# Effect of arthroscopic surgery and post-surgical triamcinolone acetonide administration on synovial fluid, serum, urine and kinetic biomarkers in an equine metacarpophalangeal osteochondral injury model

A thesis submitted to the faculty of the Graduate School of the University of Minnesota

By

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# Abstract

**Objective-** The purpose of this pilot study was to investigate the effects of arthroscopic surgery and post-surgical administration of triamcinolone acetonide (TA) on biomarkers and lameness.

**Animals**- Seven adult Quarter Horses had an osteochondral (OC) fragment arthroscopically created on the first phalanx of one metacarpophalangeal joint (MCPJ).

**Procedures-** Lameness exams, force plate data, SF from both MCPJs, serum, and urine, were collected on weeks 0 (fragment creation), 16 (fragment removal), 17 (1 week post removal), 18 (1 week post injection, 2 weeks post removal) and 20 (3 weeks post injection, 4 weeks post removal). After week 17 fluid collection, horses were randomly divided into 2 intra-articular treatment groups: 10 mg TA (n=4) or 1 ml saline (n=3). Biomarkers of joint metabolism (BAP, CP II, C1,2C, C2C, CTX II, NO, and HMGB1) were evaluated in SF, serum, and/or urine using ELISAs. Repeated measures ANOVAs were used for analysis, significant at $P < 0.05$.

**Results-** OC fragment creation resulted in collagen degradation as seen by SF increases of CTX II and C2C at week 16 from week 0 ($P=0.048$ and $P<0.001$). After TA administration, SF C2C concentrations increased, but CTX II concentrations decreased compared to saline controls ($P<0.001$). Bone production (SF BAP concentrations) in TA treated horses increased compared to saline controls at week 20 ($P=0.028$). All horses became more lame at week 17 according to trained observers ($P=0.003$) and force plate analysis ($P=0.007$), but by week 20, lameness had returned to pre-OC fragment removal (week 16) levels. There were no significant differences in SF, serum, urine and inflammatory biomarkers.

**Conclusions and Clinical Relevance-** Arthroscopic surgery appears to have no effect on cartilage metabolism with little resultant SF inflammation. TA administration is not

indicated after surgery for removal of OC fragments with superficial cartilage damage since collagen degradation increases compared to controls.

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# List of Abbreviations

BAP - Bone alkaline phosphatase

C1,2C - Carboxy-terminal neoepitope after collagenase cleavage of type I and II collagen

C2C - Carboxy-terminal neoepitope after collagenase cleavage of type II collagen

CP II - Procollagen C-propeptide of type II collagen

CTX II - Carboxy-terminal telopeptide of type II collagen

GAGs – Glycosaminoglycans

HA- Hyaluronan

HMGB1 - High mobility group box 1 protein

IL-1- Interleukin 1

MCPJ - Metacarpophalangeal joint

MMP- Matrix metalloproteinase

MPA- Methylprednisolone acetate

NO - Nitric oxide

OA – Osteoarthritis

PGE2- Prostaglandin E2

SCB – Subchondral bone

SF - Synovial fluid

TA - Triamcinolone acetonide

TNF $\alpha$- Tumor necrosis factor alpha

uCTXII - Urinary CTXII

# CHAPTER I

# INTRODUCTION AND LITERATURE REVIEW

Many performance horses sustain osteochondral (OC) injury to one or more joints during training and competition. If OC fragments are not removed, horses may develop osteoarthritis (OA) secondary to the injury. OA has been documented within a few months of the initial OC injury in both equine clinical cases[1-4] and in equine models of OC fragmentation in the carpal[5] and metacarpophalangeal joint (MCPJ).[6]

Arthroscopic removal of OC fragments has become the standard of care to maintain optimum joint function and comfort. Arthroscopy is minimally invasive surgery. As such, using rigid arthroscopes through small incisions causes minimal systemic inflammation (serum amyloid A and fibrinogen) compared to more invasive surgeries such as an open approach surgery for ovarian tumor removal.[7] In addition, arthoscopy has been shown to be advantageous over arthrotomy for joint surgery because of shorter recovery time and better prognosis for soundness.[8] Horses that had arthroscopic removal of naturally-acquired dorsal first phalanx OC fragments returned to a pre-OC fragment level of work 73.6% to 82% of the time.[2,9] In humans, removal of OC fragments has also yielded satisfactory results.[10,11] Although arthroscopic surgery to remove OC fragments gives good clinical outcomes, there is a little information regarding the effects of arthroscopy or post-surgical intra-articular medications on the joint environment.

*Components of a synovial joint*

A normal synovial joint has many components, including synovial membrane, articular cartilage, bone, and ligaments that work together to distribute mechanical forces to allow pain free movement.[12] However, because of its poor capability for repair, articular cartilage is arguably the most critical component of the joint.

Synovial membrane is composed of the intimal and subintimal layers. The intimal layer regulates the content of the synovial fluid, while the subintimal layer provides blood supply and innervation.[12] The intimal layer has type A phagocytic synoviocytes, type B secretory synoviocytes, as well as type C synoviocytes that perform both functions. Type B cells secrete hyaluronan (HA), collagen, lubricin, interleukins (ILs) and matrix

metalloproteinases (MMPs) into the SF.[12] HA serves as the framework to which proteoglycans can attach via a link protein.[12]

Articular cartilage is composed of small number of chondrocytes that produce a surrounding extracellular matrix comprised of primarily of type II collagen, with lesser amounts of type IX and XI collagen, proteoglycans, glycoproteins, and water.[12] Proteoglycans (aggrecan being the most common) are interspersed amongst collagen in the extracellular matrix to attract and hold water within the matrix. Proteoglycans have negatively charged glycosaminoglycan side chains (found on the core protein) that attract water between and within both proteoglycans and collagen.[12] When water is present, collagen is stretched under tension allowing the cartilage to deform but maintain stability.

The subchondral bone (SCB) underlies the articular cartilage, giving shape and stability to the overlying articular cartilage.[12] Remodeling and sclerosis of the SCB are changes associated with advanced osteoarthritis, and SCB is exposed to the synovial fluid when an intra-articular microfracture occurs.[13]

*Joint function and osteoarthritis*

In the superficial layer of articular cartilage, type II collagen is organized parallel to the joint surface to help counteract tensile and sheer stresses, but becomes organized in a more perpendicular manner in the deepest layers.[12] This collagen structure, along with interspersed proteoglycans, help provide the main function of cartilage which is to resist compressive forces. However, once the superficial layer of articular cartilage becomes damaged, it is much easier for damage to progress down to SCB due to the collagen alignment.[13]

Acute joint injury, such as occurs with OC fragmentation, can lead to synovial membrane inflammation.[13] Inflamed synovium releases MMPs and pro-inflammatory cytokines (such as IL-1 and TNF-$\alpha$) into the synovial fluid.[14] These inflammatory mediators can cause joint effusion and degradation of type II collagen.[14] If these molecules are not regulated, collagen degeneration will continue. This can lead to less collagen tension and release of water when loading that ultimately decreases the ability of

the joint to counteract compressive loads potentiating progressive damage to deeper layers of cartilage until SCB is exposed.[13,14]

*Osteochondral fragment models*

OC injury creates an incongruent surface that often leads to cartilage damage on the opposing cartilaginous surface, leading to OA.[15] As such, horse models for induced osteoarthritis have been created by causing OC injury in the carpal or metacarpophalangeal joints (MCPJ).[6,16] The carpal model has been well established and has been used to test the effect of various medications on the development of OA.[5,16,17] However, this carpal model results in advanced OA requiring euthanasia at the end of the study for humane reasons. In our opinion, the severity of the carpal OA model rendered it inappropriate to investigate the effects of arthroscopic removal of OC fragments and post-operative administration of intra-articular medications on horses with mild OA. A novel non-terminal model of MCPJ OA has been recently described.[6] Horses in this model have clinical, morphologic, and histological evidence of early OA that is less severe than the carpal OC model. Using this MCPJ model of OC injury, fragments can be removed via arthroscopy, as would occur in a clinical setting. This allows the horses to survive, enabling evaluation of surgery and post-operative treatment.

*Post-operative intra-articular joint medications*

Understanding the joint environment post-operatively is vital to case management. Many surgeons treat the joint for relief of pain and restoration of joint health with various medications post-operatively. Hyaluronan (HA), methylprednisolone acetate (MPA), triamcinolone acetonide (TA), bupivacaine, ketamine, and morphine have been administered post-operatively in humans.[18-22] HA, corticosteroids, and morphine are among the most common post-operative drugs administered to horses.[23] Although there are many in vitro and in vivo studies on the effects of these drugs on the extracellular matrix of cartilage,[24-28] and as treatments for OA and OC fragments, [5,17,29-32] few drugs have been examined in horses post arthroscopy. During arthroscopy only the macroscopic health of the joint can be determined, but the biochemical balance between anabolism and

catabolism is unknown immediately after surgery. Therefore, the effect of these medications on joint metabolism is not well understood. They may promote healing of the joint, they may be completely unnecessary, or, at worst, be detrimental to joint healing.

*Corticosteroids mechanism of action and intra-articular use in humans post-arthroscopy*

The two main corticosteroids used intra-articularly are MPA, a longer acting corticosteroid, and TA, an intermediate acting corticosteroid. These drugs may be used alone or in combination with HA. Corticosteroids work by binding to a cell cytoplasmic receptor and forming a complex that affects the transcription of mRNA of certain genes. mRNA translation results in the production of lipocortins, proteins which inhibit phospholipase $A_2$. These lipocortins prevent mobilization of arachadonic acid from the membrane phospholipid, stopping the cyclooxygenase and lipoxygenase pathways of inflammation.[33] In humans, MPA and HA administered at the end of knee arthroscopy decreased inflammation scores in the synovium.[26] By decreasing inflammation after surgery, it is presumed that there will be a secondary decrease in joint effusion. This was supported in a human study where MPA was administered at the end of arthroscopic knee surgery and a trend towards less joint effusion was demonstrated.[34] By decreasing effusion, pain should ultimately decrease because of less stimulation of distention receptors in the joint capsule. This decrease in pain was demonstrated by Wang et al.[35] where 10 mg TA administered at end of arthroscopic knee surgery decreased pain from 6 to 24 hrs post-operatively.

*Corticosteroids intra-articular use in OA*

Corticosteroids have long been used in horses and humans to alleviate clinical lameness even though they have been shown to have both positive and negative effects on cartilage metabolism. For example, steroids can decrease inflammation via inhibition of various MMPs and prostaglandins,[5,26,33] and result in clinical improvement in lameness.[29,31] However they can also result in increased stromelysin-induced cleavage of proteoglycan leading to increased GAGs in the SF,[29,36] and may cause decreased type II

collagen synthesis.[37] These varied effects are likely dependent upon the steroid chosen, the clinical situation, and the dose used.

MPA mostly decreases synovial membrane inflammation but reportedly has more deleterious effects on cartilage. Using the carpal model of OA, MPA resulted in decreased synovial membrane inflammation, but greater cartilage erosion than controls.[5] This decrease in inflammation was also seen with intra-articular administration of MPA in an equine synovitis model.[38] In this study, no deleterious effects of MPA on collagen and protein synthesis were seen in the inflamed joints.[38] In contrast, synovial membrane inflammation was found to be initially increased in another clinical study, while cartilage repair was inhibited.[39] In an ex vivo experiment where equine cartilage was exposed to IL-1 and then incubated with MPA, GAG synthesis levels were decreased further after the addition of the corticosteroid.[28]

TA has been reported to have more protective effects on cartilage than MPA, with positive effects on lameness.[24,29] In an ex vivo study, TA and HA decreased the negative effects of inflammation on cartilage after exposure to IL-1.[24] This is in contrast with another ex vivo study that found TA, regardless of dose (clinical ranges used), resulted in decreased GAG synthesis.[28] When TA was examined in the carpal OC fragment model,[29] lameness decreased, HA concentrations were higher, but there was also a higher amount of free GAGs within the SF. Similar to the results of MPA in this model,[5] the synovial membrane had less inflammation compared to controls, with some evidence of decreased inflammation in other joints that were distant from the injection site. Horses treated intra-articularly with TA at 14 and 28 days post carpal OC fragment creation demonstrated no altered bone remodeling or increased carpal bone fragility (microcracking).[17]

Even though TA has been reported to be protective of articular cartilage, the literature reviewed above demonstrates that TA has mixed effects on joint metabolism. This depends upon the environment at the time of administration (degree of inflammation), while consistently demonstrating pain relieving effects (decreased lameness). Nonetheless, TA is generally perceived by equine practitioners to be one of the best therapeutic options for pain relief in high motion joints with OA, and for these

reasons, was selected for this pilot study. Using horses in a non-terminal MCPJ model of OA will allow us to see the effects of TA on joint metabolism post-arthroscopy.

*Biomarkers of joint metabolism*

A biomarker is a natural by-product of joint metabolism, be it a protein, enzyme, epitope, or cytokine, which is measureable in the synovial fluid, urine, or blood using ELISAs or other technology. These biomarkers are associated with specific aspects of the joint, such as synovial membrane (for inflammation), cartilage or bone. Many of the by-products released into the SF and subsequently into the systemic circulation can be related to specific aspects of the development or degradation of the main components of the cartilage extracellular matrix. For example, the C2C ELISA identifies the C-terminal neoepitope after collagenase cleavage of type II collagen. This epitope can only be identified after cleavage of the collagen triple helix by MMPs 1, 8, and 13 and is therefore a good example of a degradative biomarker of type II collagen (Figure 1).[40] On the other hand, the CP II ELISA identifies the C-propeptide of type II procollagen triple helix. After release from the nucleus, the N- and C- terminal propeptides are cleaved off the procollagen molecule allowing the newly formed triple helix to incorporate into a collagen fibril. The C-terminal propeptide is released into the SF and is thus indicative of recent type II collagen production making it a good biomarker of synthesis (Figure 1).[41] Identification and quantification of these specific degradative or synthetic biomarkers of the components of cartilage help identify what types of pathways are engaged after joint interventions such as injury, exercise, or therapeutic administration[3,4,6,42].

Synovial fluid is considered the ideal fluid for assessing biomarker concentrations because it is most representative of the local joint environment. SF will collect most of the cartilage debris that is produced in a joint since there is no blood supply within articular cartilage. In addition, there should be less metabolism of the epitope of interest in SF compared to the systemic circulation. Systemic fluids (serum and urine) are still important to evaluate, although they are likely of less benefit when examining changes in one joint since they get contributions from all other joints in the body. In addition, obtaining serum and urine (in horses and humans) is less invasive than obtaining SF and

would be ideal for future clinical and research use if they can be shown to be representative of the local joint environment.

*Overview of chosen biomarkers*

*Biomarkers of inflammation*

Biomarkers in this study were chosen for their ability to indicate joint inflammation, degradation and synthesis of collagen, and bone production (Table 1) and could be identified in the SF, serum, and/or urine from horses that sustained OC injury. SF was collected from joints in which OC fragments were created and removed to determine the effect of the fragment, arthroscopy and post-operative therapy on biomarker concentrations. In addition, SF was collected from sham operated joints in an attempt to distinguish what biomarker concentrations are truly attributable to our interventions of either surgery or TA administration versus being the result of synovial membrane and articular cartilage biopsies. Sham joint biomarker concentrations can also help assess possible systemic effects of TA administration, since there have been effects reported on collagen biomarkers in distant, non-treated joints.[43]

Joint inflammation is one of the first steps toward development of joint effusion and cartilage damage that can ultimately lead to development of pain and OA. Joint inflammation has been recently assessed in the horse using high mobility group box- 1 protein (HMGB-1). HMGB-1 is a protein that normally resides within cells, but can become extracellular and act as a proinflammatory cytokine that can activate the receptor for advanced glycation end products as well as nuclear factor κβ.[42] In horses, HMGB-1 was found in the synovial membrane of diseased joints only, and near OC fragments with fibrous tissue repair.[44] HMGB-1 concentrations have also been shown to be increased in synovial fluid in Thoroughbreds with OC injury in MCP and carpal joints.[45]

Joint inflammation has also been measured by analyzing nitric oxide (NO) concentrations. NO is a highly reactive cytotoxic free radical by-product formed from L-arginine after it is acted on by NO synthase. NO is thought to play a role in OA by activating MMPs and decreasing proteoglycan and collagen synthesis.[46-49] In the horse, NO end products were seen to be higher in SF from MCPJs affected by OA.[50]

Therapeutic intervention has been shown to have variable effects on chondrocyte NO gene expression. Dexamethasone and polysulfated glycosaminoglycans decrease NO gene expression, whereas HA and phenylbutazone have no consistent effects on NO gene expression.[46]

*Biomarkers of cartilage degradation and synthesis*

Since type II collagen has a limited capacity for repair, OA is thought to commence when type II collagen degradation occurs without appropriate collagen synthesis.[51] C1,2C and C2C have been well established biomarkers of collagen degradation,[4,40,51-53] with CTX II being a more recently studied collagen degradation biomarker in humans and the horse[3,6,51,52] Conversely, CP II is the only biomarker for type II collagen synthesis that has shown reactivity in the horse.[53] In horses, C2C[52] and CTX II[3] SF levels in MCPJs increased with OC injury. However, CTX II concentrations in the serum were lower in OC injured horses.[3] In the carpal OC injury model, OA affected joints showed increases in SF concentrations of collagen synthesis (CP II), cartilage type II degradation, and inflammatory biomarkers.[53]

Ibuprofen administered to patients with knee OA caused decreased CTX II concentrations in the urine.[54] CTX II urine concentrations were also shown to be decreased in humans with OA treated with Tibolone (a synthetic steroid hormone).[55] Urinary CTX II (uCTX II) concentrations were predictive of clinical response after intra-articular administration of HA in humans with knee OA.[56] Studies describing the effects of intra-articular steroid treatment on biomarker levels post OC fragment removal in horses are lacking. Repeated injections of MPA into OA affected radiocarpal joints were associated with increases in cartilage degradation products and with significant decreases in CP II.[57] Repeated TA administration into one radiocarpal joint of healthy horses increased biomarker concentrations of C1,2C, CP II and others of aggrecan degradation and synthesis (keratan sulfate and CS 846) in the injected joint. C1,2C and CP II also increased in a control joint, suggesting systemic effects of TA administration.[43]

*Biomarkers of bone production*

BAP, a biomarker of bone production, is formed by osteoblasts in large amounts[58] and is believed to help with calcification.[59] BAP ratio in the SF to the serum of ≥ 0.5 was predictive of OC injury in horses.[60] Increased BAP SF concentrations within the joint may be indicative of bone production associated with osteophytosis or osteoarthritic changes.[60,61] The C1,2C assay measures both type I and II collagens since the short amino acid sequence identified is shared between both collagens.[40] Therefore, it may also help quantify bone breakdown since type I collagen is located primarily within bone and fibrous tissue.

*Influences on biomarkers concentrations*

Age, time of day, season, metabolism of the amino acid sequence of interest, systemic contributions (from multiple joints, spine and respiratory tract), and therapeutics can all play a part in affecting biomarker concentrations.[51,52,62] Younger horses and samples taken earlier in the day tend to be higher in concentration than that found in older horses and samples obtained later in the day. [51,52,63] Bone biomarkers tend to be higher in concentration in winter.[63] There are unknown effects of metabolism on biomarker concentrations, with systemic contributions being animal and disease dependant.[51] In addition, any deleterious effects of surgery itself may be masked by the routine use of systemic anti-inflammatories, such as oral phenylbutazone,[64] and intra-articular morphine.[23] These medications decrease joint inflammation by inhibition of the cyclooxygenase pathway for prostaglandin production[65] which may reduce cartilage destruction.

*Lameness evaluation*

In this study, clinical lameness grades and objective lameness data were used to evaluate treatment response.

Clinical lameness examinations are primarily subjective in practice and research. The American Association of Equine Practitioners grading scheme is used for clinical exams, with 0 indicating a sound horse, and 5 indicating a horse that is non-weight bearing lame on a limb.[66] This grading system has issues in that agreement among

observers can be as low as 56%,[67] part of which can be due to experience of the observer.[68] However, it remains the most common way in which equine clinicians report lameness.

Objective lameness information can be gathered using force plate gait analysis of ground reaction forces (kinetics) at the trot. Forces can be measured in each limb over time in the vertical (up and down), horizontal (side-to-side), and longitudinal (braking and propulsion) directions. The peak vertical force indicates the maximal load a limb takes when striking the ground in the stance phase and provides the highest sensitivity and specificity in detecting lameness.[69] In a study of induced synovitis in the MCPJ of 32 horses, significant decreases in peak vertical force were associated with subjective lameness, with the objective measure seen as most reliable.[69]

*Objective/Hypotheses*

The purpose of this study was to investigate the effects of arthroscopic surgery to remove an OC fragment and post-surgical intra-articular administration of TA on joint metabolism and lameness. The central hypothesis was that joint inflammation, as well as collagen synthesis, collagen degradation, and bone production biomarker concentrations would increase in SF, serum, and urine after arthroscopic removal of OC fragments. After TA administration, biomarkers would reflect less inflammation but greater articular cartilage degradation than in horses treated with only saline after surgery. In addition, it was hypothesized that lameness (subjective and objective) would improve more rapidly within the TA treated group than the saline treated group.

# CHAPTER II

# Effect of arthroscopic surgery and post-surgical triamcinolone acetonide administration on synovial fluid, serum, urine and kinetic biomarkers in an equine metacarpophalangeal osteochondral injury model

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**Objective-** The purpose of this pilot study was to investigate the effects of arthroscopic surgery and post-surgical administration of triamcinolone acetonide (TA) on biomarkers and lameness.

**Animals-** Seven adult Quarter Horses had an osteochondral (OC) fragment arthroscopically created on the first phalanx of one metacarpophalangeal joint (MCPJ).

**Procedures-** Lameness exams, force plate data, SF from both MCPJs, serum, and urine, were collected on weeks 0 (fragment creation), 16 (fragment removal), 17 (1 week post removal), 18 (1 week post injection, 2 weeks post removal) and 20 (3 weeks post injection, 4 weeks post removal). After week 17 fluid collection, horses were randomly divided into 2 intra-articular treatment groups: 10 mg TA (n=4) or 1 ml saline (n=3). Biomarkers of joint metabolism (BAP, CP II, C1,2C, C2C, CTX II, NO, and HMGB1) were evaluated in SF, serum, and/or urine using ELISAs. Repeated measures ANOVAs were used for analysis, significant at $P < 0.05$.

**Results-** OC fragment creation resulted in collagen degradation as seen by SF increases of CTX II and C2C at week 16 from week 0 ($P=0.048$ and $P<0.001$). After TA administration, SF C2C concentrations increased, but CTX II concentrations decreased compared to saline controls ($P<0.001$). Bone production (SF BAP concentrations) in TA treated horses increased compared to saline controls at week 20 ($P=0.028$). All horses became more lame at week 17 according to trained observers ($P=0.003$) and force plate analysis ($P=0.007$), but by week 20, lameness had returned to pre-OC fragment removal (week 16) levels. There were no significant differences in SF, serum, urine and inflammatory biomarkers.

**Conclusions and Clinical Relevance-** Arthroscopic surgery appears to have no effect on cartilage metabolism with little resultant SF inflammation. TA administration is not indicated after surgery for removal of OC fragments with superficial cartilage damage since collagen degradation increases compared to controls.

Many performance horses sustain osteochondral (OC) injury to one or more joints during training and competition. If OC fragments are not removed, horses may develop osteoarthritis (OA) secondary to the injury. OA has been documented within a few months of the initial OC injury in both equine clinical cases[1-4] and in equine models of OC fragmentation in the carpal[5] and metacarpophalangeal joint (MCPJ).[6] Arthroscopic removal of OC fragments has become the standard of care to maintain optimum joint function and comfort. Horses that had arthroscopic removal of naturally-acquired dorsal first phalanx OC fragments returned to a pre-OC fragment level of work 73.6% to 82% of the time.[2,9]

In human medicine, anti-inflammatory treatments, such as corticosteroids or hyaluronan (HA) are often administered intra-articularly by surgeons after arthroscopic surgery in an attempt to minimize pain and/or inflammation.[26,34,35] This practice is commonplace in veterinary medicine as well. One of the most commonly used treatments for joint inflammation and/or OA in horses is intra-articular administration of a corticosteroid such as triamcinolone acetonide (TA). TA has demonstrated beneficial effects on lameness outcomes[16,28] even though it can have negative effects on cartilage, such as cartilage erosion, decreased cartilage repair, and chondrocyte death.[27-29] However, some studies have shown that TA has less negative effects on cartilage metabolism and bone than other steroids.[17,24,27] Therefore, TA is often used for injection of high motion joints with OA and for post-operative relief of inflammation and pain. Little is known about the inflammatory status of the joint, as well as collagen, and bone metabolism in the joint after surgery or how anti-inflammatory drugs affect this metabolism on a local and systemic level.

Biomarkers of joint metabolism, may identify changes that occur to the synovium, cartilage and bone after joint injury that can potentially enable early diagnosis, identify therapeutic targets,[51] and allow assessment of these therapies.[43,70] Metabolic responses of the joint to naturally-occurring and experimentally-induced OC injury in horses have been well studied using biomarkers identified in the serum, urine, and synovial fluid.[3,4,45,51-53,60,61,71] Collectively, these studies have demonstrated that after OC injury horses show significant differences in inflammation (HMGB-1, NO), collagen type II

degradation (C1,2C, C2C, CTX II), collagen type II synthesis (CP II), and bone turnover (BAP), compared to controls. However, these studies have evaluated concentrations of these joint biomarkers of metabolism longitudinally after creation or natural occurrence of an OC injury, not after the fragment has been removed. Therefore, there is a gap in knowledge about joint metabolism after surgery to remove an OC fragment and what effects post-arthroscopic intra-articular treatment may have on that joint.

The purpose of this pilot study was to investigate the effects of arthroscopic removal of an OC fragment and post-surgical intra-articular administration of TA has on the joint. A novel non-terminal equine model of OC injury in the metacarpophalangeal joint (MCPJ)[6] was used to examine the clinical response (lameness) and biomarker concentrations in the SF, serum, and urine after surgical removal of the OC fragment and follow-up TA treatment. The central hypothesis was that joint biomarkers of inflammation, collagen synthesis and degradation, and bone production would increase in SF, serum, and urine after arthroscopic removal of OC fragments. After TA administration, biomarkers would reflect less inflammation but greater articular cartilage degradation than joints treated with only saline after surgery. In addition, it was hypothesized that lameness (subjective and objective) would improve more rapidly within the TA treated group compared to the saline treated group.

*Materials and methods*

*Horses*

Seven adult Quarter Horses (age range 4-9 years, median 5 years; 3 mares, 4 geldings) were used in this study. All study procedures were approved by the University's Institutional Animal Care and Use Committee.

*Data collection*

At all time points (weeks 0, 16, 17, 18 and 20), subjective lameness analysis, force plate data collection, as well as SF from both MCPJs, serum and urine were collected before any interventions (surgery or intra-articular medications) (Figure 2). For a brief overview, at week 0 an OC fragment was created in one MCPJ.[6] This fragment

was subsequently removed arthroscopically at week 16. After fluid collection at week 17, horses were randomly divided into 2 groups where either 10 mg of TA (4 horses) or 1 ml of saline (3 horses) was administered into the injured MCPJ.

*Osteochondral fragment osteoarthritis model*

All horses had been part of a non-terminal OC fragment OA model.[6] Briefly, at week 0, horses had a dorsomedial proximal first phalanx OC fragment created in one randomly chosen MCPJ under arthroscopic guidance, synovial biopsies from dorsomedial and dorsolateral locations were also obtained at that time in all horses. The other MCPJ was arthroscopically explored, and synovial biopsies were obtained from the dorsomedial aspect of the joint in 5 of the 7 horses, but no fragment was created (sham). After 2 weeks of stall rest and a 10 day course of phenylbutazone[a] (5 days at 2.2 mg/kg orally twice a day and 5 days at 2.2 mg/kg orally once a day), horses were exercised on a treadmill 5 times a week for a total of 14 weeks to induce osteoarthritis. At week 16, horses were again placed under general anesthesia for arthroscopic removal of the fragment. To document creation of osteoarthritis, arthroscopic biopsies (totaling approximately 2 x 10 mm) of dorsomedial and dorsolateral synovial membrane and full thickness articular cartilage from both MCPJs were collected. Cartilage biopsies in all but one horse were collected from the third metacarpal condyle directly opposite the osteochondral fragment in injured joints and from the same location in the sham leg. Morphine[b] was injected into all OC injured joints after fragment removal and any sham joint where biopsies were taken. Horses were recovered from anesthesia, and followed a post-operative course of phenylbutazone as stated above. Horses were stall rested for 10 days before being turned out into a paddock for the remainder of the study. One horse suffered from radial nerve paralysis post anesthesia, but recovered by 2 weeks post-operatively after appropriate treatment, which included additional anti-inflammatories.

*Fluid Collection and effusion scoring*

All samples were collected between 7:45 AM and 9:45 AM. Horses were sedated with intravenous detomidine[c] (0.01-0.02 mg/kg), acepromazine[d] (0.02-0.04 mg/kg), and

butorphanol[e] (0.01mg/kg) after collecting 20 mls of whole blood from the jugular vein. Both MCPJs were subjectively evaluated for effusion (0=none, 1=mild, 2=moderate or 3=severe). Both MCPJs were then aseptically prepared, and SF was obtained without lavage using a lateral approach while the joint was held in flexion. Urine was obtained via catheterization. Whole blood (once clotted) and SF samples with blood contamination were centrifuged after collection at 2,000 x g for 10 minutes and then decanted. All samples were aliquoted into 200-300 µl samples and stored at -80C until analysis.

*Fluid Biomarker Evaluation*

Commercially available ELISAs, previously validated for use in the horse, [3,4,45,52,60] were used to measure inflammation, type II collagen synthesis, type I and/or II collagen degradation, and bone turnover (Table 1). Samples were analyzed for all biomarkers at weeks 0, 16, 17, 18 and 20, with the exception that sham leg samples for NO were not run on weeks 17, 18 and 20 due to financial constraints. All samples were measured in duplicate and absorbance was measured using a FLUOstar Optima plate reader[f] with MARS Data Analysis Software v.2.0[f]. Samples were not pretreated unless specified by manufacturer instructions. ELISAs for bone turnover (BAP[g]), type II collagen degradation (CTX II[h], C1,2C[i], C2C[i]), and synthesis (CP II[i]), inflammation (NO[j] and HMGB-1[k]), and creatinine[g] were used to evaluate SF, serum, and/or urine concentrations where applicable (Table 1).

Inflammation in the joint was monitored via measurement of HMGB-1. SF samples were assayed neat. The intra- and inter-assay coefficient of variation (CV) for HMGB1 was less than 5% and 8%, respectively. Inflammation was additionally monitored by measuring NO concentrations. NO analysis was not performed on samples from sham joints in weeks 17, 18 and 20 due to financial constraints. SF samples were assayed at neat or up to a 1:4 dilution. The intra- and inter-assay CV for NO was less than 8% and 11%, respectively.

Type II collagen synthesis was evaluated using the CP II assay. SF samples were diluted 1:2 to 1:8, and serum samples were diluted from 1:3 to 1:6. The intra- and inter-assay CV for CP II was less than 7% and 16%, respectively for SF and serum combined.

Type II collagen degradation via collagenase cleavage was evaluated using the C2C assay. SF samples were diluted 1:4, and serum samples were diluted 1:2. The intra- and inter-assay CV for C2C was less than 9% and 10%, respectively for SF and serum combined.

Combined type I and type II collagen degradation via collagenase cleavage was evaluated using the C1,2C assay. SF samples were diluted 1:4, and serum samples were diluted from 1:3 to 1:4. The intra- and inter-assay CV for C1,2C was less than 1% and 16%, respectively for SF and serum combined.

Degradation products of the carboxy terminal telopeptides of type II collagen were measured using the CTX II assay. SF and urine samples were assayed at neat or up to a 1:2 dilution, and serum samples were diluted 1:2. The intra- and inter-assay CV for CTX II in SF and serum combined was less than 8% and 13%, respectively. The intra- and inter-assay CV for CTX II in urine was less than 2% and 10% respectively, and the urine creatinine dilutions intra- and inter- assay CV was less than 3% and 7%, respectively.

Bone production was assessed by measuring using a BAP assay. SF and serum samples were assayed at neat. The intra- and inter-assay CV for BAP was less than 6% and 19%, respectively for SF and serum combined.

*Subjective and objective lameness examination*

Subjective lameness examinations were performed using video recordings of the horses walking and trotting in a straight line on a hard, even surface. Bilateral palmar digital nerve blocks were performed on all horses to remove any possible influence from sore heels. Videos of all horses post palmar digital nerve block were assigned AAEP lameness grades[66] by experienced lameness clinicians who were blinded to the date of collection and treatment of each horse (TNT, MB) and one (JMM) who was blinded to the date of collection. For further analyses, the assigned grades of all reviewers were averaged. Ground reaction forces were obtained by a force plate.[l,m] Data was used only if the ipsilateral front and hind feet struck the force plate within the appropriate speed (2.8-3.3 m/s) and acceleration ranges (± 10%)[n]. Five passes at the trot for each fore and hind

limb pair were obtained. Force plate and lameness data could not be collected from one horse during week 17 as he had suffered from a post anesthetic radial nerve paralysis and was too lame at that time. A percent symmetry score was calculated from the peak vertical force in the following manner: (lowest average peak vertical force between the forelimbs/highest average peak vertical force between the forelimbs) * 100.

*Statistics*

Repeated measures ANOVA with Tukey's adjustment for pairwise differences were performed[o] to determine differences in clinical lameness grades, force plate forelimb peak vertical force percent symmetry scores, effusion scores, age, gender and inflammatory, collagen, and bone biomarker concentrations from SF, serum, and urine over time and between groups. Spearman correlations were performed on age and gender data with biomarker concentrations that had been indicated as being significant with the repeated measures ANOVA. Post-hoc power calculations were performed via t-tests.[p] Significance was set at $P < 0.05$ for all analyses.

*Results*

*Response to OC fragmentation*

There were no significant differences in inflammatory biomarkers (HMGB-1 and NO) from week 0 to week 16 (Figure 3).

Horses exhibited increased concentrations of collagen type II degradation biomarkers in the SF 16 weeks after OC injury as compared to week 0 concentrations, while collagen and bone synthesis were unchanged (Figures 4 and 5). C2C concentrations were significantly increased ($P = 0.048$) at week 16 compared to week 0 in OC injured joints, but concentrations were not different than sham joints at week 16. CTX II concentrations in OC injured joints were significantly increased ($P<0.001$) at week 16 compared to week 0, and concentrations were significantly higher ($P=0.001$) than sham joints at week 16 (Figure 4). Serum and urine concentrations of biomarkers showed no significant differences between week 0 and 16 (Figures 5, 6 and 7).

Subjective and objective lameness scores at week 16 were not significantly different from week 0 (Figure 8).

Effusion scores indicated an increased amount of effusion from week 0 to week 16 (P= 0.002) (Figure 9).

*Response to arthroscopic removal of OC fragments*

Inflammatory biomarkers had mixed results with a trend towards increased concentrations of NO one week (week 17) after arthroscopic removal of the OC fragment (P=0.12) but no differences with HMGB-1 (Figure 3). There were no significant differences in collagen degradation or synthesis and bone synthesis biomarker concentrations in the SF at week 17 (OC injured joints), or in weeks 18 and 20 of the saline treated horses (Figure 4 and 5). Additionally, serum and urine biomarkers did not show any significant differences during this time (Figures 5, 6 and 7).

Subjective lameness increased (P=0.027) 1 week after arthroscopic surgery to remove the OC fragment, but returned to week 16 grades by weeks 18 and 20 (Figure 8). Compared to week 16, peak vertical force percent forelimb symmetry was decreased at week 17 in all horses (P=0.007) and at week 18 in saline treated horses (P=0.009).

There was no increase in effusion from week 16 to 17, or from week 16 to weeks 18 or 20 in the saline treated horses (Figure 9).

*Response to intra-articular administration of TA post-operatively*

For the inflammatory biomarkers, NO and HMGB-1, there were no significant differences over time or between groups (Figure 3) by 1 or 3 weeks after TA treatment.

CTX II concentrations in SF were significantly decreased in week 18 and 20 (P= 0.001) in TA treated joints compared to OC injured joints in week 17 such that they were no different from sham joints. At week 18, CTX II concentrations in TA treated joints were significantly lower than saline treated joints (P< 0.0001), but by week 20 there was no difference between TA and saline treated joints (Figure 4).

TA treated joints had significantly higher C1,2C concentrations (P<0.001) in SF than OC injured joints prior to TA administration in week 17. In addition, TA treated joints had higher C1,2C concentrations at week 18 compared to saline treated (P< 0.001) and sham joints (P<0.0001). C1,2C concentrations decreased in week 20 from week 18 in TA treated joints (P< 0.001) but remained significantly higher than sham joint concentrations in that week (P =0.03) (Figure 4).

TA treated joints had significantly higher C2C concentrations (P<0.001) in SF than OC injured joints prior to TA administration in week 17. In addition, TA treated joints had higher concentrations at week 18 compared sham joints, as well as to saline treated joints (both P< 0.0001). TA treated joints had significantly higher SF C2C concentrations (P=0.045) than sham joints (P<0.001) and saline treated joints (P= 0.045) at week 20, but had decreased concentrations from TA treated joints in week 18 (P= 0.044) (Figure 4).

With collagen synthesis biomarker CP II, there were no significant differences over time or between groups (Figure 4) by 1 or 3 weeks after TA treatment.

There was a significant increase in BAP (bone production) concentrations in SF of the TA treated joints at week 18 compared to week 17 (P=0.037) and sham joints at week 18 (P= 0.01). During week 20, there was a significant increase in BAP concentrations in the SF of TA treated joints as compared to joints at week 17 (P =0.002), sham joints (P=0.001) and the saline treated joints (P=0.029) at week 20 (Figure 5).

Serum and urine biomarker concentrations showed no significant differences between groups or weeks 1 and 3 weeks post TA administration (Figure 5, 6 and 7).

Subjective and objective lameness at week 18 did not differ from week 17 regardless of treatment (Figure 8). Subjective lameness in TA treated horses decreased in week 20 compared to week 17 (P=0.007), although there was no significant difference from the saline treated horses at this time point (Figure 8). Objective lameness evaluation demonstrated a trend (P=0.08) for TA treated horses to be more symmetric at week 20 compared to week 17, although TA treated horses were not more symmetric than saline treated horses at week 20.

There was a trend (P= 0.058) towards less effusion in the TA treated joints one week post TA administration as compared to joints in week 17 and as compared to saline treated joints in week 18 (P=0.091) (Figure 9).

*Age and gender*

Age was a significant factor in SF C1,2C (P =0.003), SF C2C (P <0.0001), serum C1,2C (P= 0.03), serum BAP (P= 0.002), and urine CTX II (P< 0.001). Gender was a significant factor in SF C1,2C (P= 0.02), SF C2C (P= 0.01), serum CTX II (P=0.02) and urine CTX II (P= 0.003). uCTX II and serum BAP were negatively correlated for age (R= -0.87; P< 0.0001 and R=-.565; P < 0.001, respectively), while serum CTX II concentrations were higher in geldings than mares (R= 0.394, P= 0.0007).

*Discussion*

Analysis of biomarkers 16 weeks after creation of an OC fragment indicated that the joint environment was experiencing increased type II collagen degradation compared to baseline with a lack of a significant repair response. Based on the lack of significant increases in collagen degradation or synthesis biomarkers, surgery to remove the OC fragments had little effect on joint metabolism out to 1 month post-operatively. Post-operatively administered TA demonstrated a mixed effect on collagen degradation, with minimal collagen repair and an increased production of bone within the joint, possibly due to healing subchondral bone or osteophytosis. Serum and urine biomarkers evaluated in this study were not representative of the SF concentrations of type II collagen degradation biomarkers within the joint due to acute OC injury, surgery to remove the OC fragment, or TA administration post-operatively. Lameness increased immediately after arthroscopic removal of the OC fragment, but subjectively returned to pre-surgical (week 16) lameness grades by week 18 in all horses. Based on objective lameness assessment, TA treated horses had returned to week 16 symmetry scores by week 18.

The horses in this study were part of a previous study which created a MCPJ OC fragment model of early OA.[6] Our shared results at week 16 are similar in seeing the increases in some of the collagen degradation biomarkers (CTX II and C2C), but not others (C1,2C). Our levels of synthesis were also not significant; whereas week 16 synthesis levels compared to baseline were significantly increased in the model.[6] Inflammatory and bone production biomarkers lacked significance in either study by week 16.[6] The differences seen in the biomarkers above may be due to the decreased number of horses enrolled in this current study (n=7 vs. 11). There was increased joint effusion but not significant lameness by week 16 after OC fragment creation. However, the similar biomarker pattern between the studies, a low grade of lameness, and mild changes in the synovial and SCB biopsies, and arthroscopic and radiographic scores,[6] all support that horses in this project by week 16 had early OA.

Arthroscopic surgery to remove OC fragments appeared to have minimal effects on joint metabolism and inflammation. Inflammation was minimally present at 7 days post-operatively, with no significant increases in HMGB-1 or NO concentrations. It is possible that that earlier evaluation of NO and HMGB1 may have revealed peaks not identified in this study. Examination of NO concentrations in sham joints may have helped provide a clearer picture if the trend towards an increase of inflammation at week 17 from week 16 was real, but were not performed due to financial limitations. Ex vivo studies have shown NO concentrations from articular chondrocytes in culture exposed to IL-1 beta were reduced from controls after 6 hours of exposure to anti-inflammatories.[46] Therefore, it is possible that phenylbutazone administration for 1 week may have significantly decreased NO concentrations. The use of anti-inflammatory drugs such as phenylbutazone is considered the standard of practice after arthroscopic surgery in horses. Therefore, it is unrealistic to evaluate horses post-operatively without them, even though they may affect biomarker concentrations. The one horse who suffered a post-surgical radial nerve paralysis received the high dose of phenylbutazone for a longer time period, in addition to intravenous diluted DMSO, which may have further decreased any joint inflammation present. His data was still included as there were such a small number of horses enrolled in this study. Joint lavage associated with arthroscopic surgery can also

dilute biomarker concentration such that an inflamed joint may appear to have lower concentrations, though it is unknown how long post-surgery these effects may be seen. Additionally, the insignificant increases in inflammation may have been because of the low numbers of horses with high variability in individual NO levels. Arthroscopic surgery to remove OC fragments resulted in an increase in subjective and objective lameness 1 week post-operatively with no further increase in effusion. The increase in lameness in week 17 is suggestive that some degree of inflammation is still occurring at the joint level, or it could be due to capsular or subchondral bone pain. Further evaluation of other inflammatory biomarkers such as PGE2 and various MMPs[72-74] should be undertaken before conclusively stating that there are minimal inflammatory effects associated with surgery that would not benefit from TA therapy post-operatively.

Intra-articular administration of TA after arthroscopic removal of OC fragments resulted in no significant change in inflammatory biomarkers (HMGB-1 and NO), mixed effects on type II collagen degradation, increased bone production, and minimal improvements on lameness. Inflammation did not decrease with TA administration as would be expected, although there was a trend towards decreased joint effusion. On the other hand, these biomarkers also did not increase post-operatively regardless of treatment. Therefore, there may be no clinical indication for intra-articular corticosteroid administration post-operatively, particularly if such administration has any detrimental effects.

After TA administration, type I and II collagen degradation increased via the MMP driven catabolic pathway (C2C and C1,2C). Type II collagen telopeptide degradation significantly decreased with no concomitant significant increase in type II collagen synthesis. Even though one in vitro study suggested that TA would decrease MMP-1,-3, and -13 activity,[25] in our study post-operative TA increased collagenase activity over saline treated horses for the remaining 3 weeks of the study. However, TA may have altered ratios in which individual MMPs were expressed, resulting in lower CTX II concentrations. In other words, the CTX II degradation pathway may be different than the collagenase cleavage pathway that creates C2C and C1,2C neoepitopes. Further support of a different pathway for these biomarkers was demonstrated in the MCPJ OC

fragment model where C1,2C and C2C concentrations increased earlier than CTX II.[6] CTX II telopeptide is formed after exposure to IL-1,[75] TNF-alpha[76] as well as after exposure to cathepsin K,[76] cathepsin B and MMPs -1,-3,-7,-9 and-13.[77,78] Therefore, since TA effectively down regulated the CTX II concentration, but not collagenase cleavage (C2C and C1,2C), it is likely that TA may not suppress MMP-13 (a common MMP in pathways to create both CTX II and C2C/C1,2C). TA may suppress either the cytokines, cathepsins, or other MMPS such as MMP 7 or the gelatinases (MMP-9) that create cleavage exposing the CTX II epitope but not the C1,2C or C2C neoepitopes.[76,77,79,80] Drugs that inhibit cathepsin K reduce CTXII concentrations, although to date, corticosteroids have not been identified as one of these drugs.[81,82] Nonetheless, our study demonstrates that it is important to measure both C2C and CTX II over time if a medical intervention with steroids is to occur, as their levels may reveal different effects on collagen degradation.

TA administered into one joint can have systemic effects on other remote non-injected joints.[43] However, this was not seen within this study, as biomarker concentrations were not significantly increased or decreased in sham joints over time after TA administration. However, in the horses used in our study, all but one sham joint had synovial membrane and full thickness articular cartilage biopsies taken at week 16.[6] Therefore, both sham and OC injured joints had a surgical injury at week 16 which could result in biomarker concentration elevations; however, significant increases in OC injured joint biomarker concentrations as compared to the shams were still apparent.

Collagen synthesis was not increased in any of the joints from either saline or TA treated horses. In a rabbit model of arthritis, with intra-articular administration of either MPA or saline, genes for type II collagen synthesis were down regulated only in MPA treated joints, indicating decreased synthesis.[83] Likewise in a horse model of repeated intra-articular injections of TA or saline, changes in cartilage synthesis (CP II) in TA treated joints were not increased from saline controls.[43] A previous study with the MCPJ OA model[6] was able to detect dynamic changes in CP II concentrations in synovial fluid collections occurring every 2 weeks, indicating that CP II can change in a short period of time after injury. This was not seen in this study, and may be because we only used 7 of

the 11 horses enrolled in that previous study, and there were large standard deviations in biomarker concentration within our group.

An increase in bone production (BAP) was seen in the synovial fluid of TA treated joints throughout the duration of the study, though increased BAP was not seen in the serum. This finding is in contrast with Fuller et al.[61] who saw an increase in BAP in the serum of racehorses with osteochondral injury, but in agreement with Trumble et al.[60] who saw a trend towards increased levels of BAP in the synovial fluid of OC injured MCPJs. It is unknown if horses in those studies received any intra-articular steroids since they were samples collected from clinical cases. As previously suggested, this increased bone production may be linked to early osteophyte formation or the reparative function of subchondral bone.[60,61] Within the time frame and interventional confines of this study, distinction between the two was not possible. Later follow up with radiographs and/or arthroscopy would be needed to distinguish between the two, as increasing SF BAP concentrations have been positively correlated to increasing levels of articular cartilage damage seen during arthroscopy in OC injured horses.[61] It appears that increased BAP concentration was due to TA because there was minimal change in BAP in response to OC fragment creation or arthroscopic surgery in sham and saline treated joints. In humans, steroids have been responsible for osteoporosis rather than bone production, although these doses administered to humans in this study were higher than those normally administered in horses.[84]

Subjective and objective lameness increased in all horses one week (week 17) after OC fragment removal. Previous studies have shown improvement in lameness associated with intra-articular steroid administration and this was partially seen in this study.[17,31] In our study, TA treated horses by one week post TA administration had returned to pre-surgery (week 16) lameness levels subjectively and objectively; whereas, it took saline treated horses until week 20 to be objectively evaluated as not significantly different than week 16. However, TA and saline treated horses were not significantly different from each other in either week 18 or 20 either subjectively or objectively.

The post-hoc power to see an 8.5% change in percent symmetry between horses between weeks 16 and 17 was 80%. However, once the horses were subdivided by

treatment, the post-hoc power was only 10% to detect a 5% difference in percent symmetry score between the saline treated horses in week 20 as compared to horses in week 17. For TA treated horses, the power to see a difference in objective lameness between weeks 17 and 20 was 20%. Regardless, by four weeks after surgery for OC fragment removal, lameness in all horses had returned to the level that was present before the surgery.

Serum and urine biomarkers evaluated in this study were not representative of the SF concentrations of type II collagen degradation biomarkers within the joint due to acute OC injury, surgery to remove the OC fragment, or TA administration post-operatively when compared to actual SF concentrations. This is in agreement with some and in contrast to other studies findings.[17,60,72] Systemic biomarker concentrations could have been low for several reasons. There could be increased metabolism rates, increased binding of the epitope to large proteins in the systemic circulation (preventing assay antibodies from binding),[85] or they could have been sequestered in the SF (from decreased diffusion due to capsular and synovial membrane thickening after repeated surgeries and arthrocentesis). Additionally, it is important to note that the largest amount of cartilage in the body is in the spine and respiratory system,[51] and biomarkers present in the serum or urine are indicators of metabolism in those areas as well. Therefore concentrations emanating from one joint that is not severely injured may be minimal when combined into the pooled concentrations in the systemic circulation.[51] Ratios of systemic to synovial fluid concentrations of biomarkers may help to elucidate actual metabolism by helping to minimize individual animal variation in concentrations.[60] Also, other markers of collagen type II degradation are continually being developed, such as C2C in the urine, which may be more indicative of what is occurring at the joint level,[86] providing a source for future studies.

The major limitation in this study was the number of horses enrolled due to the pilot nature, especially once subdivided into treatment groups. Biomarker changes after TA administration (such as increased BAP, C2C, C1,2C and decreased CTX II) as compared to pre-treatment (week 17) all demonstrated power at 80%. This provides evidence that even with this low number of horses, these results are less likely to be due

to chance. However, when trying to determine the effect of surgery, there was not enough power; NO, C1,2C, C2C, CP II, HMGB1 demonstrated power of 25-50% for comparing saline treated horses to week 16 concentrations. Following all horses for one more week before administering the intra-articular TA may have allowed us to see more effects of surgery once the administration of systemic drugs had ceased. In addition, some positive effects of TA administration on lameness may have been missed as power to detect lameness in both groups was low.

Age and gender were also sources for variation with some of our biomarker concentrations. Younger horses have been reported to have increased concentrations of many biomarkers as compared to older horses, as was seen here with uCTX II.[52] Age effects were also seen in some biomarkers in the MCPJ OC fragment model study using some of these same horses, with males and younger horses having higher concentrations.[6] With serum CTX II, geldings had higher concentrations in this study. On average, TA treated horses were 5.8 years old, while the saline treated horses average age was 8 years. Of the TA treated horses, 1 was a mare and 3 were geldings, while in the saline treated group there were 2 mares and 1 gelding. Although these trends in urine and serum CTX II did appear to hold true, there was no significant effect of age or gender on SF CTX II concentrations. Enrolling age and gender matched horses would have helped to minimize the subsequent effect on biomarker variations. The other relationships that were significant as indicated by the ANOVA were not seen to be linear. Repeated arthrocentesis has been linked to alterations in some synovial fluid biomarkers,[43] although the recommendation that samples be taken at least a week apart was followed in this study. Following the horses out over a significantly longer time period would have helped further our understanding on which biomarker(s) may have the best predictive value for advancing OA when coupled with clinical and radiographic evaluations.

Overall, arthroscopic surgery to remove a first phalanx OC fragment resulted in a lack of significant post-operative inflammation as seen by SF biomarkers. However, an increase in lameness one week post-arthroscopic removal of the OC fragment suggests that inflammation was in fact present, but just undetected by these biomarkers. If in fact inflammation isn't present, perhaps there is no need to administer corticosteroids into the

joint post arthroscopy. TA itself had a mixed effect on joint metabolism, with an exacerbation of some pathways type II collagen degradation, and inhibition of others, without a significant increase in reparative collagen synthesis. There was increased bone production, possibly related to subchondral bone repair or osteophyte formation within TA treated joints. Lameness improvement was similar in TA and saline treated horses, having returned to baseline by four weeks post-operatively, although subjective lameness grades were more improved with TA at that point. TA administration is not indicated after surgery for removal of OC fragments with superficial cartilage damage since collagen degradation increased compared to controls. TA may be indicated for horses that need a faster return to work, or those who have more pronounced lameness and effusion at presentation and/or post-operatively.

*Footnotes*

a. Phenylbutazone (1 gram tablets), Vedco, St Joseph, MO, USA

b. Morphine (15 mg/ml), Baxter Healthcare Corp, Deerfield, IL, USA

c. Dormosedan (10 mg/ml), detomidine hydrochloride, Pfizer Animal Health, Madison, NJ, USA

d. Acepromazine maleate (10 mg/ml), Vedco, St Joseph, MO, USA

e. Butorphanol tartrate, Butorphic injection (10 mg/ml), Lloyd labs, Shenandoah, IA, USA

f. BMG Labtech, Chicago, IL, USA

g. Quidel Corporation, San Diego CA, USA

h. Immunodiagnostic Systems, Inc. Scottsdale, AZ, USA

i. IBEX Technologies, Inc. Montreal, Quebec, Canada

j. Enzo Life Sciences Inc., Farmingdale, NY, USA

k. IBL International Corp., Toronto, ON, Canada

l. Force Plate Model #BP900900-8K-3200, AMTI (Advanced Mechanical Technology, Inc), Watertown, MA, USA

m. DMAS 6 Suite, Motion Imaging Corporation, Simi Valley, CA, USA

n. Polaris Wireless Timer with 3 photo-transmitter/-receiver pairs,Timers: FarmTek, Inc., Wylie, TX , USA

o. SAS 9.2, SAS Institute Inc., Cary, NC, USA

p. GraphPad InStat, GraphPad Software, Inc., La Jolla, CA, USA

| Biomarker | Synthesis, degradation or inflammation | Representative Joint structure |
|---|---|---|
| HMGB1 | inflammation | Synovial Fluid and membrane |
| NO | inflammation | Synovial Fluid and membrane |
| CPII | synthesis | Cartilage-type II collagen |
| C2C | degradation | Cartilage-type II collagen |
| C1,2C | degradation | Cartilage-type I and II collagen |
| CTXII | degradation | Cartilage-type II collagen |
| uCTXII | degradation | Cartilage-type II collagen |
| BAP | bone production | Bone |

Table 1. Commercial biomarkers (left column) listed by their overall actions (middle column) and the joint structure they represent (right column). HMGB1= High mobility group box 1 protein, NO= Nitric oxide, CPII= Procollagen C-Propeptide of type II collagen, C2C= C-terminal neoepitope after collagenase cleavage of type II collagen, C1,2C= C-terminal neoepitope after collagenase cleavage of type I and II collagen, CTXII= C-terminal telopeptide of type II collagen, uCTXII= Urinary CTX II, BAP= Bone alkaline phosphatase

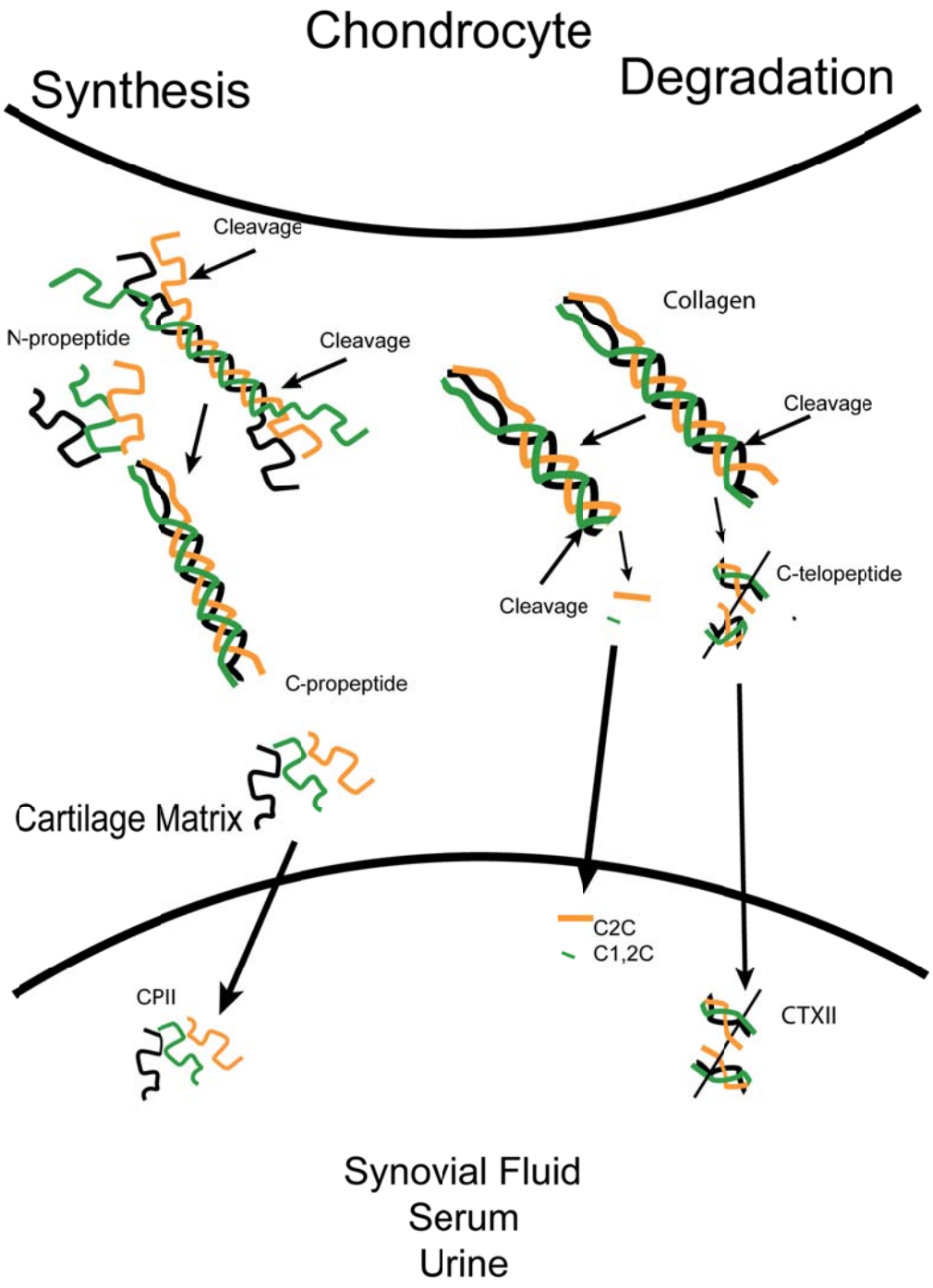


Figure 1. Formation of cartilage biomarkers from collagenase cleavage and collagen synthesis. Adapted from kit insert in ELISA from IBEX[i] (June 2010).

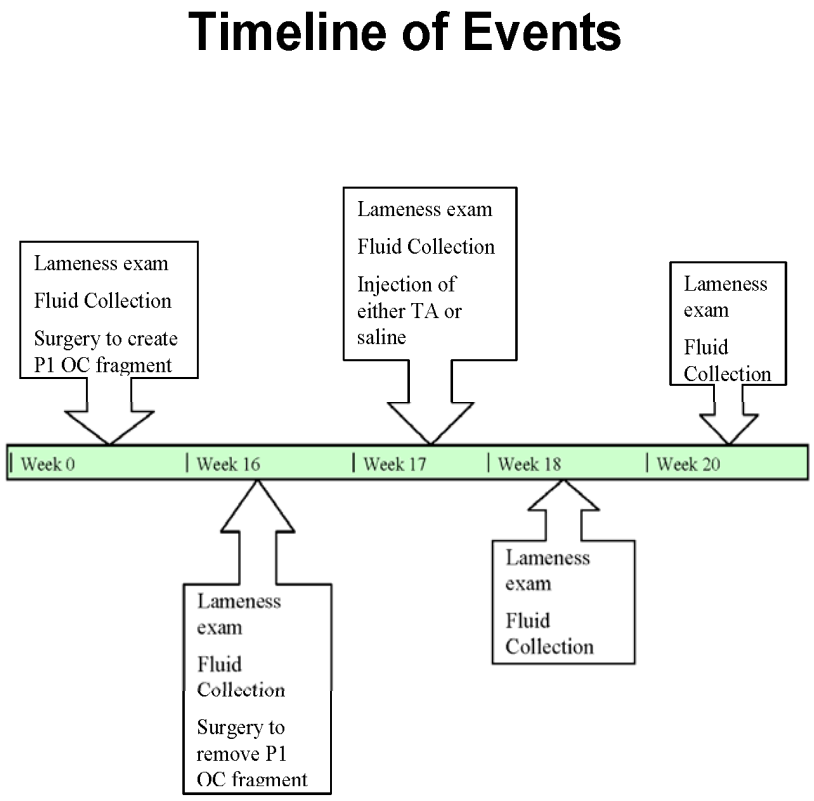


Figure 2. Timeline of events for the study listed by week. Procedures performed during each week are listed in the box.

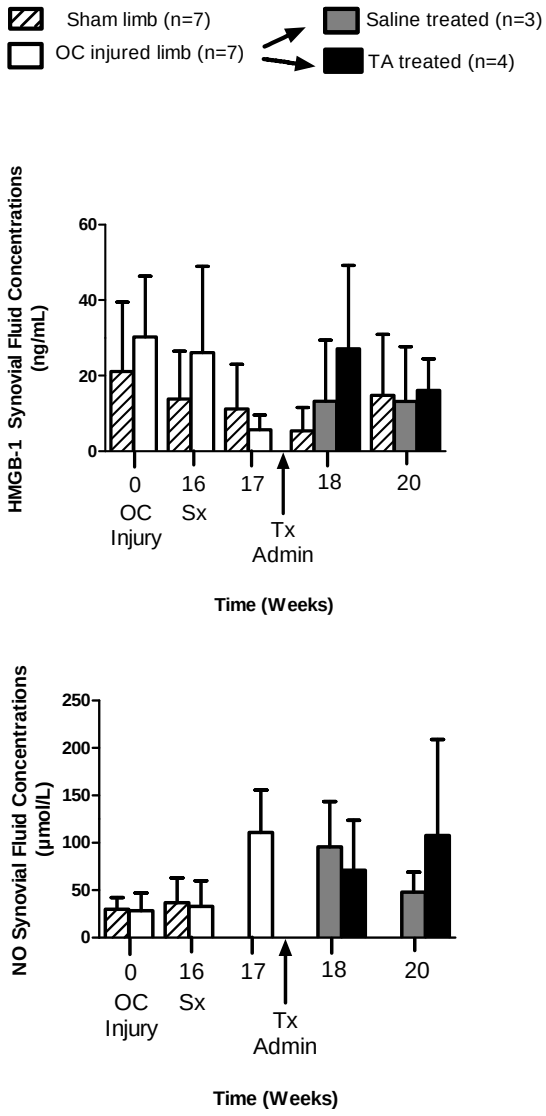


Figure 3. Mean (± SD) concentrations of inflammatory biomarkers, HMGB1 (top) and NO (bottom) in synovial fluid from Sham operated limbs (diagonally striped bars), OC injured limbs (white bars), saline treated (gray bars), and triamcinolone acetonide (TA) treated (black bars) groups for weeks 0, 16, 17, 18, and 20. Week 0 = time of osteochondral fragment creation (OC Injury); week 16 = OC fragment removal (Sx); week 17 = 1 week post OC fragment removal followed by intra-articular injection into the previously injured joints (arrow = Tx Admin) of either TA (n = 4 horses) or saline (n = 3 horses); week 18 = 2 weeks post OC fragment removal and 1 week post saline or TA injection; week 20 = 4 weeks post OC fragment removal and 3 weeks post saline or TA injection. NO sham limb samples were not run for weeks 17, 18 and 20 due to financial constraints. There are no significant differences ($P<0.05$).

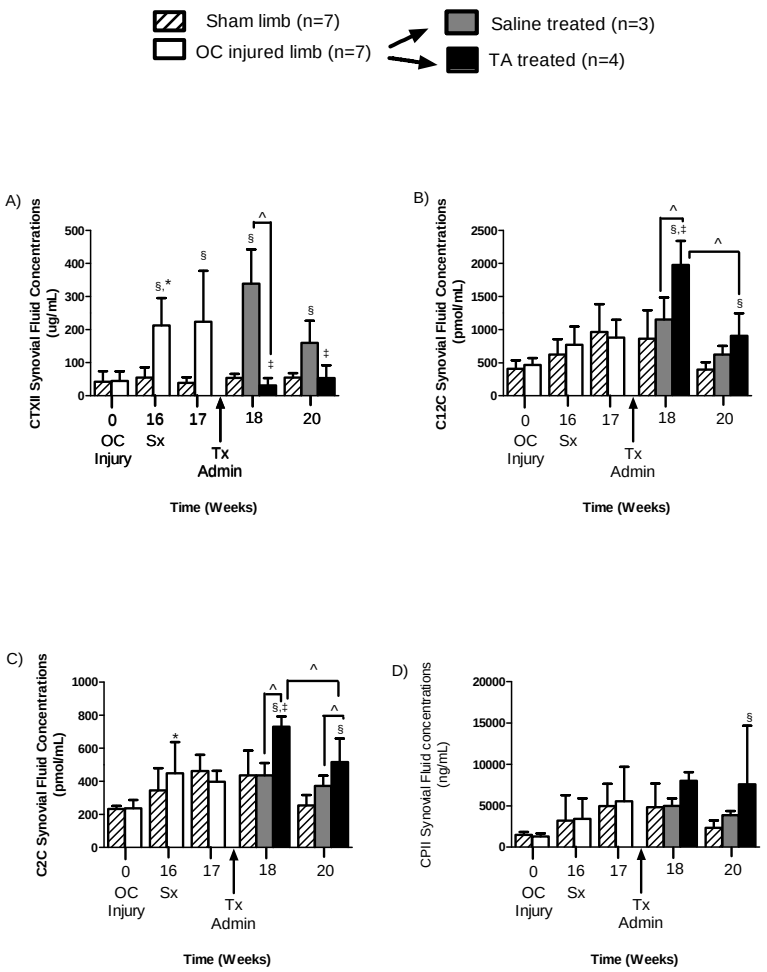


Figure 4. Mean (± SD) concentrations for collagen degradation biomarkers CTX II (A), C1,2C (B), C2C (C) and the cartilage synthesis biomarker CP II (D) in synovial fluid from Sham operated limbs (diagonally striped bars), OC injured limbs (white bars), saline treated (gray bars), and triamcinolone acetonide (TA) treated (black bars) groups for weeks 0, 16, 17, 18, and 20. Week 0 = time of osteochondral fragment creation (OC Injury); week 16 = OC fragment removal (Sx); week 17 = 1 week post OC fragment removal followed by intra-articular injection into the previously injured joints (arrow = Tx Admin) of either TA (n = 4 horses) or saline (n = 3 horses); week 18 = 2 weeks post OC fragment removal and 1 week post saline or TA injection; week 20 = 4 weeks post OC fragment removal and 3 weeks post saline or TA injection.

* different from week 0; ‡ significantly different from week 17; § significantly different from sham joint at the given time point; ^ and bar indicates connected groups are significantly different. All significant differences are $P<0.05$.

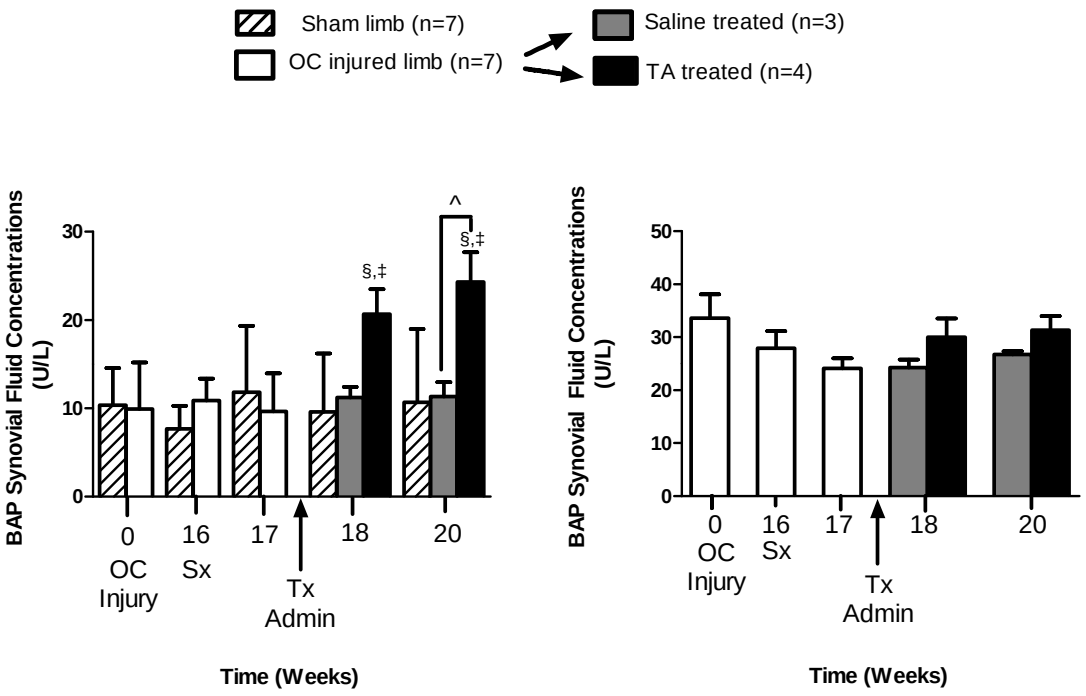


Figure 5. Mean (± SD) concentrations for bone synthesis (BAP) in synovial fluid (left) and serum (right) from Sham operated limbs (diagonally striped bars-SF only), OC injured limbs (white bars), saline treated (gray bars), and triamcinolone acetonide (TA) treated (black bars) groups for weeks 0, 16, 17, 18, and 20. Week 0 = time of osteochondral fragment creation (OC Injury); week 16 = OC fragment removal (Sx); week 17 = 1 week post OC fragment removal followed by intra-articular injection into the previously injured joints (arrow = Tx Admin) of either TA (n = 4 horses) or saline (n = 3 horses); week 18 = 2 weeks post OC fragment removal and 1 week post saline or TA injection; week 20 = 4 weeks post OC fragment removal and 3 weeks post saline or TA injection. ‡ significantly different from week 17; § significantly different from sham joint at the given time point; ^ and bar indicates connected groups are significantly different. All significant differences are $P<0.05$.

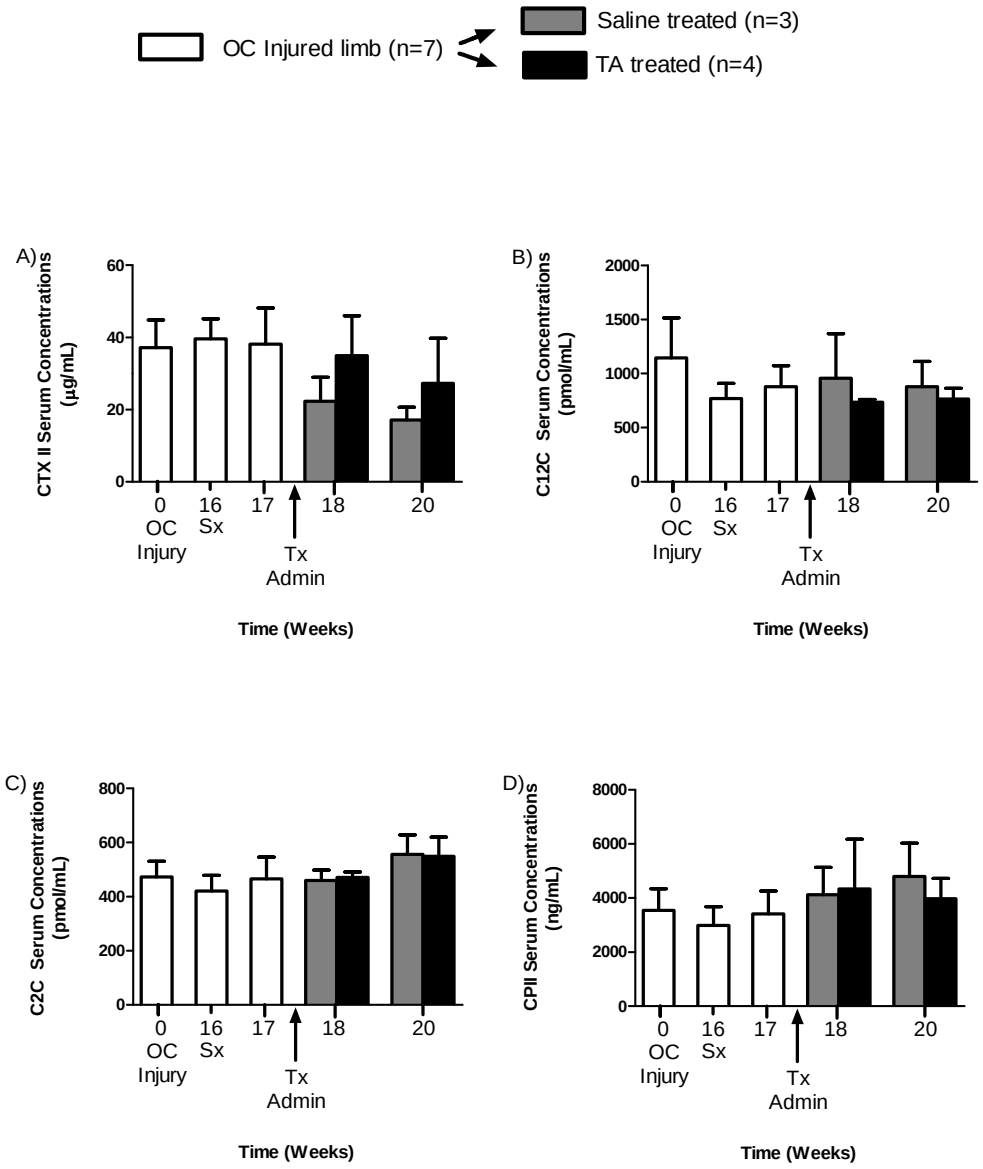


Figure 6. Mean (± SD) concentrations for CTX II (A), C1,2C (B), C2C (C) and CPII (D) in serum from OC injured horses (white bars), saline treated horses (gray bars), and triamcinolone acetonide (TA) treated horses (black bars) for weeks 0, 16, 17, 18, and 20. Week 0 = time of osteochondral fragment creation (OC Injury); week 16 = OC fragment removal (Sx); week 17 = 1 week post OC fragment removal followed by intra-articular injection into the previously injured joints (arrow = Tx Admin) of either TA (n = 4 horses) or saline (n = 3 horses); week 18 = 2 weeks post OC fragment removal and 1 week post saline or TA injection; week 20 = 4 weeks post OC fragment removal and 3 weeks post saline or TA injection. There are no significant differences ($P < 0.05$).

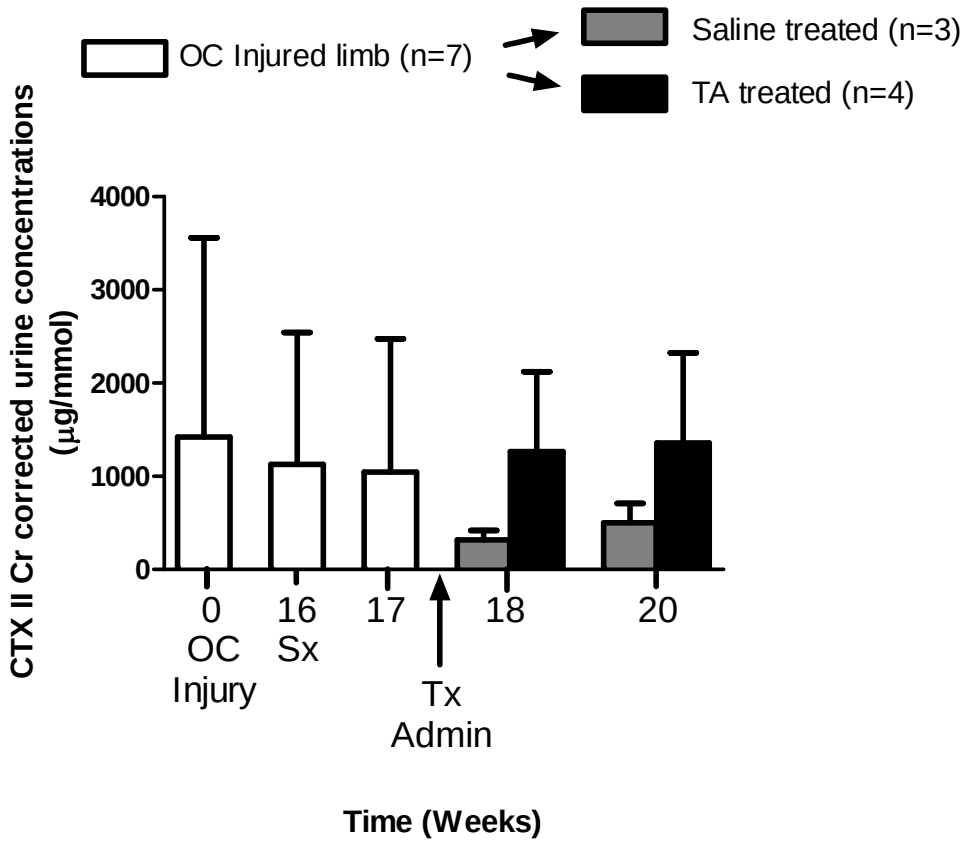


Figure 7. Mean (± SD) concentration of creatinine (Cr) corrected CTXII concentration in urine from OC injured horses (white bars), saline treated horses (gray bars), and triamcinolone acetonide (TA) treated horses (black bars) for weeks 0, 16, 17, 18, and 20. Week 0 = time of osteochondral fragment creation (OC Injury); week 16 = OC fragment removal (Sx); week 17 = 1 week post OC fragment removal followed by intra-articular injection into the previously injured joints (arrow = Tx Admin) of either TA (n = 4 horses) or saline (n = 3 horses); week 18 = 2 weeks post OC fragment removal and 1 week post saline or TA injection; week 20 = 4 weeks post OC fragment removal and 3 weeks post saline or TA injection. There are no significant differences ($P < 0.05$).

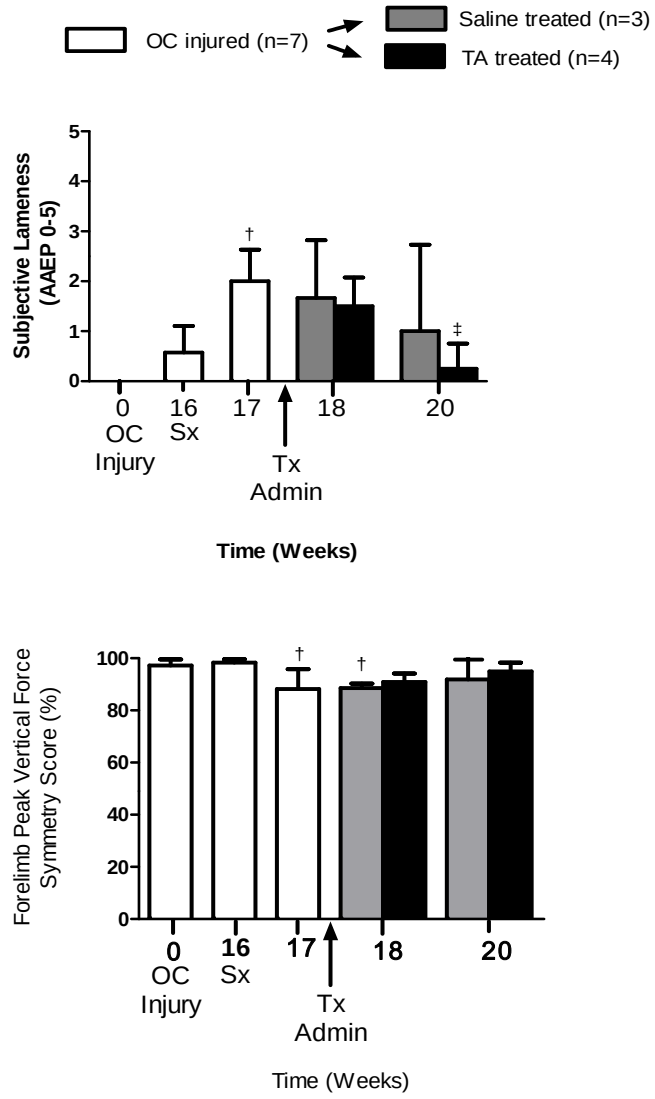


Figure 8. Mean (± SD) subjective lameness grade (top) and percent forelimb peak vertical force symmetry score (bottom) over time from OC injured horses (white bars), saline treated horses (gray bars), and triamcinolone acetonide (TA) treated horses (black bars) for weeks 0, 16, 17, 18, and 20. Week 0 = time of osteochondral fragment creation (OC Injury); week 16 = OC fragment removal (Sx); week 17 = 1 week post OC fragment removal followed by intra-articular injection into the previously injured joints (arrow = Tx Admin) of either TA (n = 4 horses) or saline (n = 3 horses); week 18 = 2 weeks post OC fragment removal and 1 week post saline or TA injection; week 20 = 4 weeks post OC fragment removal and 3 weeks post saline or TA injection. All significant differences are $P<0.05$. † significantly different from week 16; ‡ significantly different from week 17.

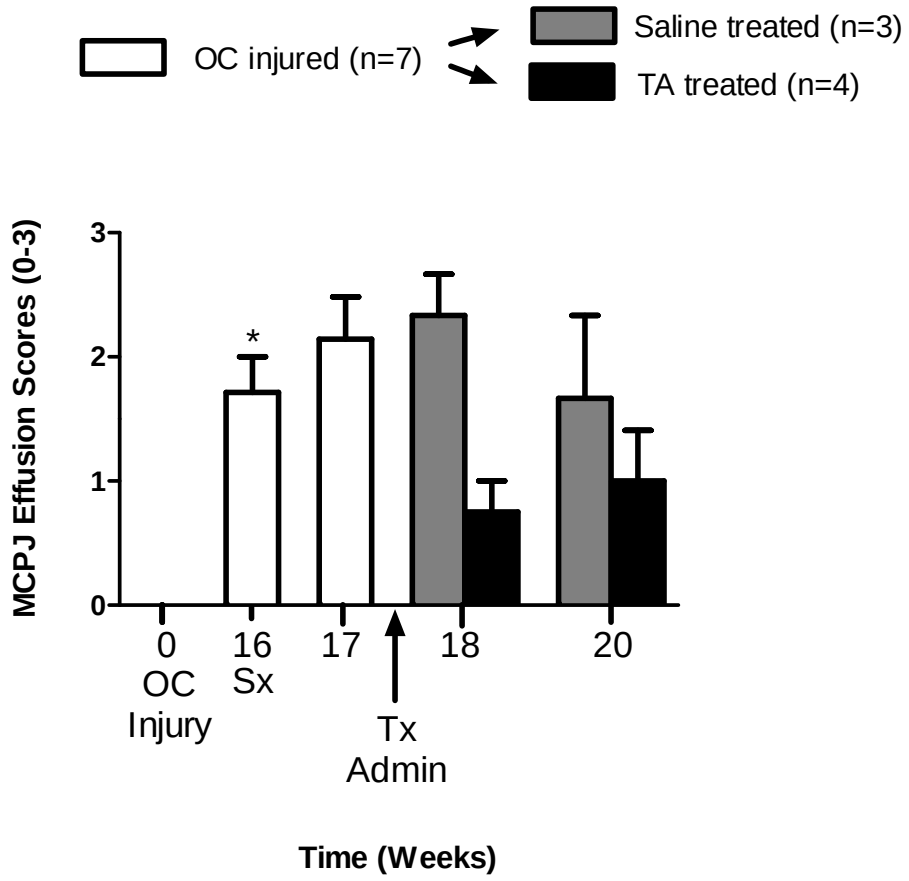


Figure 9. Mean (± SD) MCPJ effusion scores (0=none, 1=mild, 2=moderate, 3=severe) over time from OC injured horses (white bars), saline treated horses (gray bars), and triamcinolone acetonide (TA) treated horses (black bars) for weeks 0, 16, 17, 18, and 20. Week 0 = time of osteochondral fragment creation (OC Injury); week 16 = OC fragment removal (Sx); week 17 = 1 week post OC fragment removal followed by intra-articular injection into the previously injured joints (arrow = Tx Admin) of either TA (n = 4 horses) or saline (n = 3 horses); week 18 = 2 weeks post OC fragment removal and 1 week post saline or TA injection; week 20 = 4 weeks post OC fragment removal and 3 weeks post saline or TA injection. All significant differences are $P<0.05$. * = significantly different from week 0.

# CHAPTER III

# Conclusions and Future Directions

*Conclusions*

This study demonstrated that arthroscopic surgery itself to remove an OC fragment from a MCPJ with early OA had little effect on joint metabolism in the light of concurrent systemic and local medication. There was a lack of significant post-operative inflammation as seen by SF biomarkers. However, an increase in lameness one week post-arthroscopic removal of the OC fragment calls into question whether inflammation was in fact present, but just undetected by these biomarkers. If in fact inflammation isn't present, perhaps there is no need to administer corticosteroids into the joint post arthroscopy. With TA administration, some pathways for type II collagen degradation were altered, and as a consequence, there was less degradation of the C-telopeptide (CTXII) but more of other collagen cleavage biomarkers (C2C and C1,2C) without a significant increase in reparative collagen synthesis. There was increased bone production, possibly related to subchondral bone repair or osteophyte formation within TA treated joints. Urine and serum biomarkers in this study were not good representatives of collagen degradation, collagen synthesis and bone production occurring in the SF in acute OC injury, after arthroscopic surgery or after TA administration. Lameness improvement was similar in TA and saline treated horses, having returned to baseline by four weeks post-operatively. TA administration in cases of recent OC injury with mild degrees of lameness and effusion is not indicated as increased collagen degradation results. TA may be indicated for horses that need a faster return to work, or those who have more pronounced lameness and effusion at presentation and/or post-operatively.

*Future directions*

As this was a pilot study, it was encouraging to see that changes could be identified in even a small number of horses in regards to lameness after surgery and in biomarker concentrations after a drug intervention. Future studies developing from and improving on this work can occur in many areas: structure of the study, drug or therapy choice, biomarkers studied and further investigation into kinetic and kinematic analysis.

*Structure of the study*

As a pilot study, there were only 7 horses enrolled in this study. This was the number of horses available from the MCPJ model OA study performed just prior.[6] While significance was achieved in some cases, lower than desirable power was present in some instances where there was a greater variability in the data (for example clinical lameness grades and some of the biomarkers) and having 16-20 horses per group, for a total of 36-40 (resulting in an approximately 80% power), enrolled in this study would have helped to alleviate this issue, with the understanding that financially this may not be reasonable.

Additional joints, or joints without biopsies taken, may be also evaluated to see if differences exist between them in regards to the effects of local TA administration or systemic diffusion of TA.

*Drug or therapy choice*

While triamcinolone acetonide (TA) was chosen in this study, other drugs are also commonly used for post-arthroscopic injection, and new therapeutics are continually developed for intra-articular use. HA is perhaps one of the most commonly administered intra-articular drugs post-arthroscopy. For this reason, it was a tough decision on whether to investigate it or TA for my Master's project. Due to the small numbers of horses and the limitations of a pilot study, TA was felt to offer the best chances for seeing significant differences between horses on both a clinical and joint metabolism level. However, understanding what benefits or disadvantages HA either alone or in combination with triamcinolone would obtain is clinically relevant and the logical next step with this research.

Other drugs (such as Adequan, Polyglycan, amikacin and morphine) are also administered intra-articularly with an unknown effect on the joint metabolism and clinical soundness post-arthroscopy. Newer biologicals (platelet rich plasma and stem cells) have been clinically used as treatments for osteoarthritis, and it is only a matter of time before these too are injected after surgery.

Other direct or indirect treatments for osteoarthritis have been employed such as Extra Corporeal Shock Wave therapy, chiropractic manipulations and acupuncture. This model can provide a more standardized way to investigate these modalities as well.

*Biomarkers*

The field of biomarkers is rapidly expanding, and there is a continual search for new and/or ideal biomarkers that will help move the field forward. Many of the biomarkers examined here evaluated different parts of cartilage metabolism, including synthesis and degradation of collagen, in an attempt to understand if either of these paths was favored within the cartilage of the affected joint post-arthroscopy and after intra-articularly injected medication. It is unknown which of these biomarkers is more predictive of future OA development. Following horses out over longer periods of time, with radiographs as well as serum, urine and synovial samples for biomarker analysis will be important in determining which biomarkers are the most reliable prognostically. Further evaluation of ratios of degradation to synthesis or synovial fluid to serum and/or urine may also add insight into what is occurring at a joint level, and can be performed with this data.

The types of biomarkers in this study were primarily focused on cartilage metabolism, but evaluating inflammatory mediators directly (IL-1, TNF alpha, capthepsin K and B, various MMP's, etc.) may help elucidate the degree of direct anti-inflammatory properties of TA may have had. These additional inflammatory markers could also be useful in the evaluation of other drugs selected to be studied using this model in the future.

*Kinetics and kinematics*

Horses in this study were evaluated for lameness by subjective assessment as well as by force plate analysis utilizing peak vertical force forelimb symmetry at the trot. There is much more kinetic evaluation that can be performed than what was examined in the current study. For example, impulse at the trot has been shown to have good potential for reflecting lameness[69] as it accounts for the time the leg is on the ground as well as the

forces exerted against the ground. Breaking and propulsion forces can also provide further insight into the lameness that occurs. While the trot is most commonly studied, ground reaction forces at the walk may also be impacted by different treatments, and could therefore be measured.

The horses in this study had light reflective markers placed on bony landmarks for kinematic evaluation. This data which was acquired simultaneously with the force plate data, so that the data can be directly compared; however, no evaluation of this data has occurred to date. Analysis of this data may be beneficial as joint angles and/or head and neck movements may have altered in response to surgery or treatment. Future studies could focus more on specific kinematic adaptations and responses to treatment post-arthroscopically with different medications.

Overall, the addition of more horses, different drugs, and further kinetic and kinematic analysis can be expected to provide more insight into the joint environment in post-arthroscopy, guiding practitioners to the most appropriate post-surgical intra-articular therapeutic options.

# References

1. McIlwraith CW, Yovich JV, Martin GS. Arthroscopic surgery for the treatment of osteochondral chip fractures in the equine carpus. *J Am Vet Med Assoc* 1987;191:531-540.

2. Kawcak CE, McIlwraith C.W. Proximodorsal first phalanx osteochondral chip fragmentation in 336 horses. *Equine Vet J* 1994;26:392-396.

3. Cleary OB, Trumble TN, Merritt KA, et al. Effect of exercise and osteochondral injury on synovial fluid and serum concentrations of carboxy-terminal telopeptide fragments of type II collagen in racehorses. *Am J Vet Res* 2010;71:33-40.

4. Trumble TN, Scarbrough AB, Brown MP. Osteochondral injury increases type II collagen degradation products (C2C) in synovial fluid of Thoroughbred racehorses. *Osteoarthritis Cartilage* 2009;17:371-374.

5. Frisbie DD, Kawcak CE, Baxter GM, et al. Effects of 6alpha-methylprednisolone acetate on an equine osteochondral fragment exercise model. *Am J Vet Res* 1998;59:1619-1628.

6. Boyce M. Biomarker Response to Acute Joint Injury in a Non-terminal Animal Model of Post-traumatic Osteoarthritis. Ph.D. Dissertation, *Veterinary Population Medicine*. University of Minnesota, St Paul: Minnesota, 2012;190.

7. Jacobsen S, Nielsen JV, Kjelgaard-Hansen M, et al. Acute phase response to surgery of varying intensity in horses: a preliminary study. *Vet Surg* 2009;38:762-769.

8. Fubini SL, Erb HN, Freeman KP, et al. Prognostic factors affecting survival of 507 horses with joint disease: (1983 to 1990). *Can J Vet Res* 1999;63:253-260.

9. Yovich JV, McIlwraith CW. Arthroscopic surgery for osteochondral fractures of the proximal phalanx of the metacarpophalangeal and metatarsophalangeal (fetlock) joints in horses. *J Am Vet Med Assoc* 1986;188:273-279.

10. Yamamoto Y, Ide T, Ono T, et al. Usefulness of arthroscopic surgery in hip trauma cases. *Arthroscopy* 2003;19:269-273.

11. Parisien JS. Arthroscopic treatment of osteochondral lesions of the talus. *Am J Sports Med* 1986;14:211-217.

12. Poole AR. Chapter 3: The Normal Synovial Joint: OARSI Primer, 2010. http:\\primer.oarsi.org.
13. Salter DM, Buckwalter JA, Sandell LJ. Chapter 4: Pathology of OA. 2010 ed: OARSI Primer, 2010. http:\\primer.oarsi.org.
14. van der Kraan PM, van den Berg WB. Chapter 5: OA Pathogenesis: OARSI Primer, 2010. http:\\primer.oarsi.org.
15. Fjordbakk CT, Strand E, Milde AK, et al. Osteochondral fragments involving the dorsomedial aspect of the proximal interphalangeal joint in young horses: 6 cases (1997-2006). *J Am Vet Med Assoc* 2007;230:1498-1501.
16. Foland JW, McIlwraith CW, Trotter GW, et al. Effect of betamethasone and exercise on equine carpal joints with osteochondral fragments. *Vet Surg* 1994;23:369-376.
17. Kawcak CE, Norrdin RW, Frisbie DD, et al. Effects of osteochondral fragmentation and intra-articular triamcinolone acetonide treatment on subchondral bone in the equine carpus. *Equine Vet J* 1998;30:66-71.
18. Thein R, Haviv B, Kidron A, et al. Intra-articular injection of hyaluronic acid following arthroscopic partial meniscectomy of the knee. *Orthopedics* 2010;33:724.
19. Hempfling H. Intra-articular hyaluronic acid after knee arthroscopy: a two-year study. *Knee Surg Sports Traumatol Arthrosc* 2007;15:537-546.
20. Townshend D, Emmerson K, Jones S, et al. Intra-articular injection versus portal infiltration of 0.5% bupivacaine following arthroscopy of the knee: a prospective, randomised double-blinded trial. *J Bone Joint Surg Br* 2009;91:601-603.
21. Raj N, Sehgal A, Hall JE, et al. Comparison of the analgesic efficacy and plasma concentrations of high-dose intra-articular and intramuscular morphine for knee arthroscopy. *Eur J Anaesthesiol* 2004;21:932-937.
22. Batra YK, Mahajan R, Bangalia SK, et al. Bupivacaine/ketamine is superior to intra-articular ketamine analgesia following arthroscopic knee surgery. *Can J Anaesth* 2005;52:832-836.
23. Lindegaard C, Frost AB, Thomsen MH, et al. Pharmacokinetics of intra-articular morphine in horses with lipopolysaccharide-induced synovitis. *Vet Anaesth Analg* 2010;37:186-195.

24. Schaefer EC, Stewart AA, Durgam SS, et al. Effects of sodium hyaluronate and triamcinolone acetonide on glucosaminoglycan metabolism in equine articular chondrocytes treated with interleukin-1. *Am J Vet Res* 2009;70:1494-1501.

25. Garvican ER, Vaughan-Thomas A, Redmond C, et al. MMP-mediated collagen breakdown induced by activated protein C in equine cartilage is reduced by corticosteroids. *J Orthop Res* 2010;28:370-378.

26. Guidolin DD, Ronchetti IP, Lini E, et al. Morphological analysis of articular cartilage biopsies from a randomized, clinical study comparing the effects of 500-730 kDa sodium hyaluronate (Hyalgan) and methylprednisolone acetate on primary osteoarthritis of the knee. *Osteoarthritis Cartilage* 2001;9:371-381.

27. Bolt DM, Ishihara A, Weisbrode SE, et al. Effects of triamcinolone acetonide, sodium hyaluronate, amikacin sulfate, and mepivacaine hydrochloride, alone and in combination, on morphology and matrix composition of lipopolysaccharide-challenged and unchallenged equine articular cartilage explants. *Am J Vet Res* 2008;69:861-867.

28. Dechant JE, Baxter GM, Frisbie DD, et al. Effects of dosage titration of methylprednisolone acetate and triamcinolone acetonide on interleukin-1-conditioned equine articular cartilage explants in vitro. *Equine Vet J* 2003;35:444-450.

29. Frisbie DD, Kawcak CE, Trotter GW, et al. Effects of triamcinolone acetonide on an in vivo equine osteochondral fragment exercise model. *Equine Vet J* 1997;29:349-359.

30. Murray RC, Znaor N, Tanner KE, et al. The effect of intra-articular methylprednisolone acetate and exercise on equine carpal subchondral and cancellous bone microhardness. *Equine Vet J* 2002;34:306-310.

31. Labens R, Mellor DJ, Voute LC. Retrospective study of the effect of intra-articular treatment of osteoarthritis of the distal tarsal joints in 51 horses. *Vet Rec* 2007;161:611-616.

32. Kay AT, Bolt DM, Ishihara A, et al. Anti-inflammatory and analgesic effects of intra-articular injection of triamcinolone acetonide, mepivacaine hydrochloride, or both on lipopolysaccharide-induced lameness in horses. *Am J Vet Res* 2008;69:1646-1654.

33. Masferrer JL, Seibert K. Regulation of prostaglandin synthesis by glucocorticoids. *Receptor* 1994;4:25-30.

34. Smith MD, Wetherall M, Darby T, et al. A randomized placebo-controlled trial of arthroscopic lavage versus lavage plus intra-articular corticosteroids in the management of symptomatic osteoarthritis of the knee. *Rheumatology (Oxford)* 2003;42:1477-1485.
35. Wang JJ, Ho ST, Lee SC, et al. Intraarticular triamcinolone acetonide for pain control after arthroscopic knee surgery. *Anesth Analg* 1998;87:1113-1116.
36. Saari H, Tulamo RM, Konttinen YT, et al. Methylprednisolone acetate induced release of cartilage proteoglycans: determination by high performance liquid chromatography. *Ann Rheum Dis* 1992;51:214-219.
37. Chunekamrai S, Krook LP, Lust G, et al. Changes in articular cartilage after intra-articular injections of methylprednisolone acetate in horses. *Am J Vet Res* 1989;50:1733-1741.
38. Todhunter PG, Kincaid SA, Todhunter RJ, et al. Immunohistochemical analysis of an equine model of synovitis-induced arthritis. *Am J Vet Res* 1996;57:1080-1093.
39. Carter BG, Bertone AL, Weisbrode SE, et al. Influence of methylprednisolone acetate on osteochondral healing in exercised tarsocrural joints of horses. *Am J Vet Res* 1996;57:914-922.
40. Billinghurst RC, Dahlberg L, Ionescu M, et al. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. *J Clin Invest* 1997;99:1534-1545.
41. Nelson F, Dahlberg L, Laverty S, et al. Evidence for altered synthesis of type II collagen in patients with osteoarthritis. *J Clin Invest* 1998;102:2115-2125.
42. Loeser RF, Yammani RR, Carlson CS, et al. Articular chondrocytes express the receptor for advanced glycation end products: Potential role in osteoarthritis. *Arthritis Rheum* 2005;52:2376-2385.
43. Celeste C, Ionescu M, Robin Poole A, et al. Repeated intraarticular injections of triamcinolone acetonide alter cartilage matrix metabolism measured by biomarkers in synovial fluid. *J Orthop Res* 2005;23:602-610.
44. Ley C, Ekman S, Roneus B, et al. Interleukin-6 and high mobility group box protein-1 in synovial membranes and osteochondral fragments in equine osteoarthritis. *Res Vet Sci* 2009;86:490-497.

45. Brown MP, Trumble TN, Merritt KA. High-mobility group box chromosomal protein 1 as a potential inflammatory biomarker of joint injury in Thoroughbreds. *Am J Vet Res* 2009;70:1230-1235.

46. Tung JT, Venta PJ, Caron JP. Inducible nitric oxide expression in equine articular chondrocytes: effects of antiinflammatory compounds. *Osteoarthritis Cartilage* 2002;10:5-12.

47. Abramson SB. Nitric oxide in inflammation and pain associated with osteoarthritis. *Arthritis Res Ther* 2008;10 Suppl 2:S2.

48. Bird JL, May S, Bayliss MT. Nitric oxide inhibits aggrecan degradation in explant cultures of equine articular cartilage. *Equine Vet J* 2000;32:133-139.

49. Murrell GA, Jang D, Williams RJ. Nitric oxide activates metalloprotease enzymes in articular cartilage. *Biochem Biophys Res Commun* 1995;206:15-21.

50. van der Harst M, Bull S, Brama PA, et al. Nitrite and nitrotyrosine concentrations in articular cartilage, subchondral bone, and trabecular bone of normal juvenile, normal adult, and osteoarthritic adult equine metacarpophalangeal joints. *J Rheumatol* 2006;33:1662-1667.

51. Garvican ER, Vaughan-Thomas A, Innes JF, et al. Biomarkers of cartilage turnover. Part 1: Markers of collagen degradation and synthesis. *Vet J* 2010;185:36-42.

52. Nicholson AM, Trumble TN, Merritt KA, et al. Associations of horse age, joint type, and osteochondral injury with serum and synovial fluid concentrations of type II collagen biomarkers in Thoroughbreds. *Am J Vet Res* 2010;71:741-749.

53. Frisbie DD, Al-Sobayil F, Billinghurst RC, et al. Changes in synovial fluid and serum biomarkers with exercise and early osteoarthritis in horses. *Osteoarthritis Cartilage* 2008;16:1196-1204.

54. Gineyts E, Mo JA, Ko A, et al. Effects of ibuprofen on molecular markers of cartilage and synovium turnover in patients with knee osteoarthritis. *Ann Rheum Dis* 2004;63:857-861.

55. Karsdal MA, Byrjalsen I, Leeming DJ, et al. Tibolone inhibits bone resorption without secondary positive effects on cartilage degradation. *BMC Musculoskelet Disord* 2008;9:153.

56. Conrozier T, Balblanc JC, Richette P, et al. Early effect of hyaluronic acid intra-articular injections on serum and urine biomarkers in patients with knee osteoarthritis: An open-label observational prospective study. *J Orthop Res* 2012;30:679-685.
57. Robion FC, Doize B, Boure L, et al. Use of synovial fluid markers of cartilage synthesis and turnover to study effects of repeated intra-articular administration of methylprednisolone acetate on articular cartilage in vivo. *J Orthop Res* 2001;19:250-258.
58. Lepage OM, Carstanjen B, Uebelhart D. Non-invasive assessment of equine bone: an update. *Vet J* 2001;161:10-22.
59. Beertsen W, van den Bos T. Alkaline phosphatase induces the mineralization of sheets of collagen implanted subcutaneously in the rat. *J Clin Invest* 1992;89:1974-1980.
60. Trumble TN, Brown MP, Merritt KA, et al. Joint dependent concentrations of bone alkaline phosphatase in serum and synovial fluids of horses with osteochondral injury: an analytical and clinical validation. *Osteoarthritis Cartilage* 2008;16:779-786.
61. Fuller CJ, Barr AR, Sharif M, et al. Cross-sectional comparison of synovial fluid biochemical markers in equine osteoarthritis and the correlation of these markers with articular cartilage damage. *Osteoarthritis Cartilage* 2001;9:49-55.
62. Kong SY, Stabler TV, Criscione LG, et al. Diurnal variation of serum and urine biomarkers in patients with radiographic knee osteoarthritis. *Arthritis Rheum* 2006;54:2496-2504.
63. Woitge H S-NHW, and others. Seasonal variation in biochemical indexes of bone tunrover: results of a population based study. *Journal of Clinical Endocrinology and Metabolism* 1998;83:68-75.
64. Raekallio M, Taylor PM, Bennett RC. Preliminary investigations of pain and analgesia assessment in horses administered phenylbutazone or placebo after arthroscopic surgery. *Vet Surg* 1997;26:150-155.
65. Doucet MY, Bertone AL, Hendrickson D, et al. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc* 2008;232:91-97.
66. Swanson T. Guide for Veterinary Service and Judging of Equestrian Events 3rd ed. Golden,CO: Am Assoc Eq Pract, 1984;24.

67. Hewetson M, Christley RM, Hunt ID, et al. Investigations of the reliability of observational gait analysis for the assessment of lameness in horses. *Vet Rec* 2006;158:852-857.

68. Parkes RS, Weller R, Groth AM, et al. Evidence of the development of 'domain-restricted' expertise in the recognition of asymmetric motion characteristics of hindlimb lameness in the horse. *Equine Vet J* 2009;41:112-117.

69. Ishihara A, Bertone AL, Rajala-Schultz PJ. Association between subjective lameness grade and kinetic gait parameters in horses with experimentally induced forelimb lameness. *Am J Vet Res* 2005;66:1805-1815.

70. Fradette ME, Celeste C, Richard H, et al. Effects of continuous oral administration of phenylbutazone on biomarkers of cartilage and bone metabolism in horses. *Am J Vet Res* 2007;68:128-133.

71. Ley C, Ekman S, Elmen A, et al. Interleukin-6 and tumour necrosis factor in synovial fluid from horses with carpal joint pathology. *J Vet Med A Physiol Pathol Clin Med* 2007;54:346-351.

72. Frisbie DD, Ray CS, Ionescu M, et al. Measurement of synovial fluid and serum concentrations of the 846 epitope of chondroitin sulfate and of carboxy propeptides of type II procollagen for diagnosis of osteochondral fragmentation in horses. *Am J Vet Res* 1999;60:306-309.

73. Brama PA, van den Boom R, DeGroott J, et al. Collagenase-1 (MMP-1) activity in equine synovial fluid: influence of age, joint pathology, exercise and repeated arthrocentesis. *Equine Vet J* 2004;36:34-40.

74. Fietz S, Einspanier R, Hoppner S, et al. Determination of MMP-2 and -9 activities in synovial fluid of horses with osteoarthritic and arthritic joint diseases using gelatin zymography and immunocapture activity assays. *Equine Vet J* 2008;40:266-271.

75. Christgau S, Garnero P, Fledelius C, et al. Collagen type II C-telopeptide fragments as an index of cartilage degradation. *Bone* 2001;29:209-215.

76. Sondergaard BC, Henriksen K, Wulf H, et al. Relative contribution of matrix metalloprotease and cysteine protease activities to cytokine-stimulated articular cartilage degradation. *Osteoarthritis Cartilage* 2006;14:738-748.

77. Charni-Ben Tabassi N, Desmarais S, Bay-Jensen AC, et al. The type II collagen fragments Helix-II and CTX-II reveal different enzymatic pathways of human cartilage collagen degradation. *Osteoarthritis Cartilage* 2008;16:1183-1191.

78. Fosang AJ, Stanton H, Little CB, et al. Neoepitopes as biomarkers of cartilage catabolism. *Inflamm Res* 2003;52:277-282.

79. Vinardell T, Dejica V, Poole AR, et al. Evidence to suggest that cathepsin K degrades articular cartilage in naturally occurring equine osteoarthritis. *Osteoarthritis Cartilage* 2009;17:375-383.

80. Dejica VM, Mort JS, Laverty S, et al. Increased type II collagen cleavage by cathepsin K and collagenase activities with aging and osteoarthritis in human articular cartilage. *Arthritis Res Ther* 2012;14:R113.

81. Lindstrom E, Vrang L, Sedig S, et al. Effect of the Selective Cathepsin K Inhibitor MIV-7H on Markers of Cartilage Degradation and on Gross and Histopathology Scores in Dogs Subjected to Partial Medial Meniscectomy, an Experimental Model of Osteoarthritis. *Osteoarthritis Cartilage* 2011; 19:S 59.

82. McDougall JJ, Schuelert N, Bowyer J. Cathepsin K inhibition reduces CTXII levels and joint pain in the guinea pig model of spontaneous osteoarthritis. *Osteoarthritis Cartilage* 2010;18:1355-1357.

83. Kydd AS, Reno CR, Tsao HW, et al. Early inflammatory arthritis in the rabbit: the influence of intraarticular and systemic corticosteroids on mRNA levels in connective tissues of the knee. *J Rheumatol* 2007;34:130-139.

84. Maricic M. Update on glucocorticoid-induced osteoporosis. *Rheum Dis Clin North Am* 2011;37:415-431

85. Poole. NIH White Paper: The Osteoarthritis Initiative, 2000.

86. Trumble TN, Groschen DM, Ha N, et al. New urine biomarker assay compares to synovial fluid changes after acute joint injury in an equine model of osteoarthritis. *Osteoarthritis Cartilage* 2012; 20:S 91