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The prevalence of arthritis in the United States has determined the need for increased evaluation of pathophysiologic mechanisms of the disease. Dynamic evaluation of the vascular and transsynovial fluid of osteoarthritis (OA) joints is critical to the understanding and anticipation of the response to medication and physiologic challenges in humans and animal species. In addition to basic hemodynamic interactions, the control of the alterations between the OA and control joint must be established on both the organ and cellular levels.

Multiple models of OA have been developed and used. Despite the common athletic injuries leading to OA in the equine, no such model representing these changes has been documented. Initially, a novel model of instability induce OA was established by transecting the lateral collateral and lateral collateral sesamoidean ligaments of the metacarpophalangeal (MCP) joint in the horse. Basic criteria for lameness, increased joint circumference and decreased range of motion, similar to that of naturally-occurring OA, was met with this model. This model provided clinical and gross features of classic OA, making it useful for additional studies including physiologic, diagnostic and therapeutic studies. Consistency of clinical signs and gross lesions indicated the comparable nature of these horses to those with naturally-acquired OA.

This model of OA was induced in horses for the measurement of codependent hemodynamic and transsynovial fluid flow parameters with the isolated, stationary preparation. A significant increase in total vascular resistance, a decrease in joint blood
flow and a decrease in synovial fluid production in OA joints compared to control joints. The mechanism behind the increased resistance is unknown, but thought to be related to either changes in sympathetic stimulation or increased fibrosis of the joint capsule in the OA joints. These data suggest OA caused significant alterations in baseline and physiologic responsiveness to hemodynamic manipulation.

Following this study, we challenged OA and control joints with vasoactive, constrictive and dilatory, agents to document hemodynamic alterations or effects of these agents. The vasoactive drugs (dopamine, norepinephrine, esmolol, nitroglycerin and LNAME) were administered in random order intraarterially in the isolated OA or control joint. Vascular and transsynovial parameters were measured after each drug was administered. Isolated OA joints maintained and increased vascular resistance, but were less responsive to vasoconstrictive agents when compared to control joints. In addition, OA joints maintained higher pressures for longer duration following vasoconstriction.

Synovial membrane from OA and control joints were placed in modified flux chambers to evaluate ion and protein transport. Radioactive Na and Cl were added to the nonvillous side without drug, or with the addition of 1 of 4 vasoactive agents used in the previous study. Timed samples were collected from the villous side to determine flux across the membrane, similar to the filtration process in vivo. Membrane from the OA joints was slower to initiate flux with all drugs except esmolol. Esmolol demonstrated an increased flux in OA membrane compared to control. This is attributed to a non-receptor-mediated change. This study indicated a need for additional studies of solute transport and protein movement across synovial membrane under specific cellular control.
Dedicated to Mom and Grandpa Frank
ACKNOWLEDGMENTS

I wish to thank my adviser, Dr. Alicia Bertone, for scientific support and encouragement, making this endeavor possible. I realize I needed a bit of extra “molding” and appreciate the time and effort put towards my success. Also, I thank Dr William Muir for great ideas and the use of his equipment. Dr Weisbrode for his encouraging attitude and Dr Cooke for her understanding!

I thank Dr. Rick Sams for his countless hours of advice and coffee. I thank (and curse) Dr. Joanne Hardy for everything (like designing the prep in the first place), especially all of her surgical expertise! I thank Bonnie Bragdon for her early mornings, and Holly for the daily kick in the pants! I would especially like to thank my Mother and Anne, Bryan, Kenner and Zachary for every aspect of support and encouragement that they have given me. I wish Grandpa could have been here to tell me how this paralleled his time at the University of California at Davis. But I know He has been with me throughout this endeavor!! Thanks Grandpa!
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**Abstracts**


Bertone AL, Hardy J, **Simmons EJ.** Muir WW. Physiologic responses of a novel isolated joint model. *Advances in Veterinary Medicine Abstracts,* 1995:16

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Proceedings and abstracts


Published Abstracts


Simmons EJ, Bertone AL, Weisbrode SE. Instability model of osteoarthritis in the horse. Trans 44th Annual Meeting Orthopedic Research Society, 1998:


**Poster sessions**


Bertone AL, Hardy J, Simmons EJ, Muir WW. Joint physiologic response to vascular manipulation. 2nd Life Sciences Research Exposition. The Ohio State University, Columbus, OH, Feb 28, 1997.


FIELDS OF STUDY

Major Field: Equine Orthopedics
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CHAPTER 1

REVIEW OF THE LITERATURE

Arthritis is the most common joint disease in man and the most crippling disease in the United States.\(^1\) Arthritis is still the leading cause of chronic painful, debilitating disease in the aging human population, and joint injuries are among the leading causes of morbidity and lost performance of human athletes. An estimated 40 million Americans have arthritis and one out of 3 families have their lives affected by someone with arthritis. The prevalence is greater in people over 45, and in women. The Centers for Disease Control reports that arthritis now limits major activities such as working or housekeeping for 6.7 million Americans. The overall most common type of arthritis is osteoarthritis (OA) which affects one out of 12 people at a given time and almost everyone at some time in their life.\(^1\) The estimated costs of OA to the US economy is 54.6 billion (1\% GNP) in medical care and lost work with 427 million days of restricted activity, 156 million days in bed and 45 million days lost from work. Osteoarthritis is the leading cause of industrial absenteeism and the second leading cause of disability payments after heart disease. Expenses for medical care and treatment are estimated at $150,000 per person lifetime.\(^1\)

Osteoarthritis is a species nonspecific condition in which the degradation process of cartilage exceeds the synthesis process.\(^2\) Osteoarthritis represents deterioration of the articular cartilage combined with osteophyte formation and sclerosis of the subchondral
bone. Enzymatic degradation accompanied mechanical wear to produce the clinical and histopathologic alterations in the joint tissues. Fibrillation of the cartilage and sclerosis of the subchondral bone leads to decreased shock absorbing capability of these tissues. Histologically, OA joints show evidence of fibrillation of the articular cartilage at various depths, chondrone formation, and surface eburnation. Bony changes that accompany the cartilage alterations include subchondral sclerosis and osteophyte formation. Biochemically, increased degradative enzymes decrease the proteoglycan content and alter collagen formation. Eventually this degradation exceeds the chondrocytes ability to repair the cartilage constituents, leading to breakdown of the cartilage.

Muscloskeletal disorders, including OA, are the leading causes of morbidity in the equine racing industry. Research completed on all breeds of racehorses found 84.6% of all injuries to be musculoskeletal in nature. Most of these injuries (90.2%) occurred in the forelimb. 85.5% of which involved the leg from the carpus to the pastern. The metacarpophalangeal joint (MCP) had the largest number of degenerative lesions of any limb joint in the racing horse. Often these injuries are related to excessive joint motion and excursion due to uneven ground, overtraining, immaturity, poor conformation, or improper shoeing, similar to human athletes. With repeated use (age) or excessive use (athletes) of human and horse joints, the tissues will wear, become inflamed and produce pain ie, OA develops.

There are currently a multitude of animals used for research of various arthritides. Some models are specifically used for OA research while others are used to mimic rheumatoid-type arthritis. These OA models can be categorized into instability models and non-instability models. To date, the primary instability model, the canine Pond-Nuki model, has been used to mimic classic OA by surgically simulating anterior cruciate ligament (ACL) rupture in the human athlete, thereby creating exaggerated mechanical joint
trauma. Mechanical, biochemical and metabolic changes of both articular cartilage and synovial membrane contribute to the breakdown of the joint components in this model. The mechanical instability following transection of the ACL produces joint laxity, loss of joint congruency and abnormal cartilage loading that can directly and indirectly induce abnormal cartilage wear. Subsequent changes in the articular cartilage and synovium worsen the damage by increasing the rate of degradation via stimulation of synthesis and release of cytokines and other inflammatory mediators such as collagenase, stromelysin and interleukins. This model produces minimal synovial changes including a mild monocytic infiltrate, lining cell hyperplasia and lymphoid aggregation. Other models of instability-induced OA include the medial meniscectomy model used in sheep and rabbits. Both models assess the biochemical and biomechanical changes of the OA joint and are used to evaluate the efficacy of pharmacologic agents. Until now, no such instability model has been documented in the horse, despite the wide clinical occurrence of OA in horses, especially equine athletes.

Noninstability animal models of OA include intraarticular injection of various irritants and antibiotics. Intraarticular monoiridoacetate (MIA) has been used in horses, dogs and guinea pigs. This irritant inhibits glycolysis in the chondrocyte, inducing cell death and matrix destruction. The result is fibrillation, decreased uronic acid content and decreased chondrocyte numbers. In addition, fibrillation and osteophyte formation are evident within 6 weeks of injection. These changes are attributed to mechanical factors superimposed on compromised cartilage. Joint space is noticeably diminished on radiographs of MIA injected joints. The primary limitations of this model include pain, extensive and unpredictable chondrocyte loss and a marked synovial inflammatory response not typical of spontaneous OA. Carrageenan, another irritant, produces
synovial inflammation that is substantially greater than that of MIA. Intraarticular injection produces local activation of the complement pathway with subsequent rupture of the lysosomes, releasing enzymes that degrade the joint. This irritant causes a decreased proteoglycan synthesis, initially, that eventually recovers and exceeds normal synthesis. Multiple, painful injections, requiring anesthesia, is a disadvantage of this model, as is the intense synovitis, not typical of naturally-occurring OA.

Two different antibiotics have been used as models of OA. Intraarticular filipin initially causes an acute synovitis followed by degenerative changes in the articular cartilage. Arthritis is induced by disrupting lysosomes primarily affecting the synovial membrane, thereby inducing cartilage destruction, stiffness and thickening of the joint capsule and periarticular tissue. Filipin must be injected multiple times with careful monitoring of the animals to ensure that the joint destruction is not inhumane. Intraarticular injection of amphotericin B develops a greater synovitis than filipin. Cartilage changes with this antibiotic are not consistent. Freuds complete adjuvant causes primarily a synovitis with carpal swelling, decreased range of motion and lameness of a two month duration. Lipopolysaccharide is primarily inflammatory in nature, developing systemic signs of inflammation (fever) and requiring repeated injections for chronic effects.

Cartilage fragments have been injected into the synovial cavity of normal joints to induce changes consistent with natural OA. These fragments tend to induce the release of inflammatory mediators and enzymes such as collagenase at 3 months after injection. Within 5 months, there was documentable pannus with macroscopic cartilage changes. Repeated injection, however, induced an undesirable antibody formation to Type II collagen.

Equine fracture models show variable signs of OA with changes that were inconsistent due to the position and size of the fracture. Cartilage erosion occurred around
the chip fractures and lameness was variable. This model induced an inflammatory component associated with the fracture, unpredictable motion of the fragment and physical damage of the fragment that are not typical of most naturally-occurring or post-injury OA in humans.

Ideally, an equine model of OA could be produced in a short period of time without injection of an exogenous agent or material, to most appropriately mimic naturally-occurring joint disease. Such a model would exhibit classic clinical signs of OA including mild to moderate lameness, mildly decreased range of motion (stiffness), and slightly increased joint circumference. This model should induce classic gross features of OA including marginal osteophytosis, articular surface score lines and surface erosions and a mild inflammatory response of the synovium. Classic histological changes would support the gross findings with altered quality and quantity of staining of cartilage sections. Such a model would provide an excellent resource to evaluate the effects of OA on the physiology of the joint. Also, this model would be beneficial for the study of pharmacological agents.

Extensive research has focused on the role of articular cartilage in OA, but the contribution of the synovium and other soft tissues has received less emphasis. Synovial pathology and malfunction is present in all forms of painful joints disorders. The synovial membrane influences the initiation and perpetuation of articular degradation leading to chronic OA. The synovial membrane is responsible for the composition of synovial fluid through ultrafiltration of plasma and hyaluronate production. In acute inflammation, perfusion of the synovial membrane is altered through changes in capillary permeability, joint distension, and distribution of blood flow. These changes affect synovial fluid quality and quantity, therefore altering cartilage nutrition. Despite its important role in the pathogenesis of articular pathology, knowledge is limited on synovial transcapillary exchanges, perfusion, capillary permeability and sequential inflammatory...
mediator production associated with these transsynovial fluid movements. The role of the synovial membrane physiology and function in normal and OA joints deserves greater investigation.

The basic physiology of joint disease is thought to be related to a number of general hemodynamic concepts. To this point, the principles defining circulation in the isolated OA joint have not been established. Poiseuille's law relates blood flow to the change in pressure (P), vessel length (l) and radius and viscosity (η) of the blood traveling through this vessel. Small changes in vessel radius (r) play the most important role in flow (Q) as determined by this equation:

\[ Q = \frac{\pi (P_1 - P_0) r^4}{8 \eta l} \]

This equation defines the degree to which a small change in vessel radius, changes flow to the fourth power. Here \( P_1 \) is the current pressure, \( P_0 \) is the initial pressure. Therefore vasoconstriction and dilation are very significant in blood flow.

Ohms law is an electrical term defining a relation of voltage (pressure) as a product of current (flow) times resistance. Pressure has a two-fold effect on the vessels and on flow. First, blood pressure "pushes" on the blood within the vessel to propel it forward. Second the pressure "pushes" on the vessel wall, distending its wall and decreasing resistance, thereby, increasing flow. \(^{29}\)

Control of blood flow occurs acutely and over the long term, depending on the situation. With direct increases in tissue metabolism, there is a decrease in oxygen availability. This stimulates the release of vasodilators to increase flow and oxygenate the smooth muscle that surround vessels. The circulatory system maintains homeostasis by autoregulation. Two theories behind this action exist. \(^{30}\) One is related to metabolism. As
pressure increases, flow increases flushing vasodilators out of the system, allowing the vessels to constrict, decreasing flow. Alternatively, the myogenic theory centers around the thought that stretching of the vessel wall will stimulate immediate constriction in response to the wall tension. Flow will then decrease back to normal. This is not thought to be a powerful method of regulation, but is considered to protect the capillaries from excessive pressure.

Perfusion of tissues is dependent on blood flow and oxygen delivery to the tissues. Studies comparing cerebral perfusion following cardiopulmonary bypass with pulsatile and nonpulsatile blood flow have found a few discrepancies. Nonpulsatile flow has been accused of causing increased sympathetic activity through a carotid sinus reflex thereby increasing cerebral vasoconstriction and decreasing flow. This is restored to baseline after approximately 20 minutes, yet an overall decrease in tissue perfusion and flow was found with nonpulsatile flow. In contrast, Shevde et al found a decreased total vascular resistance with improved capillary perfusion and tissue metabolism with pulsatile flow. In addition, pulsatile flow improved liver and kidney blood flow while increased lymph flow, due to the pulsatile expansions of the blood vessels, stimulating the lymph. Hindman et al. found no difference between the two types of flow during cardiopulmonary bypass. There were two theories proposed to explain this finding. The first was that nitric oxide was released when flow was changed in their subjects from one form to another, disrupting the endothelium by shear stress factors. The second theory was based on hemodilution of the blood through the bypass machinery.

Changes in flow, as mentioned previously, affect oxygenation of tissues in both OA and normal joints. Diseased joints are at a more severe disadvantage with decreased flow because of several factors: increased intercapillary distance due to synovial
inflammation or chronic fibrosis, increased risk of tissue hypoxia due to higher intraarticular pressures and increased metabolism due to inflammation. It has been shown that increased interstitial edema, as that found in inflamed synovial membrane, will likely decrease oxygen delivery but will not change oxygen consumption or extraction ratio. Interruptions of the normal pattern of blood flow may have a severe effect on these joints as noted by the above factors. In addition, these changes will increase free radicals within the joint, increasing inflammatory mediators, and possibly worsening the disease state.

The joint is innervated with large myelinated afferent and efferent sympathetic postganglionic nerve endings and small unmyelinated C-fibers. C-fibers store substance P after it is synthesized in the dorsal root ganglion. Substance P, released from unmyelinated C-fibers is proinflammatory in arthritic synovial membrane causing plasma extravasation via increased vascular permeability, vasodilation and mast cell release of inflammatory mediators, including eicosanoids such as prostaglandin E₂ (PGE₂). Such changes lead to protein accumulation and increased effusion that further magnify joint distension, joint pain and lameness, all primary features of acute articular inflammation. The activation threshold of the pain receptors in arthritic synovial membrane may be sensitized by PGE₂, thus enhancing the mechanosensitivity to noxious stimuli. PGE₂ correlates directly with severity of the synovitis, therefore acting as a clinical marker of synovial pathology.

The sympathetic nervous system plays a crucial role in and richly innervates the synovial joint. Levine et al demonstrated that sympathectomy in a rat OA model prevented the development of joint pain and OA. This was further supported by the finding that hypertensive rats, characterized by increased sympathetic activity, had significantly more severe joint pain and arthritis. Sympathetic postganglionic fibers
indirectly increase plasma extravasation through activation of the cyclooxygenase pathway and synthesis of prostaglandins. Beta₂ adrenergic receptors have been shown to be pro-arthritic, where alpha₂ receptors had an anti-arthritic effect.⁴³,⁴⁴ The mechanisms of interest in this study are related to the sympathetic nervous system (alpha₁(α₁) and alpha₂ (α₂) receptors) influence at the cellular and physical (hemodynamic fores) level. Articular blood flow has been shown to be regulated by the sympathetic receptors and neurotransmitters in the synovial membrane of the joint. General information regarding such receptors are tissue specific.⁴⁸-⁴⁹ The densities and distributions of these entities within the joint are not known. In particular, alterations in distribution, density and activity of these elements in arthritic joints have not been identified. Investigations of articular diseases have rarely centered on the joint as an organ, possibly overlooking the interaction of several mechanisms present during the inflammatory and degradative process. Demonstrations of hemodynamic and transsynovial fluid flow alterations following administration of drugs that affect such receptors, may increase our understanding of the interactive mechanisms responsible for changes in light of joint disease.

Vasoreactive agents, that affect such receptors, produce differential responses depending on dose, mechanism of action, receptor availability, substrate availability and length of action. Dopamine (α₁ adreno-receptor mediated vasoconstriction) was used for initial vasoconstriction because its effects resolve quickly as the drug is rapidly metabolized once infusion is stopped. Dopamine has been documented to be effective in equine tissue.⁴⁵ Dopamine primarily stimulates alpha receptors at low and moderate dosages. We administered a dose in the moderate range to affect the alpha receptors. Norepinephrine (α₁ and α₂ receptor mediated vasoconstriction) is a postsynaptic α₁, α₂ adrenergic receptor
agonist that increases blood pressure by inducing vasoconstriction. Norepinephrine
directly stimulates alpha receptors, increasing peripheral vascular resistance and mean
arterial blood pressure following continuous systemic infusion. Esmolol is a rapid-onset,
short acting β1 selective antagonist that induces vasodilation, decreasing total peripheral
resistance.

Nitric oxide (NO), another endogenous vasoactive agent, is synthesized by nitric
oxide synthase (NOS) in the vascular endothelium and is responsible for the regulation of
blood pressure. In equine tissue, NO has been documented in the endothelium,
specifically acting as an endogenous control factor of the vasomotor tone of the
arterioles. It is overproduced in rheumatoid arthritis and OA with a significant role as an
inflammatory mediator in these arthritic conditions. Nitrite, an indirect measure of NO
production, was increased in patients with rheumatoid arthritis and OA compared to human
controls. The powerful vasodilating effects of NO enhance cellular injury in the articular
tissues.

Nitroglycerin stimulates the release of NO, to decrease total peripheral resistance,
by primarily acting on venous capacitance vessels. As the dose is increased there is an
additional increase in the relaxation of arterial vascular smooth muscle. Acting as a
competitive antagonist for the endothelial enzyme NOS, N^G-nitro-L-arginine methyl ester
(L-NAME) blocks NO synthesis, increasing blood pressure via vasoconstriction.
Physiologic responses to nitroglycerin and L-NAME may represent the contribution of
endothelial-dependent vasodilators to the hemodynamics of arthritic joints. Study of nitric
oxide may have important consequence on its emerging pharmacologic actions in arthritic
joints.
It has been widely presumed that Starling forces govern transsynovial fluid flow as in other connective tissue spaces. Starling’s hypothesis predicts that the rate of fluid filtration is a linear function of capillary pressure and interstitial colloid osmotic pressure and a negative linear function of plasma colloid pressure and interstitial pressure. The basic laws of hemodynamics are governed by the following equation:

$$J_v = K_{fc} [(P_c - P_t) - \sigma d(\pi_p - \pi_t)]$$

where $J_v$ describes flow across synovial capillary walls, $K_{fc}$ is the filtration coefficient, $P_c$ is hydrostatic pressure of the capillary, $P_t$ is the interstitial tissue pressure, $\pi_p$ is the plasma osmotic pressure, $\pi_t$ is the osmotic pressure of the interstitial tissue and $\sigma d$ is the osmotic reflection coefficient.

Due to the presence of a large third space (joint cavity), a simple two-compartment model of fluid exchange with the interstitium does not necessarily apply. Many sophisticated and elaborate studies have been performed to indirectly measure or theoretically calculate the main coefficients modifying the flow from plasma to synovial fluid. Unfortunately, most of the studies that have investigated trans-synovial fluid flow and fluid absorption from the synovial cavity infused electrolyte solutions (saline) or oil. Characteristics of infusate were noted to yield differing results. We have subsequently demonstrated that synovial fluid infusate yields significantly lower elastance curves and greater hysteresis in joints than saline.

Fewer studies have evaluated other physiologic parameters separately, including lymphatic flow. Clearance studies from the synovial cavity following intraarticular injection of $^{133}$Xenon or $^{123}$I and $^{131}$I labeled albumin can estimate blood flow, but are limited by the fact that an intraarticular injection of even balanced electrolyte solution
will induce a transient but significant synovitis that would alter blood flow. Metabolic parameters in synovial fluid have been measured to estimate indirectly blood flow relative to oxygen demand, and estimate joint ischemia, but do not provide direct and simultaneous measurements of fluid exchange. In virtually all studies, measured values were either theoretically calculated, inherently accurate, or limited by the number of physiologic parameters that could be evaluated. Presumption of the physiologic responses of OA joints have been based on these indirect or singular supporting observations. Lymphatic drainage from synovitic rabbit stifles was reduced, but the importance of synovial pressure, vascular flow or synovial permeability could not be determined. In a traumatic OA rabbit model, xenon clearance from the joint was significantly increased for greater than 16 weeks, but a relationship to joint effusion, inflammation or blood flow could not be concluded. In evaluation of iodide clearance, rheumatoid joints showed reduced clearance compared to OA joints, but results were highly limited by the inability to control effusion, intra-articular pressure or iatrogenic inflammation. These studies suggest that arthritic joints demonstrate altered hemodynamics and transsynovial fluid management but improved methods for measurement are needed.

An isolated, perfused hindquarter preparation had been used to describe transsynovial flow, but pressure changes and blood flow were not representative of only the joint. Presumption or theoretical calculation of physiologic parameters, therefore, is required due to a lack of an isolated joint model as has been described and repleely investigated for the heart, lung, intestine, hoof and laminae. Recently, we have described a reliable method to create an isolated, autoxygenated, pump-perfused preparation using the equine MCP joint. This model permits simultaneous measurement of codependent changes in blood flow, synovial fluid production and composition, and
interstitial fluid accumulation that is expected to occur in arthritis and acute joint inflammation. To the best of our knowledge, this has not been previously described. The horse is required for this preparation due to the anatomical considerations, including the well-developed singular digit (MCIII) with a simple neurovascular network and lack of musculature. The joint is large enough to allow for ample quantities of synovium, articular cartilage and synovial fluid for multiple analyses. As previously mentioned, the MCP joint had the largest number of degenerative lesions of any limb joint in the racing horse, therefore, providing a good model of degenerative joint disease.

Two components determine transsynovial fluid flow: the capillary endothelium and the synovial intima. Evaluation of the capillary function can be performed in vivo by using the previously described isolated joint model. Differences in transsynovial fluid flow characteristics of OA joints may be controlled by receptors at the synoviocyte level in addition to vasoregulatory control. Use of an Ussing flux chamber has permitted scientists to study the effects of solute transport at the cellular level within the gastrointestinal tract. At this time, no such work has been documented for joint tissue, primarily the synovial membrane. Determination of solute transport control by the synovial membrane intima would enhance the understanding of the regulatory mechanisms of hemodynamics within normal and OA joints. Studies involving radiolabelled sodium and chlorine have allowed for measurement of active and passive transport across intestinal membranes.

Increasing knowledge of the hemodynamic and cellular physiology together with the basic pathology of the osteoarthritic joint, will likely enhance the ability to diagnose, prevent and treat this condition more effectively. A combination of the results documented in this manuscript will tie together some basic principles concerning hemodynamics of the osteoarthritic compared to the normal joint.
References


CHAPTER 2

INSTABILITY-INDUCED OSTEOARTHRITIS IN THE METACARPOPHALANGEAL JOINT OF HORSES

Summary

The objective of this study was to establish an instability model of osteoarthritis (OA) that mimics the early changes of naturally-acquired OA thought to be initiated by joint instability followed by mechanical degradation and articular erosion. Six healthy, mature, radiographically normal horses underwent transection of the lateral collateral and lateral collateral sesamoidean ligaments through a stab incision in a randomly selected metacarpophalangeal (MCP) joint in each horse. Lameness examinations were performed every 7 days after surgery for 8 weeks. Craniocaudal stress radiographs were taken immediately before and after desmotomy and at 8 weeks after surgery. All horses were euthanized 8 weeks after instability surgery. Bilateral MCP joints were evaluated for osteophytes, score lines and surface erosions. Specimens of articular cartilage were harvested for histology and tissue culture. Synovial membrane was harvested for histology.

Lameness scores were significantly increased over time with an average score of 1.6/4 for the 8-week period following surgery. Joint circumference was significantly greater in the OA joint versus the contralateral joint. Range of motion was significantly
decreased over time in the OA joint versus the contralateral joint. The number and size of osteophytes determined radiographically and grossly were significantly greater for the OA limb when compared to the contralateral limb. Newly synthesized proteoglycan was significantly greater at 18 and 72 hours of cartilage explant culture in the OA joints as compared to the contralateral joints. A difference in total PG content and PG degradation was not detected between OA and contralateral joints.

Desmotomy of the lateral collateral and lateral collateral sesamoidean ligaments induced an instability model of naturally-acquired OA in the horse as documented by lameness, clinical signs of osteoarthritis, osteophyte formation, and articular cartilage surface erosions and scorelines in the OA joint.
Introduction

Osteoarthritis (OA) is the most common disease of joints. It is estimated to affect 37 million people in the United States (15% of the population) resulting in 427 million days of restricted activity and 45 million days on which people are unable to work.¹

Musculoskeletal disorders, including OA are leading causes of morbidity in the equine racing industry. Research for all breeds of racehorses found that 84.6% of injuries were musculoskeletal in nature. ² About nine-tenths of those injuries were in the forelimbs, and 85% involved the area of the limb extending from the carpus to the pastern. ² One of the joints most frequently affected in horses by arthritis is the metacarpophalangeal (MCP) joint.⁴ These injuries are related to excessive joint motion and excursion from uneven ground, excessive training, immaturity, poor conformation, or improper shoeing.³ Damage is initiated by mechanical trauma leading to progressive cartilage deterioration. Disruption of cell membranes of articular tissues stimulates release of cytokines and increases production of destructive metalloproteinases such as collagenases and proteoglycanases, the amount of which relate directly to the severity of disease in humans with osteoarthritic cartilage.⁴⁵ Ultimately, proteoglycan (PG) depletion is attributable to an imbalance between PG degradation and PG synthesis.⁵ Synovium participates in the degeneration by the release of additional degradative enzymes and the increase in protease synthesis.⁴⁵

In one model,⁶ dogs have been used to mimic classic OA by surgically creating excessive mechanical trauma to the joint, thereby simulating rupture of the cranial cruciate ligament in the humans. In this model, mechanical, biochemical and metabolic changes of articular cartilage and synovial membrane contribute to the breakdown of joint
components. Mechanical instability after transection of the cranial cruciate ligament produces joint laxity, loss of joint congruency and abnormal cartilage weight bearing and trauma that can directly and indirectly induce abnormal cartilage wear. Subsequent changes in the articular cartilage and synovium worsen the damage. Synovial membrane are believed to release cytokines, including interleukin-1β (IL-1β), that increase progression of articular cartilage degradation by stimulating the synthesis of matrix metalloproteinases such as collagenase and stromelysin. Transection of the cranial cruciate ligament causes minimal changes in the synovium, including infiltration of monocytes, hyperplasia of cells lining the synovium and lymphoid aggregation. Other models of instability-induced OA have been achieved by performing medial meniscectomy in rabbits and sheep. Each model has been used to assess biomechanical and biochemical changes of joints and to provide a model of OA for evaluating efficacy of various pharmacologic agents. A similar instability model has not been developed for horses, despite the widespread clinical prevalence of OA in horses, particularly horses used for athletic activities. The purpose of the study reported here was to develop an instability model of OA in horses that would mimic naturally acquired OA.

In summary, an instability model of OA is needed in the horse, and could mimic naturally-acquired equine and human OA and other well accepted models of OA.

Materials and Methods

Horses and clinical assessments —Six healthy, mature (> 3 years old) horses that were considered normal on the basis of results of physical examination (temperature, pulse and respiration), CBC, lameness examination, and palpation of both MCP joints were included in the study. Complete physical examinations were performed every 30 days. At
the start of the study (baseline values), all horses were given a lameness examination including radiographic evaluation and measurement of joint circumference and range of motion for both MCP joints. Lameness examinations were performed at 7-day intervals. Scoring was on a scale from 1 to 4 (1 = lameness detectable only when horse was trotting; 4 = horse unable to bear weight\(^9\)). Clinically sound horses were not assigned a lameness score. Joint circumference was measured by placing an orthotic measuring tape around the widest part of the MCP joint. Range of motion was measured by a goniometer placed on the lateral aspect of the MCP joint, with one arm of the goniometer extending along the first phalanx and the other arm of the goniometer extending along the metacarpus, with the midportion located over the center of the MCP joint. The joint was then flexed until resistance was met, as evidenced by elevation of the horse's head above the initial neutral starting position. Joint circumference and range of motion were measured on MCP joints of each horse before surgery and for 8 weeks after surgery at 7-day intervals. All horses were euthanatized 8 weeks after surgery by IV administration of an overdose of pentobarbital \(^\text{a}\).

**Creation of MCP joint instability** -- Each horse was prepared for desmotomy of a randomly selected MCP joint. A catheter was placed in the left jugular vein for administration of anesthetics. The selected limb was clipped from the middle of the third metacarpal to the junction between the first and second phalanx. General anesthesia was induced by IV injection of xylazine hydrochloride (0.4 mg/kg of body weight), ketamine hydrochloride (1.1 mg/kg IV), and a 5% solution of guaifenesin (to effect ). Anesthesia was maintained by use of halothane in oxygen. Each horse was placed on a surgery table in lateral recumbency with the surgical limb uppermost. The limb was prepared for aseptic surgery. A 1-ml sample of synovial fluid was collected to enable evaluation of number of
WBC and concentration of total protein (TP). A 1-cm incision was made over the distal palmar MCP pouch immediately proximal to the prominence of the first phalanx. An arthrotomy was created to enable insertion of a bistoury into the joint. The bistoury was directed proximally into the palmar pouch to transect the lateral collateral sesamoidean ligament, then redirected dorsally to transect the lateral collateral ligament from the joint surface outward (Fig 2.1). All subcutaneous tissue and skin remained intact over the desmotomies. One or two sutures were placed to close the skin incisions made for introduction of the bistoury. Horses recovered from anesthesia in a Farley boot and were housed in a box stall (4 x 4 m) for 24 hours. After surgery, horses were walked on a high speed treadmill (5 min/d., 3x/week, for 2 weeks), after which they trotted (15 min/d; 3d/wk, for 6 weeks). Immediately before horses were euthanatized, synovial fluid was collected for analysis of number of WBC and TP concentration.

**Radiographic measurements**—Craniocaudal radiographic views were taken immediately before and after ligamentous transection and at time of euthanasia. With the horse in lateral recumbency, a 4.5 kg weight was suspended from the center of the distal aspect of the horizontally suspended forelimb to compress the joint medially and distract the joint laterally. A goniometer was used to measure the joint space on the radiographs to enable us to compare values among the 3 time points. A standard series of radiographs were taken at the start of the study and the day of euthanasia. Each radiographic view was used to determine number of locations of osteophytes, length of osteophytes, and width of joint spaces.
Postmortem examination—Immediately after each horse was euthanized, both MCP joints were aseptically prepared and incised to allow tissue collection. Photographs were taken to document gross abnormalities including score lines, surface erosions and osteophytes. Number of score lines per cm² of the articular surface and number of osteophytes were recorded, and specimens were then harvested. The number of osteophytes were counted and recorded. Specimens were then harvested. Articular cartilage plugs were taken from the medial and lateral condyles of the distal third metacarpal bone and the medial and lateral articular fovea of the proximal phalanx, placed in a cassette with OCT® media, frozen immediately in liquid nitrogen, and stored in a -70°C until histologic examination. Remainder of the articular cartilage was harvested and pooled for tissue culture. Synovial membrane was harvested from the palmar and lateral surfaces of the joint capsule and placed in neutral-buffered 10% formalin until histologic examination.

Cartilage explant culture -- Pooled cartilage shavings from each joint were placed in plates containing Dulbecco's Modified Eagle's Media® with 10% fetal calf serum, penicillin (50 units/ml), streptomycin (50 µg/ml) and 40 µCi [35S] Na₂SO₄. Cartilage from each joint was divided into 4 wells, 2 wells per plate (100mg/well) and cultured in duplicate. Plates were incubated in 5% carbon dioxide at 37°C with constant stirring. Techniques for these procedures have been reported elsewhere.¹⁰

PG extraction and dialysis -- After incubation for 18 and 72 hours for plates 1 and 2, respectively, plates were removed from the incubator, media was discarded, and the explants (plate 1) or aliquots of explants (plate 2) were washed four times with phosphate-buffered saline (Fig 2.2). Specimens were extracted overnight at 4°C with 4M guanidine
hydrochloride in 0.05M sodium acetate (pH 5.8) with protease inhibitors, EDTA, 6-aminohexanoic acid and benzamidine hydrochloride. Extracts were then dialyzed overnight at 4 C against distilled water (molecular weight cutoff, 6000 to 8,000 kD) to remove unincorporated [\(^{35}\)S] and promote aggregation of PG to available hyaluronic acid in the guanidine hydrochloride extraction. Volume of the nondialyzable extract was recorded, and radioactivity was counted for each horse, using a scintillation counter.

**PG synthesis** -- Explants for plate 1 were removed from the incubator after 18 hours, extracted, and dialyzed as described previously. An aliquot was counted, using the scintillation counter, to determine counts per minute ([CPM]/µg of protein) of newly synthesized PG. The remaining extract was frozen and stored at -70 C for biochemical assays.

**PG degradation** -- Aliquots from plate 2 were removed from the incubator at 72 hours and divided (50 mg/well) into 2 additional plates (2a and 2b). Cartilage of plate 2a was extracted and dialyzed as described previously. An aliquot was counted, using the scintillation counter, to determine incorporation of radiolabelled material before degradation studies were performed. For cartilage in plate 2b, media was removed, fresh media was added, and plates were incubated for an additional 72 hours (total incubation time, 144 hours) to determine 72-hour degradation characteristics. Media from plate 2b was harvested and replaced with fresh media at 24, 48 and 72 hours after the initial media change. Harvested media was stored frozen until analyzed. Samples were thawed to ambient temperature, dialyzed, and counted by use of the scintillation counter to determine quantity of released radiolabelled PG.
Biochemical analysis -- Determination of endogenous glycosaminoglycan (GAG) content of articular cartilage specimens was determined using the dimethylmethylene blue assay on the extracts. Dimethylmethylene blue reagent was prepared by adding 3.04 g of glycine, 2.37 g NaCl and 16 mg of 1,9-dimethylmethylene blue to one liter of deionized water, with the pH adjusted to 3.5 with HCl. Dimethylmethylene blue reagent (2.5 ml) was added to an aliquot of each sample. Absorbance was determined by use of a spectrophotometer at 525 nm. Total GAG concentrations were calculated from the standard chondroitin sulfate curve. After addition of 100 µl of 0.25 units/ml chondroitinase ABC lyase, samples were incubated for 1 hour at 37 C to digest the chondroitin sulfate in each sample. A second aliquot (300 µl) was removed and added to 2.5 ml of dimethylmethylene blue solution to determine absorbance. Difference between the total GAG value and the new concentration was considered the portion of chondroitin sulfate in the sample. We added 50 µl of 0.1 units/ml keratanase to the remainder of each sample and allowed digestion at 37 C for 1 hour. Absorbance after this digest was associated with the cleavage of keratan sulfate. Values were reported as µg/g of protein. Articular cartilage protein concentration was determined by a dye-binding assay using bovine serum albumin as a standard.

Histology -- Synovial membrane was removed from formalin, placed in paraffin, sectioned at a thickness of 6 µm, and stained with H&E. One section per specimen was graded by two investigators (EJS, SEW) for evidence of cellular hyperplasia (scored 0 to 4, semi-quantitatively; based on continuity of synoviocytes on the surface of one side of a villous, 0 is the absence of hyperplasia and 4 is > 76% of surface having a continuous layer of >3 cell thickness, vascularity of the villi (scored 0 to 4; based on the percentage of area of vascularity within the villous with 0 defined as <24% of area as vascular to 4 defined as
> 76% of area as vascular), and inflammation (scored 0 to 4 based on percentage of interstitium within the villi with inflammation; 0 defined as absence of interstitial inflammation to 4 representing > 76% of interstitium of the villi with inflammation.) Investigators unaware of previous treatment (surgical MCP joint vs nonsurgical MCP joint) for each specimen. Mean score was calculated and recorded.

Articular cartilage specimens were cryosectioned (8-μm thick) at -30 C with a microtome. Sections were fixed in cold acetone for 5 minutes and stored at -20 C overnight. Toluidine blue was applied to the sections for 10 minutes, followed by clearing with xylene. Slides were evaluated by the investigators at 10X and 40X magnification for intensity and pattern of toluidine blue stain, cellular morphology and gross disruption of architecture. Routine scoring was not performed. Subjective evaluations were made on individual slides and compared with gross necropsy findings.

**Statistical analysis** -- Quantitative serial data (joint circumference, range of motion, laxity measurement) were compared by a two-factor ANOVA with repeated measures followed by use of a post-hoc Dunnett’s t-test. Serial data for lameness was compared among time periods for each group, using a Friedman ANOVA and Kendall coefficient of concordance test. Quantitative terminal and baseline data for results of radiographic measurement and synovial fluid analysis were compared with a paired t-test. Quantitative data collected only at the time of euthanasia (PG synthesis, PG content, gross measurements) were compared with that for the contralateral MCP joint of the same horse, using a Student’s t-test. Histologic data was compared with that for the contralateral MCP joint of the same horse, using Mann-Whitney rank test. Significance for all tests was P <0.05.
Results

Clinical assessment -- Results for rectal temperature, pulse and respiratory rate, and CBC were within reference range values for all horses during the 8-week period. All horses were clinically sound before surgery. After surgery, lameness scores increased significantly (P<0.0001), with a mean score of 1.6 for the 8-week period (Fig 2.3). Joint circumference significantly (P<0.0001) increased for the OA joints (32.8 +/- 1.4 cm) compared with baseline values (26.8 +/- 0.7 cm) and values for contralateral joints (26.0 +/- 0.8 cm) during the 8-week period (Fig 2.4). Range of motion for the OA joints during the specifies time period significantly (P<0.0001) decreased (mean, 20°), compared with contralateral joints (Fig 2.5).

Synovial fluid analysis -- Analysis of synovial fluid yielded WBC and TP values within reference ranges. Values before surgery (600 +/- 124 cells/µl; 2.0 g/dl) were similar to those at 8 weeks after surgery (267 +/- 99 cells/µl; 2.0 g/dl).

Radiographic measurements -- Stressed laxity measurements were significantly (P<0.0001) greater immediately after surgery and at 8 weeks after surgery for the OA joints (7.2 +/- 0.6 mm; 6.2 +/- 0.7 mm, respectively; Fig 2.6), compared with the contralateral joints (2.1 +/- 0.0 mm). Number (mean, 2) and size (mean, 2.67 mm) of osteophytes detected on radiographs obtained at time of euthanasia were significantly (P<0.0001; Fig 2.7) greater than for OA joints before surgery. Osteophytes did not develop in any of the contralateral joints.

Gross findings -- Gross evaluation revealed the number of score lines was significantly (P<0.0001) greater for OA joints (mean, 2.8 lines/cm), compared with contralateral MCP.
joints (mean, 0.0 lines/cm; Fig 2.8). Number (mean, 2.6) and size (mean, 3mm) of osteophytes detected during gross examination were also significantly (P<0.0001) greater than for the contralateral joints. Osteophytes were found on the dorsolateral aspect of the proximal phalanx and along the sesamoids (Fig 2.8). Erosions of the articular surface were found on the dorsomedial aspect of the lateral plateau of proximal phalanx in most of the OA joints. Score lines, osteophytes, and erosions were not found in the contralateral joints.

Biochemical analysis -- The amount of newly synthesized PG was significantly (P<0.0001) greater for the OA joints, compared to the contralateral joints, after 18 and 72 hours of incubation with S^{35} (Table 2.1). Proteoglycan degradation did not differ for articular cartilage from OA joints, compared with cartilage from the contralateral joints, at any time point (Table 2.2).

Total GAG content was similar between articular cartilage from OA and contralateral joints in explants incubated for 18, 72, or 144 hours. Specific chondroitin sulfate and keratan sulfate content was similar between OA and contralateral joints (Table 2.3).

Histology -- Hyperplasia, vascularity, or inflammation of synovium did not differ between OA and contralateral joints. In most specimens, articular cartilage from OA joints had reduced staining of interterritorial matrix and reduction in PG staining in the territorial zone immediately adjacent to the chondrocytes in most specimens. Sites of fissures and adjacent chondrone formation were identified (Fig 2.9, arrow on chondrone). Osteophytes revealed typical surface fibrocartilage and woven bone formation (Fig 2.10).
Discussion

Analysis of results of the study here indicated that our instability model can be used to establish OA in the MCP joint of horses. Classic clinical signs and necropsy findings for lameness, joint enlargement, stiffness and osteophyte formation were evident within 8 weeks. All horses were lame for the entire 8 week period and had the same lameness score for the last 2 weeks of the study. This model produced minimal synovial membrane and fluid changes, similar to naturally acquired OA in human beings. In contrast, marked synovial inflammation is produced by chemically-induced OA. Articular cartilage degeneration in the horses of our report also was typical, with formation of score lines, osteophytes and articular surface erosions.

Typical clinical signs of OA induced by use of this technique were lameness, decreased range of joint motion, and increased joint circumference. Lameness in naturally acquired OA is attributable to pain associated with the capsule and subchondral bone early during the course of the disease in affected joints as well as the more chronic mechanical restriction of fibrosis. Pain associated with soft tissues and subchondral bone also contributes to decreased range of motion of OA joints. Chronic restriction of movement is further evidence of fibrosis of the joint capsule. Increased joint circumference of OA joints during the early part of our study could be attributed to surgical trauma and effusion, but fibrosis of the joint capsule was evident at necropsy. Chronic active joint inflammation and effusion was not detected by palpation, and results of synovial fluid analysis at 8 weeks were similar between OA and contralateral joints.

Gross features of OA including surface erosions, osteophytes, and score lines were induced by use of this surgical technique, predominantly on the side of instability (ie, the lateral side of OA joints). In naturally acquired OA of humans, such lesions are documented more frequently in weight-bearing areas that have an increased load. In the unstable MCP joint of our horses, lesions were also evident in areas of increased load and
instability (ie, the distolateral portion of the metacarpus and the proximal portion of the proximal phalanx. Erosions were found on the dorsal aspect of proximal part of the proximal phalanx and on the sagittal ridge of the third metacarpal bone (Fig 2.8). Irregular loss of articular cartilage (erosion) is a common finding in joints affected by naturally acquired OA. Pathogenesis of the surface erosions in not completely understood, but is believed to be initiated by mechanical insult in an area of high load or shear forces. In horses, mechanical causes are related to abnormal forces on a normal articulation or normal to abnormal forces on an abnormal articulation. These insults are associated with excessive joint motion, running on uneven ground, immaturity, excessive training or poor conformation. Abnormal stresses or strains on the articular surfaces modify the normal chondrocyte physiologic processes, altering nutrient availability of synovial fluid and mechanical influence on cellular health.

Alterations in the synovial fluid, early synovial membrane inflammation or abnormal stretching of the joint capsule at its insertion are possible contributors to the formation of osteophytes. Radiographic osteophytes were most apparent on the lateral aspect of proximal PI at the junction of the synovial membrane and perichondrium. Grossly, osteophytes were most apparent laterally along the articular margin of the sesamoid and lateral PI (Fig 2.8). Due to the instability created by lateral collateral desmotomy, increased friction and wear occurred at these areas, likely causing osteophyte formation. These osteophytes might be formed to help restore stability of the joint by pressing directly against a slackened or fibrotic collateral ligament and increasing joint surface area and congruency. The collateral ligament is transected in this model but the osteophyte is likely to provide the same action against the fibrous tissue formed intracapsularly in place of the collateral ligaments.
Score lines were significantly evident on gross (Fig 2.8) and histological specimens (Fig 2.9) from OA joints. Scorelines were diffusely distributed across the surface of all OA joints but were found in conjunction with other changes typical of OA (ie-lameness, osteophytes, erosions). Osteophytes were most prominent laterally, but erosions and scorelines were evident throughout the joint. The score lines represent an uneven wear and use of the articular cartilage in the OA joint as compared to the more typical wear of cartilage in the contralateral joint. The cause of score line formation is uncertain. One theory associates their production with turbulence of the synovial fluid in the joint. It is possible that the alterations in the quantity and quality of synovial fluid and decreased congruency in motion cause the formation of the score lines. The biomechanical changes described are attributed in part to the biochemical alterations in OA joints.

Instability in the MCP joint of horses significantly increased PG synthesis (CPM/μg protein. Table 2.1). Newly synthesized PG on a wet weight of cartilage basis was less significant suggesting an increase in water content of the OA cartilage. This is consistent with the increased water content in cartilage of spontaneous OA. We were unable to detect a significant difference in degradation of newly synthesized PG between cartilage from OA and contralateral joints. We also were unable to detect a significant difference in total PG content of OA joints, compared with contralateral joints. However, total PG content tended to be less in OA cartilage, when compared with that for contralateral joints. This has been documented by other investigators. Increased PG synthesis without a significant change in total PG content early during the development of OA indicates an increased breakdown of macromolecules of the matrix. Additional PG is synthesized, but total PG remains consistent, indicating increased PG degradation. These factors induce a metabolic change in the chondrocytes. In early stages of arthritic environments, chondrocytes increase synthesis in an attempt to repair damaged or traumatized cartilage, similar to the
results for our study. Subsequent release of mediators such as interleukin-1 can influence chondrocytes to increase PG degradation. Increased degradation was not detected in our study and was attributed to dilution of cartilage specimens during pooling of samples from the entire joint. Articular cartilage lesions were most obvious on the lateral aspect of affected joints; however cartilage was harvested from the entire joint. Similar influence of pooling was reported in the sheep lateral meniscectomy model. Total PG is depleted in OA, because degradation (mechanical and chondrocyte) exceeds synthesis. Proteoglycan synthesis will decrease as chondrocytes become damaged or degenerate. By pooling cartilage from all areas of each joint, biochemical effects that were probably most prominent in the areas with greater gross lesions were likely diluted. Future investigations should use specimens for biochemical study harvested only from the more-affected side of the OA joint.

Histological assessment of the synovial membrane of OA joints revealed mild, though nonsignificant, increases in hyperplasia without an increase in inflammation. This illustrated the noninflammatory nature of this technique, similar to OA in other species. Specific sections of articular cartilage had lesions typical of OA, but sections were not routinely scored because of the multifocal nature of the lesions. Focal score lines were lined with chondrones and adjacent areas of decreased territorial staining, indicating a decreased PG content within the matrix (Fig 2.9). Osteophytes had a dramatic decrease in staining and were consistent with fibrocartilage (Fig 2.10). These changes are similar to those described as typical of naturally acquired OA.

The instability model for horses described here provided clinical and gross features of classic OA. Similarities documented between horses in this study model and those developing naturally acquired OA enhance the usefulness of this technique for additional studies including diagnostic, therapeutic and physiologic studies. Lameness reached a
steady-state at 7 weeks after surgical desmotomy, and osteophytes were radiographically
evident at that time. Consistency of the clinical signs and gross lesions indicate the
comparable nature of these horses to those with naturally-acquired OA. Further studies that
involve use of this technique should concentrate on cartilage obtained from the lateral side
of the affected joints.
a- Schering-Plough Animal Health Corp., Kenilworth, NJ.
b- Equine Orthotics Inc.; Indian Harbour Beach, FL.
c- Highspeed treadmill, Sato AG, Uppsala, Sweden.
d- Tissue-Tek. Miles. Elkhart, IN.
e- Gibco BRL, Gaithersburg, MD.
f- New England Nuclear Research Products, Boston, MA.
g- Sigma Chemical CO., St. Louis, MO.
h- Serva Feinbiochemica, Heidelberg, Germany.

References


Table 1  Mean ± SEM articular cartilage proteoglycan synthesis (CPM/µg protein and CPM/gm wet weight) after 18 hours and 72 hours of incubation with 35SO4

<table>
<thead>
<tr>
<th>Joint</th>
<th>18 hrs</th>
<th>72 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPM/µg protein</td>
<td>CPM/gm wet weight</td>
</tr>
<tr>
<td>OA</td>
<td>133.3 ± 8.2</td>
<td>13251 ± 2855</td>
</tr>
<tr>
<td>Contralateral</td>
<td>59.0 ± 12.1</td>
<td>7355.8 ± 1325.7</td>
</tr>
<tr>
<td>OA/contralateral ratio</td>
<td>2.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (P>0.05).
Means followed by different letters are significantly different (P<0.05) in columns down.
* denotes a trend towards significance.
Table 2 Mean ± SEM proteoglycan degradation expressed as radioactive proteoglycan release (CPM $^{35}$SO$_4$ x 10$^3$) in 24, 48 and 72 hours of incubation.

<table>
<thead>
<tr>
<th>Joint</th>
<th>Total $^{35}$SO$_4$ incorporation in 72 hrs</th>
<th>Amount $^{35}$SO$_4$ released in 24 hrs</th>
<th>Amount $^{35}$SO$_4$ released in next 24 hrs</th>
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<th>Total $^{35}$SO$_4$ released at 72 hrs</th>
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<td>71.8 ± 2.2</td>
</tr>
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<td>36.6 ± 1.0</td>
<td>24.8 ± 0.6</td>
<td>21.9 ± 0.6</td>
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<tr>
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<td>6.0 ± 1.5</td>
<td>5.0 ± 1.5</td>
<td>3.6 ± 1.2</td>
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<tr>
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<td>0.8</td>
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<tr>
<td><strong>Chondroitin sulfate</strong></td>
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<tr>
<td>OA point</td>
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<td>2.0 ± 1.3</td>
<td>0.79 ± 0.3</td>
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<td><strong>Keratan sulfate</strong></td>
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<tr>
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<td>OA/contralateral</td>
<td>0.5</td>
<td>0.9</td>
<td>0.7</td>
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2.1 Diagram demonstrating transected lateral collateral and lateral collateral sesamoidean ligaments to induce instability in the metacarpophalangeal (MCP) joints of horses.
2.2 Diagram of cartilage culture time table demonstrating specific steps to measure synthesis and degradation.
2.3 Graph depicting lameness scores (mean +/- SEM) for all horses at baseline and continued over the 8-week period following surgically-induced instability. Lameness was graded on a scale of 1 to 4 with 1 demonstrating lameness evident at the trot only, and 4 as the inability of the horse to bear weight.
2.4 Joint circumference (cm) of OA and contralateral MCP joints at baseline and for the 8-week period following surgically-induced instability.
2.5 Range of flexion (degrees) of OA and contralateral MCP joints at baseline and for the 8-week period following surgically-induced instability.
2.6 Radiographic stressed laxity produced immediately before (left) and after (right) desmotomy of the lateral collateral and lateral collateral sesamoidean ligaments of the MCP joint.
2.7 Radiographic evidence of osteophyte formation in an OA joint at 8-weeks following surgically-induced instability, prior to euthanasia.
Photographic evidence of articular lesions on the proximal first phalanx in a MCP joint. 8-weeks following surgically-induced instability.
2.9 Fissure formation and adjacent clusters of chondrone formation were evident on histologic evaluation of OA joints. Contralateral joints showed no evidence of such degenerative changes.
Histologic assessment of osteophyte formation demonstrated surface fibrocartilage and woven bone formation.
CHAPTER 3

VASCULAR, TRANSSYNOVIAL FLUID, AND OXYGEN METABOLISM ALTERATIONS OF THE ISOLATED EQUINE OSTEOARTHRITIC JOINT

Summary

This study defined the transsynovial forces and hemodynamics in the unloaded, stationary equine osteoarthritic (OA) metacarpophalangeal (MCP) joint compared to the isolated normal, control joint. Twelve healthy, sound horses were used. Osteoarthritis was induced in 6 horses by transection of the lateral collateral and lateral collateral sesamoidean ligaments of a randomly chosen MCP joint. Increased joint circumference and lameness, decreased range of motion, osteophytosis and articular scorelines documented OA. Control horses were sound with palpably and radiographically normal MCP joints. All horses were anesthetized and the selected MCP joint isolated and placed on arterial and venous pumps as previously described. Baseline measurements were taken at an isogravimetric state. Arterial pressure was adjusted to 150, 200, and 250 mm of Hg and hemodynamic parameters recorded. Horses were euthanatized at the termination of the isolation.

A significant increase in total vascular resistance, decrease in blood flow and decrease in transsynovial flow were documented in isolated OA joints compared to isolated normal joints. Precapillary resistance was greater in OA joints than control joints and greater than postcapillary resistance.
In conclusion, isolated OA joints had greater baseline vascular tone as compared to isolated normal joints, decreasing transsynovial fluid flow and hemodynamic responsiveness.
Introduction

Osteoarthritis (OA) is a chronic, painful, debilitating disease in the aging human population and in human and equine athletes. Extensive research has been reported on the role of articular cartilage in OA, with less information on the changes in and the contribution of the synovium to the disease process. Synovial pathology and malfunction are present in most forms of painful joint disorders. The synovial membrane influences the initiation and perpetuation of articular degeneration, the hallmarks of OA. In acute joint inflammation, perfusion through the synovial membrane is altered through changes in capillary permeability, joint distention, and distribution of blood flow. Although the synovium of the OA joint shows minimal inflammation, it is known to contribute to OA. Trauma and mild changes of the OA synovial membrane stimulate release of cytokines and degradative enzymes, such as interleukins and metalloproteinases, that initiate degeneration of articular cartilage. Direct trauma to the articular cartilage and subsequent wear particles contribute to the enzymatic release within the joint, increasing the activation factors for further degradation.

Transsynovial fluid flow is governed by Starling forces on both sides of the synovial membrane. Altered synovial membrane function may affect transsynovial flow. As synovial fluid volume increases in joint disorders, there are alterations in compliance (viscous creep) to accommodate this increase in fluid volume. In joint inflammation, permeability is affected and both small and large molecules move into the synovial fluid creating increased osmotic pressure and protein values within the joint cavity. In chronic OA joints, the effusion, increase in total protein, and increase in WBC count are less severe than acute synovitis, but joint capsular thickening, fibrosis and altered synovial function likely influence fluid dynamics in a similar manner. In OA, there is a general increase in
total joint circumference that is attributed to thickening of the joint capsule. Capsular fibrosis and enlargement would be expected to produce a decreased tissue compliance with a corresponding increase in intraarticular pressure with even slight increases in joint effusion or synovial fluid production. Alteration in fluid flux through the joint would affect nutritional exchange between the articular cartilage and the synovium. The avascular articular cartilage is dependent on synovial fluid exchanges to provide all nutrients and remove all wastes. Reduced gas electrolyte and nutrition exchange to articular cartilage exacerbates articular surface fibrillation and degenerative changes found in OA.4,10

In OA joints, alterations in capillary permeability, vascular dynamics and mechanisms of transsynovial fluid flow have not been studied. Our laboratory has studied these hemodynamic and fluid exchanges in normal and inflamed (IL-1 induced) equine joints using an isolated metacarpophalangeal (MCP) model, evaluating values for the isogravimetric state, response to incremental pressure manipulation, permeability and oxygen metabolism.11,12 The purpose of this study reported here was to document the hemodynamic, transsynovial fluid flow and oxygen metabolism responses to hemodynamic manipulations in isolated equine OA MCP joints and to compare these values to those of isolated normal joints taken at similar times in the same laboratory.

Materials and Methods

Horses and inclusion criteria -- Twelve healthy (normal physical examination, complete blood count (CBC), serum chemistry profile) horses (age > 3 years; 300-450 kg body weight; mares and geldings) were used in this study. Six horses were used as controls. In 6 horses, OA was induced in a randomly assigned MCP joint by surgical transection of the lateral collateral and lateral collateral sesamoidean ligaments and exercised for 8 weeks.12 Inclusion criteria for OA in the 6 horses were: lameness greater than 1 out
of 4 (1/4); greater than 45% decrease in pain-free range of motion; greater than 5% increase in joint size; and radiographic evidence of osteophyte formation. Inclusion criteria for the 6 normal horses were: soundness at the walk and trot; normal radiographic examination, normal appearance, range of motion and palpation of both MCP joints. All horses were managed in accordance with the Animal Care and Use Guidelines at The Ohio State University. At the termination of the study, all OA joints had gross evidence of articular cartilage degeneration, including score lines, erosions and osteophytes. All normal joints had no evidence of articular changes on gross examination.

Anesthesia and systemic hemodynamic measurements -- A single horse was studied on a given day due to the complexity of the preparation. All horses were anesthetized with xylazine (0.5 mg/kg, i.v.) and sodium thiopental (2 mg/kg, i.v.) and maintained on sodium pentobarbital (5-15 mg/kg/hr to effect) with 100% oxygen via mechanical ventilation through out the procedure. Horses remained in lateral recumbency with anesthesia monitored by continuous display of direct arterial blood pressure, hourly cardiac output and arterial blood gas measurements. Mean systemic arterial pressure was maintained at > 70 mm Hg and ventilation was adjusted to maintain $P_{a\text{O}_2} \geq 150$ mm of Hg and $P_{a\text{CO}_2} \leq 55$ mm of Hg. Heparin* (50,000 units IV) was administered every 90 minutes following completion of joint isolation.

Joint isolation and instrumentation -- Isolation of clinically normal MCP joints has been reported previously (Fig 3.1). In this study, the OA joint in the OA horses, or a matched control joint of the control horses was isolated. Arterial and venous blood flow could be controlled and was measured by calibrated peristaltic pumps. Arterial pressure of
the circuit was measured by in-line pressure transducers\(^e\) in both the arterial and venous tubing, connected to a multichannel recorder\(^d\). An 18 gauge intraarticular catheter was attached to a three-way stopcock for the withdrawal of synovial fluid, to measure synovial fluid production. The weight of the preparation was continually measured by a FT03 force transducer\(^e\) connected to a physiograph\(^f\).

**Baseline measurements and calculations** -- Baseline measurements and calculations were completed as described previously.\(^{11}\) Briefly, the joint preparation was maintained at an isogravimetric state (no loss or gain of weight) for 30 minutes before baseline measurements and pressure manipulations were initiated. The last 5 of the 30 minutes were utilized to take the baseline measurements including; circuit arterial blood flow \((Q_{\text{a,cir}}; \text{ml/min})\), venous blood flow \((Q_{\text{v,cir}}; \text{ml/min})\), circuit arterial pressure \((P_{\text{a,cir}}; \text{mm of Hg})\), circuit venous pressure \((P_{\text{v,cir}}; \text{mm of Hg})\), synovial flow \((Q_s; \mu\text{l/min})\), and weight of the preparation. Capillary pressure \((P_{\text{cap}})\) was measured by venous occlusion at this baseline venous pressure. Total vascular resistance \((R_v)\), pre capillary resistance \((R_{\text{pre}})\), and postcapillary resistance \((R_{\text{post}})\) were calculated by dividing the arterial-venous, arterial capillary, and capillary-venous pressure gradients, respectively, by blood flow. Pre- and post-capillary resistance ratios were calculated from the \(P_{\text{cap}}\). Plasma and synovial fluid samples were collected and placed in an eppendorf tube for total protein and albumin measurements and calculation of oncotic pressure, osmotic reflection coefficient and transitional microvascular pressures. Vascular and tissue compliances were calculated as described previously.\(^{11}\)
Oxygen metabolism -- Arterial and venous blood was collected from the isolated circuit to determine PO$_2$, PCO$_2$ and pH. Samples were collected at baseline in the isolated normal joints and at each pressure for the OA joints. These measurements were corrected for the horse’s body temperature at the time of blood collection. A co-oximeter$^8$ was used to measure total hemoglobin (Hb) and arterial oxygen saturation (SaO$_2$) from which arterial oxygen content (CaO$_2$) could be calculated for each sample (CaO$_2$ [ml/dl] = Hb x SaO$_2$ x 1.36 + PaO$_2$ x 0.003). Calculations were performed to determine oxygen delivery (DO$_2$), oxygen consumption (VO$_2$) and the oxygen extraction ratio (O$_2$ER). Oxygen delivery was calculated as DO$_2$ (ml/min/g) = CaO$_2$ (ml/dl) x Qa$_{cir}$ (ml/min)/W where Qa$_{cir}$ is the arterial flow and W is the weight (grams). Oxygen consumption was calculated as VO$_2$ (ml/min/g) = (CaO$_2$ - CvO$_2$) x Qa$_{cir}$/W. The oxygen extraction ratio was calculated as O$_2$ER = (CaO$_2$ - CvO$_2$)/CaO$_2$.

Pressure manipulations -- Circuit arterial pressures were manipulated after measurements were taken at an isogravimetric state. Circuit arterial pressure was adjusted to 150, 200 and 250 mm of Hg for 30 minute intervals, with measurements recorded in the last 5 minutes at each pressure. Isolated joints were returned to the baseline isogravimetric state for 30 minutes before venous manipulations. In normal joints, the isogravimetric state did not return to the original baseline, therefore, in OA joints, thirty mls of 25% dextrose solution was injected intraarterially to return the weight of the isolated joint to baseline. Circuit venous pressure was then adjusted from 10 to 20 and 40 mm of Hg for 30 minutes each with measurements collected in the last 5 minutes. Horses were humanely euthanized via an overdose of pentobarbital at the end of the study.
Measurement of synovial fluid and plasma total protein concentration -- A dye-binding assay was used to determine protein concentration as reported previously.\textsuperscript{3} Total protein was then used to calculate oncotic pressures for plasma ($\pi_p$) and synovia ($\pi_s$), osmotic reflection coefficient ($\sigma_d$), net filtration pressure and transitional microvascular pressure (TMP) as reported previously.\textsuperscript{11} Briefly, net filtration pressure was calculated as $[P_{\text{cap}} - P_s] - \sigma_d[\pi_p - \pi_s]$. Transitional microvascular pressure for synovial fluid flow (TMP$_s$) was calculated using the formula $\text{TMP}_s = \pi_p - \pi_s + P_s$ that was reduced to $\text{TMP}_s = \pi_p - \pi_s$ since $P_s$ was zero in this isolated OA preparation, similar to the isolated normal preparations. In normal and OA joints, a permeability coefficient was calculated for the isogravimetric state. Synovial membrane permeability coefficient was estimated at $P_{\text{cap}}$ for the OA joints as described previously.\textsuperscript{3} Briefly, the protein flux across the membrane was calculated from the volume of synovial fluid produced between $P_{\text{cap}}$ and $P_{250}$ multiplied by the difference in synovial fluid protein concentration between the same pressures. The protein flux was then divided by the difference in protein concentration between plasma and synovial fluid at $P_{250}$.

Statistical analysis -- Statistical analyses performed with a non-parametric Friedman ANOVA with repeated measures (time) were used to determine differences among pressure manipulations. An ANOVA was used to compare values between isolated normal and OA joints. A value of $P < 0.05$ was considered significant.
**RESULTS**

**Horses** -- For the horses with OA, the mean lameness grade was 1.5/4, joint circumference was increased by 5% over baseline and painfree range of motion was decreased by 45% over baseline. At the termination of the project, all OA joints demonstrated score lines, osteophytes and surface erosions and all normal horses were sound at the walk and jog, with no gross evidence of articular degeneration.

**Anesthesia and systemic hemodynamics** -- All horses met the anesthesia inclusion criteria (mean systemic arterial blood pressure ≥ 70 mm of Hg, PaO₂ ≥ 150 mm of Hg and PaCO₂ ≤ 55 mm of Hg) for the duration of the study.

**Baseline hemodynamics** -- Baseline hemodynamic parameters ($Q_{a_{cir}}$, $P_{a_{cir}}$, $P_{v_{cir}}$, $P_{cap}$) were documented at an isogravimetric state in the OA joint prior to pressure manipulation (7.92 ml/min, 137.2 mm of Hg, 10 mm of Hg, 19.33 mm of Hg, respectively; Table 3.1). In comparison, baseline $Q_{a_{cir}}$, $P_{a_{cir}}$, $P_{v_{cir}}$, $P_{cap}$ for the isolated normal joints were 37.17 ml/min, 133.57 mm of Hg, and 10.5 mm of Hg, 22.6 mm of Hg, respectively. The baseline $Q_{a_{cir}}$ for the OA joint (7.92 +/- 1.52) was significantly less than the isolated normal joint (37.17 +/- 13.65; P < 0.002). Baseline $R_t$ for the OA joint (18.43 mm of Hg/ml) was determined primarily by $R_{pre}$ (16.98 mm of Hg/ml) compared to $R_{post}$ (2.15 mm of Hg/ml). The isolated normal joint baseline $R_t$ (6.41 +/- 2.62 mm of Hg/ml) was significantly less than the values for the OA joint (18.4 +/- 3.4; P < 0.0001). No lymph flow was demonstrated in any of the 12 horses studied. Transsynovial fluid flow was documented to be significantly less at an isogravimetric state in the OA joints (140 µl/min) than in the isolated normal joints (300 µl/min; P < 0.001).
Hemodynamic response to pressure manipulations of OA joints -- Circuit arterial flow significantly increased in the OA joint with incremental increases in $P_{a\text{cir}}$ from 137.0 to 250 mm of Hg (7.92 to 26.9 ml/min; $P < 0.0008$; Fig 3.2). The peak $Q_{a\text{cir}}$ in the OA joint was 26.9 ml/min at a $P_{a\text{cir}}$ of 250 mm of Hg as compared to the peak $Q_{a\text{cir}}$ in the isolated normal joint (mean = 46.83 ml/min). There was no significant change documented in the OA joint $Q_{a\text{cir}}$ with changes of $P_{v\text{cir}}$. Circuit arterial blood flow of the OA joints moved in a relative manner compared to the circuit venous blood flow with arterial and venous pressure manipulations.

Circuit arterial pressures were measured with each pressure manipulation and were found to be maintained within an acceptable range compared to the predetermined values set (Table 3.1). The $P_{v\text{cir}}$ values averaged 11.93 +/- 1.31 mm of Hg during the $P_{a\text{cir}}$ manipulations. This was not significantly different than the $P_{v\text{cir}}$ value determined at an isogravimetric state (10.00 +/- 1.7 mm of Hg). Similarly, during $P_{v\text{cir}}$ manipulations, $P_{a\text{cir}}$ remained steady at 134.6 +/- 1.8 mm of Hg that was not different from the isogravimetric $P_{a\text{cir}}$ of 137.2 +/- 7.6 mm of Hg. Capillary pressure was not significantly increased across pressure manipulations in the OA joints (Fig 3.3).

The weight of the isolated OA joint significantly increased during incremental increases in $P_{a\text{cir}}$ from isogravimetric conditions ($P_{a\text{cir}} = 137.0$ mm of Hg; $P_{v\text{cir}} = 10.0$ mm of Hg; $P < 0.0005$ ). Increases in $P_{v\text{cir}}$ from 10 to 20 and from 20 to 40 mm of Hg demonstrated a significant increase in weight compared to isogravimetric state ($P < 0.01$).
Tissue compliance in the OA joints increased significantly across arterial and venous pressure manipulations ($P < 0.0001$; Fig 3.4). Vascular compliance in the OA joint increased with arterial and venous pressures changes ($P < 0.002$; Fig 3.5). The vascular compliance increase was greater with the venous manipulations than with the arterial manipulations.

**Oxygen metabolism of isolated osteoarthritic joints** -- Oxygen delivery, oxygen consumption and oxygen extraction ratio were not significantly different at the isogravimetric state between isolated OA joints (0.244 +/- 0.06 ml/min/g) and the isolated normal joints (0.195 +/- 0.2 ml/min/g).

Oxygen delivery in OA joints increased significantly with incremental increases in $P_{a_{\text{cir}}}$ from a mean of 0.197 ml/min/g at an isogravimetric state to 0.665 ml/min/g at $P_{a_{\text{cir}}}$ 250 mm of Hg ($P < 0.01$). There was no significant difference in oxygen delivery with increases in $P_{v_{\text{cir}}}$. There was a trend towards a decrease in oxygen consumption ($P < 0.07$; Fig 3.6) in OA joints with increased $P_{a_{\text{cir}}}$ and $P_{v_{\text{cir}}}$. There was no significant difference in oxygen extraction ratio with any pressure alteration in the OA joints ($P = 0.21$).

**Hemodynamic and transsynovial parameters as compared between OA and normal joints** -- A significant increase in $R_t$ ($P < 0.0001$), a decrease in joint blood flow ($P < 0.02$) and a decrease in transsynovial flow ($P < 0.03$) were documented in isolated OA joints compared to isolated normal joints. The incremental increase in $R_t$ in OA
joints (18.2 to 22.0 mm of Hg/ml) was less than normal joints (5.1 to 16.2 mm of Hg/ml) as $P_{acir}$ was increased from 137 to 200 mm of Hg (Fig 3.7). Precapillary (arterial) resistance (92.8%) was greater than post capillary (venous) resistance (7.6%). Precapillary resistance was also greater in OA joints (92.8%) than in normal joints (72.9%). The increase in joint blood flow was less in OA joints (10.1 to 28.4 ml/min) than normal joints (13.6 to 48.5 ml/min) as $P_{acir}$ was increased from 150 to 250 mm of Hg (Fig 3.2).

Synovial fluid production was less in OA than normal joints, and did not increase until a threshold $P_{acir}$ of 250 mm of Hg was reached, as also occurred in normal joints (Fig 3.8). Synovial fluid production did not significantly increase with $P_{vcir}$ in the OA joint. The osmotic reflection coefficient for synovial fluid from OA joints (0.23) was not significantly different than normal joints (0.22). The permeability surface area product for the OA joints (0.00031 +/- 0.00017 ml/min) was less than that reported for the isolated normal joints (0.00057 +/- 0.00014 ml/min).4

Tissue compliance was significantly greater at $P_{a200}$ and $P_{a250}$ in isolated normal joints compared to OA joints (Fig 3.4). After removal of synovial fluid, intitial tissue compliance was greater in OA joints (0.0173 +/- 0.004 ml/mm of Hg) than normal joints (0.0114 +/- 0.001 ml/mm of Hg). However, as circuit areterial pressure increased, the OA joints became less compliant. In contrast, the tissue compliance in OA joints was significantly greater than normal joints with increasing $P_{vcir}$. Vascular compliance was not significantly different between groups with arterial pressure manipulation, but was significantly less in OA joints than normal joints with initial venous pressure manipulations, i.e $P_{v20}$. 
DISCUSSION

This study evaluated the hemodynamic, transsynovial fluid and metabolic responses to pressure manipulations of an OA equine joint, documenting greater vascular resistance, lower tissue compliance and lower synovial fluid production values than isolated, normal joints. One reason to explain both the increased resistance and lower tissue compliance could be alterations of the joint capsule. Abnormal joint capsule may be thicker and have increased sympathetic tone to joint vasculature. Altered vascular tone may be attributed to increased receptor sensitivity, responsiveness, number or affinity.

Specifically, isogravimetric $P_{\text{a}_{\text{cir}}}$ for the OA joint (137.2 mm of Hg) was greater than both the normal MCP joint (121.5 mm of Hg) and previously reported values for the isolated digit (100.0 mm of Hg). The measured $P_{\text{v}_{\text{cir}}}$ (10.0 mm of Hg) and $P_{\text{v}_{\text{ap}}}$ (19.33 mm of Hg) for the OA MCP joint were less than that reported for the isolated equine digit as were the isolated normal MCP joints. Isolated joint blood flow, under these conditions, was significantly less in OA joints compared to the normal joints reflecting a significantly greater vascular resistance (Fig 3.7). This greater resistance may be attributable to fibrosis of the joint capsule as occurs in naturally-occurring OA. In addition, the OA joint may have higher sympathetic tone of the precapillary arterioles. Our study documented a greater percentage of vascular resistance that was attributed to precapillary tone in OA joints (92.8%) as compared to normal joints (72.8%). The arteriole is the primary vessel responsible for autoregulatory changes of the circulatory tree and maintains constant tone via mild, constant vasoconstriction. Changes in vessel diameter depend on the stimulus applied, receptor density and reactivity. One of these three components may contribute to the documented increase in arteriolar tone in isolated OA joints.
Although the $Qa_{cir}$ increased with increases in $P_a_{cir}$, as would be anticipated, the increase under isogravimetric conditions and across pressure manipulations was attenuated in the OA compared to the normal joints (Fig 3.2). Just as under isogravimetric conditions, this may be attributed to the increased vascular tone and tissue response of the OA joints. Poiseuille’s law describes flow as the product of a change in pressure over resistance of a given vessel. The incremental changes in pressure were the same for the OA and normal joints, therefore attributing the difference in flow to an increased precapillary resistance in the OA joints (Fig 3.7). The myogenic theory of autoregulation states that acute increases in arterial pressure stretch the vessel walls thereby stimulating local vasoconstriction to return blood flow back to its normal state. For example, the abrupt increase in $P_a_{cir}$ from 150 to 200 mm of Hg causes an initial increase in blood flow that passively distends the vessel, stretching the vascular smooth muscle, causing a vasoconstrictive response to attempt to decrease the higher flow. In OA joints the increase in arterial pressure was likely unable to passively distend the vessels due to increased tone, thereby producing lower arterial blood flow.

The transsynovial fluid flow was significantly lower in OA joints as compared to normal joints under isogravimetric conditions using the Starling equation \[ Jv = Kc[(P_{cap} - P_s) - \sigma_d (\pi_p - \pi_s)] \] where $Jv$ is the rate of fluid filtration and $Kc$ is the capillary filtration capacity. A slightly lower net filtration pressure (NFP) was present in OA joints and was attributed to the difference in osmotic pressure, plasma ($\pi_p$) and synovial fluid ($\pi_s$), with OA joints less permeable than normal joints. This decreased permeability to protein in the OA joints was confirmed by the lower permeability coefficient, as compared to the normal.
isolated joints. The OA joint demonstrated increased thickening and fibrosis of the synovial membrane interstitium, creating a greater distance of permeation, increasing the opportunity for uptake by the lymphatics and altering synovial function.

In both normal and OA joints, synovial fluid production did not significantly increase until a threshold $P_{a_{\text{cir}}}$ of 250 mm of Hg was reached, indicating a "breaking pressure" phenomena, similar to normal joints (Fig 3.8). The association of increased synovial fluid production and $P_{a_{\text{cir}}}$ may have caused an increased capillary permeability or surface area necessary to demonstrate an increased synovial fluid flow. The increased $P_{a_{\text{cir}}}$ likely overrides the $T_{M_{p}}$ thereby offsetting the fluid forces and increasing production. The $T_{M_{p}}$ and $Q_{s}$ for the OA joint was significantly lower than the control $T_{M_{p}}$ ($P < 0.001$) and was attributed to the overall decrease in arterial blood flow to the OA joint as compared to the control joint (Fig 3.8).

The weight of the isolated OA joint was significantly increased during incremental increases in $P_{a_{\text{cir}}}$ and $P_{v_{\text{cir}}}$. This change was attributed initially to a change in vascular volume followed by a change in tissue volume as the pressures were allowed to equilibrate for 30 minutes. Vascular compliance changes caused by increased arterial pressure were minimal, compared with those caused by increased venous pressure owing to the greater elastance of arteries and the larger muscular arterial wall (Fig 3.5). The vessels became more compliant with increases in blood flow and blood pressure. Synovial tissue was less compliant in the OA joints, compared to control joints, specifically with arterial pressure changes. The lower tissue compliance of the OA joint was related to the increased arterial resistance and attributed to fibrosis of the joint capsule. Baseline tissue compliance in the OA joints was greater than in the normal joints, reflecting greater distension of the OA joints with a larger initial synovial fluid volume. Increases in vascular compliance were
demonstrated and attributed to pooling of blood in the venules of the isolated preparation. Similar fibrosis surrounds the venules as the arterioles.

Oxygen delivery is a measure of blood flow to tissues and the tissue’s need and ability to use oxygen. Pressure and blood flow to the synovium changed, resulting in expected changes in DO2, reflecting blood flow. Oxygen extraction ratio is also a measure of the tissue’s need for and ability to use oxygen. Both DO2 and VO2 were not significantly different from control joints under isogravimetric conditions. Oxygen delivery was significantly increased in the OA joints with incremental increases in PaO2. There were no significant increases in DO2 with increased PvO2. The OA joint appeared to have increased O2 demand with increased pressure increments.

These data suggest OA caused significant alterations in baseline and physiologic responsiveness to hemodynamic manipulations, and the responses were attenuated in OA joints compared to normal controls. Baseline R1 was significantly increased in OA joints due to an increased baseline precapillary (arterial) tone. This increase was presumably responsible for decreased blood flow and transsynovial flow as defined by Starling forces. In conclusion, OA joints had an increased baseline vascular tone which probably decreased transsynovial fluid flow and hemodynamic responsiveness.
a- Sigma Chemical Co.; St. Louis, MO
b- Masterflex, Cole Parmer Int; Chicago, IL
c- Spectramed P23XL; Oxnard, CA
d- VR-12, Honeywell Corp; Pleasantville, NY
e- FTO3, Grass Instruments; Quincy, MA
f- Grass Model 70 Polygraph, Grass Instruments; Quincy, MA
g- ABL 500, Radiometer, Copenhagen, Denmark

References


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Table 3.1 - Isolated metacarpophalageal joint (control and OA) vascular and fluid values (mean +/- SEM) at isogravimetric conditions

<table>
<thead>
<tr>
<th>Measured Variables</th>
<th>Control Joint Values Obtained</th>
<th>OA Joint Values Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joint Blood Flow (ml/min)</td>
<td>16.33 +/- 5.41 *</td>
<td>7.92 +/- 2.4</td>
</tr>
<tr>
<td>Joint Arterial Pressure (Pacir mmHg)</td>
<td>121.5 +/- 9.49</td>
<td>137.2 +/- 8.2</td>
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<tr>
<td>Joint Venous Pressure (PvCir mmHg)</td>
<td>9.0 +/- 2.90</td>
<td>10.0 +/- 1.72</td>
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<tr>
<td>Joint Capillary Pressure (Pcap mmHg)</td>
<td>22.60 +/- 4.07</td>
<td>19.33 +/- 2.61</td>
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<td>Joint Resistance (Rt mmHg/ml)</td>
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<td>Joint Arterial Resistance (Rpre mmHg/ml)</td>
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<td>Contribution R post (%)</td>
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<td>Osmotic reflection coefficient</td>
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<td>Synovial fluid production (ul/min)</td>
<td>83.0 +/- 14.5 *</td>
<td>265.0 +/- 95.0</td>
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* Asterisk denotes significant difference P<0.05.
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*Note: The table above represents the schedule or data of the specified dates.*
3.1 Diagram depicting the pump-perfused isolated metacarpophalangeal joint preparation.
Figure 3.2 - Circuit arterial blood flow alterations with increasing arterial and venous pressure manipulations.
Capillary pressure ($P_{\text{cap}}$ mmHg) alterations of isolated OA joints with incremental pressure manipulations. ISO denotes the isogravimetric state. $P_a$ denotes arterial pressure and $P_v$ denotes venous pressure.
Figure 3.4 - Tissue compliance (ml/mmHg) in isolated OA and control MCP joints with arterial and venous manipulation.
Figure 3.5 - Vascular compliance (ml/mmHg) in isolated OA and control MCP joints with arterial and venous pressure manipulations.
3.6 Oxygen consumption (VO$_2$: ml/min/g) and oxygen delivery (DO$_2$: ml/min/g) of the isolated OA joint in response to changes in $P_{a_{cir}}$ and $P_{v_{cir}}$. ISO denotes the isogravimetric state. $P_a$ denotes arterial pressure and $P_v$ denotes venous pressure.
Figure 3.7 - Total vascular resistance of the OA joint as compared to the control joint.
Figure 3.8 - Synovial fluid production was significantly different in OA joints compared to control joints.
3.9 Precapillary ($R_{pre}$: mmHg/ml) and postcapillary ($R_{post}$: mmHg/ml) resistance in isolated OA joints with arterial and venous pressure manipulations.
CHAPTER 4

NITRIC OXIDE SYNTHASE ACTIVITY IN HEALTHY AND INTERLEUKIN 1- B EXPOSED EQUINE SYNOVIAL MEMBRANE

Summary

The objective of this phase of studies was to quantitate nitric oxide synthase (NOS) activity in healthy and interleukin 1β (IL-1β) exposed equine synovial membrane. Six healthy horses, free from lameness, ages 2 to 8 years were used in this study. Recombinant human IL-1β (0.35 ng/kg) was injected intraarticularly into a randomly chosen metacarpophalangeal (MCP) joint of each horse. The contralateral joint served as an unexposed control. All horses were euthanatized 6 hours after injection of IL-1β, and synovial membrane specimens were assayed for NOS activity by measuring conversion of arginine to citrulline. Severity of inflammation was semi-quantitated by analysis of synovial fluids and histologic examination of synovial membrane.

Equine synovial membrane had minimal NOS activity. A significant difference in NOS activity between control and IL-1β exposed specimens was not detected. Histologic examination revealed a neutrophilic infiltrate in synovial membrane exposed to IL-1β.
Synovial fluid from IL-1β exposed joints had a moderate inflammatory response and significantly greater concentrations of IL-1β and interleukin 6 than fluid from healthy joints.

In conclusion, healthy equine synovial membrane had low NOS activity that was not affected by exposure to IL-1β.
Introduction

Nitric oxide (NO) is an uncharged free radical that is released from various tissues and cells. It is a product of the reaction between L-arginine and oxygen that is mediated by nitric oxide synthase (NOS) and related enzymes. There are 2 predominant isoforms of NOS. The inducible isoform (iNOS), is found in neutrophils, macrophages, and vascular smooth muscle, releases large amounts of NO, and can be induced by incubation of cell cultures with interleukin 1β (IL-1β), endotoxins and tumor necrosis factor-α (TNF-α). The constitutive isoform (cNOS) is found in endothelium, brain and platelets, and causes production of small amounts of NO. In synovial joints, iNOS may be more important than cNOS in production of NO. Rabbit chondrocytes cultured with IL-1β and endotoxin were induced to synthesize NO. However, synovial fibroblasts cultured with IL-1β and TNF-α synthesized a third to a fourth as much NO as chondrocytes that were cultured similarly. These results suggest that articular cartilage may be the predominant producer of NO, in synovial joints. The direct activity of iNOS in intact synovial membrane has not been determined.

Nitric oxide is highly volatile and reacts quickly with oxygen in air, and with hemoglobin in blood. Thus, NO is likely synthesized locally, and diffuses out of cells for short distances. Murrell et al documented the importance of NO for articular cartilage catabolism by demonstrating its role in inflammation and its activation of metalloproteases. Farrell et al documented an increased concentration of synovial fluid nitrite, compared with serum nitrite, in patients with rheumatoid arthritis and osteoarthritis, a finding that suggested synthesis of NO within joints. Two sites of synthesis, articular cartilage and synovial membrane, have been proposed. Endothelial cells lining synovial membrane capillaries may produce NO and, infiltrating leukocytes from inflamed synovial membrane
and synovial fibroblasts may produce high concentrations of NO within joints. However, direct production of NO by synoviocytes, alone, has not been documented. Models of acute inflammation for several species, including horses, have used IL-1 to incite inflammation in various tissues, such as equine chondrocytes.\textsuperscript{3,7,8} The purpose of the study reported here was to determine iNOS activity in healthy equine synovial membrane and in synovial membrane after exposure to IL-1β.

**Materials and Methods**

**Horses** -- Six horses, free from lameness, 2- to 8- years old, and of various breeds, were determined to have healthy metacarpophalangeal (MCP) joints by means of radiographic evaluation and joint palpation. Recombinant human IL-1β\textsuperscript{a} (0.35 ng/kg of body weight {b.w.}) was injected intraarticularly into a randomly chosen MCP joint, whereas the contralateral joint received no injection. Six hours later, all horses were humanely euthanatized with pentobarbital\textsuperscript{b} administered IV. Within 30 minutes, synovial fluid samples and synovial membrane specimens were aseptically harvested from each MCP joint. One-ml aliquots of synovial fluid were placed in sterile tubes containing EDTA for cytological examinations, and in sterile eppendorf tubes for determination of cytokine concentrations. The synovial intima was separated from the underlying fibrous capsule, using a dissection microscope. Portions of each specimen were snap frozen in liquid nitrogen and stored at −70 C until assayed and fixed in neutral-buffered 10% formalin for histologic examination. Brain tissue from mice was pooled and frozen at −70 C as a positive control.
iNOS activity -- Activity of iNOS in synovial tissue was determined by measuring incorporation of radioactive arginine in the conversion of L-arginine to citrulline. Synovial intima specimens were thawed to room temperature (24° C) and washed several times in ice cold (4 C) tris-hydrochloric acid (HCl) buffer (52mM), homogenized at 4 C using a glass tissue homogenizer and approximately 1ml Tris-HCl buffer per 0.25 g tissue, and transferred to microcentrifuge tubes that were spun at 20,000g for 20 minutes. The supernate was removed and the pellet (enzyme extract) was resuspended in 250μl of Tris-HCl buffer. A buffer was prepared in glass vials coated with 5 mg NADPH, with 100mM Hepes, 2.5 mM CaCl₂, calmodulin 20 μg/ml, 2 mM dithiothreitol, 0.02 mM Na acetate, 6 μM tetrahydrobiopterin, 2 μM flavin adenine dinucleotide, 2 μM flavin mononucleotide, and 1,130 μl distilled water to equal a volume of 3 ml. In the order listed, the following reagents were added to a microfuge tube: 50 μl buffer, 23 μl water, 2.3 μl of arginine labeled with radioactive carbon (¹⁴ C; 300mCi/mmol) and 25 μl of enzyme extract. Tubes were placed in a 37 C water bath for 20 minutes, and then the assay was stopped with 2-ml stop solution (25 ml 200 mM Hepes (pH 5.5), 1 ml 0.5 M EDTA (pH 8.0), and 224 ml water, to equal 250 ml). Positive controls (mouse brain) were processed in a similar manner. Negative controls contained no enzyme extracts.

Cation exchange columns (2 ml) were changed from H+ form to Na+ form, before use, by addition of 1 M NaOH, and equilibrated with stop solution. Test solution (1.125 ml) was placed on top of the column, and bed volume (750 μl) was discarded. The column was then washed twice with 2 ml stop solution and 4 ml of eluate was collected for scintillation counting. Each sample was counted 3 times, and mean values (as nmol citrulline/g protein/minute) were calculated. A dye-binding protein assay with bovine
serum albumin as a standard was used to express citrulline concentration as a function of protein content (nmol/g min). The equation used to calculate these data was derived as follows:

\[
\frac{(\text{mean DPM} - \text{background DPM}) \times 4 \times 1 \mu \text{Ci} \times 1000^2 \mu g \times 1 \text{mmol} \times 1 \text{mCi} \times 1000^2 \text{nmol}}{\text{Time (minutes)} \times \text{protein (g)} \times 2.2 \times 10^6 \text{DPM} \times 1 \mu g \times 304 \text{mCi} \times 1000 \mu \text{Ci} \times 1 \text{nmol}}
\]

**Synovial fluid analysis** -- Total WBC count (cells/μl) of synovial fluid was determined. Assays for IL-1β and interleukin 6 (IL-6) concentrations were performed with an ELISA kit\(^d\) that used monoclonal antibodies against human IL-1β and IL-6.

**Histologic examination** -- Formalin-fixed synovial membrane specimens were embedded in paraffin, sectioned at 6 μm, and stained with H&E. One section per specimen was graded semi-quantitatively, in a blind fashion, by 3 investigators (SEW, EJS, ALB), and a score was assigned for the following characteristics: synovial cell hyperplasia (scored 0 to 4 on the basis of continuity and numbers of synoviocytes on villi, with 0 = absent; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; and 4 = >76% of surface with continuous layer of >3 cell thickness.); percentage vascularity (scored 0 to 4 on the basis of percentage of each villus that had vascularity, with 0 = absent; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; and 4 = >76% of villus was vascular); intensity of inflammation (scored 0 to 4, with 0 = none; 1 = minimal; 2 = mild; 3 = moderate; 4 = severe); and extent of inflammation (scored 0 to 4, on the basis of percentage of interstitium within the villi with inflammation with 0 = none; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; and 4 = >76%). Mean values for scores were determined. Type of inflammation was evaluated qualitatively.
Statistical analyses — Activity of iNOS (nmol/g min) in specimens from healthy and IL-1β exposed equine joints were reported as mean +/- SEM and were compared by use of a one-way ANOVA. A Mann-Whitney U test was used to compare scored data. A value of $P < 0.05$ was considered significant.

Results
Substantial iNOS activity (mean +/- SEM, 328,660.6 +/- 51,909.13 nmol/g min) was detected in mouse brain positive control tissue. No difference in iNOS activity was detected between specimens from healthy joints (1226.29 +/- 271.83 nmol/g min) and specimens from IL-1β exposed joints (847.31 +/- 181.70 nmol/g min).

The WBC count of synovial fluid from IL-1β exposed joints (7,800 +/- 1,200) was significantly ($P < 0.001$) greater than counts for fluid from healthy joints (1,040 +/- 250).

Concentration of IL-6 in synovial fluid from IL-1β exposed joints (43.73 +/- 31.61 pg/ml) was significantly ($P < 0.01$) greater than concentration in fluid from healthy joints (6.18 +/- 2.17 pg/ml).

Intensity of inflammation was significantly greater in the IL-1β exposed specimens (mean +/- SEM score, 1.07 +/- 0.21) compared with healthy specimens (0.26 +/- 0.10; $P < 0.001$). Extent of inflammation was significantly greater in IL-1β exposed specimens (1.80 +/- 0.38) than healthy specimens (0.37 +/- 0.16; $P < 0.002$). No significant difference was detected for synovial cell hyperplasia or percentage vascularity between groups.
Discussion

Results of the study reported here documented that intraarticular injection of IL-1β (0.35ng/kg) did not significantly increase synovial membrane iNOS after 6 hours despite a significant increase in WBC count for synovial fluid, and inflammation in synovial tissues.

Interleukin 1β is a documented mediator of articular inflammation, and, in combination with other cytokines, can induce release of NO from articular cartilage, including IL-6. In equine chondrocytes, IL-1β increased production of a NO metabolite, nitrite, in vitro. In our study, IL-1β induced inflammation and release of additional cytokines as documented by production of IL-6 in synovial fluid from the exposed joints. The type of stimulus, either direct or via subsequent cytokine stimulation, may influence induction of NOS. Nitric oxide production by synoviocytes and chondrocytes from human arthritic joints was up-regulated when cells were treated with IL-1β (1ng/ml), TNF-α (10 U/ml), and LPS (10 μg/ml), for 48 hours, but not when cells were treated with IL-1β alone. Up-regulation was attributed to activation of macrophages or fibroblasts in response to the combination of cytokines. Neutrophils cultured in vitro were able to generate nitrite anion, an indirect measure of nitric oxide production. However, enzymatic iNOS activity of neutrophils alone has not been documented. In this present study, the IL-1β exposed synovial membrane did not produce detectable iNOS levels despite the neutrophilic infiltration. The synovial fluid values corresponded to typical values associated with moderate, acute joint inflammation documenting an inflammatory challenge produced by IL-1β. It is possible that other challenges of synovial membrane, such as longer challenge of IL-1β, combinations of cytokines or greater challenge may be necessary to produce an effect from synovium.
Our study evaluated the effects of intraarticular administration of IL-1β on equine synovial membrane iNOS activity. Equine synovial membrane had low iNOS activity, and no increase in activity was detected despite IL-1β exposure and resulting inflammation.
References


Table 1. Nitric oxide synthase activity in equine synovial intima in normal control and IL-1β stimulated joints as compared to a positive control. (Mean +/- SEM; nmol/gmin)

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<th>GROUP</th>
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<td>Normal control</td>
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<td>IL-1β stimulated</td>
<td>847.31 +/- 181.70a</td>
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<td>Positive control</td>
<td>328660.6 +/- 51909.13b</td>
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4.1 Histologic assessment of synovial membrane from MCP joints exposed to IL-1β.
3.2 Histologic assessment of synovial membrane from control joints.
CHAPTER 5

RECEPTOR MECHANISMS FOR ENHANCED VASCULAR RESPONSIVENESS OF ISOLATED OSTEOARTHRITIC JOINTS

Summary

The vasoactive agents investigated in this study stimulated known receptor mechanisms that affect blood flow and vascular resistance which has been previously reported to be altered in osteoarthritic (OA) joints. Specifically, the objective of this study was to assess the receptor responsiveness in vasoreactivity of isolated OA and control metacarpophalangeal (MCP) joints after administration of 5 specific vasoactive agents: 1) Dopamine (α1 adrenoceptor - vasoconstriction); 2) Norepinephrine (α1, α2 adrenoceptor - vasoconstriction); 3) Esmolol (β1 antagonist - vasodilation); 4) Nitroglycerin (nitric oxide stimulator - vasodilation); 5) LNAME (nitric oxide synthase inhibitor - vasoconstriction).

Six healthy, radiographically and clinically sound horses had OA surgically induced by desmotomy of the lateral collateral and lateral collateral sesamoidean ligaments of a randomly chosen MCP joint per horse. Six similar horses served as controls and had normal MCP joints. Osteoarthritis was documented in the 6 horses by lameness, increased joint circumference, decreased pain-free range of motion and osteophytosis as has been previously reported for this model.

All horses were anesthetized for surgical isolation and vascular perfusion of the selected MCP joint as has been reported previously. An isogravimetric state (no loss or gain of joint weight of the isolated joint preparation) was established for 30 minutes.
followed by serial intraarterial infusion of the vasoreactive agents in random order. An isogravimetric state was re-established between each infusion. Hemodynamic and transsynovial parameters were recorded or calculated before and after each drug administration. At the termination of the study, all horses were euthanized and isolated joint preparations were disassembled.

At baseline, isolated OA joints demonstrated an increased vascular resistance and decreased arterial blood flow, compared to control joints, as has been previously reported. Dopamine and norepinephrine-induced vasoconstriction demonstrated a significant increase in arterial pressure and resistance (precapillary) that was dampened in OA joints when compared to control joints. Vasoconstriction following these two drugs, persisted significantly longer following termination of infusion in OA joints compared to control joints as reported by increased time to return to an isogravimetric state. The baseline (resting) arterial vasoconstriction of the OA joints was greater and, therefore, less responsive to further stimulation.

Nitroglycerin stimulated a significant decrease in vascular resistance in both control and OA joints. Esmolol and nitroglycerin demonstrated significant weight loss in OA compared to control joints. This was coupled with lower tissue and vascular compliances following vasodilation in OA joints compared to control joints. The decreased vascular resistance was not associated with a significant increase in blood flow, therefore synovial fluid production was significantly less in the OA joints following dilation compared to norepinephrine constriction.

Both OA and control joints were less responsive to the vasoconstrictor, LNAME, compared to dopamine and norepinephrine. In both OA and control joints, vasodilation was more pronounced following NO stimulation (ie, nitroglycerin administration) than esmolol.
administration. We were able to document an equivalent, and opposite change in pressure by blocking this activity with LNAME, yet this vasoconstriction was not equal to that of dopamine or norepinephrine.

In conclusion, α receptor (dopamine and norepinephrine-induced) stimulation produced intense vasoconstriction which was slower to relax and return to baseline in OA joints. The baseline (resting) arterial vasoconstriction of isolated OA joints was greater and, therefore, less responsive to further stimulation, particularly vasoconstriction. These data indicate that the increased vasomotor tone documented in OA joints is at least, in part, sympathetic and dopaminergic receptor mediated.
Introduction

Joint disorders, such as osteoarthritis (OA), are among the most debilitating diseases of humans and horses, resulting in days off of work or training, and decreased income or monetary reward as in racing. Alterations in blood flow and total vascular resistance have been documented in an isolated, stationary metacarpophalangeal (MCP) joint model comparing basic hemodynamics between OA and normal, control joints. Decreased blood flow was attributed to an increase in arterial and total vascular resistance in the OA joints compared to normal, control joints. Transsynovial fluid flow was decreased in OA compared to normal, control joints following incremental arterial pressure changes. Mechanisms responsible for these differences are unknown but are predicted to be important in determining responses to medications, drug distribution and joint physiology, and therefore, may alter symptoms and the progression of joint disease.

The sympathetic nervous system plays a crucial regulatory role in and richly innervates the synovial joint. Sympathectomy in a rat arthritis model prevented the development of joint pain and arthritis. This was further supported by the finding that hypertensive rats, with increased sympathetic activity, had significantly more joint pain and arthritis. The finding that vascular resistance is significantly increased in OA joints suggests that sympathetic activity is functionally altered in arthritis. Sympathetic postganglionic stimulation indirectly increases plasma extravasation through activation of the cyclooxygenase pathway and synthesis of prostaglandins, such as PGE_2. Some adrenergic receptors (β) have been shown to be pro-arthritic, where α^2 receptors had an
anti-arthritic effect.\textsuperscript{6,7} The mechanisms of interest in this present study are related to the sympathetic nervous system (\(\alpha 1,2\) and \(\beta\) receptors) influence at the cellular and physical (hemodynamic forces) level.

Nitric oxide (NO), another endogenous vasoactive agent, is synthesized by nitric oxide synthase (NOS) in the vascular endothelium and is known to regulate capillary blood pressure.\textsuperscript{8} In equine tissue, NO has been documented in the endothelium, specifically acting as an endogenous control factor of vasomotor tone in arterioles.\textsuperscript{9} The powerful vasodilating effects of NO are thought to enhance cellular injury in articular tissues. Nitrite, an indirect measure of NO production, was increased in human patients with rheumatoid arthritis and OA compared to controls.\textsuperscript{10} Articular cartilage has been shown to produce increased levels of NO as compared to other tissues in both normal and osteoarthritic joints. Synovial membrane, normal or IL-1 stimulated, demonstrated relatively low levels of NOS activity in the isolated equine joint model.\textsuperscript{11} Nitric oxide is expected to have a vasodilatory effect on synovial vasculature that is blocked by L-NAME.

Normal vascular beds predictably respond to known vasodilator and vasoconstrictor substances.\textsuperscript{12,13} These responses can be sensitively measured in isolated, controlled organ models, such as the isolated equine metacarpophalangeal joint preparation.\textsuperscript{14,15} Specifically, this study defined alterations in vasoreactivity of OA and normal, isolated MCP joints following the administration of 5 vasoactive agents selected to increase or block sympathetic activity and nitric oxide production. The drugs selected here have been independently linked to increased arthritic activity. The vasoactive agents chosen represent two categories, vasoconstrictors: 1) Dopamine (\(\alpha 1\) adrenoceptor); 2) Norepinephrine (\(\alpha 1, \alpha 2\) adrenoceptor); and vasodilators: 1) Esmolol (\(\beta 1\) antagonist); 2) Nitroglycerin (nitric oxide stimulation); 3) LNAME (nitric oxide synthase inhibitor).
We have previously documented baseline differences between OA and control joint hemodynamics. We were interested in determining the vasoactive properties of OA and control joints with respect to substances normally found as endogenous or exogenously administered vasoregulators. Documentation of these properties provide further insight into the vascular alterations found in OA joints.

Materials and Methods

Horses -- Twelve horses, mares and geldings, 393 to 530 kg body weight, 3 to 8 years old, normal and sound as determined by results of physical examination, lameness examination, and complete blood count, were used in this study (Table 1). In 6 of the 12 horses, OA was induced in a randomly chosen MCP joint. Inclusion criteria for OA in these 6 horses were: a lameness score of 1/4, a 5% increased joint circumference, a 15% decrease in the range of motion, and evidence of osteophyte formation on radiographs. The remaining 6 horses were used as normal controls that were sound at the walk and jog, with normal radiographic appearance and palpation of both MCP joints. All horses were managed in accordance with the Animal Care and Use Guidelines at The Ohio State University. At the termination of the study, all OA joints had gross evidence of articular cartilage degeneration, including score lines, erosions and osteophytes, similar to that previously reported. No articular changes were present in the 6 normal control joints.

Anesthesia and systemic hemodynamic measurements -- A single horse was studied per day. Horses were sedated with xylazine hydrochloride (0.5 mg/kg, IV) and anesthesia was induced with guaifenesin (25 mg/kg, IV to effect) and sodium thiopental (2 mg/kg, IV to effect). Anesthesia was maintained by administering sodium pentobarbital (5-15 mg/kg/hour, IV to effect). All horses were mechanically ventilated with 100%
oxygen in order to maintain a PaCO₂ between 30 and 55 mmHg. Horses remained in lateral recumbency with anesthesia monitored by continuous display of direct arterial blood pressure, hourly cardiac output and pH and arterial blood gas (PaO₂, PaCO₂) measurements. Heparin was infused every 90 minutes (50,000 IU IV) following isolation of the MCP joint. All horses were euthanized at the end of the study period.

**Joint isolation** -- Isolation and preparation of the equine MCP joint has been described previously.¹⁵ Arterial and venous blood flow was controlled and measured by calibrated peristaltic pumps. Extracorporeal circuit pressures were maintained and adjusted by measuring and recording in-line pressure⁶ in both the arterial and venous tubing.⁶ An 18 gauge intraarticular catheter was attached to a three-way stopcock to collect synovial fluid and measure intraarticular pressure. The weight of the preparation was continually measured by a FT03 force transducer connected to a physiograph.

**Baseline measurements and calculations** -- Baseline measurements and calculations were calculated as described previously.¹⁵ Briefly, the joint preparation was maintained in an isogravimetric state (no loss or gain of weight) for 30 minutes. During the last 5 of the 30 minute equilibration phase, baseline measurements including: circuit arterial blood flow (Qₐ₁, ml/min), circuit arterial pressure (Pₐₙ₁; mm of Hg), circuit venous pressure (Pᵥₐ₁; mm of Hg), synovial fluid flow (Qₛ; µl/min), and weight were recorded. Capillary pressure (P₉) was measured by venous occlusion.¹⁵
Total vascular resistance ($R_v$), precapillary ($R_{pre}$) and postcapillary resistance ($R_{post}$) and pre- and postcapillary resistance ratios were calculated from the $P_{cap}$. Vascular and tissue compliances were calculated as described previously.\textsuperscript{3,15}

**Drug administration** -- All drugs were infused in a random order (Table 2) except for LNAME, which was infused last due to it's long duration of action: dopamine\textsuperscript{f} (0.2 μg/ml); norepinephrine\textsuperscript{g} (4 μg/ml); esmolol \textsuperscript{h} (0.05 mg/ml); nitroglycerin\textsuperscript{i} (0.2 μg/ml); LNAME\textsuperscript{j} (100 μg/ml). All drugs were prepared in 5\% dextrose to their final infusion concentration immediately prior to administration. Drug dosages were: dopamine (15 μg/kg/min); norepinephrine (0.15 μg/kg/min); esmolol (50 μg/kg/min); nitroglycerin (1μM); L-NAME (300μM). Each drug was infused for 15 minutes. Final drug volume (ml) and amount (μg) were tabulated per horse and meaned.

**Hemodynamic response to drugs**

Each drug was individually assessed for it's effect on the measurements listed previously including $Q_{acir}$, $P_{acir}$, $P_{vcir}$, $Q_s$, $P_{cap}$, $R_t$, $R_{pre}$ and $R_{post}$. The time of return to an isogravimetric state following drug administration was recorded. Additional calculations were performed to evaluate the amount of drug required to produce each mmHg change in pressures, both arterial and venous.

**Measurement of total protein concentration**

A dye-binding assay was used to determine total protein concentration of plasma and synovial fluid samples as reported previously.\textsuperscript{3,15}
Total protein was then used to calculate oncotic pressure for plasma ($\mu_p$) and synovia ($\mu_s$).

Protein flux and permeability surface area calculations were performed to evaluate permeability alterations produced by each drug.\textsuperscript{14}

**Statistical Analysis**

Values were reported as mean +/- SEM and compared using an ANOVA for repeated measures to determine significant differences between groups over time. An LSD post-hoc test was used to specify the exact interactions responsible for any significant changes. A value of $P < 0.05$ was considered significant.

**Results**

**Horses** -- All 6 horses in which surgical instability of the MCP joint was produced met the inclusion criteria for OA. Mean lameness grade was 1.5/4, joint circumference increased by 5%, and pain-free range of motion decreased to 45%. At the termination of the study, all OA joints demonstrated scorelines, osteophytes and surface erosions. The control horses remained radiographically and clinically sound throughout the study.

**Anesthesia and systemic hemodynamics** -- All horses met the inclusion requirements for anesthesia (mean systemic arterial pressure > 70 mm of Hg, $\text{PaO}_2 > 150$ mm of Hg, and $\text{PaCO}_2$ (30 - 55 mm of Hg) for the duration of the isolation.

**Baseline hemodynamics** -- Baseline hemodynamic parameters ($\text{Pa}_{\text{cir}}$, $\text{Pv}_{\text{cir}}$, $\text{Qa}_{\text{cir}}$, $\text{P}_{\text{cap}}$, synovial fluid volume) measured at an isogravimetric state averaged 135.2 mm of Hg, 12.2 mm of Hg, 14.0 ml/min, 29.5 mm of Hg, 3.8 ml respectively, in the OA joints compared
to 124.0 mm of Hg, 10.8 mm of Hg, 19.0 ml/min, 25.2 mm of Hg, and 1.5 ml, respectively, in the normal joints (Table 3). This resulted in a baseline Rt of 13.1 +/- 2.9 mmHg/ml for the OA joints which was greater than the control joints 7.1 +/- 0.81 mmHg/ml (P < 0.045). Synovial fluid volume was significantly greater in the OA joints (3.8 +/- 1.1 ml) compared to control joints (1.5 +/- 0.5 ml) at baseline (P<0.001; Table 3).

**Hemodynamic response to drug administration**

All vasoconstrictor agents, regardless of group, increased Pacir from baseline (Fig 5.1). Dopamine and norepinephrine demonstrated a percent increase in $P_a^{cir}$ that was significantly less in the OA joints (36.4%; 69.4%; respectively; Fig 5.2) compared to the control joints (62.1%; 112.22%; P < 0.0001). The percent increase in $P_a^{cir}$ produced by norepinephrine was greater in both groups compared to the percent increase in $P_a^{cir}$ produced by dopamine (P < 0.0001) or L-NAME (P < 0.0001). LNAME increased Pacir in both OA (39.4%) and control (43.28%) joints from baseline, but no significant difference was present between the two groups. The OA joints took a significantly longer time to return to an isogravimetric state for dopamine (P<0.006; Fig 5.3), norepinephrine (P<0.002), and LNAME (P<0.003), compared to control joints. Norepinephrine took longer to return to an isogravimetric state in OA joints (P<0.05) compared to dopamine and LNAME, which were not different from each other.

Esmolol and nitroglycerin produced arterial vasodilation in all joints. Their administration produced a progressive decrease in $P_a^{cir}$ which was not different between OA and control joints (Fig 5.1). The percent change from baseline was significantly greater for nitroglycerin (34.5%) compared to esmolol (20.0%; P < 0.002; Fig 5.2).
Esmolol and LNAME decreased $P_{vcir}$ more in the OA joints (8.00 +/- 1.93 mmHg) compared to control joints (13.17 +/- 2.75 mmHg; $P < 0.0001$) at 15 minutes (Fig 5.4). LNAME $P_{vcir}$ was also less in OA joints (10.0 +/- 2.64 mmHg) than the controls (11.83 +/- 0.95 mmHg; $P < 0.022$; Fig 5.2). There was no difference between group for time to return to baseline.

Baseline arterial flow was significantly less in OA than control joints ($P<0.04$) and there were no significant differences between groups or for any drug for circuit blood flows (Fig 5.5). Administration of LNAME induced a significant increase in capillary pressure for OA joints compared to control joints ($P<0.02$; Fig 5.6). No other differences were noted between groups for capillary pressure.

Baseline total vascular resistance was significantly greater in the OA joints compared to the control joints (13.1 +/- 2.9 mmHg/ml; 7.1 +/- 0.8 mmHg/ml; $P<0.04$; Fig 5.7). The percent change in total vascular resistance ($%R_t$) was less in the OA joints for dopamine (18.7%, 48.5%; respectively) and norepinephrine (39.3%, 16.3%) than control joints (58.7%, 152.4%; 71.5%. 83.1%; $P < 0.0001$;Fig 5.8). Following the administration of LNAME, there was a trend for a percent decrease in $R_t$ in OA joints (17.4%) compared to control joints (34.7%; $P < 0.10$). There as no difference between $R_t$ percent changes between esmolol and nitroglycerin. Total vascular resistance following vasoconstrictor administration was significantly greater than following vasodilators, as expected ($P < 0.0001$). The total change in precapillary resistance ($R_{pre}$; Fig 5.9) was significantly greater in the OA joints (9.6 +/- 1.2 mmHg/ml) compared to control joints (5.6 +/- 1.5 mmHg/ml) when esmolol was administered ($P<0.05$). Postcapillary resistance was also changed significantly greater in OA than control joints during norepinephrine and LNAME administration ($P<0.044, P<0.05$; Fig 5.10).
Synovial fluid volume was significantly greater at baseline for the OA joints (3.84 +/- 1.18 ml) than for control joints (1.48 +/- 0.48 ml; P < 0.002; Fig 5.11). Administration of norepinephrine (P<0.05), LNAME (P<0.02), nitroglycerin (P<0.04), and esmolol (P<0.05), the OA joints had significantly less synovial fluid production than control joints. Osteoarthritic joints demonstrated significantly greater TMP following norepinephrine, LNAME and esmolol administration (P<0.05; P<0.004; P<0.05; Fig 5.12).

Weight gain following administration of the vasoconstrictive agents was greater for the isolated OA joints than for control joints (Fig 5.13). In contrast, the OA joints demonstrated greater weight loss related to the vasodilatory agents when compared to the control joints. Both vasodilators (esmolol and nitroglycerin) caused weight loss in OA joints compared to controls. Vascular compliance was lower in control joints following vasoconstriction than vasodilation (Fig 5.14). The OA joints demonstrated a significantly lower vascular compliance than the control joints following vasodilation with nitroglycerin (P<0.004) and esmolol (P< 0.03). In contrast, following vasoconstriction, both groups demonstrated tissue compliance that was significantly greater than after vasodilation. With dopamine-induced vasoconstriction, the OA joints tissue compliance was significantly greater than the control joints (P<0.001; Fig 5.15).

**Synovial fluid and plasma protein analysis** - Synovial fluid protein concentration was less in the OA joints following esmolol and LNAME administration (2.34 +/- 0.07 g/dl; 2.45 +/- 0.16 g/dl;respectively) compared to control joints (3.45 +/- 0.5 g/dl; 3.55 +/- 0.10 g/dl;P < 0.002, P < 0.002; Fig 5.16).

Synovial fluid oncotic pressure following esmolol administration was less in the OA joints ( 4.8 +/- 0.31 mmHg) than in the control joints (7.96 +/- 0.15 mmHg; P<0.0016). LNAME administration followed the same pattern of significance with OA (5.2 +/- 0.4 mmHg) being less than the control joints (8.3 +/- 0.2; P<0.0016). Protein
flux was less in the OA joints compared to the control joints with the exception of
norepinephrine administration, where the difference was not significant (Fig 5.17).
Similarly, permeability surface area product was less in the OA joints compared to the
control joints following administration of dopamine, LNAME, nitroglycerin and esmolol
(Fig 5.18).

Discussion

Results of this document provide insight into the vascular responsiveness of the
isolated OA and control joint vasculature to challenge by known vasoactive agents
administered ex vivo. A primary goal of this study was to evaluate the possible
mechanisms related to the reported physiological alterations of the isolated OA joint
including, increased arterial and total vascular resistance.\(^3\) Isolated vascular ring models,
from the lower limb, have been used to measure the response of normal vessels and vessels
from OA joints to known vasodilators and constrictors, but these studies did not investigate
vessel response in situ.\(^{18-20}\) Vasoreactive agents produce different responses depending on
dose, mechanism of action, receptor availability, substrate availability and length of action
and might be expected to produce different responses in normal and diseased joints.

Dopamine is an adrenergic (\(\alpha_1, \alpha_2\)) and dopaminergic receptor agonist that
increases blood pressure by vasoconstriction. Dopamine was used for vasoconstriction
because it primarily stimulates alpha receptors at moderate dosages and its effects resolve
quickly as the drug is rapidly metabolized upon termination of the infusion. Dopamine has
been documented to produce vasoconstriction in equine tissue.\(^21\) We administered a dose
in the moderate range that should have stimulation of the alpha receptors. Osteoarthritic
joints demonstrated a significantly muted response to dopamine induced vasoconstriction as
evidenced by a lower percent change in Pacir, Rt and synovial fluid production. This was
likely due to the significantly higher baseline resistance of the OA joint vasculature. These vessels may be near to maximal constriction, and unable, with increased stimulation, to constrict to a similar percent change as a relaxed arterial vessel such as those found in the control joints. Interestingly the OA joints took significantly longer to return to an isogravimetric state than the control joints following termination of infusion.

Norepinephrine is a postsynaptic $\alpha_1$, $\alpha_2$ adrenergic receptor agonist that also increases blood pressure by inducing vasoconstriction. Norepinephrine directly stimulated alpha receptors, intensely increasing peripheral vascular resistance and mean arterial blood pressure following the 15 minute infusion. This vasoconstriction demonstrated a significant increase in Pacir, Rt, percent change in Rt and a precapillary resistance that was muted in the OA joints as noted by the significantly lower increase in percent change from baseline as compared to the controls. As proposed for dopamine, this decreased response by OA joints to the intense vasoconstriction is probably related to the higher vascular resistance already present at baseline in OA joints. The vessels of these joints may be maximally contracted, or at least, less responsive. Higher vascular resistance in OA joints has been previously documented and reconfirmed in this study. Recently, it has been reported that digital vessels from horses with induced laminitis demonstrated diminished responsiveness to vasoconstrictors, compared to healthy vessels. The authors believed this altered responsiveness to be related to the increased vascular resistance in the diseased vessels, attributed to diminished response to endogenous endothelium-dependent relaxation.

Esmolol is a rapid-onset, short acting $\beta_1$ selective antagonist that induces vasodilation, decreasing total peripheral resistance. Esmolol demonstrated lower vascular compliance and more weight loss in OA joints compared to control. This can be attributed to decreased Pvcir in the OA joints compared to the control joints. In addition, a decreased synovial fluid protein level and oncotic pressure were documented in the OA joints.
following administration of esmolol. This diminished the permeability of these joints compared to controls. This was, again, likely due to the increased baseline resistance in the OA joints.

Nitroglycerin stimulated the release of NO, to decrease total peripheral resistance, by primarily acting on venous capacitance vessels. As the dose increased there was an additional increase in the relaxation of arterial vascular smooth muscle. Nitroglycerin showed a significantly greater percent change from baseline for Pacir and Rt compared to esmolol, yet, there was no difference between the two for Pvcir. Interestingly, both vasodilators demonstrated weight loss in OA joints compared to a decreased weight gain in control joints. In the OA joints, this was coupled with lower tissue and vascular compliances, likely attributed to fluid not remaining in the tissue but flowing into the venous system. Despite the lower arterial pressures due to arterial vasodilation, and lower permeability, these drugs stimulated weight loss related to the decreased vascular compliance that showed a diminished ability for filtration. The decreased vascular resistance caused by these drugs stimulated relaxation in the OA joint vasculature, slightly increasing blood flow. These changes increased synovial fluid production slightly in the OA joints following dilation. This was not significantly greater than controls, but was greater than synovial fluid production in OA joints following norepinephrine vasoconstriction.

Acting as a competitive antagonist for the endothelial enzyme NOS, LNAME blocks NO synthesis, increasing blood pressure via vasoconstriction. Physiologic responses to L-NAME may represent the contribution of endothelial-dependent vasodilators to the hemodynamics of arthritic joints. Both OA and control joints were less responsive to LNAME when compared to dopamine and norepinephrine, both known potent vasoconstrictors. This suggests increased responsiveness to sympathetic stimulation.
compared to NO mechanisms, particularly in the OA joints. Interestingly, we were able to
document a similar, yet opposite response in joints with nitric oxide stimulation
(nitroglycerin administration) and by blocking this activity with LNAME. This
demonstrates a general vasoactive response to NO within the joint vasculature.

Vasoconstriction increased arterial pressure and vascular resistance without
significantly increasing blood flow. Therefore changes in weight and synovial fluid
production are linked to alterations in the protein concentration and compliance conditions
within the two groups of joints. Normal isolated joints gained weight with increased
pressure increments, that were achieved via increased blood flow. Blood flow was not
significantly altered in our study. However, tissue compliance in both studies did increase
with pressure manipulation, allowing fluid to accumulate within the joint tissues, increasing
weight of the isolated joints. This was more prominent in the OA joints of this study than
the control joints following vasoconstriction. Capillary hydrostatic pressure increased
slightly with vasoconstriction, following the arterial pressure increase, thus increasing net
synovial fluid flow. As synovial fluid flow and arterial pressure increased, compliance of
the synovial tissues increased causing the isolated joint to gain weight. These weights
returned to baseline as the infusion of the vasoconstrictive drug was terminated, and the
pressures returned to baseline. In addition, the permeability was lower in the OA joints
compared to control joints, reflecting diminished fluid movement in the OA joints. This
finding, coupled with increased synovial oncotic pressure and net filtration in the control
joints, increased synovial fluid production in the control joints compared to the OA joints.

In general, vasodilators demonstrated a precapillary resistance that was
significantly less than the vasoconstrictors. However, there was no difference between
both vasodilators or joint groups for postcapillary resistance. Therefore, arterial
responsiveness appears to be predominant following administration of the selected
vasoactive agents. Tissue compliance was significantly lower in OA joints following vasodilation and lower for both groups following vasodilation compared to vasoconstriction and may represent capsular fibrosis associated with the OA. There were minimal changes in the remaining variables between groups.

In conclusion, we were able to define and compare the vasoactive properties of OA and control joints following administration of known vasoactive agents. Osteoarthritic joints demonstrated increased baseline vascular resistance and decreased flow compared to isolated control joints. This finding was similar to previous reports.\textsuperscript{3} Norepinephrine, or alpha-stimulation, was most effective in vasoconstriction for both groups, although, these changes, again, were muted in the OA joints. In addition, responsiveness to dopamine was significant in the hemodynamic changes of the OA joint. It appears that nitric oxide and sympathetic stimulation are prominent factors in basic joint physiology.
a- Masterflex, Cole Parmer Int; Chicago, IL.

b- Spectramed P23XL; Oxnard, CA

c- VR-12, Honeywell Corp; Pleasantville, NY.

d- FTO3, Grass Instruments; Quincy, MA

e- Grass Model 70 Polygraph, Grass Instruments; Quincy, MA

f- Sigma Chemical Co; St. Louis, MO

g- Sigma Chemical Co; St. Louis, MO

h- Brevibloc (esmolol hydrochloride); The Ohio State University Pharmacy.

I- Tridil (nitroglycerin); The Ohio State University Pharmacy.

j- Sigma Chemical Co; St. Louis, MO

References


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5.1 Signalment and group designation of horses used for OA and control isolations.
### Random order of drug administration for OA and control isolated joints.

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Baseline hemodynamic values for isolated OA and control joints prior to drug administration.

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<td>124.0 +/- 7.3</td>
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<tr>
<td>Pvcr (mmHg)</td>
<td>12.2 +/- 2.4</td>
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<tr>
<td>Qacr (mL/min)</td>
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<td>Pcap (mmHg)</td>
<td>29.5 +/- 2.3</td>
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<td>Synovial fluid volume (mL)</td>
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<td>1.5 +/- 0.5</td>
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<td>Rt (mL/mmHg)</td>
<td>13.1 +/- 2.9*</td>
<td>7.1 +/- 0.8</td>
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5.1 General trends of Pacir (mmHg) at baseline and following administration of vasoactive agents at 10 and 15 minutes. Different letters signify differences between time points for both OA and control joints.
Joint arterial pressure (mmHg) following drug administration

- Baseline
- Dopamine T=0
- Dopamine T=10
- Dopamine T=15
- Norepi T=0
- Norepi T=10
- Norepi T=15
- LNAME T=0
- LNAME T=10
- LNAME T=15
- Nitro T=0
- Nitro T=10
- Nitro T=15
- Esmolol T=0
- Esmolol T=10
- Esmolol T=15
5.2 Arterial pressure of the isolated joints as expressed on a percent change from baseline basis at 10 and 15 minutes after administration of vasoactive agents. Asterick represents a significant difference for both groups between time points.
Arterial pressure (% of baseline)

- Dopamine-10
- Dopamine-15
- Norepi-10
- Norepi-15
- LNAME-10
- LNAME-15
- Nitro-10
- Nitro-15
- Esmolol-10
- Esmolol-15

Figure 5.2
5.3 Time (minutes) to return to baseline isogravimetric state following administration of vasoactive agents. Different letters signify differences between time points for both OA and control joints.
Figure 5.3
5.4 General trends of Pvcir (mmHg) at baseline and following administration of vasoactive agents at 10 and 15 minutes.
Joint venous pressure (mmHg) after drug administration

- Baseline
- Dopamine T=0
- Dopamine T=10
- Dopamine T=15
- Norepi T=0
- Norepi T=10
- Norepi T=15
- LNAME T=0
- LNAME T=10
- LNAME T=15
- Nitro T=0
- Nitro T=10
- Nitro T=15
- Esmolol T=0
- Esmolol T=10
- Esmolol T=15

Figure 5.4
5.5 General trends of Qacir (ml/min) at baseline and following administration of vasoactive agents at 10 and 15 minutes.
Joint arterial flow (ml/min) following drug administration

Baseline
Dopamine T=0
Dopamine T=10
Dopamine T=15
Norepi T=0
Norepi T=10
Norepi T=15
LNAME T=0
LNAME T=10
LNAME T=15
Nitro T=0
Nitro T=10
Nitro T=15
Esmolol T=0
Esmolol T=10
Esmolol T=15
5.6 Capillary pressure (mmHg) at baseline and following administration of vasoactive agents.
Figure 5.6
5.7 General trends of total vascular resistance (mmHg/ml) at baseline and following administration of vasoactive agents at 10 and 15 minutes.
Percent change from baseline values of total vascular resistance of the OA joints compared to the control joints following administration of vasoactive agents.
5.9 Precapillary resistance (mmHg/ml) of OA compared to control joints following administration of vasoactive agents.
Figure 5.9

Dopamine  Norepinephrine  LNAME  Nitroglycerin  Esmolol

Precapillary resistance (mmHg/ml)

Control  OA

P<0.05
5.10 Postcapillary resistance (mmHg/ml) of OA joints compared to control joints following administration of vasoactive agents.
5.11 Synovial fluid production (ul/min) of OA joints compared to control joints following administration of vasoactive agents.
Figure 5.11
5.12 Transitional microvascular pressure (mmHg) following administration of vasoactive agents at 10 and 15 minutes.
Figure 5.12

Transitional microvascular pressure (mm Hg)

Dopamine
Endotoxin
Nitro
LNAME
Norepinephrine
5.13 Weight gain (g) of the isolated OA and control joints following administration of vasoactive agents.
5.14 Vascular compliance (ml/mmHg) of the isolated OA and control joints following administration of vasoactive agents.
**Figure 5.14**

![Graph showing vascular compliance for different drugs with significance levels P<0.004 and P<0.03.](image)

- **Control**
- **OA**

**Drugs tested:**
- Dopamine
- Norepinephrine
- LNAME
- Nitroglycerin
- Esmolol
5.15 Tissue compliance (ml/mmHg) of the isolated OA and control joints following administration of vasoactive agents.
Figure 5.15

Control □ OA

P < 0.001

Tissue compliance (mmHg/ml)

Dopamine  Norepinephrine  LNAME  Nitroglycerin  Esmolol

P < 0.001
5.16 Synovial fluid protein (g/dl) for OA and control joints following administration of vasoactive drugs.
5.17 Protein flux across synovial capillaries and tissues in isolated OA and control joints following vasoactive agent administration.
Figure 5.17
Permeability surface area product (ml/min) of isolated OA and control joints following administration of vasoactive agents.
Figure 5.18

Comparison of permeability surface area product (ml/min)

- Control
- OA

Drugs: Dopamine, Norepinephrine, LNAM, Nitroglycerin, Esmolol
CHAPTER 6

EX VIVO TRANSSYNOVIAL FLOW IN THE
OSTEOARTHRITIC JOINT

Summary

The purpose of this study was to evaluate the presence and mechanism of transsynovial flow of solute transport and fluid flow using synovium receptor agonism and antagonism. Ten healthy, sound adult horses, free from lameness were used in this study. Osteoarthritis (OA) was induced in one metacarpophalangeal (MCP) joint of 5 of the horses using an instability model. The remaining five horses had normal MCP joints and were used as controls. At euthanasia, synovial membrane from the OA and control joints were harvested for physiologic, histologic and viability studies. Physiologic studies were performed in modified flux chambers containing radioactive ions ($^{22}$Na, $^{36}$Cl) and protein to measure flux across the OA and control isolated membranes. Four vasoactive agents (dopamine, L-NAME, nitroglycerin and esmolol) were introduced into separate chambers with membrane from the same joint to evaluate receptor action with respect to solute flux. One chamber did not have any agent added (baseline). Albumin was added to one chamber to measure protein flux. Membrane specimens from each chamber were evaluated histologically for evidence of increased vascularity, inflammation and cellularity. Viability of synovial membrane was documented from OA and control membrane from baseline.
chambers. Control and OA synovial membrane demonstrated significantly greater sodium and chloride flux in chambers with receptor agonist or antagonist added compared to baseline chambers. Osteoarthritic membrane showed significantly lower Na and Cl flux than normal control synovium at 15 minutes but were not different at 30 minutes. Membrane from OA joints demonstrated increased Na and Cl flux after the addition of esmolol and decreased flux after the addition of L-NAME, dopamine and nitroglycerin, compared to control membrane.

Synovial membrane from OA joints were less responsive, taking a longer time to initiate ion flux as the control joints. Esmolol demonstrated the most significant difference between OA and control joints and compared to other additives. There were no differences in protein flux in OA or control membrane over time. Membrane sections maintained greater than 70% viability after 30 minutes in the chambers. Further studies demonstrating steady-state will enable documentation of stimulation and inhibition of transsynovial flux using these agents.
Introduction

Synovial membrane pathology and malfunction is present in the majority of painful joint disorders and plays a key role in initiating and perpetuating articular degradation leading to conditions such as chronic osteoarthritis (OA). The synovial membrane is responsible for the composition of synovial fluid through ultrafiltration of plasma and hyaluronate production. The synovial intima is generally considered to be 1-3 cells deep. It is comprised of two primary types of cells, the phagocytic A cells and the secretory B cells. The membrane lacks distinct cellular junctions as well as a basement membrane. Certain segments of the membrane maintain spaces between cells that are 1-2 μm wide, creating a substantial interstitial area for transsynovial exchange. The intercellular regions consist of a fibrous matrix, through which, most fluid exchange occurs. In acute inflammation, perfusion of the synovial membrane is altered through changes in capillary permeability, joint distension and distribution of blood flow. These changes affect synovial fluid quality and quantity, altering cartilage nutrition. Despite its important role in the pathogenesis of articular pathology, knowledge is limited on synovial transcapillary exchanges, perfusion, capillary permeability and sequential inflammatory mediator production associated with these transsynovial fluid movements. This is particularly true with respect to the solute and small ion transport across the membrane. The role of synovial membrane physiology and function in normal and OA joints deserves greater investigation.

Changes in capillary pressure or fluid protein levels affect the hemodynamics of the fluid flow. We have identified that OA equine MCP joints have significant alterations in baseline hemodynamic properties, in vivo, when compared to normal joints and that they respond differently to physical manipulations such as changes in arterial pressure, venous...
pressure and blood flow. The contributions of possible levels of control for the mechanisms altering transsynovial fluid flow are unstudied. In addition, there may also be local control at the synovial membrane intimal level, independent of capillary dynamics, that further influence solute movement and fluid flow. Further investigations of the synovial membrane transport would be necessary to determine the contributions to control at the cellular membrane level, rather than the arterial level.

Flux chambers have been used extensively to monitor changes in active and passive transport of sodium and chloride in colonic mucosa before or after stimulation. Such studies allow for precise measure of neurally mediated alterations in ionic current, indications of net active ion transport, with immediate measurement of the ensuing response. The chambers are designed to allow addition of various compounds and subsequent removal of tissue bathing fluid to perform analytical tasks. Intestinal tissue has been studied extensively in this system. This tissue differs significantly in the cellular composition of the membrane as compared to synovial membrane. Membrane from the intestine, is composed of a continuous layer of cells that are closely adhered at the apical portion of the cells by tight junctions. Intestinal membrane also has a distinct basement membrane compared to the synovial membrane which lacks constant, cell to cell interaction and a basement membrane. Transport across the intestinal membrane can occur through three primary routes: 1) aqueous route; 2) lipid route; and 3) carrier-mediated route. Each of these methods of transport has limitations. The aqueous route is composed of water-filled pores for the movement of hydrophilic substances into the cell, down a concentration gradient. Molecular size of the substance, pore size and the reflection coefficient are of great importance with this route. The lipid route limits movement in relation to the lipid solubility of the substance, with less importance on the molecular size. The carrier-mediated route assists the movement of hydrophilic substances of larger size,
too big to move through the aqueous pores and of charged substances. This route is limited by the affinity of the substance for a specific carrier and the number of carriers available in a specific tissue to transport that substance. These routes have been studied in intestinal membrane by use of the Ussing flux chamber. Measurement of the electrophysiology of this tissue allows determination of active transport of solutes across the membrane in addition to the measurable ion fluxes, representing gradient diffusion. The potential difference across the membrane would represent increased net active transport of the sodium ion. Synovial membrane, as mentioned earlier, lacks the distinct basement membrane and cellular junctions, allowing substantial area for transsynovial exchange in the interstitium of the membrane. Synovial membrane has not been evaluated in flux chambers to study similar influences on transmembrane transport or electrophysiological activity.

The studies performed in the intestine have closely evaluated multiple aspects of this tissue. The influence of neurotransmitters, reflexes and their responses to physical stimulation have been documented in various species with respect to ion and water transport across intestinal membrane. It is known that transport in the intestine is regulated by neurohumoral signalling. This signalling is mediated by messengers, altering apical and basolateral transport. Adrenergic agonists have been shown to increase ion and water transport across the intestine in vivo and in vitro. To support this, evidence of adrenergic nerve fibers and receptors have been documented in small and large intestine. The absorption of Na and Cl was increased in the rat colon with stimulation by alpha and beta agonists in vivo. This was determined to be a direct cellular effect due to the fact that nerve inhibitors did not alter this response. Acetylcholine was documented to have an opposite response, decreasing absorption of ions, or increasing secretion, in vivo and in
Intestinal mucosal preparations studied in flux chambers respond to stimulation with reduced absorption or increased secretion of chloride. This effect was prevented with the neurotoxin, tetrodotoxin, maintaining the evidence that neurotransmitter release mediates the flux response to stimulation. The intestinal membrane and synovial membrane are similar with respect to the underlying capillary and nerve beds.

This study was performed to compliment studies on vascular control of transsynovial flux already performed in OA joints and to further explore the influence of sympathetic and NO pathways in solute and transsynovial fluid flow in synovial membrane. Dopamine, esmolol, LNAME and nitroglycerin were selected for study in these modified tissue flux chambers. Dopamine is a short-acting sympathomimetic that causes vasoconstriction produced by action at vascular postsynaptic $\alpha_1$ and $\alpha_2$ receptors. In isolated equine MCP joints, this dose of dopamine was documented to produce vasoconstriction. Esmolol is a short acting $\beta_1$ antagonist that decreases blood pressure via vasodilation, thereby decreasing peripheral resistance. These agents were chosen to evaluate the relative influence of these receptor activations at the cellular level in joint physiology.

Nitric oxide (NO) is overproduced in rheumatoid arthritis and OA. It plays a significant role as an inflammatory mediator in arthritic conditions. Nitrite, an indirect measure of NO production, was increased in patients with rheumatoid arthritis and OA compared to human controls. The powerful vasodilating effects of NO enhance cellular injury in the articular tissues. Nitroglycerin, acting as a NO agonist, decreases total peripheral resistance by stimulating the release of NO. L-NAME blocks NO synthesis by acting as a competitive antagonist for the endothelial enzyme NOS. Therefore, L-NAME increases blood pressure via vasoconstriction. Physiologic responses
to L-NAME may represent the contribution of endothelial-dependent vasodilators to the hemodynamics of arthritic joints. The influence of these compounds on synovial membrane control of fluid flow has not been studied but NOS activity in equine MCP synovium is low and minimally stimulated by an inflammation model of arthritis.\textsuperscript{20}

Intestinal membrane has been evaluated in Ussing chambers to also determine the transport of bovine serum albumin.\textsuperscript{21} Intact albumin was evaluated in two different studies, one focusing on mucosal to serosal transport and one on serosal to mucosal transport. Both studies determine albumin transport to be an energy-dependent process that utilized the microtubular network on the membrane for transport. This process was also determined to be carrier-mediated as it was defined as a saturable process.\textsuperscript{22}

Our goal of this study was to investigate the presence and control mechanisms of synovial membrane solute transport and fluid flux at the tissue receptor level devoid of arterial hemodynamic contributions. The tissue flux chambers can be used to evaluate electrophysiology and basic diffusion across a membrane. While this would potentially yield more information, our goal was to evaluate general transport to determine if further studies would be indicated. These chambers provided assessment of synovial membrane solute (Na, Cl) and protein transport by intimal cells and associated fluid flux across dissected synovial membrane of OA and control joints with and without added vasoactive agents. This study also provided an assessment of viability following 30 minutes in the chambers fluid bath.

**Materials and Methods**

**Horses and clinical assessments**

Ten healthy horses (geldings and mares; age 3-8 years; 300-450 kg body weight) with no clinical or radiographic signs of MCP inflammation or degenerative joint disease...
were used. Each horse was required to be normal on physical examination, complete blood count (CBC), lameness examination. MCP joint circumference and range of motion measurements, radiographic examination (4 standard views) and synovial fluid analysis (white blood cell count [WBC] and total protein [TP]) of the MCP joints to be included in this study. Five horses underwent surgical transection of the lateral collateral and lateral collateral sesamoidean ligaments of a randomly selected MCP joint to create OA, as described previously. The remaining 5 horses served as normal controls. Inclusion criteria for the OA horses were: lameness greater than 1 out of 4 (1/4); greater than 45% decrease in pain-free range of motion; greater than 5% increase in joint size; and radiographic evidence of osteophyte formation. All OA horses were euthanatized with an overdose of pentobarbital 8 weeks after surgical induction of OA. All control horses were managed similarly to OA horses and euthanized at the same time period so studies were performed at the same time frame. All horses were managed in accordance with the Animal Care and Use Guidelines at The Ohio State University.

Experimental Design

Following euthanasia of all horses, synovial membrane was dissected aseptically from the palmar pouch of the OA or matched control MCP joint, and stored in the following manner: 1) one section (1 cm²) in 10% formalin for histology; 2) one large section (6-8 cm²) in Dulbecco's modified eagle media (DMEM) on ice to be transported to the lab for in vitro membrane physiology. The initial large section of membrane was placed in a petri dish with continuous bathing by Kreb's solution. The corners of the membrane were pinned down onto the gel of the petri dish to aid in dissection. A pair of fine ophthalmic scissors and forceps were used to separate the synovial intima from the underlying fibrous tissue using the help of a dissection microscope. Once the intima was
separated, the membrane was split into six 1 cm$^2$ sections. Each of the 6 sections, from the same joint, were placed into 6 separate chambers, prepared in a similar manner.

Radioactive ions ($^{22}$Na, $^{36}$Cl) were used to measure flux across the membrane in 5 of the chambers. The vasoactive agents (baseline, dopamine, esmolol, L-NAME, nitroglycerin) were used to evaluate alterations in equilibration to steady-state in the separate chambers. Albumin was used in the sixth chamber to evaluate protein transport across baseline OA and control synovium, independent of ion transport.

**Synovial membrane physiology**

The method used for basic membrane physiology of gastrointestinal specimens has been published previously and was duplicated for these studies. The 5 sections from one horse were studied on a given day due to the complexity of the study. Five sections of synovial membrane (1 cm$^2$ each) from either the OA joint in the OA group or a control joint from the control group were placed into individual flux chambers with the villous side to the right. It was attempted to obtain 5 tissue sections per joint to evaluate the 4 drugs and unstimulated baseline transport. Fluid bathing both sides of the chambers was Kreb’s Ringers solution consisting of (in mM): 120 NaCl, 6 KCl, 1.2 MgCl$_2$, 6 H$_2$O, 1.3 NaH$_2$PO$_4$·H$_2$O, 14.4 NaHCO$_3$, 2.5 CaCl$_2$, 2H$_2$O, 10 g glucose. Chambers, with specimens in place, were filled with the above Kreb’s Ringer solution (pH 7.2-7.4).

**Transsynovial ion flux**

Radiolabelled sodium ($^{22}$NaCl)$^a$ and chloride (Na$^{36}$Cl)$^b$ flux were measured across each synovial membrane from the interstitium (left) to the villous (right) surface to mimic
filtration of fluid into the joint space. Movement from the interstitium towards the intimal villous surface was evaluated using tissue from the OA joint and the control joints, simulating filtration of radioactive solute to the villous nonradioactive side of the chamber (Fig 1). A mixture of 1.5 μCi of $^{22}\text{NaCl}$ and 1.5 μCi $^{36}\text{Cl}$ were added to the interstitial (left) side of the tissue then the completed chambers were placed in an incubator (37 C, 5% humidity, 95 % CO2) for a total of 30 minutes. Drug receptor agonists/antagonists were placed into the left side, similar to radioactive agents, of separate chambers, each containing membrane sections from the same joint. The drugs tested were dopamine, esmolol, nitroglycerin and LNAME. One chamber was tested across time without addition of any drug (baseline).

Timed samples (50 μl every 5 minutes) were taken from the right side of the chamber to determine transport (filtration) across the synovium, and to evaluate the effects of the vasoactive drugs on transport (ie, permeability). Nonradioactive buffer solution, of the same volume, was placed in the right side of the chamber to replenish the chamber volume, after each sample was withdrawn. Specific activity remaining on the initial radioactive side was measured from a sample taken at the end of the experiment at 30 minutes. Combined (both Na$^{22}$, Cl$^{36}$) activity of both isotopes were determined by a liquid beta scintillation counter. Specific sodium activity (CPM) was determined by a gamma counter. Chloride activity (CPM) was then calculated by subtracting sodium activity from the beta scintillation counter activity.

**Transsynovial protein flux**

Albumin (30 ul) was placed on the interstitial side of the membrane in the baseline chambers for both OA and control joints. These chambers were separate from the baseline chambers used for ion flux, to not stearically inhibit ion transport. Samples of bathing fluid
(20 ul) were removed from the villous side of the membrane every 5 minutes for 30 minutes to assay for total protein flux across the membrane. Kreb's solution was used to replace samples removed from the chamber. Total protein (µg) was measured by the BioRad assay kit, using bovine serum albumin as a standard. Standards ranged from 0 to 50 µg protein.

**Synovial membrane histology**

Synovial membrane specimens were removed from formalin, placed in paraffin, sectioned at 6 µm and stained with hematoxylin and eosin. Slides were graded by two investigators, (SEW, EJS), in a blind fashion, for: 1) Presence of inflammation (scored 0 to 4 based on a percentage of interstitium within the villi with inflammation; 0 defined as absence of interstitial inflammation to 4 representing > 76% of villi interstitium inflamed.) 2) Presence of cellular hyperplasia (scored 0 to 4, based on continuity of synoviocytes on the surface of one side of a villous, 0 is the absence of hyperplasia and 4 is > 76% of surface having a continuous layer of > 3 cells thickness.) 3) Vascularity of the villi (scored 0 to 4; based on percentage of area of vascularity within the villi with 1 defined as <24% of area as vascular to 4 defined as >76% of area as vascular.) Graded scores were meaned and recorded.

**Synovial membrane viability**

The sections of synovial membrane from the control and OA chambers, without any additive, were removed at the termination of the experiment and assessed for viability. Each sample was rinsed with DMEM media 3 times. The samples were then incubated in 3 ml of trypsin 0.25% for 30 minutes at 37 C. Samples were rinsed in DMEM 3 times, and incubated in collagenase type IV (200 iu/ml DMEM) for 20 minutes at 37 C. The media was collected and centrifuged for 2 minutes at 6500 g. The supernatant was discarded.
Fifteen ml PBS and 15 ul of trypan blue 0.4% (diluted 1:2 with PBS) were added to the pellet. The sample was then examined at 40X magnification. Three hundred cells were counted and the percentage viability calculated. These values were meaned and reported descriptively.

Statistical analysis

A two-way ANOVA with repeated measures was used to compare each drug across time between OA and control groups. A least squared difference test for post-comparison defined any differences among specific drugs when a drug effect was identified. A Friedman’s test was performed to evaluate any significance among groups over time. Significance was set as P<0.05.

Results

Horses

All horses met the inclusion criteria for OA. The mean lameness grade was 1.5/4, joint circumference was increased by 5% over baseline, and pain-free range of motion was decreased by 45% over baseline. At the termination of the study, all OA joints demonstrated scorelines, osteophytes and surface erosions and all control joints were grossly normal.

Transsynovial ion flux

Synovial membrane specimens were successfully collected from both OA and control MCP joints and dissected to separate the fibrous from intimal layers. Chambers from the same joint were run simultaneously, under the same conditions to decrease variability. Membrane from the OA joint showed significantly less sodium flux in the baseline chamber at 15 minutes (0.1 +/- 0.04 uEq/cm²h; 0.8 +/- 0.07 uEq/cm²h,
respectively; P<0.05; Fig 2; Table 1). Dopamine decreased sodium flux significantly at 15 and 30 minutes in the OA joints compared to control joints (P<0.03; P<0.03. Table 1; Fig 3). LNAME decreased sodium flux in OA joints at 30 minutes compared to control joints (P<0.05; Fig 4). Esmolol increased sodium flux in the OA joints compared to control joints at 15 and 30 minutes (P<0.04, P<0.01; Fig 5). No differences were documented in the groups after addition of nitroglycerin (Fig 6).

Chloride flux alterations followed a similar pattern as sodium with the OA membrane significantly less (0.1 +/- 0.03 uEq/cm2h) in the baseline chambers compared to the control membrane (0.5 +/- 0.08 uEq/cm2h; Fig 7). Dopamine decreased chloride flux significantly at 15 and 30 minutes in the OA joints compared to control joints (P<0.05; P<0.04. Table 1; Fig 8). LNAME decreased chloride flux in OA joints at 15 minutes compared to control joints (P<0.05; Fig 9). Esmolol increased chloride flux in the OA joints compared to control joints at 15 and 30 minutes (P<0.04, P<0.03; Fig 10). Interestingly, both groups peaked and reached a steady state within the 30 minute period following addition of esmolol. Nitroglycerin decreased chloride flux in the OA joints compared to the control joints at 30 minutes (P<0.03; Fig 11).

**Transsynovial protein flux**

Total protein transport was evaluated across membrane from the OA and control joints in baseline chambers. There was no significant difference between OA and control membrane or over time (Fig 12).

**Synovial membrane histology**

Histological sections of synovial membrane from the OA joints demonstrated no difference in cellular hyperplasia or villous vascularity compared to the control joints.
There was, however, a significantly increased percentage of inflammation within the interstitium of the OA membrane (1.0 +/- 0.3) when compared to the specimens from the control joints (0.45 +/- 0.1; P<0.044).

**Synovial membrane viability**

Viability of the membrane from the chambers without drug was assessed. Synovial membrane specimens from the OA joint chambers yielded a mean viability of 71.67 +/- 0.88 %. The control chambers’ specimens yielded a viability of 72.0 +/- 1.53 %.

**Discussion**

This study reported here is the first documentation evaluating ion and protein transport across intact synovial membrane. In addition, we documented the ability to maintain viability of the membrane in these chambers for 30 minutes. Permeability and transport of solutes across synovial membrane are important variables in the determination of the health and well being of the joint tissues. The blood-joint barrier evaluated in vivo is essentially composed of two entities: the capillary endothelium and the synovial intima. The spaces between synovial cells in the membrane are 1-2 um wide. Therefore the spaces across the intima are up to 20% of the surface, leaving substantial area for solute exchange. Levick and McDonald demonstrated a theoretical bidirectional flow across the synovial membrane. They showed a net outflow from the joint cavity (absorption), with the addition of localized movement through the interstitium, to occur simultaneously. Synovial membrane tissue preparations remove arterial/arteriole influence on the control of solute transport and isolate the synovium control of solute transport, including filtration and absorption, placing increased emphasis on the stearic exclusion properties of this tissue. They showed the thinnest sectioning and dissection used in intestinal tissue was not achievable with the...
methods available in synovial membrane to allow for measurement of electrophysiology that would determine any active transport component in this tissue. Future studies will require microscopic dissection and possibly the use of microchambers to document active transport via electrophysiology of the synovial membrane. At this time, we focused on passive transport across the synovial membrane. We chose to evaluate selected ions (Na, Cl) and protein transport as an estimate of 2 molecules to pass across the synovial membrane. The ions selected reflect transsynovial fluid flow, while the protein reflects large pore permeability and hydraulic conductance.

This study focused on the equilibration phase of transsynovial flow with respect to the effect that known vasoactive agents have on pore size and concentration gradients of the OA and control membrane. In general, agents or compounds to be tested, are not added to the chambers until an equilibrium, or steady-state has been achieved. This allows each chamber to act as its own control compared to the measured changes following addition of compounds. By measuring samples in a steady-state, the alterations produced are direct drug effects, compared to transitory fluxes as the chamber reaches equilibrium. In this study, there was greater flux of sodium and chloride, across both OA and control membrane in baseline chambers as compared to chambers with vasoactive agent. There was no significant difference between the two groups at the end point (30 minutes). It is not known why addition of these agents decreased ion flux, although it is not thought to be specific receptor-mediated as it was noted with the addition of any drug. These findings are possibly related to indirect drug action such as irritation of the membrane, physical blockade of the channels, or cellular disruption such as swelling or shrinkage, thereby opening pores within the membrane. The OA membrane was significantly slower to initiate flux as documented by a lower value at 15 minutes compared to the control joint membrane. This is a predominant pattern across the chambers in this study, regardless of additive. Dopamine, LNAME and nitroglycerin demonstrated a decreased sodium and
chloride flux in OA membrane compared to control membrane, primarily at 15 minutes, while reaching a similar endpoint at 30 minutes. This again, demonstrated the trend for OA membranes to more slowly initiate ion flux. This can be attributed to the increased inflammation of the OA synovium, possibly decreasing access to the pores, or channels. Possibly an increased concentration of ion and fluid was necessary to break through the pore to move across the membrane. This does not appear to be a specific effect of the agent used. Further studies, using these agents in chambers to affect membrane already at steady-state, will allow documentation of a specific drug effect.

Intestinal tissue has been studied extensively with documented knowledge of active and passive transport across the membrane of this tissue. The lower level of ion flux documented in synovium transport compared to intestinal transport is attributed to the intestinal measurements being attained at a steady-state. Previous studies with intestinal tissue have shown increased flux with LNAME and an opposite response with nitric oxide stimulation. This did not occur in the present study, likely due to the difference in tissue architecture and the innate variability of flux studies. Esmolol increased sodium and chloride flux in OA joints. There is evidence of increased permeability to large solutes or proteins such as albumin (MW 69,000) in rheumatoid synovium. However, this same study demonstrated a small reduction in the permeability of small solutes, such as NaCl (MW 58.5). This was similar to the findings in the study presented here, where the OA membrane showed similar transport of protein compared to control membrane, yet showed slightly less ion flux than the control membrane, with the exception of esmolol stimulation. Increased inflammation of the OA tissue, although not as predominant as rheumatoid synovial inflammation, is the primary theory behind the decreased solute transport.
Esmolol normally has low lipid solubility, decreasing its ability to cross normal membrane. It is possible that the inflammation of the OA membrane altered the lipid structure of this membrane, increasing the ability of this agent to cross when inflamed.

This study focused on the general transport and alterations in equilibration of ions with the addition of known vasoactive agents. The findings presented here do indicate the need for future studies to determine steady state of ion flux and any possible active component of flux across the membrane. In addition, more intense histologic evaluation to document any increased thickness of the OA membrane compared to the control membrane would enhance the conclusions drawn from this data.

Synovial membrane from OA joints were less responsive, taking a longer time to initiate ion flux as the control joints. Esmolol demonstrated the most significant difference between OA and control joints and compared to other additives. There were no differences in protein flux in OA or control membrane over time. Membrane sections maintained greater than 70% viability after 30 minutes in the chambers. Further studies demonstrating steady-state will enable documentation of stimulation and inhibition of transsynovial flux using these agents.
Footnotes

a - NEN, Chicago, Ill.
b - ICN Pharmaceutical, Costa Mesa, Ca.
c - Scintillation counter
d - Gamma counter

References


6.1 Modified flux chamber demonstrating placement of membrane, radioactive ions, addition of drugs and removal of samples.
Agents added

\[ \text{Na}^{22} \text{Cl}^{36} \]

Interstitial side

Villous side

Samples withdrawn
6. 2 Sodium flux (μEq/cm²h) across the synovial membrane from OA and control joints without addition of vasoactive agents.
Figure 6.2
6. 3 Sodium flux (μEq/cm²h) across the synovial membrane from OA and control joints following the addition of dopamine.
Sodium flux with dopamine (μEq/cm²/h)

Figure 6.3

Control

OA

P < 0.03

Time (minutes)

0
10
20
30

0.1
0.2
0.3
0.4
0.5

-5
10
15
20
25
30
6.4 Sodium flux (μEq/cm²h) across the synovial membrane from OA and control joints following the addition of LNAME.
Figure 6.4

Sodium flux for LNAME (uEq/cm²/h)

Control • OA

Time (minutes)

P < 0.05
6.5 Sodium flux (μEq/cm²h) across the synovial membrane from OA and control joints following the addition of esmolol.
Figure 6.5

Control OA

Sodium flux with esmolol (UEq/cm²/h)

- control
- OA

P < 0.01

P < 0.04

Time (minutes)

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

5 10 15 20 25 30
6.6 Sodium flux (\(\mu\text{Eq/cm}^2\text{h}\)) across the synovial membrane from OA and control joints following the addition of nitroglycerin.
6. 7 Chloride flux (μEq/cm²h) across the synovial membrane from OA and control joints without addition of vasoactive agents.
Figure 6.7

- Control
- OA

P < 0.02
6.8 Chloride flux (μEq/cm²h) across the synovial membrane from OA and control joints following the addition of dopamine.
Figure 6.8

Chloride flux with dopamine (uEq/cm²/h)

Time (minutes)

OA  •  Control —

P < 0.05

P < 0.01

P < 0.001

Chloride flux with dopamine (uEq/cm²/h)
6. 9 Chloride flux (µEq/cm²h) across the synovial membrane from OA and control joints following the addition of LNAME.
Figure 6.9

Chloride flux with LNAME (µEq/cm²/h)

- Control
- OA

P < 0.05

Time (minutes)
6. 10 Chloride flux (µEq/cm²h) across the synovial membrane from OA and control joints following the addition of esmolol.
6. 11  Chloride flux (μEq/cm²h) across the synovial membrane from OA and control joints following the addition of nitroglycerin.
Chloride flux with nitroglycerin (uEq/cm²/h)

Figure 6.11

Time (minutes)

P<0.03
CHAPTER 7

SUMMARY

In conclusion, this series of studies documented a novel model of osteoarthritis utilizing the metacarpophalangeal joint of the horse. This model demonstrated classic clinical, gross and histopathologic signs of osteoarthritis as seen in naturally-occurring osteoarthritis and other well-accepted models. This model was then incorporated successfully into a published isolated joint model to evaluate hemodynamic alterations in the isolated osteoarthritic joint. This demonstrated an increased vascular resistance and decreased transsynovial fluid flow related to the diminished arterial blood flow.

To determine the basic mechanisms underlying these alterations, vasoactive agents were used to evaluate vasoreactivity of the isolated OA joint compared to the control joint. This demonstrated decreased sensitivity of the OA joints to vasoconstriction, likely due to the already enhanced resistance within these joint vessels. Further studies to determine basic tissue bath responses of these OA vessels would enable further determination of the mechanisms of these alterations.

This model of OA will likely be useful for future studies of physiology and therapeutic modalities. The similarities between this model and naturally-occurring OA and the stability of the lameness within the model make it useful for a variety of evaluations.
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