

Assessment of the effects of age and joint disease on hydroxyproline and glycosaminoglycan concentrations in synovial fluid from the metacarpophalangeal joint of horses

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Objective—To assess the effects of age and joint disease on hydroxyproline and glycosaminoglycan (GAG) concentrations in synovial fluid from the metacarpophalangeal joint of horses and evaluate the association of those concentrations with severity of osteoarthritis and general matrix metalloproteinase (MMP) activity.

Sample Population—Synovial fluid was collected from the metacarpophalangeal joints of foals at birth (n = 10), 5-month-old foals (10), 11-month-old foals (5), and adult horses (73).

Procedure—Hydroxyproline and GAG concentrations were determined in synovial fluid samples. The severity of osteoarthritis in adult joints was quantified by use of a cartilage degeneration index (CDI) and assessment of general MMP-activity via a fluorogenic assay.

Results—Hydroxyproline and GAG concentrations in synovial fluid were highest in neonates and decreased with age. Concentrations reached a plateau in adults by 4 years and remained constant in healthy joints. In synovial fluid from osteoarthritic joints, hydroxyproline and GAG concentrations were not increased, compared with unaffected joints, but hydroxyproline were significantly correlated with the CDI and general MMP activity. There was no significant correlation between GAG concentration and CDI value or MMP activity.

Conclusions and Clinical Relevance—Changes in hydroxyproline concentration in synovial fluid appeared to indicate damage to collagen of the articular cartilage. In joints with osteoarthritis, the lack of high GAG concentration in synovial fluid and the absence of a significant correlation between GAG concentration and CDI values or MMP activity may severely limit the usefulness of this marker for monitoring equine joint disease (*J Am Vet Med Assoc* 2004;65:296–302)

line cartilage, which consists of a limited number of chondrocytes in an abundant extracellular matrix made up mainly of collagen, proteoglycans, and water. On a dry-weight basis, collagen (which is mainly type II) composes 50% to 80% of the cartilage matrix,^{1,3} whereas glycosaminoglycans (GAGs) compose 7.3% to 10%.³ Type-II collagen, like all fibrillar collagens, is made up of a triple helix consisting of 3 α chains; the amino acid sequence of an α chain is glycine-X-Y, where X and Y are often proline or hydroxyproline.¹ Hydroxyproline composes approximately 10% of the amino acids in collagen, and because it is present only in very small quantities in other proteins, it is often used to estimate the amount of collagen in tissues.⁴ The proteoglycans consist of core proteins to which large quantities of GAGs are attached, mainly chondroitin-sulphate and keratan sulphate.¹ These GAGs contain negatively charged sulphate groups that strongly attract water. The core proteins of the proteoglycans are connected by link proteins to hyaluronic acid molecules that are attached to the collagen fibrils. This collagen fibril network interspersed with strongly hydrophilic proteoglycans ensures the unique biomechanical properties of articular cartilage, combining compressive stiffness and resilience.

In cartilage, matrix synthesis and degradation are balanced to achieve growth, remodeling, or equilibrium, depending on the age and disease status of the animal.¹ In mature rabbits and dogs, overall proteoglycan turnover time of articular cartilage is approximately 300 days.⁵ However, compared with turnover of proteoglycans, collagen turnover times in adult dogs and humans are long, having been estimated at more than 100 years.⁵⁻⁷ There are 2 conditions in which the metabolic rate of articular cartilage extracellular matrix is high: in young growing animals and in joints affected by disease, principally osteoarthritis. In equine neonates, there is a substantial and rapid increase in collagen content of the articular cartilage of the proximal phalanx between birth and 5 months and a further increase in yearlings. During these same periods, the GAG content of the cartilage decreases.^{2,8} Furthermore, differences in the collagen and GAG content of articular cartilage at various sites are detectable by 5 months, although these site differences are absent at birth.⁹ These changes in collagen and GAG content and the development of site differences in foals and young

The articular surfaces of the bones that form diarthrodial joints are covered with a layer of hya-

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horses indicate a much more rapid metabolism in articular cartilage, compared with that of adult horses. In osteoarthritis, there is a progressive loss of articular cartilage from the joint surface; as a consequence, the cartilaginous tissue increases extracellular matrix metabolism in an attempt to repair the damage. The extracellular matrix turnover that occurs physiologically in growing animals and the cartilage degradation associated with osteoarthritis are partially mediated by **matrix metalloproteinases (MMPs)**. This group of zinc-dependent enzymes is composed of stromelysins, collagenases, gelatinases, and membrane-bound MMPs; of these enzymes, collagenases (MMP-1, -8, and -13) cleave the interstitial collagen triple helix, gelatinases (MMP-2 and -9) act on unwound collagen, and stromelysins degrade proteoglycans.¹⁰ In synovial fluid from fetuses and young horses, MMP activities are much higher than those of adult horses, which suggests a role of these enzymes in the functional adaptation of articular cartilage.¹¹ Results of several studies in humans¹²⁻¹⁴ and in horses^{11,15-17} have suggested that MMPs are involved in the breakdown of articular cartilage in degenerative joint disease.

Osteoarthritis is one of the most common causes of lameness in horses and leads to the untimely retirement of many equine athletes.¹⁸ To a large extent, this outcome is a consequence of the lack of a means of diagnosing the disorder in an early phase when its development might be forestalled. The radiographic changes associated with osteoarthritis are only detected in advanced stages of disease; furthermore, those changes are poorly correlated with clinical signs.^{19,20} Marked articular cartilage degeneration may be present in a joint despite its normal radiographic appearance.²¹ This has led to a search for biomarkers that would be useful in the detection of early changes associated with joint disease. During physiologic extracellular matrix turnover (ie, growth and maturation) and pathologic degradation (ie, osteoarthritis) of articular cartilage, components of collagen molecules and proteoglycans (eg, hydroxyproline and GAGs, respectively) are released into the synovial fluid. Hydroxyproline and GAG concentrations in synovial fluid are therefore potentially useful as biological markers of osteoarthritis. In fact, it has been suggested that concentrations of GAGs in synovial fluid are useful markers for the severity of joint disease.^{22,23} To date, the usefulness of measurement of hydroxyproline concentration in synovial fluid as an indicator of joint disease has not been investigated.

The objectives of the study reported here were to assess the effects of age and joint disease on hydroxyproline and GAG concentrations in synovial fluid from the metacarpophalangeal joint of horses and evaluate the association of those concentrations with severity of osteoarthritis and general MMP activity. In addition, the potential value of hydroxyproline concentration in synovial fluid as an indicator of pathologic alterations in metabolic activity of the extracellular matrix of cartilage was evaluated.

Materials and Methods

Horses—Ten newborn foals, ten 5-month-old foals, five 11-month-old foals, and 73 adult horses were includ-

ed in the study. The foals were part of a large research project to evaluate the influence of exercise on the development of the musculoskeletal system of foals ≤ 11 months of age,²⁴ which was approved by the University of Utrecht's Ethics Committee and conducted in compliance with the Dutch Act on Animal Experiments. Specimens from adult horses were collected immediately after they were euthanized (shot and desanguinated) at an abattoir. The age of the horses was estimated by examination of the lower incisors, but no additional clinical data regarding these horses were available.

Collection of synovial fluid—After clipping, shaving, and disinfecting the skin of the metacarpophalangeal region, synovial fluid from both metacarpophalangeal joints of each foal was collected in 5-mL syringes following arthrocentesis performed in a sterile manner by use of a 40-mm, 21-gauge needle. If necessary, foals were restrained by use of a twitch or sedated with detomidine.^a In the adult horses, synovial fluid from both metacarpophalangeal joints was aspirated into five 5-mL syringes by use of a 40-mm, 18-gauge needle after disinfection of the skin; the samples were collected within half an hour of euthanasia. After collection, synovial fluid was centrifuged and aliquots were stored in plain tubes at -80°C until analysis (approx 1 year later). In addition to collection of synovial fluid specimens, the distal limbs of the 73 adult horses were isolated by disarticulation in the carpometacarpal joint and stored at -20°C (for approx 6 months) until further processing. For this study, only samples from the right forelimb (synovial fluid and distal limb) from each horse were used.

Hydroxyproline assay—Synovial fluid hydroxyproline concentration was determined by use of **high-performance liquid chromatography (HPLC)** following derivitization with a fluorescent label of secondary amino acids (proline and hydroxyproline), as described.²⁵ In short, synovial fluid (2 to 4 μL) was hydrolyzed in 1 mL of 6N HCl (for 20 hours at 110°C), dried overnight (approx 17 hours) under vacuum,^b and dissolved in 100 μL of water.^c An aliquot was transferred to an HPLC insert and placed in the autoinjector of an HPLC system consisting of a high-precision HPLC pump,^d an autosampler,^e and a spectrofluorometric detector.^f Automated derivitization consisted of blocking of primary amino acids with o-phthalaldehyde,^g followed by labeling of secondary amino acids with 9-fluorenylmethyl chloroformate. Derivitized amino acids were injected onto a reversed-phase HPLC column^h and eluted with a tertiary gradient of citrate, acetonitrile, and methanol, as described.²⁶ Fluorescence was monitored at 254 nm (excitation) and 630 nm (emission), and data were recorded on-line by a computing integration system.ⁱ The amino acid standard for collagen hydrolysates^g served as a reference. The intra-assay variation was 3%, and the interassay variation was 10%.

Glycosaminoglycan assay—Proteoglycan content of synovial fluid was estimated by measuring glycosaminoglycan concentration by use of the **1,9-dimethylmethylene blue (DMMB)** metachromatic dye assay (modified for use in microtiter plates). After papain digestion, GAGs were precipitated and stained with DMMB^j and staining was quantified via measurement of absorbance at 656 nm. Shark cartilage chondroitin sulfate^g served as the standard. Results were expressed as micrograms of GAG per milliliter of synovial fluid.

General MMP activity assay—Matrix metalloproteinase activity was determined in the synovial fluid of the adult horses by use of a slight modification of the fluorometric assay described by DeGroot et al.²⁷ Briefly, conversion of the internally quenched fluorogenic substrate TNO211-F (2.5 μM ; Dabcyl-Gaba-Pro-Gln-Gly-Leu-Cys[Fluorescein]-Ala-Lys-NH₂) was measured in the presence of an EDTA-free gen-

eral proteinase inhibitor cocktail^k to prevent conversion of the substrate by proteinases other than MMPs. Further improvement of the assay specificity for MMPs was achieved by determining the difference in substrate conversion in the presence or absence of MMP inhibitor BB94 (10 μ M). Because the substrate is not cleaved by aggrecanases, this approach detects only MMP-mediated substrate conversion and indicates the overall MMP activity in the synovial fluid samples.

Quantification of cartilage damage—In the metacarpophalangeal joints removed from the adult horses, the degree of cartilage damage (proteoglycan loss) was determined by use of a cartilage degeneration index (CDI) described by Brommer et al.²⁸ Briefly, the limbs were thawed for 2 days at 7°C, the metacarpophalangeal joints were opened, and the proximal third of the proximal phalanx was isolated by transecting the proximal phalanx with a band saw. All surrounding soft tissues were trimmed away, and the proximal articular surface of the proximal phalanx was stained with Indian ink. Digital images were obtained before and after staining, from which the mean gray pixel values were calculated by use of a validated algorithm. As Indian ink uptake is related to cartilage degradation, the increase in mean gray pixel value after staining is a measure of the relative amount of degenerated cartilage across the entire joint surface, represented as the CDI (%). These CDI values have been shown to have excellent cor-

relation with a macroscopic grading system for osteoarthritis. For the purposes of this study, CDI values < 25% were considered indicative of unaffected (ie, healthy) cartilage, whereas CDI values \geq 25% represented various degrees of osteoarthritis.

Statistical analyses—Differences in hydroxyproline and GAG concentrations between age groups were tested by use of a 1-way ANOVA. Correlations between hydroxyproline or GAG concentrations and MMP activity and CDI values were calculated by use of the Pearson correlation coefficient. Values of $P < 0.05$ were considered significant.

Results

Adult horses—Of the 73 adult horses used in the study, 13 were 1 to 4 years of age, 14 were 5 to 8 years of age, 15 were 9 to 12 years of age, 19 were 13 to 16 years of age, and 12 were > 16 years of age. The origin of the horses was unknown, and no additional clinical data were available.

Synovial fluid samples—The volume of synovial fluid (mean \pm SD) collected from the joints of the neonates, 5-month-old foals, 11-month-old foals, and adult horses was 0.5 ± 0.1 , 1.3 ± 0.3 , 2.5 ± 0.5 , and 4.6 ± 1.0 mL, respectively. Because the volume of synovial fluid collected per joint was variable, there was not always enough for all assays to be performed for each animal. Only a small amount of synovial fluid was obtained from the 11-month old foals, and because it had previously been used for other assays, there was

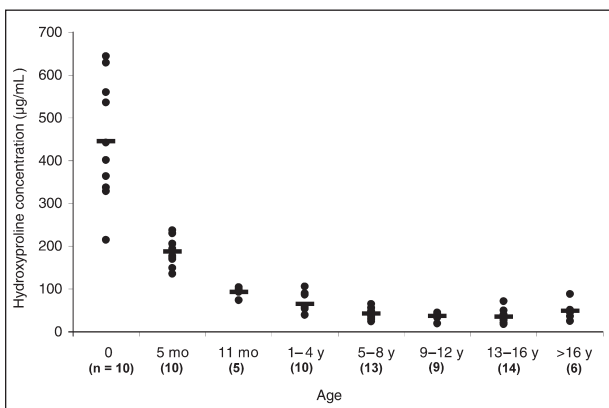


Figure 1—Hydroxyproline concentration in synovial fluid samples obtained from unaffected metacarpophalangeal joints of 77 horses grouped by age (range, 0 to 23 years). Joints were classified as unaffected (ie, healthy) on the basis of a cartilage degeneration index (CDI) assessment (CDI value, < 25%). Horizontal bar represents mean concentration of group.

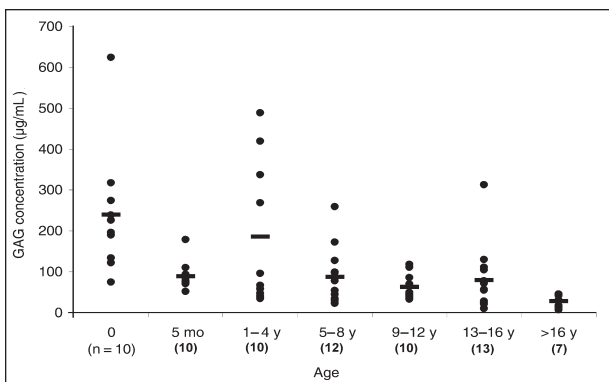


Figure 2—Glycosaminoglycan (GAG) concentration in synovial fluid samples from unaffected (CDI value, < 25%) metacarpophalangeal joints of 72 horses grouped by age (range, 0 to 23 years). Horizontal bar represents mean concentration of group.

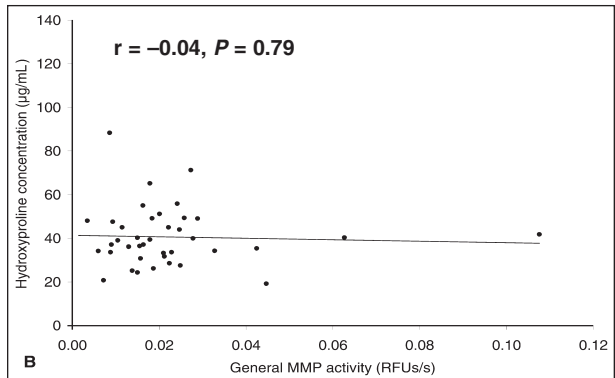
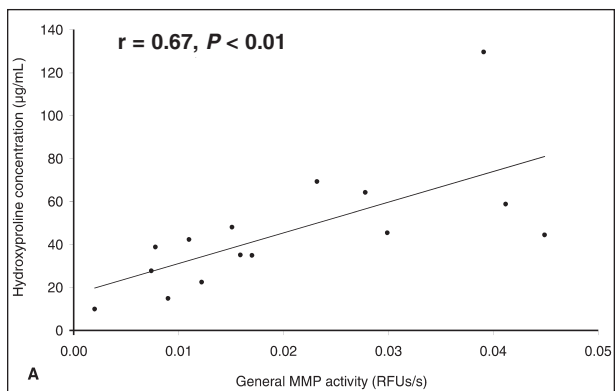


Figure 3—Hydroxyproline concentration in relation to general matrix-metalloproteinase (MMP) activity (relative fluorescence units [RFUs]/s) in synovial fluid samples from (A) osteoarthritic (CDI value, \geq 25%; $n = 15$) and (B) unaffected (CDI value, < 25%; $n = 40$) metacarpophalangeal joints in mature (> 4 years of age) horses. r = Correlation coefficient (represented by the line).

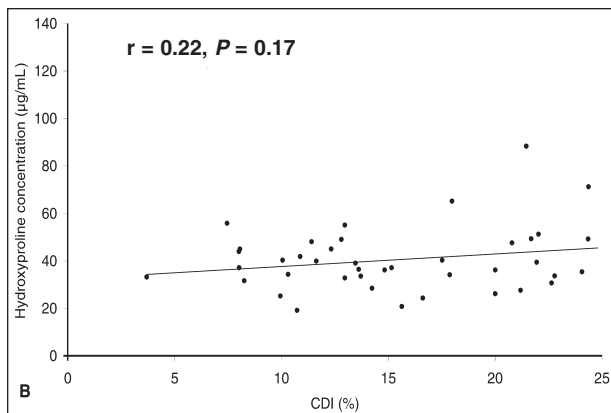
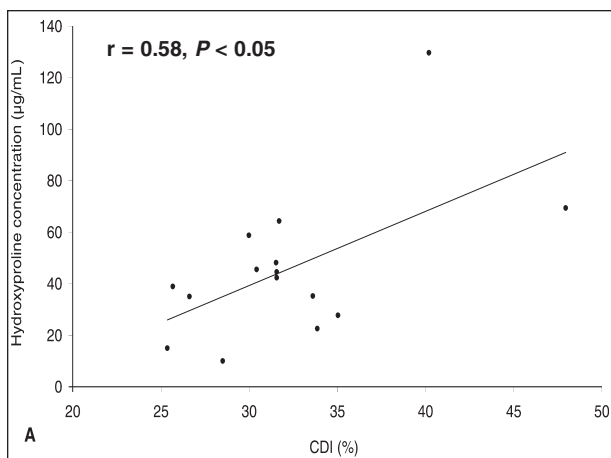


Figure 4—Hydroxyproline concentration in relation to the CDI in synovial fluid samples from (A) osteoarthritic (CDI value, $\geq 25\%$; $n = 15$) and (B) unaffected (CDI value, $< 25\%$; 41) metacarpophalangeal joints in mature (> 4 years of age) horses. See Figure 3 for remainder of key.

only sufficient volume for determination of hydroxyproline concentration (but not for the GAG assay). The hydroxyproline and GAG assays were performed on 92 (67 adults and 25 foals) and 88 (68 adults and 20 foals) samples, respectively, whereas the MMP assay was conducted on 71 adult samples.

Age effects—Of the unaffected joints, the highest hydroxyproline concentrations were detected in the synovial fluid of newly born foals (Fig 1), these being more than 11 times as great as those in mature horses ($P < 0.001$). The hydroxyproline concentrations decreased rapidly with age; the most notable decrease occurred during the first 5 months after birth. After the age of 4 years, no additional effects of ageing were observed and hydroxyproline concentrations in unaffected joints remained stable throughout the entire life span of the horses (Pearson correlation between hydroxyproline concentration and age of horses > 4 years, $r = 0.05$; $P > 0.05$). A similar pattern of change with age was detected for GAG concentrations in synovial fluid, with adult values being $<$ one-fourth of those in neonates (Fig 2).

Osteoarthritis—Of the 73 metacarpophalangeal joints examined from 73 adult horses, 56 were considered unaffected (CDI value, $< 25\%$) and 17 had osteoarthritis (CDI value, $\geq 25\%$). Hydroxyproline

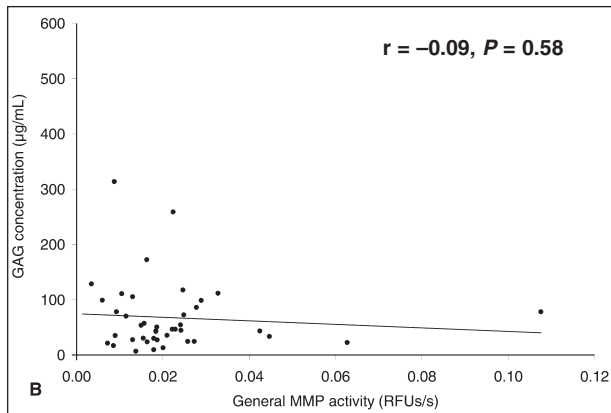
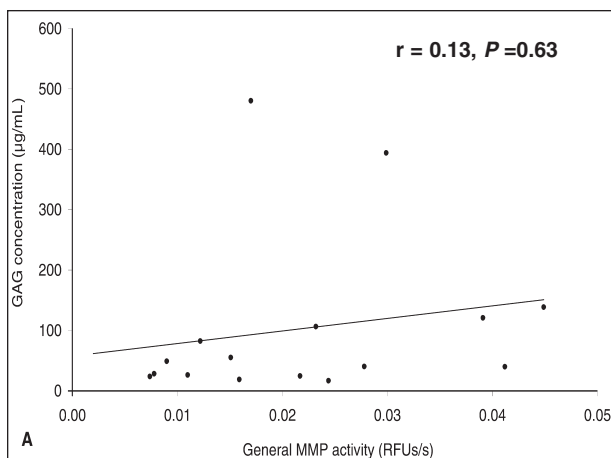


Figure 5—Glycosaminoglycan concentration in relation to general MMP activity in synovial fluid samples from (A) osteoarthritic (CDI value, $\geq 25\%$; $n = 16$) and (B) unaffected (CDI value, $< 25\%$; 41) metacarpophalangeal joints in mature (> 4 years) horses. See Figure 3 for remainder of key.

data were available for 52 unaffected and 15 osteoarthritic joints, and GAG data were available for 52 unaffected and 16 osteoarthritic joints. The mean \pm SEM age of horses from which unaffected joints and joints with osteoarthritis were obtained was 11.1 ± 0.7 and 12.8 ± 1.3 years, respectively; this age difference was not significant. The hydroxyproline concentrations in synovial fluid obtained from unaffected and osteoarthritic joints were not significantly different (41.1 ± 2.1 and 45.8 ± 7.4 $\mu\text{g/mL}$, respectively). The GAG concentrations in the synovial fluid obtained from unaffected and osteoarthritic joints were also not significantly different (84 ± 14 and 102 ± 34 $\mu\text{g/mL}$, respectively). In the group of horses with osteoarthritis (CDI value, $\geq 25\%$), there was a significant correlation ($r = 0.67$; $P < 0.01$) between hydroxyproline concentration and general MMP activity in synovial fluid (Fig 3) and a slightly weaker but significant ($r = 0.58$; $P < 0.05$) correlation between hydroxyproline content and the CDI value (Fig 4). These correlations were not present in unaffected joints from mature (> 4 years) horses (Fig 3 and 4). There were also no significant correlations between GAG concentration and MMP activity or GAG concentration and CDI value for unaffected joints or joints with osteoarthritis (Fig 5 and 6).

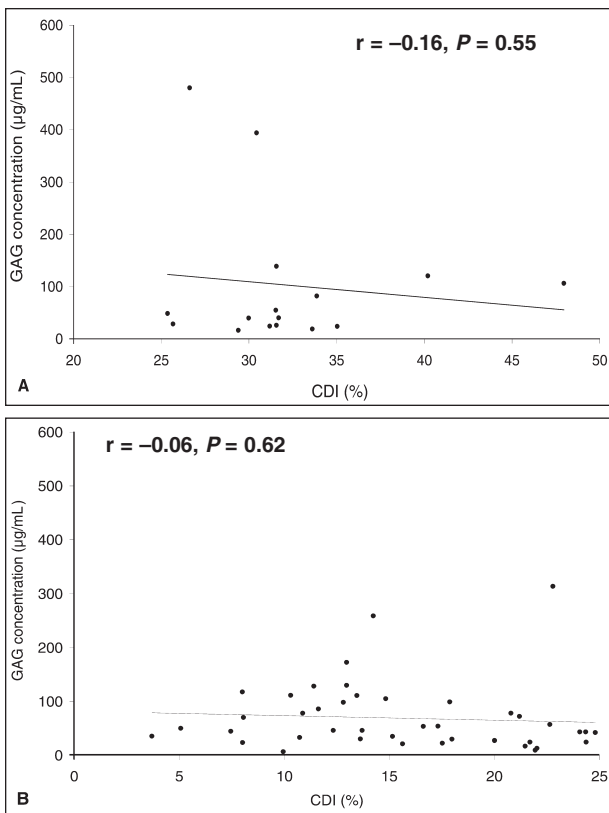


Figure 6—Glycosaminoglycan concentration in relation to CDI in synovial fluid samples from (A) osteoarthritic (CDI value, $\geq 25\%$; n = 16) and (B) unaffected (CDI value, $< 25\%$; 42) metacarpophalangeal joints in mature (> 4 years of age) horses. See Figure 3 for remainder of key.

Discussion

Results of the study reported here suggested that hydroxyproline and GAG concentrations in synovial fluid of horses are strongly related to age. The high concentrations detected in synovial fluid of equine neonates indicate the rapid turnover of the articular cartilage matrix during the first months of life. In the synovial fluid of neonates, high hydroxyproline and GAG concentrations coincided with high MMP activity, which supports the suggestion that these enzymes are involved in growth-related cartilage turnover.^{11,15}

As horses mature, the concentrations of hydroxyproline and GAG in synovial fluid decrease; at maturity (ie, > 4 years of age), these concentrations have stabilized. Hydroxyproline concentration in synovial fluid of adult horses was approximately 9% of the fetal concentration. The fact that hydroxyproline concentration was not correlated with age in adult horses is in accordance with the finding that the collagen content of articular cartilage does not significantly change with age in adult horses^{29,30}; it also corresponds with the high turnover times for collagen in mature individuals. Articular cartilage GAG concentration also remains stable in horses after maturity has been reached,³⁰ as does GAG concentration in synovial fluid from clinically normal equine joints.³¹ The lower rate of cartilage metabolism in adults probably explains much of the limited repair capability of articular cartilage and lower MMP activity detected in unaffected joints of adults,

compared with joints from juvenile horses. From our data, comparison of synovial fluid GAG and hydroxyproline concentrations in relation to age revealed that variation in GAG concentration was considerably higher than that in hydroxyproline concentration, although both putative markers had a comparable decrease in concentration with age.

As during physiologic cartilage turnover in foals, degradation products such as hydroxyproline and GAGs are released into synovial fluid during the destruction of cartilage associated with development of osteoarthritis. However, in our study, neither hydroxyproline nor GAG concentrations in synovial fluid from joints with osteoarthritis were increased, compared with concentrations measured in unaffected joints. This is believed to be a result of the lower rate of cartilage turnover in osteoarthritic joints, compared with that associated with joint remodeling during growth, and the apparent clearance of hydroxyproline and GAGs from osteoarthritic joints at a rate that prevents their accumulation to concentrations greater than those detected in unaffected joints.

Our finding regarding hydroxyproline concentration in synovial fluid is similar to that of Maldonado et al,³² who were unable to detect hydroxyproline in synovial fluid samples obtained from horses with joint disease; however, our finding regarding GAG concentration in synovial fluid conflicts with that of Alwan et al,^{22,23} who detected increased GAG concentration in synovial fluid obtained from joints with osteoarthritis, compared with concentrations in healthy joints. The definition of osteoarthritis that is used in different studies and whether inflammation was present in the joints at the moment of arthrocentesis will affect the results obtained and may (in part) explain contradictory results presented by various researchers. To classify metacarpophalangeal joints as affected or unaffected by osteoarthritis, we used an index method that has an excellent correlation with macroscopic scores of the gross extent of osteoarthritis in joints²⁸; however, most of the joints that were classified as affected had a CDI value $< 35\%$, which corresponds to mild disease that is usually not accompanied by changes that are detectable radiographically. Other investigators have mainly used radiographic criteria to assess the presence and extent of osteoarthritis, and such radiographic abnormalities are only visible in advanced stages of disease.^{19,20} Furthermore, the development of osteoarthritis is characterized as intermittent, in that the disease becomes clinically manifest as alternating periods associated with many or few signs of joint pain. It is probable that both hydroxyproline and GAG concentrations in synovial fluid vary with time, depending on the degree of inflammation associated with development of osteoarthritis. However, because of the very limited repair capability of damaged adult cartilage, neither the radiographic nor macroscopic appearance will alter notably as the inflammatory component of the osteoarthritic process subsides (albeit temporarily). Interestingly, Ratcliffe et al³³ detected high GAG concentration in synovial fluid samples in association with acute but not chronic joint disease in humans. Variable GAG release with time could explain conflicting results with respect to synovial fluid concentrations in unaffected

joints and joints with osteoarthritis. It seems probable that more GAGs would also be released into synovial fluid during active inflammation of the joint, and it would be interesting to measure GAG concentration in joints with septic arthritis, for example. In our study, the history of the adult horses used was not known; therefore, we were unable to relate our findings to clinical signs. It would appear that obtaining a single measurement of the synovial fluid hydroxyproline or GAG concentration is not a suitable determinant of the presence or extent of osteoarthritis in a joint; repeated measurements may be useful in monitoring osteoarthritis-associated inflammation, but this remains to be elucidated.

In the study of this report, the concentration of hydroxyproline in synovial fluid of osteoarthritic joints was significantly correlated with general MMP activity, whereas the concentration of GAG was not. This provides further evidence for the role of MMPs in collagen degradation in osteoarthritis and indicates that changes in synovial fluid hydroxyproline concentration may correspond with the extent of damage to collagen of the articular cartilage, which is an important aspect of osteoarthritis. The substrate (TNO211-F) employed in the general MMP assay used in our study is converted mainly by gelatinases (MMP-2 and -9), collagenase MMP-13, and, to a lesser degree, MMP-14. However, other MMPs such as MMP-1 and -3, which have a lower affinity for TNO211-F, may be major contributors to substrate turnover if their concentration is high. Substrate conversion occurs only by active MMPs (< 5% of total MMPs) and not by pro-MMPs or MMPs inhibited by tissue inhibitors of metalloproteinase. In our study, the activity of pro-MMPs could have been estimated by remeasuring MMP activity after activation of pro-MMPs by p-aminophenylmercuric acetate, but measurement of net enzyme activity was considered more appropriate for our purposes. The proteoglycans in articular cartilage are broken down not only by MMPs but also by aggrecanases, which do not cleave the substrate in the MMP-assay used in our study. This could explain the lack of correlation between GAG concentration in synovial fluid and MMP-activity because a proportion of the GAGs in synovial fluid could have been released as a result of proteolysis by aggrecanase.

In osteoarthritic joints, there was a significant correlation between hydroxyproline concentration and the severity of cartilage damage (as determined by the CDI values); such a correlation was not established for unaffected joints. A similar correlation between GAG concentration and CDI value was not detected. Some of the proteoglycans lost from damaged cartilage can be replaced, whereas the damage to the collagen network is considered permanent because of its high turnover time. It could be argued that the CDI provides a measure of proteoglycan depletion rather than collagen damage because uptake of the Indian ink used in the CDI assessment is influenced by the proteoglycan content of the cartilage^{34,35}; nevertheless, proteoglycan depletion and collagen damage can be assumed to be interrelated, with release of proteoglycans only when the collagen fibre network is disrupted.

There are a number of potential problems associ-

ated with synovial fluid sampling because various factors influence synovial fluid volume, which can cause dilution of biological markers. Evaluation of synovial fluid volume after exercise has resulted in contradictory findings; Miyaguchi et al³⁶ reported that synovial fluid volume was reduced in joints of humans after exercise, compared with pre-exercise values, whereas Otterness et al³⁷ detected less synovial fluid volume in joints of sedentary hamsters than joints of hamsters that had been exercised. Acute inflammation and most other joint injuries are associated with development of joint effusion,^{19,38} but the latter might be attenuated by medication; for example, administration of phenylbutazone and ketoprofen reduces synovial fluid volume in joints of horses with induced synovitis.^{39,40} Similarly, intra-articular administration of corticosteroids has been reported⁴¹⁻⁴⁹ to reduce synovial fluid volume in horses and cattle. In our study, we chose to investigate biomarker concentrations in synovial fluid obtained via aspiration because factors influencing synovial fluid volume are difficult (if not impossible) to control and aspiration in a clinical setting is most practical.

In conclusion, hydroxyproline and GAG concentrations in synovial fluid from growing horses were much higher than concentrations detected in samples from joints of mature horses, which corresponded with a high rate of cartilage metabolism that accompanies joint remodeling. As horses reached maturity, hydroxyproline and GAG concentrations in synovial fluid appeared to stabilize, which indicated a low level of collagen metabolism. With the development and progression of osteoarthritis, concentrations of hydroxyproline and GAGs in synovial fluid were not increased, compared with concentrations in unaffected joints, probably because of the intermittent character of the release of these substances, which makes single measurements insufficient to accurately assess the degree of pathologic changes within a joint. The relationship that was found between the extent of cartilage degeneration (as quantified by the CDI value and MMP activity) and synovial fluid hydroxyproline concentration suggests that the latter may be an indicator of damage to collagen in articular cartilage (an important aspect of osteoarthritis), but this remains to be thoroughly investigated.

^aDomosedan, Pfizer Animal Health, Capelle aan de IJssel, The Netherlands.

^bSpeedvac, Thermo Savant, Holbrook, NY.

^cMilli Q, Millipore Corp, Billerica, Mass.

^dModel 480, Gynkotec, Munich, Germany.

^eSparks Holland Triathlon, Spark Holland, Emmen, The Netherlands.

^fModel 821 FP, Jasco, Japan.

^gSigma Chemical Co, St Louis, Mo.

^hTSK ODS 80, 4.6 × 150 mm, Tosoh Biosep, Tosoh Corp, Tokyo, Japan.

ⁱChromleon, version 4.30, Dionex, Sunnyvale, Calif.

^jBlyscan kit, Biocolor Ltd, Belfast, Northern Ireland.

^kComplete, Roche Diagnostics, Almere, The Netherlands.

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