Changes in joint metabolism can be measured in serum, SF, or urine by evaluation of biomarkers.1 Biomarkers have been used in several studies2–12 of clinical and experimentally induced osteoarthritis in horses to determine the effects of injury. Type II collagen and aggrecan make up most of the extracellular matrix of articular cartilage. Disruption of the extracellular matrix is the hallmark of osteoarthritis, and as a result, biomarker development has focused on identifying by-products of type II collagen and aggrecan synthesis and degradation. Because of the availability of commercial assays, type II collagen synthesis and degradation have been commonly examined in equine studies.3–6,12–14

**Objective**—To determine the effects of horse age, osteochondral injury, and joint type on a synthesis biomarker and 3 degradative biomarkers of type II collagen in Thoroughbreds.

**Animals**—Healthy rested adult (3- to 12-year-old) Thoroughbreds (n = 19), yearling (1- to 2-year-old) Thoroughbreds (40), and Thoroughbred racehorses (2 to 7 years old) undergoing arthroscopic surgery for removal of osteochondral fragments that resulted from training or racing (41).

**Procedures**—Samples of blood and metacarpophalangeal, metatarsophalangeal, or carpal joint synovial fluid (SF) were collected from all horses. Commercially available assays were used to analyze SF and serum concentrations of type II collagen biomarkers of synthesis (carboxy propeptide of type II collagen [CPII]) and degradation (cross-linked C-telopeptide fragments of type II collagen [CTX II], neoepitope generated by collagenase cleavage of type I and II collagen [C1,2C], and neoepitope generated by collagenase cleavage of type II collagen [C2C]).

**Results**—Osteochondral injury affected concentrations of CPII, CTX II, C1,2C, and C2C in SF, serum, or both, compared with concentrations in healthy adult horses. Compared with adult horses, yearling horses had increased SF or serum concentrations of degradative biomarkers (CTX II, C1,2C, and C2C). Concentrations were higher in carpal than metacarpophalangeal or metatarsophalangeal joints for all biomarkers in osteochondral-injured horses. Variable differences in SF concentrations between joint types were detected in healthy adult and yearling horses.

**Conclusions and Clinical Relevance**—Horse age, osteochondral injury, and joint type all significantly affected type II collagen biomarker concentrations in SF and serum of Thoroughbreds. (Am J Vet Res 2010;71:741–749)

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CPII</td>
<td>Carboxy propeptide of type II collagen</td>
</tr>
<tr>
<td>CTX II</td>
<td>Cross-linked C-telopeptide fragments of type II collagen</td>
</tr>
<tr>
<td>C1,2C</td>
<td>Neoepitope generated by collagenase cleavage of type I and II collagen</td>
</tr>
<tr>
<td>C2C</td>
<td>Neoepitope generated by collagenase cleavage of type II collagen</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>SF</td>
<td>Synovial fluid</td>
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</table>

However, little is known about the effects of horse age, joint injury, and joint type on concentrations of the most commonly used type II collagen biomarkers. Type II collagen metabolism can differ depending upon the structure and function of each joint as well as in response to dynamic changes throughout life, such as rapid growth, substantial changes in exercise, and injury. Several studies7,13–18 have revealed differences in biomarkers among joint types, but more global differences in type II collagen metabolism among joints...
have not been evaluated. In horses < 1 year of age, age-related differences in serum concentrations of type II collagen synthesis13,19,20 and degradative13,19,20 biomarkers have been demonstrated. However, differences in concentrations of type II collagen biomarkers in serum and SF have not been evaluated in yearling and adult horses. Studies2,3,6,21 of experimentally induced and naturally occurring osteochondral injury in the carpus have yielded variable results with regard to serum and SF concentrations of type II collagen biomarkers in response to exercise or injury. However, only 2 type II collagen biomarkers have been examined in metacarpophalangeal and metatarsophalangeal ( fetlock) joints.6,12 which are common sites of osteochondral injury in horses.23

The purpose of the study reported here was to evaluate the effects of osteochondral injury, horse age, and joint type on SF and serum concentrations of biomarkers for type II collagen synthesis and degradation in horses. The goal was to obtain a better understanding of cartilage metabolism within various groups of horses and joints to allow more accurate clinical application of these biomarkers in the future. We hypothesized there would be greater collagen synthesis in yearlings than in adults and greater degradation in osteochondral-injured joints than in noninjured joints. On the basis of earlier findings,7 we also hypothesized that biomarker concentrations would be higher in carpal versus fetlock joints.

Materials and Methods

Animals—Three groups of horses were used in the study. The first group (adult) consisted of clinically normal, rested adult horses (n = 19; 7 females and 12 castrated males), with a median age of 4 years (range, 3 to 12 years). All were free of lameness and radiographically normal in the carpal or fetlock joints. Synovial fluid was collected from 20 metacarpophalangeal joints of 6 horses in which only fetlock SF samples were obtained. It was collected from 10 middle carpal joints and 10 radiocarpal joints of 13 horses in which only carpal SF samples were obtained.

The second group (yearling) consisted of horses purchased from yearling sales in Kentucky (n = 40; 9 females and 31 sexually intact males), with a median age of 17.5 months (range, 14 to 21 months). All were free of lameness and radiographically normal in the carpal and fetlock joints. Synovial fluid was collected from the metacarpophalangeal joints and carpal joints of all horses, but 1 sample was randomly chosen from each horse for use in this study. Selected SF samples included those from 20 metacarpophalangeal joints, 10 middle carpal joints, and 10 radiocarpal joints.

The third group (osteochondral-injured) consisted of racehorses undergoing arthroscopic surgery for removal of osteochondral fragments that resulted from training or racing (n = 41; 13 females, 18 sexually intact males, and 10 castrated males), with a median age of 3 years (range, 2 to 7 years). Osteochondral fragments were removed from dorsal articular borders of the third, radiocarpal, and intermediate carpal bones; distal radius; and proximal first phalanx. Synovial fluid was collected from 21 fetlock (16 metacarpophalangeal and 5 metatarsophalangeal), 10 middle carpal, and 10 radiocarpal joints. As determined by use of radiographic and arthroscopic examination, some horses in the osteochondral-injured group had osteochondral fragments in > 1 joint. Other joints were injured in 8 of 21 horses in which fetlock SF was examined; 6 of these horses were injured in only the contralateral fetlock joint, 1 was injured in both radiocarpal joints and the contralateral fetlock, and 1 was injured in both radiocarpal and middle carpal joints. For horses in which carpal SF was examined, 9 of 20 had osteochondral injury in other joints, including 6 horses with injury in the contralateral carpus, 1 with injury in the other carpal joint in the same limb, 1 with injury in the other carpal joint in the same limb and the contralateral middle carpal and radiocarpal joints, and 1 with injury in the contralateral fetlock and middle carpal joints.

Sample collection—Serum and SF collection from the 3 groups of Thoroughbreds was approved by the University of Florida Institutional Animal Care and Use Committee. A blood sample was collected from each horse via jugular venipuncture into serum-separating tubes. The blood was allowed to clot, samples were centrifuged, and the serum was decanted. Synovial fluid was aseptically collected by use of needle arthrocentesis (without lavage) into plain collection tubes, centrifuged (12,000 × g for 10 minutes), and decanted. Both types of sample were stored at −80°C until assayed.

Analysis of collagen biomarkers—Commercially available ELISAs were used to measure type II collagen markers CPII,a C1,2C,a C2C,a and CTX II. All have been validated for use with equine serum and SF.13,23-26 All serum and SF samples were analyzed in duplicate without digestion at appropriate dilutions (none, 1:2, 1:4, or 1:8) as suggested elsewhere.13,23-26

During synthesis of type II collagen, the procollagen molecule is secreted from a chondrocyte with its carboxy- (C-) propeptide extensions still attached. The propeptides are then cleaved and released, allowing the collagen molecule to become incorporated into the fibril.27 Identification of the cleaved CPII in SF and serum has been directly associated with type II collagen synthesis.28 The CPII assay is a competitive immunoassay that involves use of bovine CPII and a rabbit polyclonal antibody specific for CPII.

Matrix metalloproteinase enzymes such as MMP-1, -8, -13, and -14 (collagenases) expose a neoepitope by cleaving the collagen triple helix at a conserved site.29 The C1,2C assay (previously known as the Col 2-3/4C short or C2C short assay) measures the C-terminus neoepitope of the three-fourths–length peptide generated by the cleavage of types I and II collagens by collagenases. The assay is a competitive immunoassay that involves use of a rabbit polyclonal C1,2C antibody. The C2C assay (also known as the Col 2-3/4Clong mono, 234 CEQ, or Col CEQ assay) measures the C-terminus neoepitope of the three-fourths–length peptide generated by the cleavage of type II collagen by collagenases. This assay is a competitive immunoassay that involves use of a monoclonal murine antibody to identify cleavage of a
proline-leucine bond in the C-telopeptide domain of type II collagen fibrils by MMP-7, -8, and -13 and membrane type 1-MMP.

Radiographic and arthroscopic scores—Radiographic and arthroscopic images of each of the osteochondral-injured horses were evaluated and assigned scores as described elsewhere for the affected joint. Briefly, standard radiographic views of the affected joint were evaluated for joint space narrowing, osteophytes (number and size), enthesophytes (number and size), osteochondral fragments (number and size), subchondral bone changes (sclerosis and lucency), and soft tissue swelling. Each radiographic variable was assigned a score from 0 to 3, with 0 indicating nondiseased and 3 indicating severe disease. The maximal radiographic score was 30. Arthroscopic video recordings of the surgeries were reviewed, and arthroscopic scores were assigned by evaluating 11 variables reflective of synovial changes, cartilage damage, and osteochondral fragments. The maximal arthroscopic score was 37. Consensus arthroscopic and radiographic scores were obtained by 2 investigators working together (TNT and MPB). These investigators were blinded to radiographic or arthroscopic appearance when evaluating the other modality.

Statistical analysis—Statistical analyses were performed with statistical software. A value of \( P < 0.05 \) was considered significant for all analyses. Data were plotted for visual assessment of normality of distribution. Analysis of box plots identified possible outlier observations, and extreme studentized deviate tests were then performed. When a value was \( > 2 \) SDs from the mean, it was considered an outlier and was eliminated from further analysis. Differences between each group and between joints within each group were evaluated by use of the nonparametric Mann-Whitney test. Serum concentrations of each biomarker for osteochondral-injured horses with multiple joint injuries were compared with those for horses with a single joint injury by use of the Mann-Whitney test. Spearman rank correlations (age; sex; limb; joint; radiographic score; arthroscopic score; serum concentrations of CPII, CTX II, C1,2C, and C2C; and SF concentrations of CPIII, CTX II, C1,2C, and C2C) were determined for the osteochondral-injured horses. Discriminant analysis was used to classify horses into the appropriate grouping (adult, yearling, or osteochondral-injured) on the basis of the serum and SF concentrations of all biomarkers combined. A quadratic discriminant function was computed from a random sampling of the horses, with equal prior probabilities used to develop a statistical model. A subset validation study was performed with a random sampling of horses that were not used in determining the function to estimate how well the model could assign each horse to its appropriate group.

A multivariate general linear model was generated for each group (adult, yearling, and osteochondral-injured) to determine factors within each group that affected serum and SF concentrations of each biomarker (CPII, CTX II, C1,2C, and C2C). For each biomarker, the model included normalized serum and SF concentrations as dependent variables. Horse, age (3 ranges for the adult and osteochondral-injured horses: \( \leq 2 \), \( > 2 \) to \( < 5 \), and \( \geq 5 \) years of age; 4 ranges for yearlings: \( \leq 12 \), \( > 12 \) to \( \leq 16 \), \( > 16 \) to \( \leq 20 \), and \( > 20 \) to \( \leq 24 \) months of age), reproductive status (intact male, female, or castrated male), limb affected (left or right), and joint evaluated (metacarpophalangeal, metatarsophalangeal, middle carpal, or radiocarpal) were analyzed as fixed effects. For the osteochondral-injured group, total radiographic and arthroscopic scores were also analyzed as covariates. The influence of fixed effects on each model was determined by calculation of the Hotelling trace coefficient.

Results

Statistical analysis revealed there was no effect of horse on SF concentrations in the adult, clinically normal group of horses. In the osteochondral-injured group of horses, there was no difference in serum concentrations of any biomarkers when comparing horses that had single- versus multiple-joint injuries. There was no difference in SF or serum concentrations of any biomarker between samples obtained from horses with osteochondral injury in the metacarpophalangeal or metatarsophalangeal joints.

CPII—The carpal joint SF concentration of CPII was significantly lower in the osteochondral-injured group than in the adult group of horses, but fetlock joint SF concentrations were not (Figure 1). Within the osteochondral-injured group, the CPII concentration was significantly lower in carpal versus fetlock joints. Synovial fluid CPII was not significantly different between carpal and fetlock joints in the adult or yearling group. There was no significant difference between SF CPII concentrations in adult and yearling horses for either type of joint (carpal vs fetlock). In osteochondral-injured horses, the SF CPII concentration in fetlock joints was positively correlated with radiographic scores (Table 1). There was no significant difference in serum CPIII concentration between horse groups (Figure 2).

CTX II—The SF concentration of CTX II was significantly higher in carpal (\( P < 0.001 \)) and fetlock (\( P = 0.001 \)) joints of the osteochondral-injured group, compared with respective concentrations in the adult group (Figure 1). Synovial fluid CTX II concentrations were also significantly higher in carpal (\( P < 0.001 \)) and fetlock (\( P = 0.002 \)) joints of the yearling group, compared with respective concentrations in the adult group. Synovial fluid CTX II concentrations were significantly higher in carpal versus fetlock joints of the osteochondral-injured group (\( P < 0.001 \)) and the yearling group (\( P = 0.003 \)).

Serum CTX II concentration was significantly lower in horses with osteochondral-injured carpal joints, compared with that in adult horses without injury (Figure 2). There was no significant (\( P = 0.580 \)) difference in values between horses with osteochondral-injured fetlock joints and adult horses. Serum CTX II concentration was approximately twice as high in the yearling group as the adult group (\( P < 0.001 \)).
carpal joints, serum CTX II concentrations were positively correlated with radiographic scores and negatively correlated with arthroscopic scores (Table 1).

C1,2C—Synovial fluid C1,2C concentration was significantly higher in the carpal (P < 0.001) and fetlock (P < 0.001) joints of the osteochondral-injured group, compared with respective concentrations in the adult group (Figure 1). The concentration was also significantly higher in yearlings than adults in both types of joints (P < 0.001 for both). Synovial fluid C1,2C concentration was significantly (P < 0.001) higher in carpal versus fetlock joints of the osteochondral-injured group, whereas in the yearling group, the opposite was true (P < 0.001).

Compared with the value in adult horses, serum C1,2C concentration was significantly higher in horses with osteochondral-injured carpal (P < 0.001) and fetlock (P = 0.005) joints (Figure 2). Serum C1,2C concentration was also significantly (P < 0.001) higher in the yearling group than in the adult group. In osteochondral-injured horses, SF C1,2C concentration in carpal joints was positively correlated with serum concentration (Table 1).

C2C—The concentration of C2C in SF was significantly higher in carpal (P = 0.002) and fetlock (P = 0.001) joints of the osteochondral-injured group, compared with respective concentrations in the adult group (Figure 1). The concentration was significantly higher in yearlings than the adult group in fetlock joints (P < 0.001) but not carpal joints (P = 0.102). Synovial fluid C2C concentration was significantly higher in carpal versus fetlock joints of the osteochondral-injured group (P < 0.001) and the adult group (P < 0.001).

When compared with the value in adult horses, serum C2C concentration was significantly (P = 0.013) lower in horses with osteochondral-injured fetlock joints (Figure 2). Serum C2C concentration in horses with osteochondral-injured carpal joints
was significantly \( (P = 0.002) \) higher than the concentration in horses with osteochondral-injured fetlock joints. In horses with osteochondral-injured carpal joints, serum C2C concentration was negatively correlated with arthroscopic score (Table 1).

Other associations—Additional effects of horse age, osteochondral injury, and joint type on biomarker concentrations were detected by use of discriminant analysis and multivariate analysis. The type of joint (fetlock or carpal) was significantly associated with serum and SF concentrations of CPII in osteochondral-injured \( (P = 0.046) \) and yearling \( (P = 0.035) \) horses, C1,2C in adult \( (P = 0.046) \) and yearling \( (P = 0.019) \) horses, and C2C in adult \( (P < 0.001) \) and osteochondral-injured \( (P = 0.008) \) horses. Horse age was a significant contributor to the serum and SF concentrations of CPII in yearling \( (P = 0.035) \) and osteochondral-injured \( (P = 0.043) \) horses.

Synovial fluid C1,2C \( (P < 0.001) \), SF CTX II \( (P < 0.001) \), serum CTX II \( (P < 0.001) \), serum C1,2C \( (P < 0.001) \), SF C2C \( (P = 0.033) \), and serum CPII \( (P = 0.043) \) concentrations all significantly contributed to the discriminant analysis model and could be used to classify samples into the correct group (adult, yearling, or osteochondral-injured) at least 8 of 10 times, with no bias toward any particular group. To develop the discriminant analysis model, 38 samples were randomly chosen, of which 95% were correctly classified (9/9 adult, 14/15 yearling, and 13/14 osteochondral-injured samples) by the model. An additional subset of samples \( (n = 14) \) that were not part of the original 38 was randomly chosen as unknowns to test the discriminant analysis model, revealing correct classification of 86% of samples (1/1 adult, 7/8 yearling, and 4/5 osteochondral-injured samples).

Figure 2—Mean ± SD concentrations of biomarkers for collagen type II synthesis (CPII) and degradation (CTX II, C1,2C, and C2C) in serum samples from adult \( (n = 19) \) and yearling \( (40) \) horses and from horses with osteochondral-injured middle carpal or radiocarpal joints (OC-injured carpus; 20) or metacarpophalangeal or metatarsophalangeal joints (OC-injured fetlock; 21). §Value differs significantly \( (P < 0.01) \) between types of affected joints within the OC-injured group. #Value differs significantly \( (P < 0.05) \) from that of the adult group. See Figure 1 for remainder of key.
Table 1—Coefficients (P values) for correlations between SF and serum concentrations of biomarkers for collagen type II synthesis (CPII) and degradation (CTX II, C1,2C, and C2C), radiographic scores, and arthroscopic scores for horses with osteochondral-injured middle carpal and radiocarpal joints (n = 20) and metacarpophalangeal and metatarsophalangeal joints (fetlock; 21).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Fluid</th>
<th>Joint</th>
<th>Radiographic score*</th>
<th>Arthroscopic score†</th>
<th>Serum</th>
</tr>
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<tbody>
<tr>
<td>CPII</td>
<td>SF</td>
<td>Carpal</td>
<td>0.192 (0.422)</td>
<td>0.407 (0.105)</td>
<td>0.307 (0.188)</td>
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<td></td>
<td></td>
<td>Fetlock</td>
<td>0.479 (0.033)</td>
<td>0.072 (0.784)</td>
<td>0.192 (0.453)</td>
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<td>Carpal</td>
<td>0.141 (0.566)</td>
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<td></td>
<td></td>
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<td>-0.014 (0.959)</td>
<td>-0.186 (0.475)</td>
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<tr>
<td>CTX II</td>
<td>SF</td>
<td>Carpal</td>
<td>0.143 (0.559)</td>
<td>0.342 (0.179)</td>
<td>-0.358 (0.121)</td>
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<td></td>
<td></td>
<td>Fetlock</td>
<td>-0.029 (0.904)</td>
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<td>Carpal</td>
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<td>-0.362 (0.154)</td>
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<tr>
<td>C1,2C</td>
<td>SF</td>
<td>Carpal</td>
<td>-0.204 (0.402)</td>
<td>-0.031 (0.906)</td>
<td>0.556 (0.013)</td>
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<td>Carpal</td>
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<tr>
<td>C2C</td>
<td>SF</td>
<td>Carpal</td>
<td>0.052 (0.832)</td>
<td>0.194 (0.555)</td>
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<td></td>
<td>Fetlock</td>
<td>-0.258 (0.318)</td>
<td>-0.622 (0.008)</td>
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</tr>
</tbody>
</table>

*Each radiographic variable was assigned a score from 0 to 3, with 0 indicating nondiseased and 3 indicating severe disease. The maximal radiographic score was 30.arthroscopic video recordings of the surgeries were reviewed, and arthroscopic scores were assigned by evaluating 11 variables reflective of synovial changes, cartilage damage, and the osteochondral fragments. The maximal arthroscopic score was 37.

A value of P < 0.05 was considered significant.

Discussion

Results of the present study indicated that biomarkers of type II collagen synthesis and degradation are all affected by horse age, osteochondral injury, and type of joint in Thoroughbreds. Biomarker response to these factors was often different in SF versus serum (Figures 1 and 2). Findings suggested that no single fluid or biomarker is best for identifying metabolic changes related to type II collagen and that age- and joint-matched horses are necessary for further type II collagen biomarker studies.

In support of our hypothesis, our data indicated that osteochondral injury to equine carpal and fetlock joints resulted in significantly more degradation of type II collagen than in clinically and radiographically normal adult joints, as inferred through CTX II, C2C, and C1,2C concentrations. The data also suggested that when a specific joint has osteochondral injury, type II collagen biomarker responses will be identified most often when SF rather than serum is used for testing. This is likely because synthesis and degradation products of collagen metabolism will be directly deposited into the SF. However, because SF cannot always be obtained, even in horses, differences between biomarker concentrations in SF and serum require further investigation, particularly given that there was little correlation between serum and SF concentrations in our study (Table 1). In addition, serum concentrations are affected by events in multiple joints and may not be the best indicator of changes when a single joint is affected.32 On the other hand, we identified no difference in serum concentrations of biomarkers in osteochondral-injured horses that had injury in multiple versus single joints.

Higher SF concentrations of type I or II collagenase cleavage products after osteochondral injury were revealed in studies of clinical8 and experimentally induced1 disease in horses. Similarly, SF concentrations of CTX II reportedly increase with injury in dogs,31 rats,34 and humans.35 A corresponding increase in type II collagen synthesis after osteochondral injury, as measured by use of CPII concentration, appears to be inconsistent. In the present study, when compared with the concentration in adult horses, the SF CPII concentration was higher in horses with osteochondral injury to the carpal but not the fetlock joint. The finding in carpal joints is similar to that in horses with experimentally created osteochondral carpal fragments3,21 but differs from that previously reported in horses with clinical osteochondral injury of carpal joints, in which there is no increase in CPII concentrations.2 To the authors’ knowledge, there are no equine studies in which the CPII response to osteochondral injury in the fetlock joints has been examined.

Comparison of our findings to those of others suggests much variability in serum type II collagen biomarker response to osteochondral injury. In the present study, the serum concentration of 1 biomarker (CPII) did not differ according to injury status, whereas the concentration of another was higher (C1,2C) and of other biomarkers was lower (CTX II and C2C) in osteochondral-injured horses, compared with clinically normal adult horses (Figure 2). Serum biomarker variability in response to injury, as identified in our study, has also been reported in the veterinary literature. For example, 1 study21 revealed no change from preinjury serum CPII concentrations in an experimental model of osteochondral injury in horses,2 whereas other studies3,4 revealed increased concentrations after injury. In studies of experimentally induced osteochondral injury of carpal joints, an increase in serum C1,2C concentration was detected after injury,2 but serum concentrations of collagenase cleavage products of only type II collagen were unchanged.24 Studies3,4 of CTX II in other species have shown increased serum CTX II concentration with injury. These differences in serum concentrations in response to injury may be because each
biomarker responds differently to various factors such as biomarker clearance from the joint, injury duration, injury severity, exercise, and treatment history.

Horse age appeared to have an effect on concentrations of biomarkers of type II collagen. Multivariate analyses revealed that age had a significant effect on CPII serum and SF concentrations in yearling and osteochondral-injured horses. When discriminant analysis was used, the biomarker concentrations allowed appropriate assignment of horses into adult, yearling, or osteochondral-injured groups at least 80% of the time. The observation that discriminant analysis could distinguish between adult and yearling horses, with age being the only recognized difference between these clinically normal horses, suggested that age plays a role in concentrations of all type II collagen biomarkers evaluated. This effect of age appeared most pronounced with CTX II and C1,2C (Figures 1 and 2).

An example of the complexity of age effect on type II collagen biomarkers can be demonstrated when CPII concentrations are examined. We hypothesized that yearlings would have higher type II collagen synthesis (CPII), than adults. However, we did not find an effect of age on CPII concentrations in SF or serum. Other studies have shown that in horses < 1 year of age, serum CPII concentrations increase with age. In a study in which tarsocrural joints of horses 9 to 23 months old were compared with those of horses 24 to 48 months old, SF CPII concentrations reportedly decreased after 2 years of age. Whereas a difference was not detected between groups of horses in the present study, multivariate analysis revealed that within the yearling group, there was a significant effect of age for CPII. This may suggest that only a few months’ difference in age in horses < 2 years old can affect biomarker concentrations. Age-related changes identified in our study emphasized that the effect of age may not be easily identifiable for all of the type II collagen biomarkers examined, but that age of the individual horse does affect serum and SF concentrations. Consequently, age-matched controls should be used in experimental studies of type II collagen biomarkers.

Biomarker variations between joint types in clinically normal horses have been reported and attributed to intrinsic biomechanical differences (ie, joint size, cartilage and bone properties, and forces transferred across the joint) and biochemical variations such as composition and gene expression of articular cartilage. Variation between joints in clinically normal horses emphasizes the need to establish reference limits for biomarker values for each joint of interest. However, in the present study, C2C was the only biomarker that differed in concentration between carpal and fetlock joints in clinically normal adult horses, demonstrating that the difference may be biomarker dependent. In yearlings, SF CTX II concentration was higher in carpal versus fetlock joints, but the reverse was true with SF C1,2C concentration. Significant differences between joints in yearling versus adult horses suggested there may be metabolic differences between these 2 joint types during development. However, for SF CPII concentrations, there was no difference between joint types in yearlings or between yearlings and adults. This lack of synthetic difference with higher degradation in yearlings than in adults was unexpected. Differences between carpal and fetlock joints were also of interest. It is possible that there is an association between biomarker values and the susceptibility of a joint to injury at a given age; however, this supposition requires further investigation. Joint differences in yearlings varied depending upon the biomarker examined. It is therefore possible that joint-dependent variation in biomarker concentrations may be attributable to different mechanisms of joint regulation; that is, one is regulated more by collagenase cleavage and another by telopeptide degradation.

Similar to findings in other studies involving horses, SF concentrations of all biomarkers measured in our study were significantly higher in horses with osteochondral injury to carpal versus fetlock joints. An explanation for this variation may be that a different mechanism of osteochondral injury exists between joints, despite similar clinical manifestations. For example, in humans with osteoarthritis of the hip joint, different biomarker concentration profiles between patients suggest differing pathogeneses between similar clinical diseases. Another logical explanation for the difference in biomarker concentrations between carpal and fetlock joints in osteochondral-injured horses would be that, in general, carpal joints may sustain greater overall injury to the joint after osteochondral injury than fetlock joints. However, this hypothesis was not supported by results of the present study because no significant correlations were detected between biomarker concentrations and radiographic and arthroscopic scores. The lack of correlations between our radiographic and arthroscopic scores and biomarker results may reflect the lack of sensitivity of radiology and arthroscopy for identification of changes specific to type II collagen. Articular cartilage changes are not well-defined radiographically. Other than observance of the osteochondral fragment, other radiographic changes may not be seen until later in the disease process. Conversely, arthroscopy is considered to be the gold standard of articular cartilage evaluation. However, the use of different scoring systems by different investigators makes comparison of study results more difficult. Our arthroscopic scoring system was designed to be used in any joint to assess the various components of injury, including cartilage damage, inflammation, and fragment characteristics. The inclusion of noncartilaginous changes in the score may reduce the likelihood of correlations with type II collagen-specific biomarkers. Perhaps a more direct assessment of the articular cartilage via a cartilage degeneration index, magnetic resonance imaging, or histologic evaluation would have shown better correlation, but these types of tests were neither practical nor feasible in our clinical patients.

Our study had limitations related to inherent variability when evaluating naturally occurring injury. Such variability existed in the interval from onset of injury to biomarker evaluation, injury to other joints, exercise, and prior treatment. The duration of injury was not consistently known for the horses in our study, so we could not delineate this potential effect on biomarker concentrations further. Several horses had osteochon-
 did not differ between horses that had osteochondral injury in multiple versus single joints. The effect of exercise on SF and serum concentrations of type II collagen biomarkers in adult horses with naturally developing osteochondral injury is unknown, but studies of biomarkers in horses ≤ 2 years of age have demonstrated that exercise can affect concentrations. However, we were unable to determine this effect in osteochondral-injured horses because exercise history just prior to surgery was unknown. Some of the study horses were likely previously treated with systemically administered NSAIDs, intra-articularly administered corticosteroids, or other drugs. The effects of these medications on biomarker concentrations in osteochondral-injured horses are unknown, but it is likely that administration of such pharmaceuticals would affect biomarker concentrations as indicated by other reports. Although the aforementioned variables may have influenced our results, they represent typical clinical conditions and therefore help to validate the clinical usefulness of biomarkers of type II collagen synthesis and degradation.

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