>d</sup> that cross-reacted with canine serum, as determined by the manufacturer. This immunoassay used a monoclonal antibody that competitively bound to PGE<sub>2</sub> or an alkaline phosphatase molecule bound with PGE<sub>2</sub>. Before analysis, each extracted sample was resuspended in 400  $\mu$ L of the supplied assay buffer. Then, 100  $\mu$ L of assay buffer, PGE<sub>2</sub> standards, or lavaged synovial fluid samples were applied in duplicate wells that were coated with a goat antibody specific to mouse IgG. This was followed by addition of 50  $\mu$ L of assay buffer and alkaline phosphatase conjugated with PGE<sub>2</sub> or the monoclonal antibody to PGE<sub>2</sub>. The plate was sealed and incubated

ed overnight (18 to 24 hours) at 4°C. After incubation, plates were washed. Then, 5 µL of alkaline phosphatase conjugated with PGE<sub>2</sub> was added to each well, followed by 200 µL of *p*-nitrophenyl phosphate substrate solution. After incubation for 1 hour at 37°C, 50 µL of trisodium phosphate (ie, stop solution) was added to each well. Absorbance of each well was immediately measured at 405 nm in a microplate reader.<sup>c</sup> Binding percentage for each standard and sample was calculated, and the concentration of PGE<sub>2</sub> in the samples was determined from a standard curve created by use of log-logit transformation. Because of the limited sample volume, an interassay coefficient of variation was not determined.

**Force plate analysis**—A force plate<sup>f</sup> was mounted in the center of a 15-m runway. The force plate was level with the runway surface. The signal from the force plate was processed and stored by use of computer software programs.<sup>g,h</sup> As each dog trotted over the force plate, velocity was recorded by use of 2 photoelectric cells placed 2 m apart and a start-interrupt timer system. Care was taken to ensure that each dog triggered the photoelectric cells and that a constant speed was maintained across the force plate during each evaluation. Dogs trotted across the force plate before (baseline) and 14, 28, 70, and 126 days after CCL transection. An evaluation was considered valid when a dog struck the plate with its right forelimb followed by its right hind limb while traveling at a velocity between 1.45 and 2.05 m/s. When a dog was distracted during an evaluation or any other combination of limbs struck the force plate, then the data was considered invalid. Because of the severity of lameness in some dogs, each dog trotted across the force plate a maximum of 75 times/session, with a maximum of 5 acceptable evaluations collected for each dog in each session. When a dog would not bear weight on the right hind limb, then the value entered for that evaluation was zero. All sessions were recorded and archived digitally on videotape. Peak vertical force and vertical impulse were recorded for each limb at 1-millisecond intervals for 650 milliseconds after foot strike. All forces were adjusted on the basis of the body weight for each dog recorded at each measurement period.

**Subjective scoring of clinical signs of pain**—Subjective assessment of lameness or signs of pain was performed immediately before each force plate evaluation (ie, before [baseline] and 14, 28, 70, and 126 days after CCL transection). All subjective scoring was performed by the same evaluator (TNT). Lameness was graded by use of a scale of 1 to 4 (1, no lameness; 2, mild lameness while walking or trotting; 3, moderate lameness while walking or trotting; and 4, severe lameness while walking or trotting). The degree of weight a resting dog would bear on the limb was assessed by use of a scale of 1 to 3 (1, normal weight bearing at rest; 2, partial weight bearing at rest; and 3, no weight bearing at rest). For subjective assessment of signs of pain, the limb was lifted off the ground and maximally extended. The response to this extension of the limb was assessed by use of a scale from 1 to 4 (1, no response; 2, mild response such as turning the head toward the affected limb; 3, moderate response such as withdrawal of the affected limb; and 4, severe response such as vocalization or aggression). A cumulative pain score was calculated by summing the scores for lameness, weight bearing, and joint extension. In addition, the degree of joint effusion was subjectively assessed at the time of arthrocentesis on days 0, 14, 70, and 126. Scale for the effusion score was 0 to 3 (0, no effusion; 1, mild effusion; 2, moderate effusion; and 3, severe effusion).

**Statistical analysis**—All data were expressed as mean ± SD. Distribution of data for the PGE<sub>2</sub> concentration, total

protein content, and WBC count in synovial fluid; peak vertical force and vertical impulse; and subjective scores for lameness, degree of weight bearing, joint extension, cumulative pain score, and effusion score were assessed for normality by use of the Kolmogorov-Smirnov test. Peak vertical force and vertical impulse were normally distributed, whereas data for the PGE<sub>2</sub> concentrations had to be logarithmically transformed to provide normally distributed data. All of the other data sets (total protein content and WBC count in synovial fluid as well as subjective scores for lameness, degree of weight bearing, joint extension, cumulative pain scoring, and effusion score) could not be transformed to provide normally distributed data; thus, they were analyzed by use of nonparametric methods.

For peak vertical force, vertical impulse, and PGE<sub>2</sub> concentrations, the overall effect of time was analyzed by use of a repeated-measures ANOVA on the transformed data. Additional post hoc Tukey multiple-comparison tests were performed on each data set with significant results to determine significant differences from baseline values. For the nonparametric data (total protein content and WBC count in synovial fluid as well as lameness, degree of weight bearing, joint extension, cumulative pain score, and effusion score), Kruskal-Wallis analyses were performed by use of a Dunn post hoc test to determine significant differences from baseline values. Correlations among peak vertical force, vertical impulse, and PGE<sub>2</sub> concentrations were assessed by use of the Pearson correlation coefficient on transformed data. Correlations with nonparametric data (total protein content and WBC count in synovial fluid as well as lameness, degree of weight bearing, joint extension, cumulative pain score, and effusion score) were performed by use of the Spearman rank correlation. A value of  $P < 0.05$  was considered significant for all analyses. All statistical analyses were performed by use of commercially available software.<sup>1</sup>

## Results

**PGE<sub>2</sub> analysis**—Concentrations of PGE<sub>2</sub> in synovial fluid increased significantly ( $P < 0.001$ ) above baseline values throughout the entire study. Concentrations were 1.5-fold higher at day 14, 1.4-fold higher at day 70, and 1.3-fold higher at day 126 after CCL transection, compared with baseline values (Figure 1).

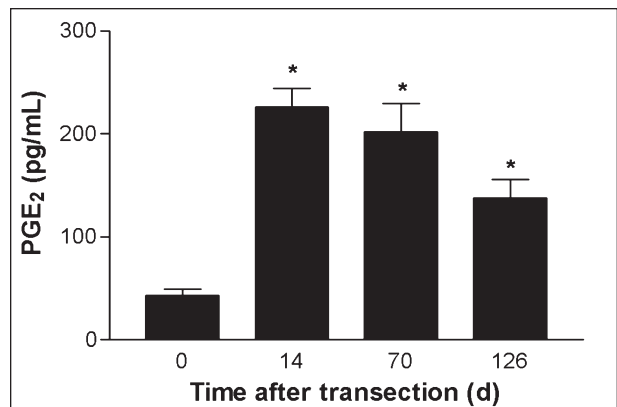


Figure 1—Mean ± SD prostaglandin (PG) E<sub>2</sub> concentrations in samples of synovial fluid obtained from the stifle joints of dogs in which the cranial cruciate ligament (CCL) was transected to create osteoarthritis. Samples were obtained before (baseline; day 0) and 14, 70, and 126 days after CCL transection. Day of transection was designated as day 0. Overall, there was a significant ( $P < 0.001$ ) effect for time, as determined by use of a repeated-measures ANOVA. \*Value differs significantly ( $P < 0.001$ ) from baseline value, as determined by use of the Tukey post hoc test.

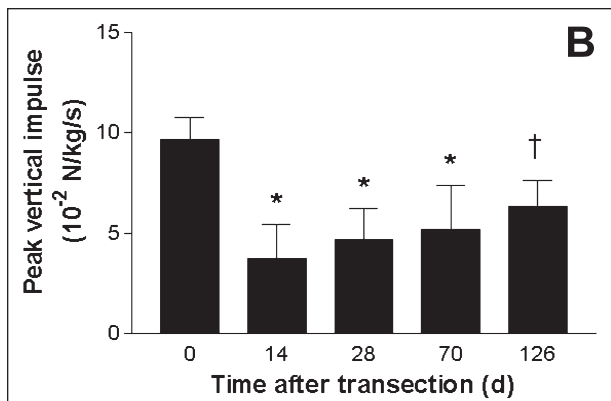
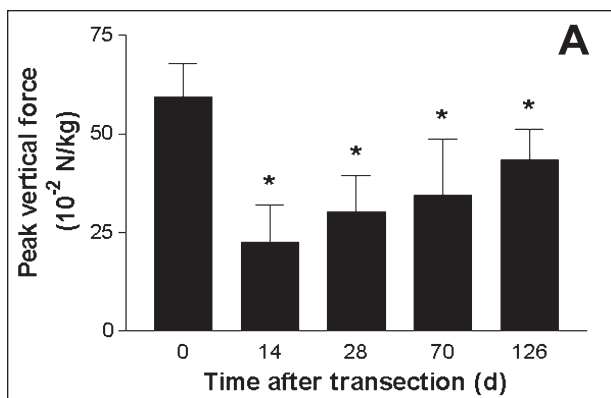


Figure 2—Mean  $\pm$  SD value for peak vertical force (A) and peak vertical impulse (B) at various time points for dogs in which the CCL was transected in a hind limb to create osteoarthritis. Overall, there was a significant ( $P < 0.001$ ) effect for time for each variable, as determined by use of a repeated-measures ANOVA. \*,†Within each variable, value differs significantly ( $*P < 0.001$ ;  $\dagger P = 0.01$ ) from baseline value for that variable, as determined by use of the Tukey post hoc test.

**Force plate analysis**—Peak vertical force for the limb with the transected CCL decreased significantly ( $P < 0.001$ ) below the baseline value throughout the entire study, with the mean force being 62% lower on day 14, 49% lower on day 28, 42% lower on day 70, and 27% lower on day 126 after CCL transection, compared with the mean force measured at baseline (Figure 2). The corresponding peak vertical impulse for the limb with the transected CCL was also significantly lower than the baseline value throughout the entire study. Mean vertical impulse was 61% lower on day 14 ( $P < 0.001$ ), 52% lower on day 28 ( $P < 0.001$ ), 46% lower on day 70 ( $P < 0.001$ ), and 34% lower on day 126 ( $P = 0.01$ ) after CCL transection, compared with the mean vertical impulse measured at baseline.

**Clinical variables**—Total protein content in synovial fluid was significantly ( $P < 0.001$ ) higher than the baseline value throughout the entire study, peaking on day 14 after CCL transection (Table 1). The WBC count in synovial fluid was significantly higher than the baseline value on days 14 ( $P < 0.05$ ) and 126 ( $P < 0.001$ ) after CCL transection. Effusion score was significantly ( $P < 0.001$ ) higher than the baseline value throughout the entire study.

Table 1—Mean  $\pm$  SD values for total protein content, WBC count, and effusion score in samples of synovial fluid obtained at various time points from the stifle joints of dogs in which the cranial cruciate ligament (CCL) was transected to create osteoarthritis.

Day	Total protein (g/dL)	WBC count (No. of cells/ $\mu$ L)	Effusion score
0	0.38 $\pm$ 0.21	192.86 $\pm$ 126.88	0 $\pm$ 0
14	2.16 $\pm$ 0.61*	536.84 $\pm$ 586.15†	2.26 $\pm$ 0.45*
70	1.63 $\pm$ 0.60*	321.05 $\pm$ 201.60	2.11 $\pm$ 0.32*
126	1.49 $\pm$ 0.73*	915.79 $\pm$ 1498.99*	2.80 $\pm$ 0.41*

Overall, there was a significant ( $P < 0.001$ ) effect for time for each variable, as determined by use of Kruskal-Wallis analysis.

\*,†Within each column, the value differs significantly ( $*P < 0.001$ ;  $\dagger P < 0.05$ ) from the baseline (day 0; day of CCL transection) value, as determined by use of the Dunn post hoc test.

Table 2—Mean  $\pm$  SD values for subjective clinical variables of lameness, degree of weight bearing, joint extension, and the total of all 3 (cumulative pain score) for the hind limb in which the CCL was transected to create osteoarthritis.

Day	Lameness	Weight bearing	Joint extension	Cumulative pain score
0	1.00 $\pm$ 0.00*	1.00 $\pm$ 0.00*	1.00 $\pm$ 0.00*	3.00 $\pm$ 0.00
14	3.50 $\pm$ 0.51†	2.00 $\pm$ 0.00†	2.32 $\pm$ 0.67†	7.89 $\pm$ 0.81†
28	3.37 $\pm$ 0.60†	2.00 $\pm$ 0.00†	2.42 $\pm$ 0.51†	7.79 $\pm$ 0.63†
70	3.16 $\pm$ 0.60†	2.05 $\pm$ 0.23†	2.47 $\pm$ 0.51†	7.68 $\pm$ 0.89†
126	2.84 $\pm$ 0.76†	1.53 $\pm$ 0.51	1.89 $\pm$ 0.57†	6.26 $\pm$ 1.33§

Overall, there was a significant ( $P < 0.001$ ) effect for time for each variable, as determined by use of Kruskal-Wallis analysis.

\*A score of 1.00 on day 0 is indicative of no lameness, typical ability to bear weight, and no response of the dog to extension of the CCL-transected joint. †,‡,§Within a column, the value differs significantly ( $\dagger P < 0.001$ ;  $\ddagger P = 0.01$ ;  $\S P < 0.05$ ) from the baseline (day 0; day of CCL transection) value, as determined by use of the Dunn post hoc test.

Lameness grade was significantly ( $P < 0.001$ ) higher than the baseline value throughout the entire study, peaking at day 14 after CCL transection and decreasing thereafter (Table 2). Degree of weight bearing on the limb with the transected CCL was significantly ( $P < 0.001$ ) worse than the baseline value on days 14, 28, and 70 after CCL transection, but it returned to the baseline value by 126 days after transection. The response to joint extension was significantly ( $P < 0.001$ ) greater than the baseline value throughout the entire study, peaking on day 70 after CCL transection before decreasing by day 126 after transection. Cumulative pain score was significantly ( $P < 0.001$ ) higher than the baseline value throughout the entire study, peaking at day 14 and decreasing thereafter.

**Correlations**—Significant ( $P < 0.001$ ) negative correlations were detected between  $PGE_2$  concentrations in synovial fluid and peak vertical force ( $r, -0.5720$ ) and between  $PGE_2$  concentrations in synovial fluid and peak vertical impulse ( $r, -0.4618$ ; Table 3). Significant ( $P < 0.001$ ) positive correlations were detected between  $PGE_2$  concentrations in synovial fluid and all clinical variables, except WBC count, including the subjective lameness score ( $r, 0.5016$ ) and effusion score ( $r, 0.6817$ ). In addition, peak vertical force and vertical impulse were significantly and negatively correlated with all clinical variables, except WBC count.



Table 3—Correlation coefficients between prostaglandin (PG) E<sub>2</sub> concentration in synovial fluid, ground reaction forces (peak vertical force and vertical impulse), and clinical variables (lameness, degree of weight bearing, joint extension, cumulative pain score, synovial fluid effusion score, total protein content in synovial fluid, and WBC count in synovial fluid) for dogs with a transected CCL.

Variable	Variable									
	PGE <sub>2</sub>	Peak vertical force	Vertical impulse	Lameness	Weight bearing	Joint extension	Cumulative pain score	Effusion score	Total protein	WBC count
PGE <sub>2</sub>	—	-0.5720*	-0.4618*	0.5016*	0.5785*	0.2955†	0.4673*	0.6817*	0.4771*	0.1336 <sup>NS</sup>
Peak vertical force	-0.5720*	—	0.9436*	-0.7846*	-0.7325*	-0.5203*	-0.7580*	-0.4775*	-0.5643*	-0.2185 <sup>NS</sup>
Vertical impulse	-0.4618*	0.9436*	—	-0.8072*	-0.7311*	-0.5139*	-0.7648*	-0.4962*	-0.5536*	-0.1968 <sup>NS</sup>
Lameness	0.5016*	-0.7846*	-0.8072*	—	0.7258*	0.5355*	0.8822*	0.6009*	0.6232*	0.3115‡
Weight bearing	0.5785*	-0.7325*	-0.7311*	0.7258*	—	0.6546*	0.8028*	0.5430*	0.6205*	0.3805§
Joint extension	0.2955†	-0.5203*	-0.5139*	0.5355*	0.6546*	—	0.8151*	0.5101*	0.4411*	0.1060 <sup>NS</sup>
Cumulative pain score	0.4673*	-0.7580*	0.8822*	0.8822*	0.8028*	0.8151*	—	0.5476*	0.5702*	0.1780 <sup>NS</sup>
Effusion score	0.6817*	-0.4775*	-0.4962*	0.6009*	0.5430*	0.5101*	0.5476*	—	0.7705*	0.4928*
Total protein	0.4771*	-0.5643*	-0.5536*	0.6232*	0.6205*	0.4411*	0.5702*	0.7705*	—	0.3504
WBC count	0.1336 <sup>NS</sup>	-0.2185 <sup>NS</sup>	-0.1968 <sup>NS</sup>	0.3115‡	0.3805§	0.1060 <sup>NS</sup>	0.1780 <sup>NS</sup>	0.4928*	0.3504	—

\* , † , ‡ , § , ¶ Correlation was significant (\**P* < 0.001; †*P* = 0.01; ‡*P* = 0.008; §*P* = 0.001; ¶*P* = 0.003). The correlation between PGE<sub>2</sub> concentration and ground reaction forces (ie, peak vertical force and vertical impulse) was determined by use a Pearson correlation coefficient. All other correlations were determined by use of a Spearman rank correlation. — = Not applicable. NS = Not significant (*P* > 0.05).

## Discussion

Production of PGE<sub>2</sub> can be stimulated through upregulation of the cyclooxygenase-2 pathway, via the proinflammatory cytokines IL-1 and TNF $\alpha$ ,<sup>6,7,30</sup> or by IL-18.<sup>31</sup> This inflammatory process stimulates primary afferent neurons within the affected joint to release neuropeptides, such as substance P, which further drives the inflammatory process by causing the release of IL-1 and TNF $\alpha$ ,<sup>32</sup> as well as initiating and enhancing PGE<sub>2</sub> production.<sup>33,34</sup> A positive correlation between substance P and PGE<sub>2</sub> has been identified in the synovial fluid from OA-affected joints of horses.<sup>18</sup> In that study, it was suggested that because of the early peak for substance P and PGE<sub>2</sub> in the early phase of OA, the synovial fluid content of PGE<sub>2</sub> likely plays a predominant role in the acute phase of the inflammatory process. Analysis of results of the study reported here supports this statement because the PGE<sub>2</sub> concentrations in the synovial fluid from joints of dogs with a transected CCL were significantly increased from the baseline value, peaking on day 14 after transection.

Additional support of the findings reported here was documented in another study<sup>1</sup> conducted by our laboratory group. In that study, we examined 3 dogs that underwent serial arthroscopic examinations 14, 28, 70, and 126 days after CCL transection. Analysis of results of that study indicated that the degree of synovitis reached a peak by 28 days after transection and then diminished over time but never completely abated. Because we did not collect synovial fluid for PGE<sub>2</sub> analysis on day 28 after transection in that study, we cannot state whether the PGE<sub>2</sub> concentration would have actually peaked at that time, as opposed to 14 days after transection reported here. However, analysis of results of both studies indicates that there is an early inflammatory peak in PGE<sub>2</sub> concentrations in the synovial fluid within the first month after CCL transection.

Lameness that develops after CCL transection is likely the result of a multifactorial process, with instability and inflammation both contributing to the process. It has been documented<sup>10,11,34</sup> in inflamed joints that afferent nerve fibers of the joint have increased sensitivity for activation of nociceptors and neuropeptides, such as substance P. When combined with joint instability, this results

in increased neural activity of the receptors (ie, hyperalgesia), which is presumably perceived as pain, especially during movement and joint loading.<sup>10</sup> However, pain can also be perceived when the joint is at rest,<sup>10</sup> which may be partially attributable to increased intra-articular volume and enhanced sensitivity of joint afferent nerves as a result of increased pressure.<sup>35</sup> The results reported here documented that PGE<sub>2</sub> concentrations in the synovial fluid are strongly associated with clinical assessments of pain in that there were significant correlations for PGE<sub>2</sub> concentrations and peak vertical force (*r*, -0.5720), vertical impulse (*r*, -0.4618), subjective lameness assessment (*r*, 0.5016), degree of weight bearing when the limb was at rest (*r*, 0.5785), and joint effusion score (*r*, 0.6817).

Our force plate results are similar to results<sup>25-29</sup> reported for dogs with naturally developing or experimentally induced CCL rupture. There was an acute decrease in peak vertical force and vertical impulse by 14 days after transection, followed by an increase in the amount of weight bearing over time, which did not return to the values obtained before CCL transection. This improvement has been attributed to the inherent increase in stability of the joint over time because of the increasing fibrous response of the joint capsule.<sup>26,28</sup> Subjective gross pathologic examination of the joints of our dogs 126 days after CCL transection certainly revealed that the dogs developed a moderate (11/19 [58%] dogs) to severe (8/19 [42%]) fibrous response of the synovium and capsule. This fibrous response likely contributed to some of the stability identified in our dogs; but often, even though many dogs improved clinically, cranial drawer instability was still evident, as has been described elsewhere.<sup>28</sup> Analysis of our results also suggests that the decrease in the inflammatory process over time after CCL transection or rupture may actually contribute to some of the improvement in weight bearing, presumably because of less pain associated with joint loading. Following this line of reasoning, any additional pathologic changes within the joint, such as meniscal tearing, may then result in increased lameness because of exacerbation of the inflammatory process because the menisci are capable of production of PGE<sub>2</sub>.<sup>30</sup> All 19 dogs in the study reported here had some

subjective degree of pathologic changes to the medial meniscal area when examined 126 days after CCL transection. Most (9/19 [47%]) had only mild fraying or incomplete tears, whereas 3 of 19 (16%) had moderate fraying or tears (longitudinal surface or small transverse tears), and 7 of 19 (37%) had severe fraying or tears (penetrating longitudinal tears). Therefore, it is likely that the decrease in peak vertical force over time after CCL transection was probably attributable to a multitude of factors, including an increase in stability as a result of fibrous tissue deposition as well as a decrease in the degree of inflammation.

It is believed that PGE<sub>2</sub> in synovial fluid originates from articular tissues, rather than blood, because no relationship has been established between PGE<sub>2</sub> concentrations and total leukocyte count in blood samples.<sup>36</sup> Even though we did not examine the total leukocyte count in blood samples, analysis of our results indicated that no correlation existed between the WBC count in synovial fluid and the PGE<sub>2</sub> concentration in synovial fluid. It has also been stated<sup>15,16</sup> that PGE<sub>2</sub> depresses proteoglycan synthesis by chondrocytes, accelerating the loss of glycosaminoglycans from articular cartilage. However, when we attempted to correlate PGE<sub>2</sub> concentrations in synovial fluid with serum glycosaminoglycan content from these same dogs, no correlation could be identified.<sup>k</sup> However, it is important to mention that we did not examine articular cartilage, and the relative lack of correlation between PGE<sub>2</sub> concentrations and serum glycosaminoglycan content in that study, compared with results for other *in vitro* studies of articular cartilage explants, is likely because our samples were obtained *in vivo* from synovial fluid and were probably not exposed to the same amounts of cytokines as those for the *in vitro* studies.

Prostaglandin E<sub>2</sub> stimulates the synthesis of degradative enzymes, such as MMPs, by chondrocytes via the promotion of plasminogen activators.<sup>13</sup> One of the primary groups of MMPs responsible for cleavage of type II collagen are the collagenases (ie, MMP-1, -8, and -13). When PGE<sub>2</sub> concentrations in the study reported here were compared with concentrations of collagenase-cleaved fragments of type I and II collagen in the same dogs,<sup>k</sup> there was a significant positive correlation ( $r, 0.6808$ ). In addition, type II collagen synthesis, as represented by the amount of carboxy-propeptide of type II collagen in the synovial fluid of these dogs,<sup>k</sup> also was significantly correlated ( $r, 0.5164$ ) with PGE<sub>2</sub> concentrations reported here. Analysis of these correlations suggested that collagenase contents are upregulated along with and perhaps as a result of PGE<sub>2</sub> concentrations, thus resulting in increased cleavage of type II collagen. As a result of this cleavage, synthesis of type II collagen likely increases to try to maintain a homeostatic balance within the extracellular matrix.

Collection of the force plate data in the study reported here was performed so that previously identified sources of variability would be minimized.<sup>21-24</sup> However, individual variation among and within each dog with regard to its response to CCL transection was difficult if not impossible to control and could have affected some of the results. For example, we had to use a rather heterogeneous population of Walker Hounds with regard to their size and movement. This required us to use a large

range of acceptable velocities (0.6 m/s) to be able to obtain a complete data set. Even though larger velocity ranges have been reported<sup>29</sup> when examining CCL-transected dogs, velocity variations of < 0.6 m/s can result in significant changes in peak vertical force and vertical impulse.<sup>23</sup> Therefore, although the subject velocity and acceptance of evaluations were carefully controlled in our study, decreasing the velocity range would probably have resulted in less variation in the data recorded. In addition, we also had other confounding factors for the determination of the subjective clinical variables in that only 1 evaluator conducted the scoring and knew the duration from time of surgery. This established consistency in the grading but added additional potential bias of our results. However, we believe that our evaluations were accurate on the basis of the significant ( $P < 0.001$ ) correlations between subjective lameness evaluation and peak vertical force ( $r, -0.7846$ ) and between subjective lameness evaluation and vertical impulse ( $r, -0.8072$ ), similar to results reported elsewhere.<sup>29</sup>

The major weakness of the study reported here was that we had to use a lavage technique to obtain sufficient synovial fluid to analyze 7 biomarkers in addition to PGE<sub>2</sub>. Although this technique has been commonly used,<sup>37</sup> it has the potential to affect the metabolism of articular tissues. In addition, the major disadvantage of the lavage technique is that it is semiquantitative because it dilutes the synovial fluid, making the actual volume of fluid within the joint unknown. It would have been ideal to correct for the dilutional factor by use of glucose or urea, as reported elsewhere,<sup>38</sup> but this was not possible in our study. We believe that these concerns were minimized as much as possible by the fact that all of the joints were treated the same for each dog at each time period. In addition, the volume of saline solution used (5 mL) and interval between collection periods (minimum of 2 weeks) minimized the affect on joint metabolism. Furthermore, we have compared concentrations of several biomarkers in synovial samples obtained by use of lavage with concentrations in synovial fluid samples obtained without use of lavage and have identified only approximately a 5- to 10-fold decrease in concentrations in the samples obtained by use of lavage while detecting the same patterns in biomarker concentrations over time (data not shown). This is similar to other results<sup>39</sup> in which investigators compared synovial fluid samples obtained with and without lavage. Nonetheless, because we used the lavage technique, there is probably more variability within our results and between the time periods than had we obtained synovial fluid without the use of lavage.

Concentrations of PGE<sub>2</sub> in synovial fluid obtained from the joints of dogs that had CCL transection were significantly increased from baseline values for 4 months after surgery, peaking acutely 14 days after transection. Correlation analyses revealed significant negative correlations between the concentration of PGE<sub>2</sub> in synovial fluid and peak vertical force and between the concentration of PGE<sub>2</sub> in synovial fluid and vertical impulse derived from force plate analysis. In addition, there were significant positive correlations of the PGE<sub>2</sub> concentrations with all clinical variables examined (eg, lameness, degree of weight bearing, joint extension, cumulative pain score, effusion score, and total protein content of synovial fluid),

except for WBC count in synovial fluid. Analysis of these results suggests that PGE<sub>2</sub> in synovial fluid probably is involved in acute inflammation and is associated with lameness and signs of pain during the early stage after CCL transection in dogs.

<sup>4</sup>Trumble TN, McIlwraith CW, Billingham RC. Technique for arthroscopically guided transection of the cranial cruciate ligament as a model for osteoarthritis (abstr). *Vet Surg* 2001;30:508.

<sup>5</sup>Amprep minicolumns, Amersham Pharmacia Biotech Ltd, Buckinghamshire, UK.

<sup>6</sup>Savant Speed Vac Plus, Thermo Savant, Holbrook, NY.

<sup>7</sup>Correlate-EIA, Assay Designs Inc, Ann Arbor, Mich.

<sup>8</sup>Dynex MRX plate reader, Dynex Technologies GmbH, Denkendorf, Germany.

<sup>9</sup>Model OR6-5, Advanced Mechanical Technologies Inc, Newton, Mass.

<sup>10</sup>IBM 486Dx, IBM Co, Boca Raton, Fla.

<sup>11</sup>Acquire, version 6.3W, Sharon Software Inc, Dewitt, Mich.

<sup>12</sup>SAS, version 8.0, SAS Institute Inc, Cary, NC.

<sup>13</sup>Trumble TN, McIlwraith CW, Billingham RC. Biomarker and arthroscopic evidence of early degradation in the canine stifle after cranial cruciate ligament transection (abstr). *Vet Surg* 2002;31:497-498.

<sup>14</sup>Trumble TN. *Early osteoarthritic changes in a canine cranial cruciate deficient model*. PhD dissertation, Department of Clinical Sciences, Colorado State University, Fort Collins, Colo, 2004.

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