DISSERTATION

EQUINE CARPAL OSTEOARTHRITIS AND THORACIC LIMB FUNCTION: EFFECTS OF AQUATIC REHABILITATION

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2011

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ABSTRACT

EQUINE CARPAL OSTEOARTHRITIS AND THORACIC LIMB FUNCTION: EFFECTS OF AQUATIC REHABILITATION

Osteoarthritis (OA) is one of the most debilitating musculoskeletal disorders among equine athletes. It is a progressive disease characterized by joint pain, inflammation, synovial effusion, limited range of motion, and a progressive deterioration of articular cartilage. Unremitting joint pain and inflammation often cause adaptive muscle guarding and altered weight bearing to protect the affected limb from further discomfort and injury. In humans, compensatory changes in posture and movement exacerbate the initial joint injury, which cause further alterations in limb biomechanics and contribute to the progression of OA. Similar compensatory mechanisms such as altered muscle activation patterns, increased joint stiffness, and a redistribution of limb loading, are likely to also occur in horses.

Physical rehabilitation has become an effective treatment option for reducing or limiting harmful compensatory gait abnormalities in humans. Rehabilitation programs that address OA and musculoskeletal injuries often incorporate some form of aquatic exercise. Exercising in water provides an

effective medium for increasing joint mobility, promoting normal motor patterns, increasing muscle activation, diminishing limb edema, and reducing the incidence of secondary musculoskeletal injuries due to primary joint pathology. Underwater treadmill exercise has become an increasingly popular therapy for the rehabilitation of equine musculoskeletal injuries; unfortunately, there is no scientific evaluation of its effectiveness for the treatment of OA. This project was established to investigate the physiologic, biomechanical, and histologic effects of aquatic therapy on diminishing the progression of OA within the equine middle carpal joint. Results from this study will provide an objective assessment of the pathologic characteristics associated with OA and the potential clinical and disease-modifying effects allied with aquatic therapy.

Sixteen horses had an osteochondral fragment created in one randomly-selected distal radial carpal bone. All horses were exercised on the high-speed treadmill to induce OA, while eight horses were randomly assigned to either daily underwater treadmill exercise or traditional hand walking protocols. In the first investigation varying stance conditions (i.e., normal square stance, thoracic limb base-narrow, and blindfolded stance positions) were used to assess the efficacy of aquatic therapy to improve postural sway variables. Underwater treadmill exercise improved postural balance in both the base-narrow and blindfolded stance conditions, whereas the control group was only effective in maintaining balance when placed in a normal stance position.

The second portion of the study assessed the progression of OA and the efficacy of aquatic therapy based on clinical examination (gait and joint range of motion measurements), radiographic and MRI morphologic changes, local and referred pain quantification, intra-articular pressures and synovial fluid total protein, prostaglandin E_2 (PGE_2), and glycosaminoglycan (GAG) concentrations, along with histologic and biochemical evaluation of the structural disease process. Underwater treadmill exercise demonstrated limited positive effects on both clinical signs and disease-modifying outcome variables.

Lastly, the quantification of muscle activation patterns, in conjunction with forelimb kinetics and kinematics provided novel insights into the adaptive and maladaptive compensatory mechanisms associated with joint pain and OA. The influence of aquatic therapy demonstrated an overall ability to improve thoracic limb weight bearing distribution, along with maintaining symmetrical timing of select thoracic limb musculature.

Overall, the precise mechanisms by which aquatic therapy positively influenced clinical signs and demonstrated disease-modifying effects is unclear. Nevertheless, results from this study indicate that underwater treadmill exercise is a potentially viable therapeutic option in managing OA in horses.

ACKNOWLEDGEMENTS

Several individuals were essential in the development and implementation of this study.

I would first like to acknowledge my graduate committee, especially Dr. C. Wayne McIlwraith for allowing me to pursue a relatively unstudied area within equine research, as well as fostering my professional development. I would also like to thank the rest of my graduate committee: Dr. Chris Kawcak, his encouragement, support, and clinical expertise were crucial in the completion of this dissertation; Dr. Kevin Haussler for his ability to help me see the 'forest through the trees' and sorting through revision after revision; and Dr. Raoul Reiser for taking a 'never ever' biomechanist under his wing and for his endless assistance with programming.

I would also like to thank Dr. David Frisbie for his surgical expertise and assisting with the histology interpretation required for this study, and Dr. Natasha Werpy for her input on the imaging portion of the study.

Several people generously gave their time to help me with this study. I would like to acknowledge Nikki Phillips, who spent numerous hours in the lab running the synovial fluid analysis, Susan James for her histologic preparations, and Dr. Christina Lee for her late-into-the-night articular cartilage preparations. I would also like to thank the amazing EORC staff, especially the gait lab crew for their endless devotion and long hours collecting data.

Many thanks also go to my parents and my brother, as they have always been there for me encouraging me to follow my dreams. I would also like to acknowledge my dear friends Dr. Myra Barrett and Carrie Adrian for their support and motivation over the last three years.

Finally, I will forever be grateful to my husband and best friend Lee and to my beautiful and incredibly bright daughter, Alissa. They have waited with incredible grace and patience while I have pursued my goals.

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CHAPTER ONE

THE ROLE OF AQUATIC THERAPY IN MANAGING EQUINE OSTEOARTHRITIS

Introduction

Osteoarthritis (OA) is one of the most debilitating musculoskeletal disorders among equine athletes.[1] It is a common cause of poor performance, early retirement, and reduced life expectancy. Medical and surgical management of equine associated lameness disorders cost more than 700 million dollars within the United States alone in 1998.[2] Osteoarthritis is a progressive disease characterized by joint pain, inflammation, synovial effusion, limited range of motion, and a progressive deterioration of articular cartilage.[3] The disease process affects not only the articular cartilage but the entire joint, including subchondral bone, joint capsule, synovial membrane, and periarticular soft tissues.[3] As joint disease progresses characteristic pathologic changes occur including fibrosis and thickening of the joint capsule, articular cartilage fibrillation and erosion, and osteophyte formation, all leading to functional impairments.[3] Unremitting joint pain and inflammation often cause adaptive muscle guarding and altered weight bearing to protect the affected limb from further discomfort and injury.[4] Compensatory muscular adaptations are characterized by inefficient muscle activity leading to muscle weakness, joint instability and altered limb loading.[5] Maladaptive musculoskeletal responses may produce additional gait alterations and predispose other articulations to increased risk of injury (i.e., compensatory lameness).[6] In humans, compensatory changes in posture and movement exacerbate the initial joint injury, which cause further alterations in limb biomechanics and contribute to the progression of OA.[5] Similar compensatory mechanisms such as altered muscle activation patterns, muscle overuse or atrophy, increased joint stiffness, and a redistribution of limb loading are likely to also occur in horses.

Physical rehabilitation has become an effective treatment option for reducing harmful compensatory gait abnormalities in humans.[7] Rehabilitation programs that address OA and musculoskeletal injuries often incorporate some form of aquatic exercise. Exercising in water provides an effective medium for increasing joint mobility, promoting normal motor patterns, increasing muscle activation, and reducing the incidence of secondary musculoskeletal injuries due to primary joint pathology.[8] Human patients with lower extremity OA show a significant gain in limb-loading parameters and improved joint range of motion following aquatic exercise.[9] Underwater treadmill exercise has become an increasingly popular therapy for the rehabilitation of equine musculoskeletal injuries; unfortunately, there is no scientific evaluation of its effectiveness for the treatment of OA and its associated alterations in musculoskeletal function in horses.

Neuromuscular responses to joint pain

Elite equine athletes undergo intense training and competition demands that often predispose horses to an increased risk of musculoskeletal injuries. Osteoarthritis and tendonitis are the two most common musculoskeletal injuries that occur within the equine forelimb.[1] Unfortunately, veterinarians and horse owners often focus on management of the pathoanatomical diagnosis of the joint or tendon injury and fail to appreciate how the presence of pain and altered limb function may cause secondary impairments in motor function within the ipsilateral and contralateral limbs. It is hypothesized that pain, inflammation, and muscle weakness associated with a primary musculoskeletal injury often cause altered weight bearing in the affected limb and compensatory changes in posture and movement that may exacerbate the initial insult or increase the risk of additional injuries.[4,7] However, the effects of these maladaptive mechanisms of the neuromuscular and musculoskeletal systems are often overlooked in both diagnosis and treatment. Biomechanical research has demonstrated decreased quadriceps muscle activation in human patients with knee OA, which leads to increased joint compression, altered limb loading, and a progression in OA.[10] Characterization of the alterations in limb use and abnormal muscle activation patterns associated with pain and OA is critical for developing targeted, clinically relevant diagnostic and treatment strategies to minimize compensatory changes that may lead to further musculoskeletal injuries in horses.

Recent human research has expanded our understanding of neuromuscular responses to joint pain.[11] Mechanoreceptors are characterized as sensory receptors within periarticular tissues that respond to changes in joint position and acceleration of movement, as well as playing an important role in regulating and maintaining neuromuscular control of joint stability.[7] Pain, inflammation and joint effusion associated with OA alters the normal sensory input from articular mechanoreceptors, which may result in decreased motor neuron excitability and reduced muscle activation.[12] A common outcome following experimentally-induced knee effusion is the presence of significant quadriceps muscle inhibition.[13-16] A linear relationship exists between increased intra-articular pressure and the level of quadriceps inhibition.[13] Electromyography studies conducted on the quadriceps muscle of patients with knee osteoarthritis often indicate a decrease in muscle strength and altered muscle timing.[17] Typically there is a delay in the onset of quadriceps muscle activation, which results in the inability of the quadriceps to stabilize the joint and attenuate the loading forces properly during locomotion.[14] The increased joint instability further alters the distribution of weight bearing forces across joint surfaces and increases the recruitment of adjacent muscles to aid in joint stability.[18] The resulting imbalances in paired agonist–antagonist muscle groups contribute to increased joint instability and altered limb biomechanics, which leads to progressive OA and chronic maladaptive compensatory mechanisms.[19]

Rehabilitation protocols used to address morbidities associated with OA often focus on improving muscle function and joint biomechanics.[10,20,21] Aquatic therapy is frequently prescribed for rehabilitation of orthopaedic injures in humans, with the goal of preventing further musculoskeletal injury and improving the overall function of the affected limb.[22] Following rehabilitation, human patients have demonstrated normalized muscle activation patterns and improved joint stability, joint range of motion, and proprioception.[7] As a profession, veterinarians are beginning to recognize that additional joint injuries may be exacerbated by decreased muscle activation, reduced joint range of motion, and fibrosis of the periarticular soft tissues. Therefore, the need to rehabilitate the entire horse instead of only focusing on the primary injury is critical for horses to return to optimum performance and overall athletic function. For professionals involved in providing equine rehabilitation, there is a basic lack of knowledge about which specific exercises or therapeutic modalities are effective for select musculoskeletal injuries and their general mechanisms of action.[23] The purpose of this review is to define the proposed mechanisms of action of aquatic therapy and to determine its efficacy in the treatment of equine OA.

Aquatic Therapy

Therapeutic aquatic interventions are often used to optimize the treatment of sensory and motor disturbances associated with the functional restoration of full athletic performance.[23] Aquatic therapies, including underwater treadmills and swimming, have been reported in humans to improve muscle strength and timing, increase cardiovascular endurance, decrease limb edema, improve range of motion, decrease pain, and reduce mechanical stresses applied to the limb.[24] Exercising in water provides a medium in which reduced gravity due to increased buoyancy, increased hydrostatic pressure, varying temperature effects, changes in salinity, and the increased viscosity of water combine to play an important role in musculoskeletal rehabilitation. The increased resistance and buoyancy inherent in aquatic exercise minimizes joint instability and weight bearing stress on muscles and joints.[25] Therefore, aquatic therapy has been an effective method of rehabilitation for individuals with OA that have difficulty with weight bearing associated with land exercise.[26]

Buoyancy

In the context of aquatic therapy, buoyancy is defined as a lifting force that acts to reduce axial loading of the joints by minimizing vertical ground reaction forces. Underwater force platform analysis of human subjects walking demonstrate a significant reduction in vertical ground reaction forces;[9] which is strongly correlated with the depth of water immersion. Humans walking at a slow pace in water at the level of the manubrium have a 75% reduction in weight bearing, but only a 25% reduction when walking in water at the level of the anterior superior iliac spine of the pelvis.[27] Walking at a fast pace in water at the level of the manubrium decreases impact forces by 1/3 to 1/2 of body weight compared to walking on land.[28] The reduction in weight bearing forces applied to the limbs allows for continued improvement in strength and joint range of motion while minimizing pain associated with impact. In horses, water at the level of the point of the shoulder produces a 50 to 60% reduction in weight.[29] Buoyancy reduces the effects of weight bearing stress placed on joints and the surrounding soft tissue structures, which reduces pain and inflammation associated with impact loading exercises. Furthermore, underwater kinematic analysis in humans demonstrated that buoyancy improves joint range of motion. Human patients with lower extremity OA show increased limb flexion while walking in water compared to decreased joint range of motion when walking on land.[30]

Viscosity

The increased viscosity of water compared to air causes an increased resistance to movement, which enhances the functional capacity (ability to perform) of both normal and pathologic neuromuscular systems. The viscosity of water is about 800 times greater than that of air; therefore, the increased effort required to move through water causes increased muscle activation and improves muscle strength, motor control and joint stability.[9] Electromyographic analysis during underwater flexion/extension exercises of the knee in human patients demonstrates increased activation of the agonist muscles during concentric contraction.[30] However, during the same concentric contraction a decrease in co-activation of the antagonist muscle occurred.[26] Increased agonist muscle activity is required to accelerate the limb in the direction of movement. During a concentric contraction the antagonist muscles are typically activated to decelerate the limb segments in preparation for foot contact. However, when exercising in water the increased resistance applied in the direction of limb motion requires minimal muscular braking of the segments.[26] Human patients with knee OA routinely demonstrate an inhibition of the quadriceps muscle group and a corresponding increase in the activity of the antagonist hamstring muscles. The increased activation of the hamstring muscles is a compensatory mechanism that helps to stabilize the joint and attenuate loading forces across the knee during locomotion.[17] Furthermore, patients with quadriceps inhibition demonstrate an increased impulsive loading of the limb, which leads to excessive or abnormal loading of the knee joint and progressive OA.[31] The increased resistance provided by aquatic therapy stimulates agonist muscle activity and reduces co-activation of antagonist muscle pairs, which enhances the neuromuscular control of muscles inhibited by the presence joint pain.

Temperature Effects

The effect of water temperature on the peripheral circulation of human patients during full body immersion demonstrates that a temperature of 32°C produces a redistribution of blood volume from the peripheral vasculature to the general circulation due to cutaneous vascular constriction.[32] This reduced blood flow decreases tissue metabolism and provides an analgesic effect by decreasing nerve conduction.[33] Conversely, during warm water immersion (36°C), reduced peripheral vascular resistance is produced by vasodilation within the peripheral vessels.[32] This increase in soft tissue perfusion may aid in dissipating inflammatory mediators associated with tissue inflammation and pain.[24] Water temperature during aquatic exercise may also play an important role in nociception by acting on local thermal receptors, as well as increasing the release of endogenous opioids.[34]

Saline Hydrotherapy

Exercising in hypertonic water has been reported to have anti-inflammatory, osmotic and analgesic effects.[35] A two week course of daily exercise in hypertonic water demonstrates improved mechanical nociceptive thresholds over the medial femorotibial joint in human patients with OA.[36] Similarly, patients with fibromyalgia report significant improvements in pain scores lasting up to three months following hypertonic hydrotherapy.[37] Horses diagnosed with distal limb injuries treated with hypertonic cold water therapy for four weeks demonstrated both clinical and ultrasonagraphic healing of both flexor tendon and suspensory lesions.[38] Visual improvements in the degree of soft tissue swelling were demonstrated within 8 days of the initiation of hypertonic cold water therapy administered for 10 minutes, three to four times a week.[38] Cases of tendonitis and desmitis that were monitored ultrasonagraphically report reduced peritendinous/periligamentous edema, decreased inflammatory infiltration, and improved fiber alignment after four weeks of hypertonic cold water therapy.[38] The mineral components within the water may work to provide an osmotic effect, which reduces soft tissue inflammation and swelling, decreases pain, and ultimately improves joint range of motion.

Hydrostatic pressure

Hydrostatic pressure facilitates an increase in neuromuscular function by stimulating cutaneous sensory nerves and joint mechanoreceptors. Under normal circumstances, as the joint capsule and surrounding ligaments stretch there is increased firing from the joint mechanoreceptors, which synapse onto the gamma-motor neuron in the ventral horn and ultimately induce fine adjustments in muscle tension that influence joint stability.[39] Joint mechanoreceptors are responsible for (i) signaling joint position and movement, (ii) aiding in the control of both timing and direction of joint movement, (iii) contributing to the initiation of reflexive muscular responses that maintain joint stability, and (iv) playing a primary role in joint nociception.[40] These specialized receptors function both as proprioceptors and modifiers of muscle activity which act to increase joint stability and protect joint structures from excessive or abnormal loading.[29] Reflex mechanisms mediated by joint receptors help to protect an injured joint from further damage via either inhibition or activation of muscular guarding in response to joint pain.[15] The joint mechanoreceptors also register mechanical deformation and changes in intra-articular pressure during joint loading. The increase in intra-articular pressure associated with joint effusion and synovitis causes afferent excitatory stimulation of 1b interneurons (located within the central nervous system), which results in inhibition of the muscles that function across the joint.[14] Afferent excitation stimulated by increased intra-articular pressure may be dampened by the effects of increased hydrostatic pressure and buoyancy provided by aquatic therapy.[24] The reduced inhibition of the 1b interneurons allows the activation of the alpha motor neurons, which results in increased muscle tone and activation. In addition, the immersion of the limb in water increases vascular and lymphatic circulation, thus reducing soft tissue swelling, increasing range of motion and decreasing pain.[24] The reduction in swelling and joint effusion may further improve synaptic information from the joint mechanoreceptors, re-establishing arthrogenic control critical for optimal soundness and athletic activity.

Effectiveness of aquatic therapy in human patients with OA

Aquatic therapy is commonly prescribed by physicians for the management of OA. The therapeutic effects of water are particularly useful for management of disabled patients with significant joint pain associated with weight bearing and land exercise. Joint replacement and arthroscopic surgery patients are also commonly referred for aquatic therapy intervention. Post-surgical patients may not be able to tolerate full weight bearing exercise soon after surgery because of increased mechanical loading on the injured joint. Aquatic exercise decreases the weight bearing stresses applied to the operated joint, allowing an early and more aggressive but less painful rehabilitation program without risk of overloading the injured joint.[41] Human patients undergoing surgical reconstruction of their anterior cruciate ligament demonstrate improved knee range of motion and quadriceps strength following aquatic therapy, compared to traditional rehabilitation.[33] Two weeks post-surgery, total hip joint replacement patients participating in aquatic therapy also demonstrate significant gains in hip abduction strength, compared to standard rehabilitation.[42]

Few randomized clinical trials assessing aquatic therapy for the nonsurgical management of OA have been conducted in patients with knee and/or hip osteoarthritic changes. These trials typically assign patients at random to either a water-based exercise group or a land-based exercise group and assess pain as the primary outcome measure. Secondary outcome measures include muscle strength, joint range of motion, and functional gait assessment measured by a timed walking test. Osteoarthritic patients in aquatic therapy programs often demonstrate improved pain scores following therapy.[43] A 50-foot walking functional test demonstrate improvements in time to completion in both land and water based exercise groups.[44] However, osteoarthritic patients in the aquatic therapy group had significant reductions in pain scores prior to and following the functional testing, compared to the land based exercise group.[44] Additional blinded, randomized controlled trials report similar findings. Patients with knee and/or hip OA enrolled in a six-week program of aquatic therapy show improved physical function, increased muscle strength, and a significant reduction in pain, compared to no intervention.[17,20] Aquatic therapy appears to have clinical benefits for the management of clinical signs associated with OA; however, controlled, randomized studies of these benefits are too few to determine if aquatic therapy reduces the progression of cartilage degradation.

Aquatic therapy in horses

While aquatic therapy is widely used in rehabilitation programs for humans, there are few investigations into the benefits of this form of exercise for equine patients. However, several studies on aquatic therapy have been conducted in dogs. Post-surgical rehabilitation of stifle injuries in dogs often incorporate swimming exercise. Aquatic therapy in dogs following cranial cruciate ligament reconstruction produces significant increases in joint range of motion, not only in the operated stifle, but also in the non-operated stifle.[45] A similar canine study assessing the efficacy of aquatic therapy following surgical repair of cranial cruciate ligament injuries demonstrated improved pelvic limb biomechanics, with no difference in peak vertical force or vertical impulse, as measured by force platform analysis between the repaired and contralateral limb at six-months follow up.[46] Kinematic analysis of dogs walking in an underwater treadmill demonstrated that joint flexion is maximized when the depth of the water is maintained above the joint of interest.[47] Clinical outcome measures of thigh circumference and stifle joint range of motion were assessed in cranial cruciate ligament-deficient dogs after tibial plateau osteotomy.[48] Underwater treadmill exercise improved stifle passive joint range of motion and increased thigh circumference, compared to cage rest and controlled walking.[40] Six weeks after surgery, there was no difference in thigh circumference and joint range of motion between the affected and unaffected limbs in the aquatic therapy group.[40] In contrast, the conventional therapy group demonstrated continued progression in joint stiffness and muscle atrophy.[40]

Unlike the canine studies, equine investigations involving aquatic therapy focus mainly on the horse's physiologic responses to exercising in water. Several equine studies have investigated cardiovascular function, blood lactate levels, and respiratory function during swimming exercise.[49-51] Swim training programs have demonstrated an improvement in cardiovascular function, a reduction in locomotor disease, and an increase in the development of fasttwitch, high-oxidative muscle fibers, reflecting improved aerobic capacity.[52,53] A recent equine study assessed changes in stride parameters while walking in various depths of water.[54] Underwater treadmill walking with water at the level of the ulna results in a longer stride length with a reduced stride frequency, compared to walking with water at the level of the pastern joint.[54] To date there are no objective studies that have determined the ability of underwater treadmill exercise to improve muscle activation patterns or thoracic limb biomechanics in the horse. There are also no studies that have objectively assessed the effects of aquatic therapy on the onset and progression of equine OA.

Conclusion

Osteoarthritis in horses is typically managed with conventional therapies aimed at reducing and maintaining a decreased level of inflammation. There are a limited number of therapies that have demonstrated disease-modifying effects however, no one therapeutic agent has effectively eliminated the progression of joint disease. Any form of treatment that can retard the onset and progression of OA is of great importance, both for continued athletic performance and for quality of life. From the canine and human literature reviewed, aquatic therapy has a significant effect on pain reduction and related outcome measurements for musculoskeletal diseases. The beneficial effects of aquatic therapy for the management of equine carpal OA have not been investigated. There is an obvious need for well-designed studies to provide sufficient evidence for the therapeutic efficacy of underwater treadmill exercise on the development and progression of OA in horses.

Purpose of Study

The purpose of this project was to identify alterations in muscle activity and thoracic limb biomechanics associated with acute joint pain and carpal OA in the horse. The acute or subclinical effects of joint pain and instability on muscle activity and compensatory gait mechanisms are unknown. The quantification of muscle activation patterns, in conjunction with thoracic limb kinetics and kinematics, provided novel insights into the adaptive and maladaptive compensatory mechanisms associated with acute joint pain and early onset OA. A better understanding of these secondary mechanisms is expected to provide the equine practitioner with improved treatment and rehabilitation protocols focused not only on maximizing treatment of the primary injury, but also slowing or preventing the development of OA and other compensatory musculoskeletal injuries.

This project was designed to assess static postural control and to identify equine muscle activation patterns, altered thoracic limb function, and articular pathology associated with a carpal OA model, as well as objectively evaluated the potential therapeutic benefits of underwater treadmill exercise, compared to traditional hand walking.

Study Goals

The goal of this project was to evaluate the effectiveness of underwater treadmill exercise on reducing morbidity associated with carpal OA, with the long term goal of reducing maladaptive compensatory lameness and secondary musculoskeletal injuries in horses.

Hypotheses

H1: Alterations in stance conditions will diminish static postural stability in horses with carpal joint pain and inflammation.

H2: Horses with experimentally-induced carpal OA exercised in the underwater treadmill will have improved postural sway characteristics.

H3: Acute joint pain correlates with alterations in thoracic limb biomechanics and muscle activation patterns.

H4: Aquatic therapy will enhance neuromuscular function and reduce thoracic limb gait abnormalities associated with carpal joint pain and inflammation.

H5: Aquatic therapy will significantly improve synovial fluid parameters along with gross and histologic morphologic scores in synovial membrane and articular cartilage.

Specific Aims

1. To assess static postural stability under varying stance conditions (i.e., normal square stance, thoracic limb base-narrow, and blindfolded) in horses with experimentally-induced carpal OA.

2. To evaluate the effects of underwater treadmill exercise on static postural sway in horses with carpal OA.

3. To determine thoracic limb motion characteristics and muscle activation patterns in horses with an experimentally-induced carpal osteochondral fragment and subsequent OA.

4. To assess the ability of underwater treadmill exercise to normalize thoracic limb locomotion and muscle activation patterns in horses with carpal OA.

5. To evaluate the effects of underwater treadmill exercise on the progression of carpal OA.

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CHAPTER TWO

EFFECT OF EQUINE CARPAL OSTEOARTHRITIS ON POSTURAL SWAY CHARACTERISTICS BEFORE AND AFTER AN AQUATIC EXERCISE INTERVENTION

Introduction

Postural stability is achieved through the precise integration of proprioceptive, vestibular, and visual information. Effective postural control also relies on the biomechanics of the musculoskeletal system, including joint stability and neuromuscular control of the extremities.[1] Muscles acting across the joints are responsible not only for motion but also for stabilization during limb loading and the maintenance of stance, thus making crucial contributions to balance control. Diminishing sensory function and slowing of sensorimotor processing compromise postural stability.[2] Specifically, the progression of osteoarthritis (OA) is of particular interest as it affects the integrity of articular cartilage, exacerbates synovial membrane inflammation, as well as induces joint capsule fibrosis, and peri articular muscle weakness.[3,4] These modifications also alter the neurophysiologic properties of joint mechanoreceptors, which aid in controlling the tonic muscular activity responsible for stabilizing joints during quiet standing.[2]

Joint mechanoreceptors provide continuous sensory input to the nervous system concerning the conditions in and around the joint. Joint mechanoreceptors are responsible for (i) signaling joint position and movement, (ii) aiding in controlling both timing and direction of joint movement, (iii) contributing to the initiation of reflexive muscular responses to maintain joint stability, and (iv) playing a primary role in joint nociception.[4] These specialized receptors function both as proprioceptors and modifiers of muscle activity which act to increase joint stability and to protect joint structures from excessive or abnormal loading.[5] Under normal circumstances, as the joint capsule and surrounding ligaments stretch there is an increase firing from the joint mechanoreceptors, which synapse onto the gamma-motor neuron in the ventral horn of the spinal cord (i.e., muscle spindle innervation) and ultimately induce fine adjustments in muscle tension that influence joint stability.[5] Reflex mechanisms mediated by joint receptors help to protect an injured joint from further damage via either inhibition or stimulation of muscle guarding in response to joint pain.[6] The persistence of joint pain and inflammation causes afferent excitatory stimulation of 1b interneurons located in the ventral horn of the spinal cord, which results in inhibition of the muscles that function across the joint.[7,8] It has also been reported that abnormal afferent articular information decreases gamma motor neuron activation, which in turn alters muscle spindle sensitivity and ultimately impairs proprioception.[9] Several human research studies have demonstrated the arthrogenic muscle response to joint mechanoreceptor afferent activity related to the presence of joint effusion, pain, and OA.[7,8,10,11] Understanding the neural networks involved in the patterned muscle responses that occur following joint effusion provides a fundamental comprehension of the compensatory adjustments made for the maintenance of postural control.

Osteoarthritis is one of the most debilitating musculoskeletal disorders among equine athletes and is a common cause of poor performance, early retirement, and reduced life expectancy.[12] Medical and surgical management of equine associated lameness disorders cost more than 700 million dollars within the United States alone in 1998.[13] Traditionally, veterinarians and horse owners or trainers often focus on management of the pathoanatomical diagnosis of the joint or tendon injury and fail to appreciate how the presence of pain and altered limb function may cause secondary impairments in motor function within the ipsilateral and contralateral limbs. It is hypothesized that pain, inflammation, and muscle weakness associated with a primary musculoskeletal injury often causes altered weight bearing in the affected limb and compensatory changes in posture and movement that may exacerbate the initial insult or increase the risk of additional musculoskeletal injuries.[4,14,15] Unfortunately, the effects of these maladaptive mechanisms of the neuromuscular and musculoskeletal systems are often overlooked in both diagnosis and treatment. Human research using force platform analysis has demonstrated that patients with knee and hip OA have significantly impaired neuromuscular function during quiet standing that negatively affects the ability to control balance.[16,17] Characterization of the alterations in postural stability associated with pain and OA is critical for developing targeted clinically relevant diagnostic and treatment strategies to minimize compensatory changes that may lead to further musculoskeletal injuries in the horse.

Subclinical musculoskeletal dysfunction associated with OA in human patients can be quantitatively measured using force platform analysis during quiet standing.[1,4,16,17] In order to stand stationary, slight adjustments in muscle tension are required to maintain positioning of the center of mass during upright stance. While standing quietly on a force platform these slight adjustments in muscle tension can be recorded as movements of an individual's center of mass in mediolateral and craniocaudal directions.[18] The force platform records the center of pressure (COP) as the point of application of the ground reaction forces under the feet.[19,20] The trajectory of the COP provides insights into the process of balance control as changes in the movement of the COP are directly related to the motion of an individual's center of mass.[19] The COP movement in craniocaudal or mediolateral directions indicates the principal direction of sway. An excessive sway in a mediolateral direction is often used to predict if human patients are at risk for falling.[21] Individuals with proprioceptive deficits will have a larger COP area due to altered signaling pathways that diminish the neuromuscular responses needed for postural stabilization.[22-24] Force platform analysis demonstrates that human patients with knee and/or hip OA have significantly greater postural sway disturbances compared to age-matched healthy subjects; concluding that OA negatively affects balance.[16,17] In addition, electromyography (EMG) studies conducted on the quadriceps muscle of patients with knee OA often demonstrate a decrease in muscle strength and delayed quadriceps muscle activation.[1] This inappropriate activation of the quadriceps leads to balance deficits; a reduction in the ability to maintain, achieve, or restore postural stability.[4] Abnormally increased COP displacement in either a craniocaudal or mediolateral direction potentially allows for identification of the neuromuscular response mechanisms primarily responsible for maintaining stance positions.

In equine clinical and research settings the assessment of balance is typically done through a series of subjective tests; the objective assessment of balance has just recently been investigated. Recent equine research has demonstrated that force platform analysis provides reliable results in the assessment of postural stability in the horse.[23,24] Healthy horses have been used to establish reliable postural sway outcome measures needed to quantify balance control.[24] A follow-up study assessed the effect of sedation on postural stability, indicating that the measured COP variables demonstrated significant changes following sedation.[23] The quantification of balance control can be a valuable tool for the diagnosis of subclinical neuromuscular and musculoskeletal pathologies and in the monitoring of the response to rehabilitation. Horses with inaccurate sensory feedback due to pain, inflammation, or inappropriate muscle activity may have an impaired ability to make motor output adjustments efficiently. Therefore, recognizing that alterations in static postural stability may influence performance on balance required dynamic tasks will further aid in targeted diagnostic and treatment strategies.

Physical rehabilitation has become an effective treatment option for reducing or limiting harmful compensatory gait abnormalities in humans.[4] Rehabilitation programs that address OA and musculoskeletal injuries often incorporate some form of aquatic exercise. Exercising in water provides an effective medium for increasing joint mobility, promoting normal motor patterns, increasing muscle activation, and reducing the incidence of secondary musculoskeletal injuries due to primary joint pathology.[25] Human patients with lower extremity OA show a significant reduction in the amplitudes of postural sway following aquatic exercise.[21] The improved muscle strength and function associated with aquatic exercise significantly reduces the abnormal postural sway characteristics typically demonstrated in osteoarthritic adults.[16] Recent postural sway studies have focused on the use of tactile stimuli as an aid for proprioceptive and postural stability. Tactile stimuli in the form of foot orthotics significantly improved postural control in human patients with ankle instability.[26] The application of water as a form of tactile stimuli may enhance motor learning through multisensory impulses, improving posture and stability.

Human postural sway assessment using force platforms often is assessed under both a double- and single-leg stance position,[21] along with testing subjects with their eyes open and closed.[27] Trials with the eyes closed are often included to assess the role of visual pathways in postural stability. There is conclusive evidence that the changes in stance and the removal of visual cues provides valuable information into the severity of balance deficits present in patients with OA and is effective in assessing the degree of improvement in postural sway variables following an exercise intervention.[16,25,27-29] Subjective assessment of a horse's response to balance related manipulative tests (i.e., crossed-limb stance and blindfolded) are routinely incorporated into clinical neurologic exams. However, the relationship between changes in stance conditions and the associated balance deficits in the horse remains inconclusive. Furthermore, the effect of aquatic exercise on postural sway variables in horses with carpal OA has yet to be investigated. Therefore, the two primary purposes of this study were (1) to assess static postural sway under varying stance conditions (i.e., normal square stance, thoracic limb base-narrow, and blindfolded), and (2) to evaluate the effects of underwater treadmill exercise on static postural sway in horses with carpal OA. Our hypotheses were that the alterations in stance conditions would diminish postural stability and that horses with experimentally-induced carpal OA exercised in the underwater treadmill would have improved postural sway characteristics compared to control horses.

Materials and Methods

Animals - Sixteen skeletally mature horses, age 2 – 4 years (mean ± SD mass, 385 ± 40 kg; height at withers, 1.46 ± 0.04 m) were included in the study.

Prior to inclusion in the study, all horses underwent a lameness examination, range of motion (flexion) testing of the carpal joints, evaluation for middle carpal joint effusion, body condition assessment, and radiography of the carpal joints; only horses without abnormal findings were permitted in the study. A minimum 14-day acclimatization period to new environmental factors and training to safely enter and use the high-speed and underwater treadmills occurred prior to baseline data collection. The Colorado State University Institutional Animal Care and Use Committee approved the study protocol.

Experimental induction of osteoarthritis - Following general anesthesia and routine preparation for surgery, each horse underwent bilateral arthroscopic surgery of the middle carpal joints to ensure that there were no pre-existing abnormalities (termed day 0). During this procedure, an osteochondral fragment was created in one randomly selected middle carpal joint. An 8-mm-wide fragment was generated by use of an 8-mm curved osteotome directed perpendicular to the articular cartilage surface of the radial carpal bone at the level of the medial synovial plica.[30] The dorsal surface of the fragment was allowed to remain adhered to the joint capsule proximally. A motorized burr was used to debride the exposed subchondral bone between the fragment and the parent bone. A 15-mm-wide defect bed was created and the debris was not actively lavaged from the joint, thereby adding in the induction of OA. This joint was designated as the osteochondral fragmented joint (OCF) and the sham-operated joint was used as the control joint. The arthroscopic portals were closed with 2-0 nylon suture in a simple interrupted pattern and cyanoacrylate glue[a] was applied to the incision. The thoracic limbs were bandaged and the horses were allowed to recover from anesthesia and surgery. All study horses received a single dose of ceftiofur[b] (4.0 mg/kg) IM just prior to surgery and 2 grams of phenylbutazone[c] orally once a day for 5 days after surgery. Bandages were changed every three to five days and maintained until suture removal at 10 days after surgery.

Exercise - Each horse was housed in a 3.7 m^2 stall. Beginning on day 15, all horses were exercised on a high-speed treadmill[d] 5 days each week, until the end of the study to promote the development of OA. Each day, the horses were trotted at 4.4 m/s for 2 minutes, galloped at 8.8 m/s for 2 minutes, and trotted again at 4.4 m/s to simulate the strenuous exercise associated with race training.

Treatment groups - One week after surgery (day 7), the horses were ranked by lameness score and randomly assigned to one of two groups with 8 horses in each group.[31] The intervention group was exposed to underwater treadmill exercise[e] (UWT) starting on day 15. The underwater treadmill exercise protocol consisted of horses exercising at a brisk walk (2.1 m/sec) for 5 minutes, once a day, for 5 days. The water level in the underwater treadmill was maintained at the point of the shoulder for the duration of the study, which provides approximately 60% reduction in weight bearing.[31] At weekly intervals the duration of underwater treadmill exercise was increased by 5 minutes, until a maximum of 20 minutes was reached. Horses then continued to be exercised for 20 minutes once a day, five days a week, for the remainder of the ten-week study (total of 8 weeks of exercise). The control group was exercised on a low-speed treadmill (without water) at the same speed, frequency and duration as the intervention group, also starting at day 15. The low-speed exercise provided within the control group simulated hand walking and light exercise as prescribed in current rehabilitation protocols.

Kinetic data collection - Displacement of the center of mass was determined from COP measurements recorded using two strain-gauge based force platforms[f] (60 x 90 cm) mounted sequentially in a concrete base at the center of a 25-m runway. The force platforms and runway were covered with a rubber mat (9-mm thick) to prevent horses from slipping during locomotion. The rubber mat was incised around the periphery of the force platforms and a 60 x 90-cm section was adhered to each force platform to provide a continuous visual field. Postural sway data was collected from each horse at four different time points during the study, prior to induction of the osteochondral fragment (day -7), after induction of osteochondral fragment and prior to initiating exercise therapies (day 14), 4 weeks after starting therapy (day 42), and at study conclusion (day 70).

Stance conditions - Postural control was assessed using three different stance conditions: (1) normal square stance with vertical placement and equal weight distribution on all four limbs, (2) base-narrow placement of thoracic limbs, and (3) removal of visual cues during a normal square stance. The placement of each hoof was marked with chalk on the surface of the force platform mat and the distance between the hooves and the lateral edges of the force platform were recorded to provide consistent hoof placement across all trials. Horses were initially positioned on the force platforms in a normal stance position, with the limbs positioned squarely underneath the trunk and the head and neck maintained in a relaxed neutral position. Each horse was positioned with their thoracic limbs squarely placed on one force platform and their pelvic limbs placed squarely on a second force platform (Fig 2.1). The horse handler was instructed to stand in front of the horse holding the lead shank loosely with no direct contact, while two assistants stood on the left and right side near the pelvic limbs to encourage a static stance. For the second stance condition the thoracic limbs were placed with the medial aspect of each hoof in contact in a base-narrow stance position. The last stance condition involved returning the thoracic limbs to a normal square stance position and placing a blinder hood over the horse's head that completely blocked vision in both eyes (Fig 2.2). For each stance condition, horses stood quietly for three consecutive, 10-second trials. The results of the three trials in each condition were averaged for analysis.

Kinematic data collection - Cyanoacrylate glue was used to adhere 2.5-cm round reflective markers on the mid-dorsum of each hoof 3 cm distal to the coronary band and on the forehead at midline of the frontal bone between the supraorbital foramen. Trials with displacement of the head and neck greater than 20 cm in any direction were excluded as movements of the head and neck cause large shifts in the COP. Kinematic data was simultaneously collected at 200 Hz using a motion analysis system[g] with eight, high-speed infrared cameras[h] distributed equally around the peripheri of the force platforms. The capture volume over the region of the force platforms was calibrated using a customized calibration frame and wand with an accuracy of 0.71 mm.

Data Processing - COP data was collected at a sampling rate of 3,000 Hz and filtered using a Butterworth filter with a cut off frequency of 15 Hz. The COP in both the craniocaudal (CrCd) and mediolateral (ML) directions were determined initially from each force platform. The individual COP force platform data was then combined to calculate a net COP in a CrCd and ML direction with respect to the equation below.[20]

$$\text{netCOP} = ((\text{COP}_i\ (\text{Plate 1})\ \text{X}\ (F_z\ \text{Plate 1} / F_z\ \text{Plate 1} + F_z\ \text{Plate 2})) +$$

$$((\text{COP}_i\ (\text{Plate 2})\ \text{X}\ (F_z\ \text{Plate 2} / F_z\ \text{Plate 1} + F_z\ \text{Plate 2}))$$

COP_i refers to the COP recorded from force platform 1 or platform 2 in either the craniocaudal or mediolateral direction. F_z Platform 1 and F_z Platform 2 are the vertical ground reaction forces recorded from each plate respectively. Stabilograms were created to demonstrate the movement of the netCOP in a craniocaudal (y) and mediolateral (x) direction for the three different stance conditions (Fig 2.3).

Postural sway variables

Several postural sway variables were calculated from the netCOP for each stance position.[26]

1. Craniocaudal sway (mm) – the movement of the COP in a craniocaudal direction relative to the horse (positive values toward the cranial aspect of the horse).
2. Mediolateral sway (mm) – the movement of the COP in a mediolateral direction (positive values toward the right side of the horse).
3. COP area (mm^2) – the extreme bounds of the COP movement in both the craniocaudal and mediolateral directions
4. COP velocity (mm/s) – the direction and amplitude with which the COM position was displaced in the following time instant and was calculated as the average velocity between each successive data point.[24]
5. COP Radius (mm) – the average displacement of each COP data point (x_i, y_i) relative to the geometric center (i.e., mean location of the COP = x_0,y_0) of the stance.

Kinematic stance position variables

The 3-D transformed coordinates calculated from the position of the reflective markers were used to determine the following hoof positions as previously described:[26]

1. Craniocaudal length (m) – midpoint distance between the front hoof markers to the midpoint distance between the hind hoof markers.
2. Mediolateral width (m) – midpoint distance between the left front and left hind hoof markers to the midpoint distance between the right front and right hind hoof markers.
3. Base of support (m^2) –the total area confined by all four hoof markers.
4. Head height (m) – vertical distance between the head reflective marker and the reflective marker on the left front hoof.

Normalization procedure

Prior to statistical analysis, the postural sway variables (Table 2.1) were normalized to the corresponding kinematic measurements as previously described[26] and reported as a normalized percent value (postural sway variable divided by stance position variable x 100 %). Normalization was used to remove the dependence of the postural sway variables on each individual horse's biomechanical factors. The craniocaudal sway was normalized to the craniocaudal length (Table 2.2). Similarly, mediolateral sway was normalized to the mediolateral width, COP area normalized to the base of support area and lastly head height was normalized to the wither height within each horse (Table 2.2).[24]

Data Analysis – COP data measured sequentially at different time points over the course of the study were statistically evaluated with an ANOVA or ANCOVA framework and a software program[i] depending on the presence or absence of a covariate, respectively. Analysis of variance was used to determine the influence of stance position on the dependant postural sway variables. The ANOVA design also involved assessing the COP variables between days -7 and day 14 (pre-treatment analysis) to demonstrate that no treatment group effect was detectable prior to the initiation of underwater treadmill exercise. The ANCOVA analysis was designed to assess the post-treatment postural sway variables using day 14 as a covariate to adjust for any influences that existed at that time point. The ANOVA tables were used to determine significant main effects and interactions between main effect variables. When individual comparisons were made, least square means were used. A value of $P < 0.05$ was considered significant. Values were reported as mean ± SEM.

Results

Craniocaudal Sway – Craniocaudal sway differed significantly ($P = 0.04$) depending on the stance positions, with the blindfolded horses having the greatest amount of craniocaudal sway (1.6 ± 0.1 normalized %), when compared to the base-narrow and normal stance conditions (1.4 ± 0.1 and 1.3 ± 0.1%, respectively) (Fig 2.4). Pre-treatment analysis (days -7 and day 14) demonstrated that craniocaudal sway did not differ significantly depending on whether the horse was in the aquatic therapy or control group (no main effect of treatment group), nor was a significant difference demonstrated between the treatment groups on either day -7 or day 14. However, post-treatment analysis (days 42 and 70) demonstrated that the craniocaudal sway was significantly (P = 0.0005) influenced by presence or absence of aquatic therapy. Horses exercised in the underwater treadmill had significantly less craniocaudal sway (1.1 ± 0.1%), compared to the control group (1.7 ± 0.1%). When specific individual comparisons were conducted, the blindfolded stance position demonstrated a significant difference in the amplitude of craniocaudal sway at days 42 and 70 between the aquatic therapy and control conditions (Fig 2.5). Horses exercised in the underwater treadmill had significantly less craniocaudal sway when blindfolded on both days 42 (1.3 ± 0.2%; P = 0.004) and 70 (1.0 ± 0.2%; P = 0.03), compared to the control group (2.2 ± 0.2 and 1.6 ± 0.2%, respectively). Similarly, horses exercised in the underwater treadmill had significantly less craniocaudal sway when standing in a base-narrow position on both days 42 (1.1 ± 0.2 %; P = 0.03) and 70 (1.0 ± 0.2%; P = 0.009), compared to the control horses (1.7 ± 0.2 and 1.8 ± 0.2%, respectively) (Fig 2.6). No significant differences were detected in the normal stance position between the two treatment groups, across either of the post-treatment study days.

Mediolateral Sway – Mediolateral sway differed significantly (P = 0.01) depending on the stance position maintained on the force platform. The base-narrow condition caused a significantly higher amplitude of mediolateral sway (6.5 ± 0.5%), as well as an increase when horses were blindfolded (6.0 ± 0.5%), compared to normal stance conditions (4.8 ± 0.5%) (Fig 2.7). No significant differences in mediolateral sway between the blindfolded and base-narrow stance positions were detected. Pre-treatment analysis (days -7 and 14) demonstrated that mediolateral sway did not differ significantly depending on whether the horse was in the aquatic therapy or control group, nor was a significant difference demonstrated between the treatment groups on either day -7 or day 14. However, post-treatment analysis demonstrated that the mediolateral sway was significantly (P = 0.0002) influenced by presence or absence of aquatic therapy. Horses exercised in the underwater treadmill had significantly less mediolateral sway (4.4 ± 0.3%) compared to control horses (6.6 ± 0.3%). When specific least square mean comparisons were conducted, the pattern of influence on the degree of mediolateral sway during the post-treatment collection days consistently changed depending on stance position and presence or absence of aquatic therapy. Horses exercised in the underwater treadmill had significantly less mediolateral sway when blindfolded on day 42 (4.8 ± 0.7%; P = 0.02), compared to the control group (7.1 ± 0.7%) (Fig 2.8). Similarly, horses exercised in the underwater treadmill had significantly less mediolateral sway when standing in a base-narrow position on day 42 (4.8 ± 0.7%; P = 0.01), compared to the control horses (8.2 ± 0.7%) (Fig 2.9). Lastly, aquatic therapy horses had significantly less mediolateral sway during normal stance position on both days 42 (3.8 ± 0.7%; P = 0.01) and 70 (3.7 ± 0.7%; P = 0.04), compared to the control group (6.3 ± 0.7 and 6.8 ± 0.7%, respectively) (Fig 2.10).

Center of Pressure Area – COP area was significantly (P = 0.02) influenced by the stance position of the horse (Fig 2.11). The removal of visual cues caused a significantly larger area of center of pressure movement (126.0 ± 17.7%; P = 0.006), compared to a much smaller area during normal stance (69.0 ± 17.7%). Similarly, the base-narrow stance position resulted in a significantly larger COP area (112.0 ± 17.7%; P = 0.04), compared to the normal stance condition. Pre-treatment analysis (days -7 and 14) demonstrated that COP area did not differ significantly depending on whether the horse was in the aquatic therapy or control group, nor was a significant difference demonstrated between the treatment groups on either day -7 or day 14. In contrast, post-treatment analysis demonstrated significantly smaller COP area's (56.4 ± 11.0%; (P < 0.0001) from horses exercised in the underwater treadmill compared, to control horses (135.2 ± 11.0%) (Fig 2.12).

Center of Pressure Velocity – The COP velocity was significantly (P = 0.02) influenced by the stance position of the horse (Fig 2.13). The lack of visual stimulation caused a significantly greater velocity in the COM displacement (16.1 ± 1.1 mm/s; P = 0.007), compared to normal stance (13.9 ± 1.1 mm/s). The COP velocity was not significantly different between the blindfolded and base-narrow stance, nor was the base-narrow stance significantly different from the normal stance position. Pre-treatment analysis (days -7 and 14) demonstrated that COP velocity did not differ significantly depending on whether the horse was in the aquatic therapy or control group, nor was a significant difference demonstrated between the treatment groups on either day -7 or day 14. The presence or absence of aquatic therapy significantly ($P = 0.05$) influenced the COP velocity in the post-treatment analysis (days 42 and 70). Specifically, horses exercising in the underwater treadmill maintained a slower COP velocity (12.5 ± 0.8 mm/s), compared to the control group (15.1 ± 0.8 mm/s) (Fig 2.14).

Center of Pressure Radius – Alterations in stance position did not significantly influence the COP radius. In addition, pre-treatment analysis demonstrated that the COP radius did not differ significantly depending on whether the horse was in the aquatic therapy or control group, nor was a significant difference demonstrated between the treatment groups on either day -7 or day 14. However, the COP radius was significantly influenced by the presence or absence of aquatic therapy in the post-treatment analysis. Horses exercised in the underwater treadmill had a significantly smaller COP radius (3.6 ± 0.2 mm; $P < 0.0001$) than that of the control group (5.5 ± 0.2 mm) across all three stance positions. Horses exercised in the underwater treadmill had a significantly smaller COP radius when blindfolded on both days 42 (4.1 ± 0.5 mm; $P = 0.02$) and 70 (3.5 ± 0.5 mm; $P = 0.04$), compared to the control group (5.8 ± 0.5 and 5.0 ± 0.5 mm, respectively) (Fig 2.15). Similarly, horses exercised in the underwater treadmill had a significantly smaller COP radius when standing in a base-narrow position on both days 42 (3.5 ± 0.5 mm; P = 0.002 and 70 (3.4 ± 0.5 mm; P = 0.03), compared to the control horses (6.0 ± 0.5 and 4.9 ± 0.5 mm, respectively) (Fig 2.16). The normal stance condition also demonstrated a significantly smaller COP radius in horses exercised in the underwater treadmill on both days 42 (3.7 ± 0.5 mm; P = 0.03) and 70 (3.3 ± 0.5 mm; P = 0.0004) compared to control horses (5.2 ± 0.5 and 6.0 ± 0.5 mm, respectively) (Fig 2.17).

Craniocaudal Length – The craniocaudal length was significantly (P = 0.006) influenced by the three varying stance positions. Specifically, the normal stance condition had a significantly smaller craniocaudal length (1.06 ± 0.01 m), compared to both the blindfolded (1.07 ± 0.01 m; P = 0.02) and base-narrow conditions (1.08 ± 0.01 m; P = 0.002). No significant differences were detected between the base-narrow and blindfolded craniocaudal length. Study day and the presence or absence of aquatic therapy did not significantly influence the craniocaudal length.

Mediolateral Width – The mediolateral width was significantly (P < 0.0001) influenced by the three varying stance positions. Specifically, the base-narrow stance condition had a significantly smaller mediolateral width (0.210 ± 0.005 m; P < 0.0001), compared to both the blindfolded (0.250 ± 0.005 m) and control conditions (0.250 ± 0.005 m). No significant differences were detected between the normal and blindfolded mediolateral width. Study day and the presence or absence of aquatic therapy did not significantly influence the mediolateral width.

Base of support – The base of support was significantly ($P < 0.0001$) influenced by the three varying stance positions. Specifically, the base-narrow stance condition had a significantly smaller base of support ($0.230 \pm 0.007\ m^2$; ($P < 0.0001$), compared to both the blindfolded ($0.270 \pm 0.007\ m^2$) and control conditions ($0.270 \pm 0.007\ m^2$). No significant differences were detected between the normal and blindfolded base of support. Study day and the presence or absence of aquatic therapy did not significantly influence the base of support measurement.

Head height – The height of the head was significantly lower in the base-narrow stance position (88.0 ± 2.0%; $P = 0.0004$), compared to the blindfolded stance conditions (92.0 ± 2.0%). Head height did not significantly differ between the normal (90.0 ± 2.0%) and base-narrow stance, nor was a difference detected between the normal and blindfolded stance conditions. Height of the head did not significantly change between study days for each stance position, and a significant difference was not detected between the treatment groups.

Discussion

Evaluation of balance control through postural sway analysis is a reliable and valid approach to determining static stability under various conditions.[25,26] This study demonstrated that underwater treadmill exercise significantly improves static balance control in horses with carpal OA, which is fundamental to providing evidence based support for equine aquatic therapy. Furthermore, this study provides the first evidence that variations in stance conditions have profound effects on the mechanics of standing balance in the horse. Direct measurement of changes in the COP movements confirmed that stance width and removal of visual stimuli significantly influence stability.

Balance control is a dynamic task in which sensorimotor systems must interact with the external environment to maintain stability.[32] In varying stance conditions, one of the most striking findings from this study occurred when the thoracic limbs were placed in a base-narrow position and a significant increase in mediolateral sway was demonstrated, but no change in craniocaudal sway was appreciated. This phenomenon may be explained by the mechanical differences in joint structure and function that contribute to motion in the two different directions. Under normal circumstances, mediolateral movement of thoracic limb articulations is very restricted due to the constraining properties of periarticular collateral ligaments.[29] However, joint motion in flexion/extension within a sagittal plane is normally achieved by the hinge joints within the thoracic and pelvic limbs.[29] The change in stance position may also have altered the direction of the ground reaction force vector, thus increasing net torque across the joint surfaces.[32] The increased joint torque may cause a counter productive increase in muscle activation, inducing excessive levels of tonic activity which leads to larger changes in the COP movement.[29,32] Interestingly, the only significant change in head height was appreciated during the base-narrow stance position. Lowering of the head may have been a strategy adopted in order to change the position of the COM, moving it forward and placing the thoracic limbs in a slightly inclined vertical axis to act as more of a mechanical strut counteracting the destabilizing motion in the mediolateral direction. The base-narrow stance condition indicates that hoof position is an important determinant of standing balance and should be considered when alteration in hoof position through shoeing application or certain terrain characteristics may force the horse to move or stand in a base-narrow position.

The visual system is a major contributor to balance control, providing both spatial and temporal information. The removal of visual cues requires a reliance on proprioceptive and vestibular sensory cues, which signal body movement relative to the hoof and head. Reduced afferent information causes balance control to be less precise as the central nervous system has less information to accurately estimate and control the center of mass position and velocity.[22] The blindfolded stance condition resulted in larger and faster displacements of the COP in both craniocaudal and mediolateral directions. The increased COP movement during the blindfolded condition demonstrates the sensitivity of postural control on visual dependence. This is consistent with the well-documented phenomenon of impaired balance control in humans standing with eyes closed.[16,27,33] The loss of visual cues increases muscle stiffness and alters postural sway thus impacting the reflexive response to alterations in COM movement.[22] Several human studies have demonstrated the compensatory effects of vision under conditions of muscular fatigue.[27,33] The destabilizing effects of muscular fatigue on balance control were significantly compensated for when patients stood with eyes open versus eyes closed. In addition, elderly human patients typically suffer from a progressive lose in their peripheral vision,[22] which causes an inability to control movement of the COM, leading to a greater number of falls.[22] The implication of our findings indicate that the removal of visual cues is an important determinant of standing balance control in the horse and should strongly be considered when visual input is restricted for the purposes of various athletic activities (i.e., application of blinders). However, this is a limitation of the current study as it only tested horses in a completely blindfolded situation and future studies may need to address various types of partial and complete alterations in visual stimuli.

This study provides the first evidence that underwater treadmill exercise improves static balance control in equine patients with carpal OA. The most notable differences occurred in the blindfolded condition when patients were deprived of sensory visual cues and had to rely on vestibular and somatosensory input to maintain balance. The underwater treadmill exercise improved balance control in both the base-narrow and blindfolded stance conditions, whereas the control group was only effective in maintaining balance when placed in a normal stance position. Underwater treadmill exercise may have improved the afferent excitation of the motor neuron pool in the muscles responsible for stabilizing the thoracic and pelvic limbs. Therefore, the sensory mechanisms (e.g., muscle spindles, golgi tendon organs), afferent feedback transmission, and the muscle actuators of the neuromuscular system may have all been positively affected through aquatic therapy. Postural control requires the coordinated action of multiple muscles, and underwater treadmill exercise may have provided an increase in muscle strength, which enhances muscle spindle sensitivity and hence improves proprioceptive acuity and postural control.[4,34]

Horses within the control group demonstrated a greater disturbance in postural stability, specifically when challenged with conditions requiring enhanced proprioceptive input needed to maintain equilibrium. The larger variations in postural sway characteristics indentified within the control group may be associated with enhanced nociception and altered afferent signaling from the joint mechanoreceptors in response to joint pain and inflammation. Inaccurate sensory feedback due to synovial effusion and inflammation results in inappropriate muscle activity, which may ultimately impair the horse's ability to make motor output adjustments efficiently during functional mobility and dynamic control of balance.

Limitations

Due to constraints within the study design, thoracic and pelvic limb COP measurements were combined to report a net COP displacement. The inability to record COP changes from each limb independently, restricted the evaluation of changes in COP due to the presence of an osteochondral fragment. This study also did not assess kinematic changes in trunk orientation and limb geometry, which may have aided in the assessment of alterations in postural stability associated with the presence of an osteochondral fragment and applied aquatic therapy. Future studies are also needed to determine the influence of alterations in static postural control on performance capabilities during balance required tasks.

Conclusion

Although the exact mechanism for improved postural control after aquatic exercise could not be determined within the present study, the finding that postural sway assessment can detect changes in balance control for horses with carpal OA may prove clinically useful. Postural sway analysis is a sensitive diagnostic modality that may aid in identifying potential balance deficits associated with neuromuscular impairments due to joint pain and inflammation. In addition, postural sway analysis provides insight into the effects that specific intervention programs have on postural stability. This study also provides clinical relevance that altering visual input and stance positions may negatively affect the horse's ability to maintain postural control.

Material Sources

a. Super glue (cyanoacrylate adhesive), Pacer Technology, Rancho Cucamonga, CA.
b. Naxcel, Pfizer Inc, New York, NY.
c. Equi-Phar, Schering-Plough Animal Health Corp, Union, NJ.
d. Equigym, Equigym LLC, Lexington, KY.
e. Equine underwater treadmill, Ferno Veterinary Systems, Wilmington, OH.
f. Bertec Force Platforms model FP6090-15, Bertec Corporations, Columbus, OH.
g. Motus Software version 9.1, Vicon, Centennial, CO.
h. Volant 200 Hz cameras, Vicon, Centennial, CO.
i. Glimmix SAS Procedure, SAS Institute Inc, Cary, NC.

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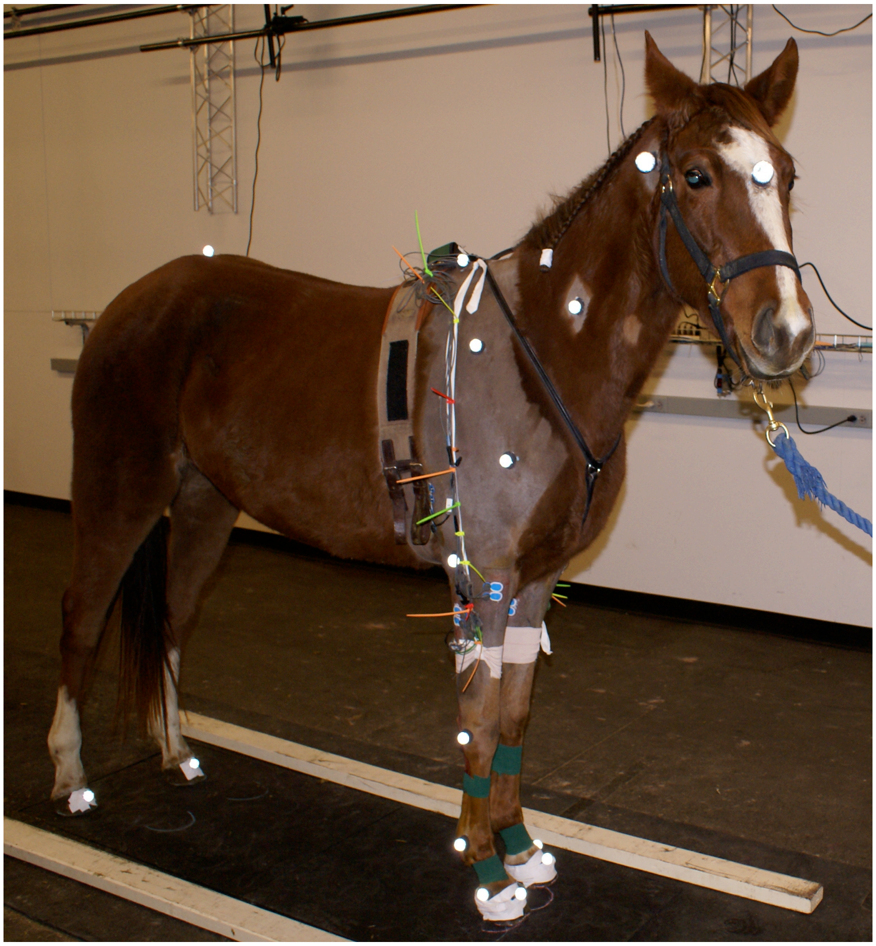

Figure 2.1: Photograph of a horse standing in a square, balanced stance position on two serial force platforms.

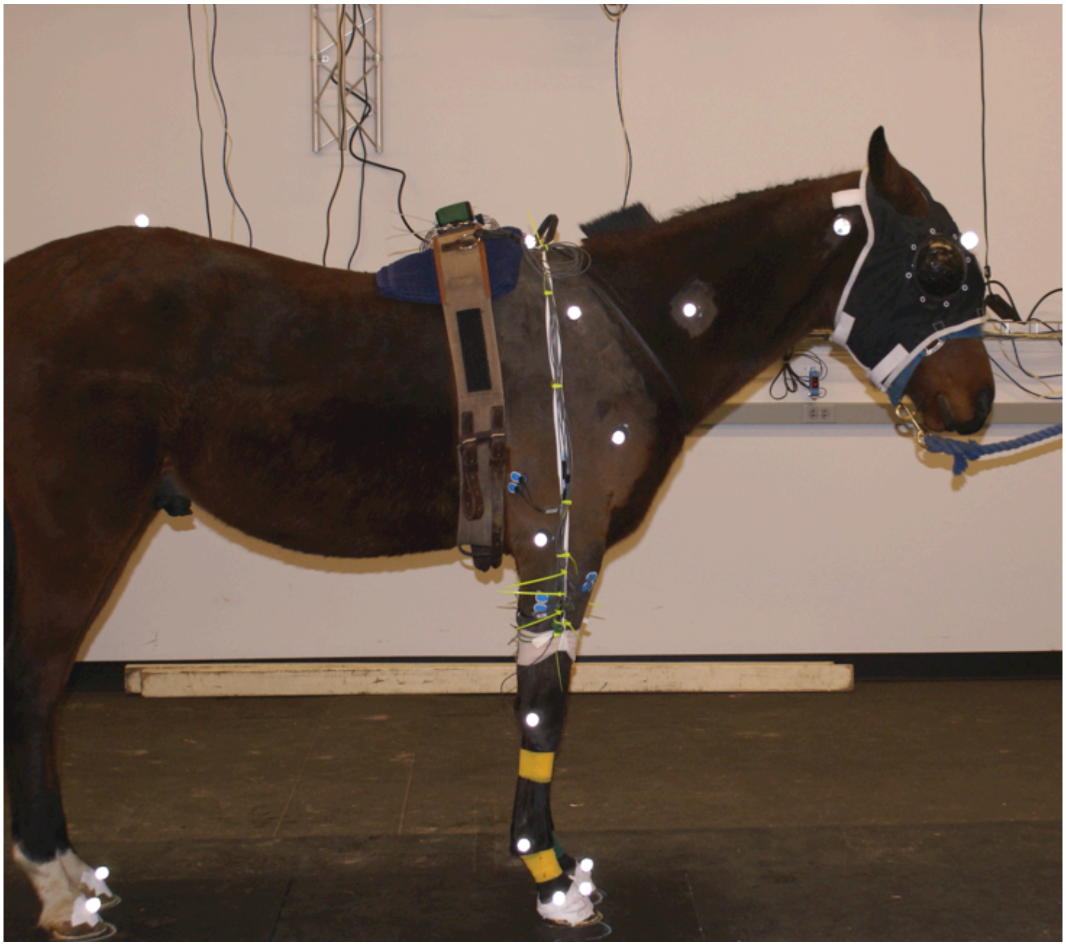

Figure 2.2: Photograph of a horse with a blinder hood applied to remove visual stimuli during postural sway analysis.

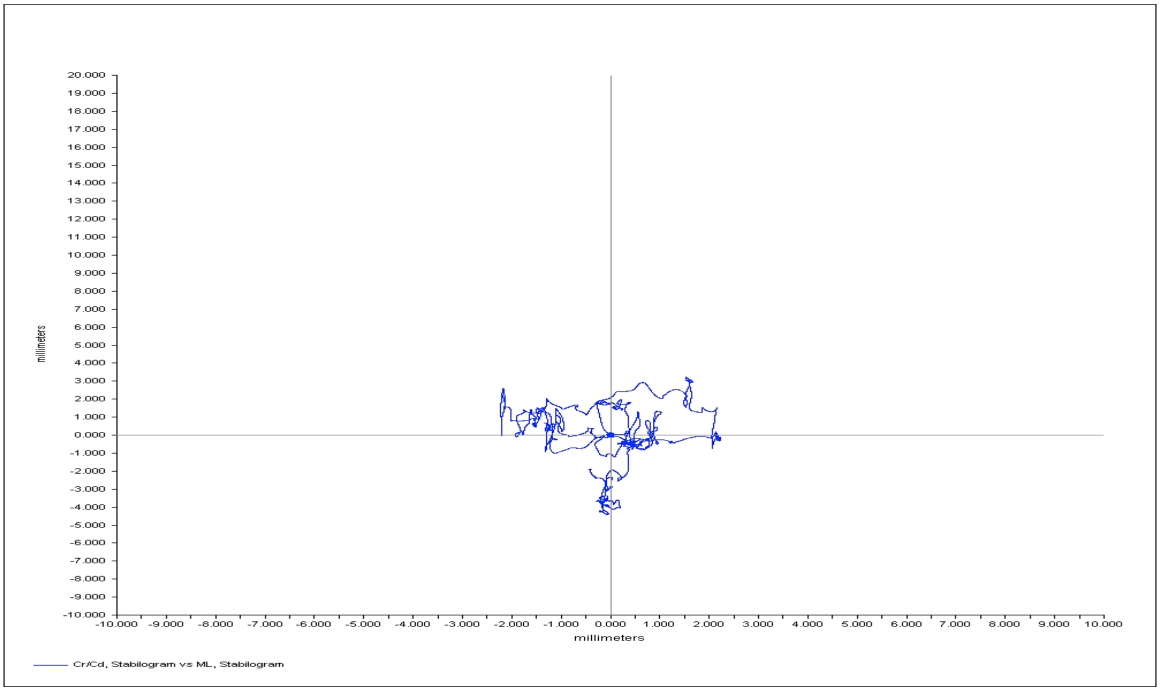

Fig 2.3A – Graph of normal square stance condition COP tracing from a single horse over a ten-second data collection time frame. Note the small amplitudes of craniocaudal and mediolateral changes in COP.

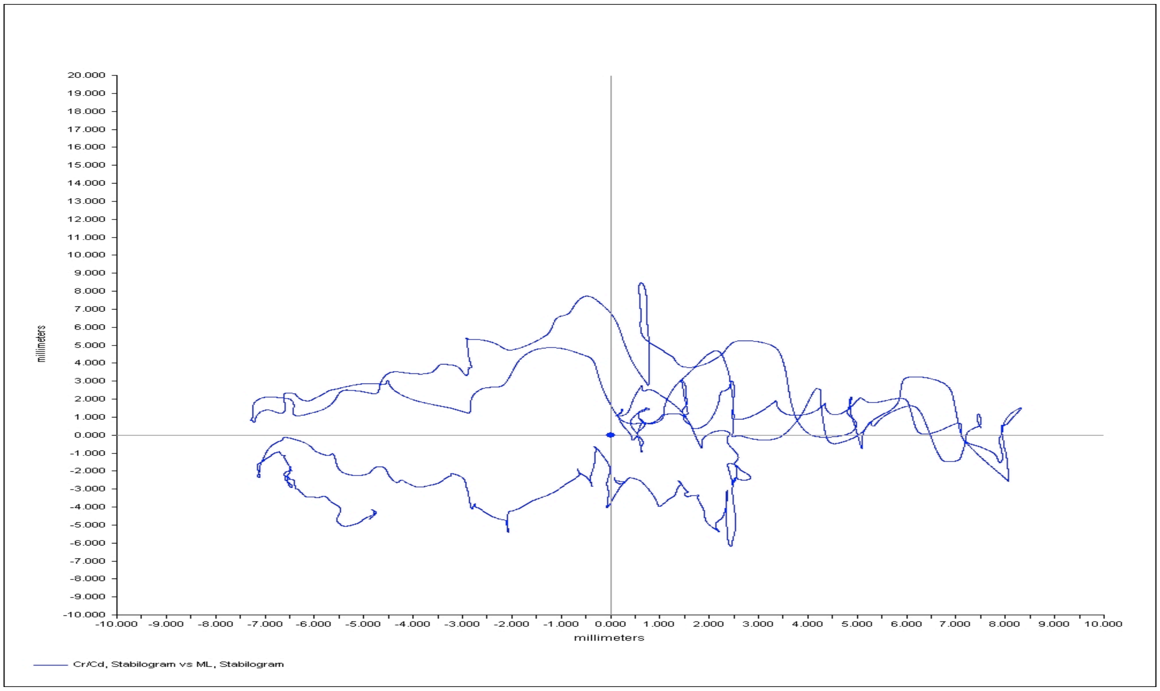

Fig 2.3B – Graph of base-narrow stance condition COP tracing from a single horse over a ten-second data collection time frame. Note the increased area of COP movement compared to the normal square stance condition.

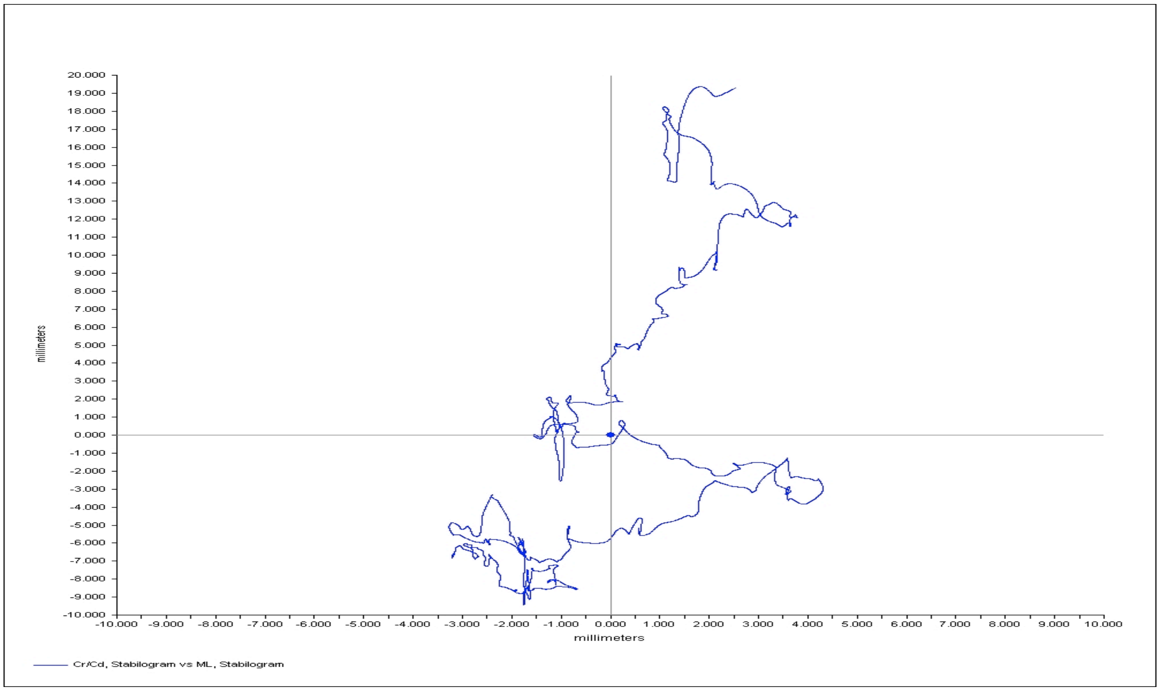

Fig 2.3C – Graph of blindfolded stance condition COP tracing from a single horse over a ten-second data collection time frame. Note the increased area of COP movement compared to the normal square stance condition.

Figure 2.3: Stabilograms depicting the movement of the center of pressure in craniocaudal (vertical axis) and mediolateral directions (horizontal axis) during a single, ten-second data collection session from one horse prior to osteochondral fragmentation. A – Normal square stance condition. B – Base-narrow stance condition. C – Blindfolded stance condition.

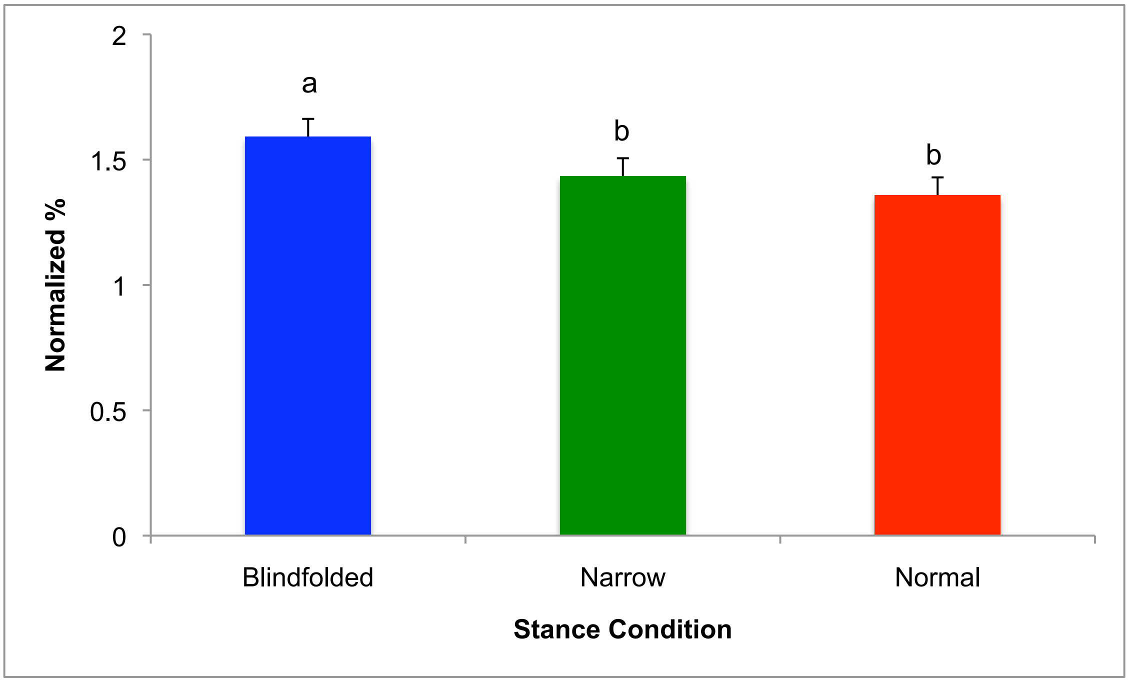

Figure 2.4: Graph of normalized amplitudes of the COP movement in a craniocaudal direction for the three different stance conditions (i.e., blindfolded, base-narrow, and normal). Different letters indicate significant ($P < 0.05$) differences between the stance conditions.

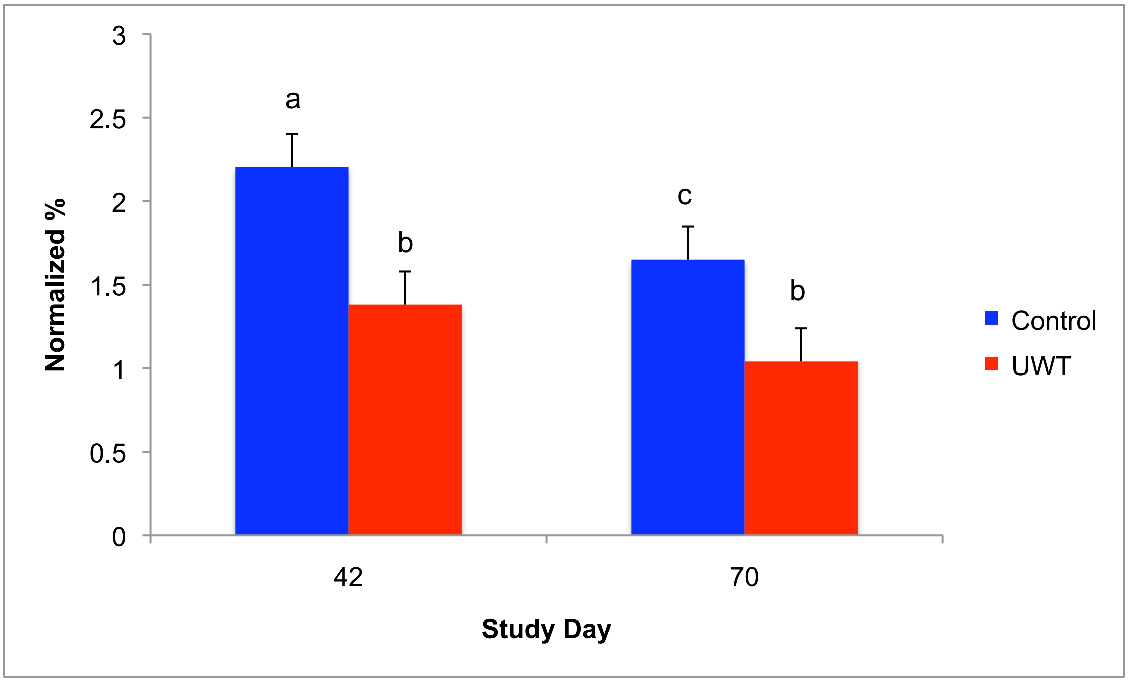

Figure 2.5: Graph of normalized amplitudes for craniocaudal COP movement during the blindfolded stance condition in the underwater treadmill exercise and control groups on days 42 and 70 (post-treatment analysis). Different letters indicate significant ($P < 0.05$) differences between the treatment groups.

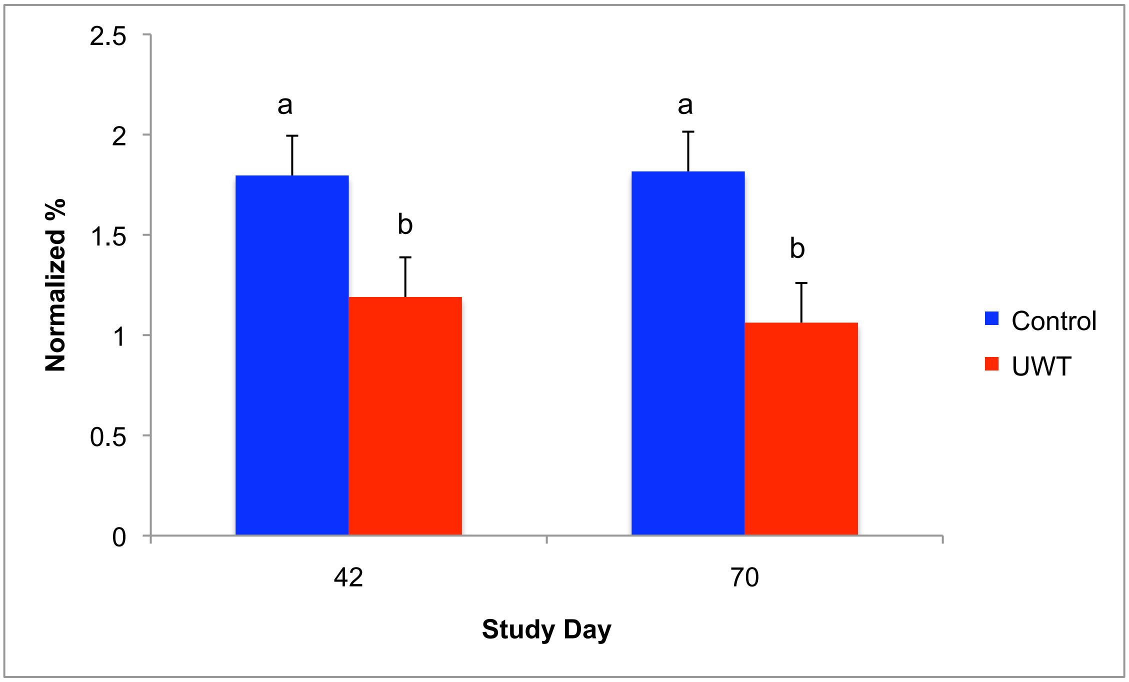

Figure 2.6: Graph of normalized amplitudes of the craniocaudal COP movement during the base-narrow stance condition between the underwater treadmill exercise and control groups on days 42 and 70 (post-treatment analysis). Different letters indicate significant ($P < 0.05$) differences between the treatment groups.

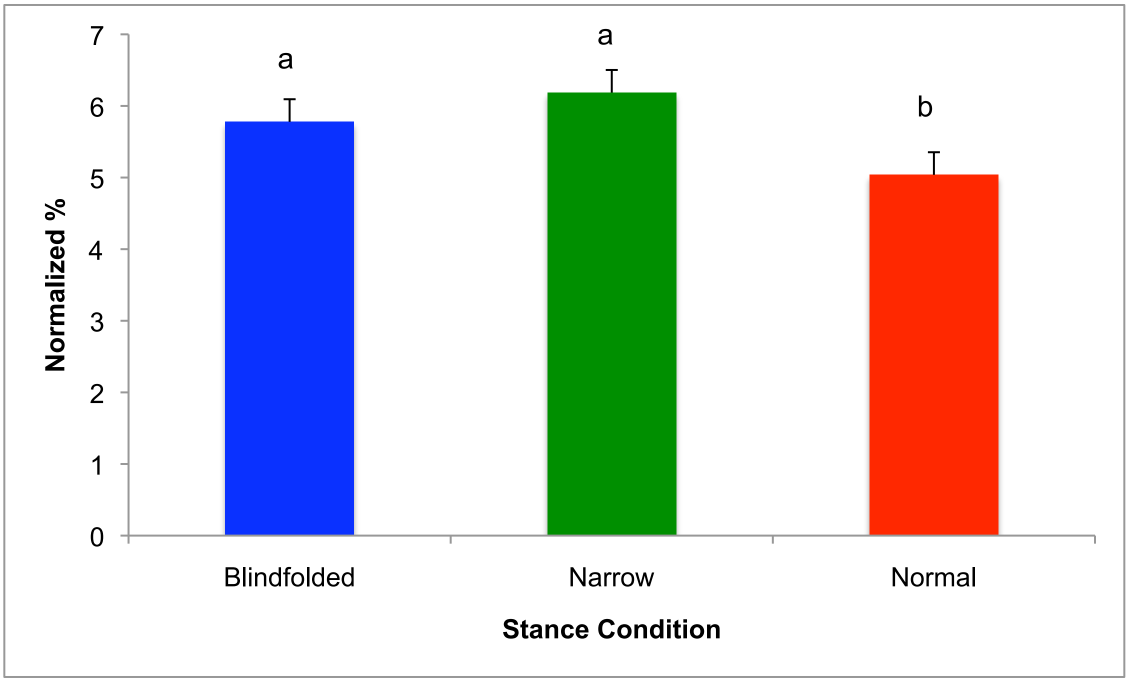

Figure 2.7: Graph of normalized amplitudes of the COP movement in a mediolateral direction for the three different stance conditions (blindfolded, base-narrow, and normal). Different letters indicate significant ($P < 0.05$) differences between the stance conditions.

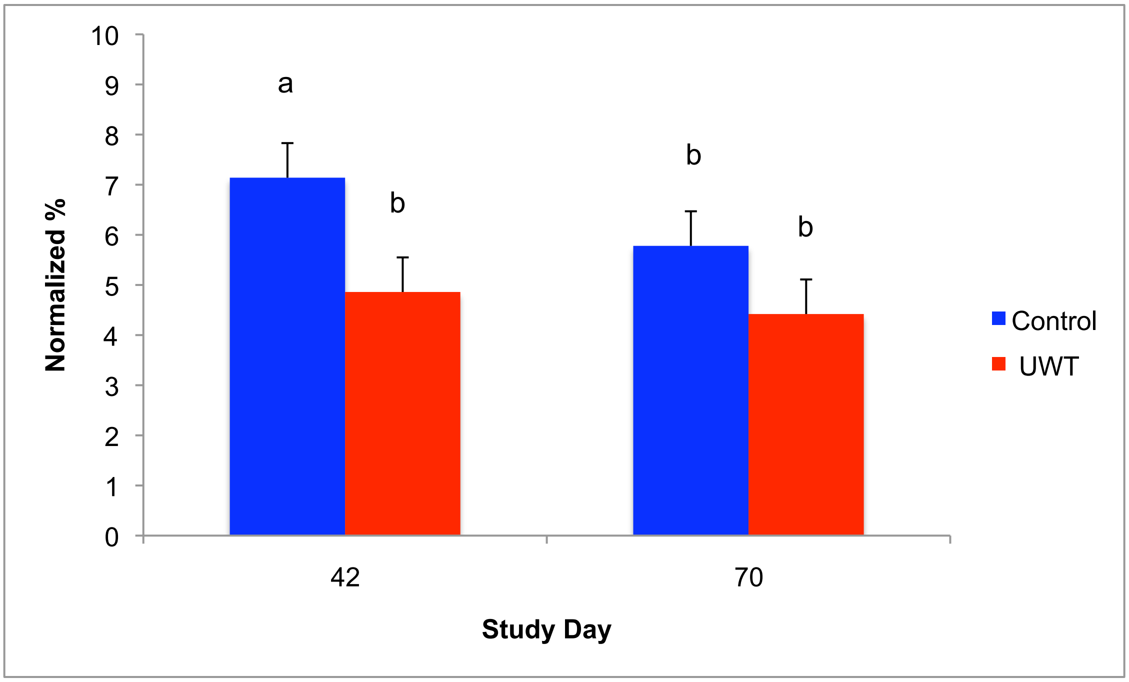

Figure 2.8: Graph of normalized amplitudes of the mediolateral COP movement during the blindfolded stance condition between the underwater treadmill exercise and control groups on days 42 and 70 (post-treatment analysis). Different letters indicate significant ($P < 0.05$) differences between the treatment groups.

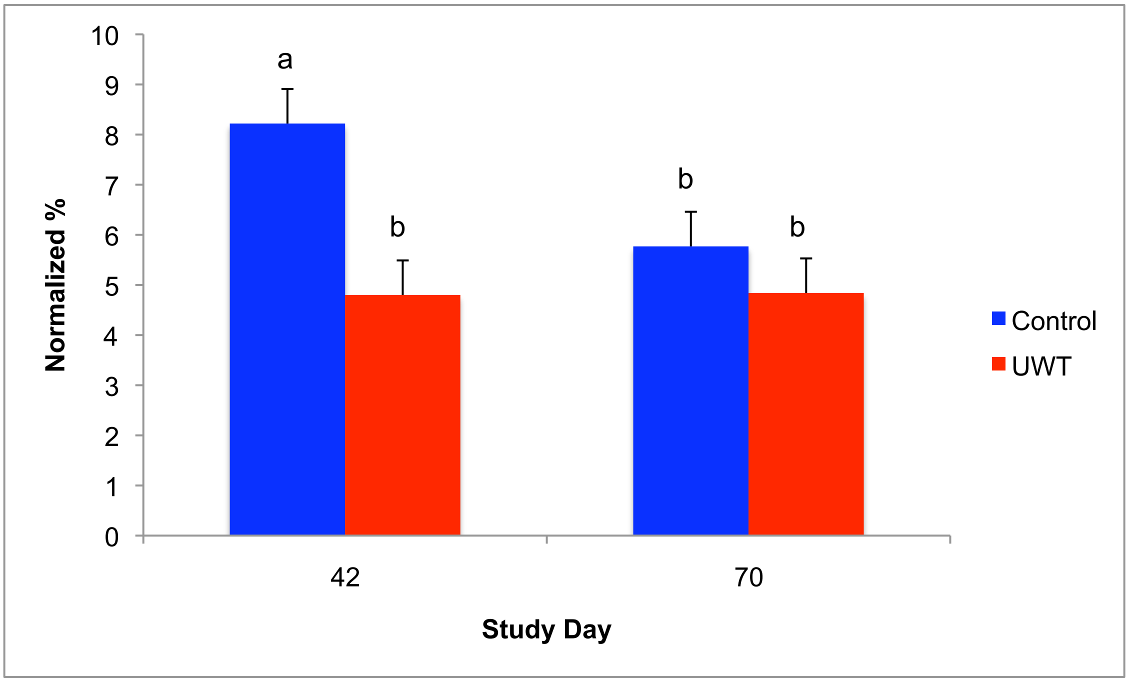

Figure 2.9: Graph of normalized amplitudes of the mediolateral COP movement during the base-narrow stance condition between the underwater treadmill exercise and control groups on days 42 and 70 (post-treatment analysis). Different letters indicate significant ($P < 0.05$) differences between the treatment groups.

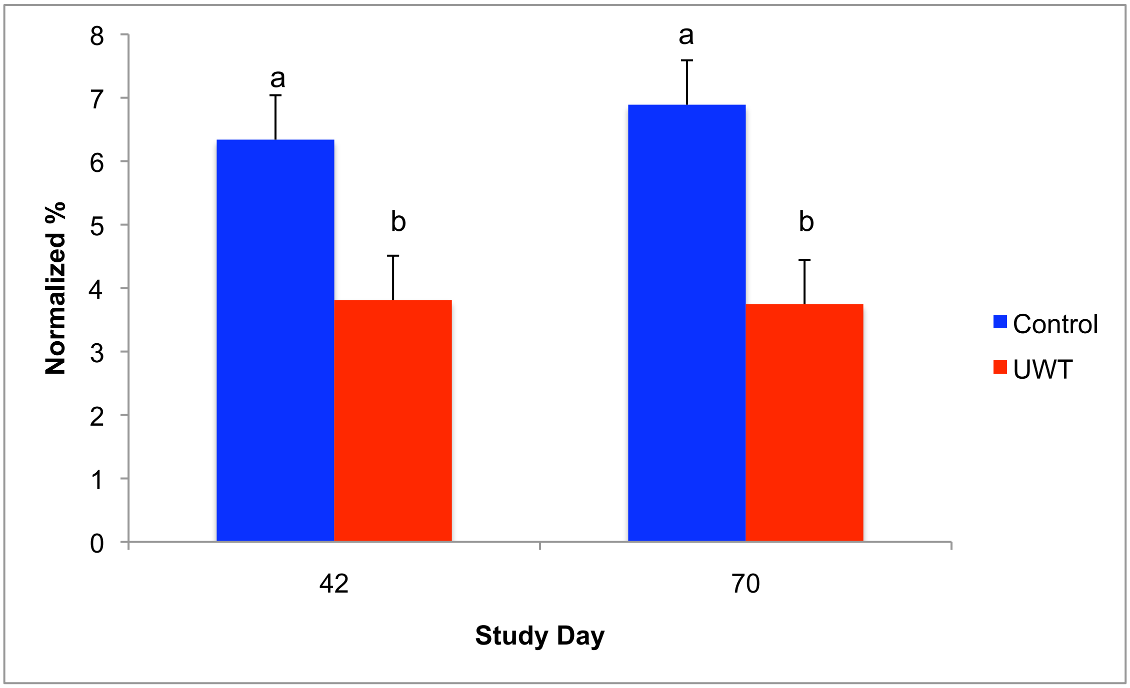

Figure 2.10: Graph of normalized amplitudes of the mediolateral COP movement during the normal square stance condition between the underwater treadmill exercise and control groups on days 42 and 70 (post-treatment analysis). Different letters indicate significant ($P < 0.05$) differences between the treatment groups.

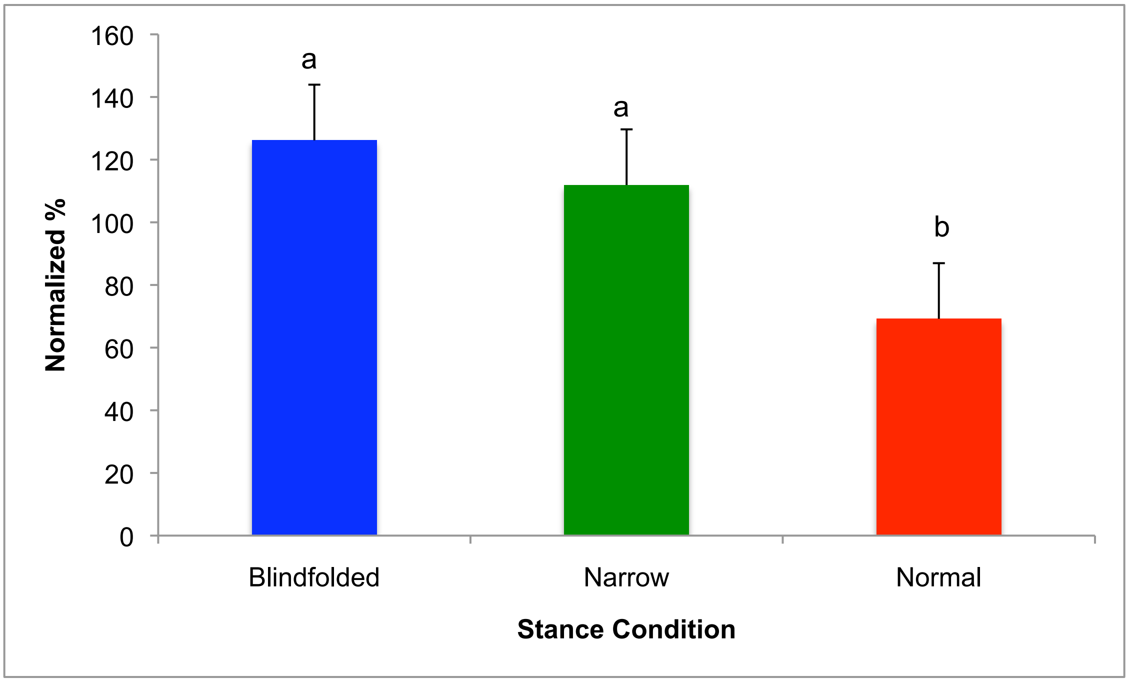

Figure 2.11: Graph of normalized amplitudes of the COP area for the three different stance conditions (i.e., blindfolded, base-narrow, and normal). Different letters indicate significant ($P < 0.05$) differences between the stance conditions.

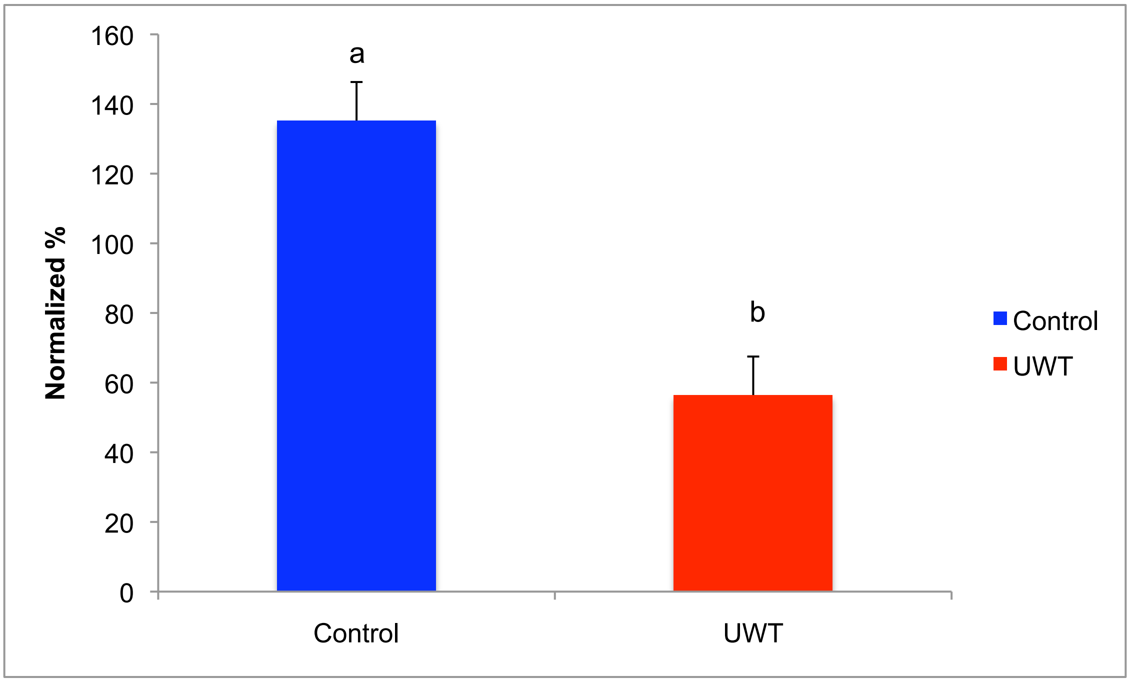

Figure 2.12: Graph of the post-treatment normalized amplitudes for the COP area between the underwater treadmill exercise and control groups. Different letters indicate significant (P < 0.05) differences between the treatment groups.

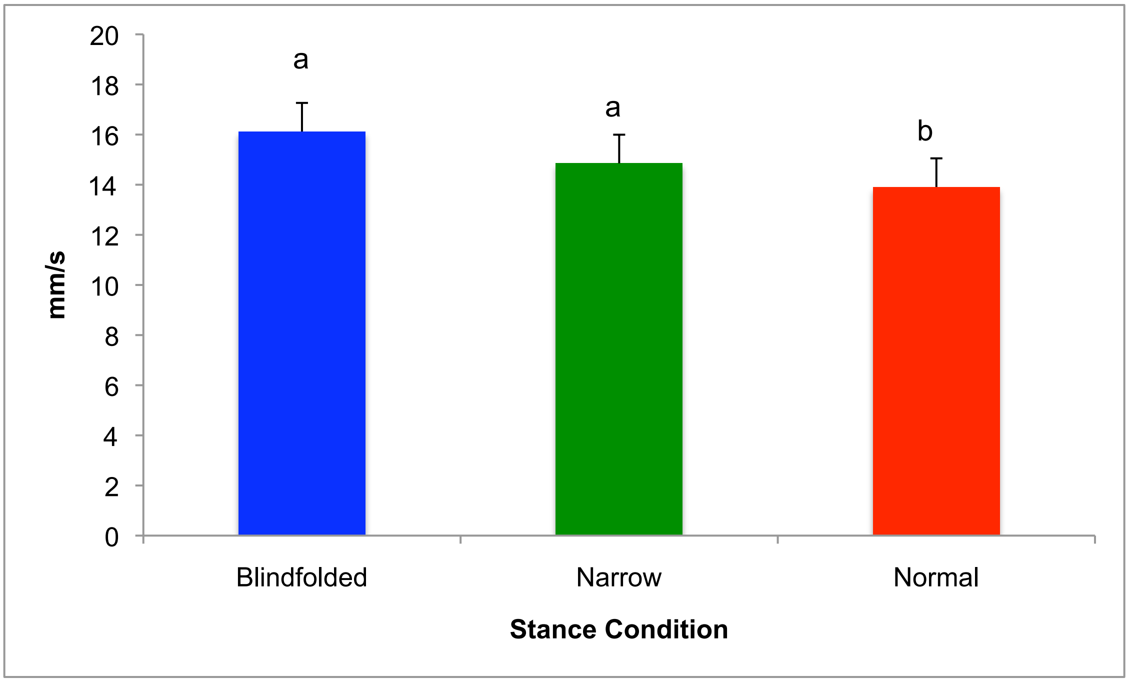

Figure 2.13: Graph of the COP velocity for the three different stance conditions (i.e., blindfolded, base-narrow, and normal). Different letters indicate significant ($P < 0.05$) differences between the stance conditions.

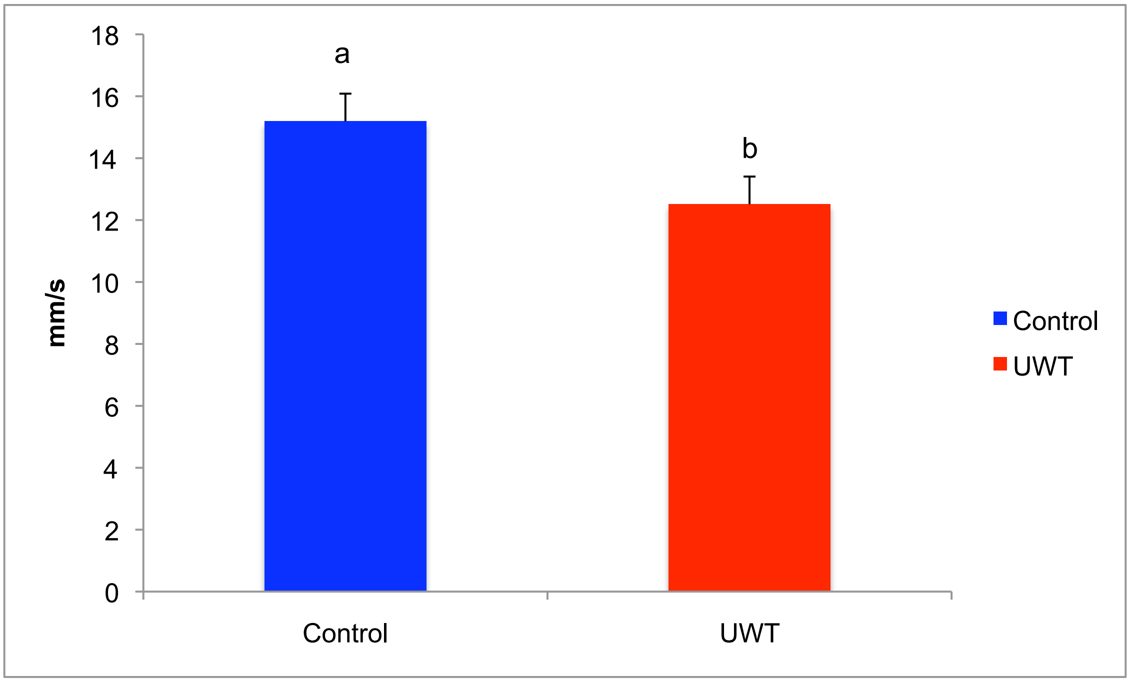

Figure 2.14: Graph of the post-treatment COP velocity between the underwater treadmill exercise and control groups. Different letters indicate significant (P < 0.05) differences between the treatment groups.

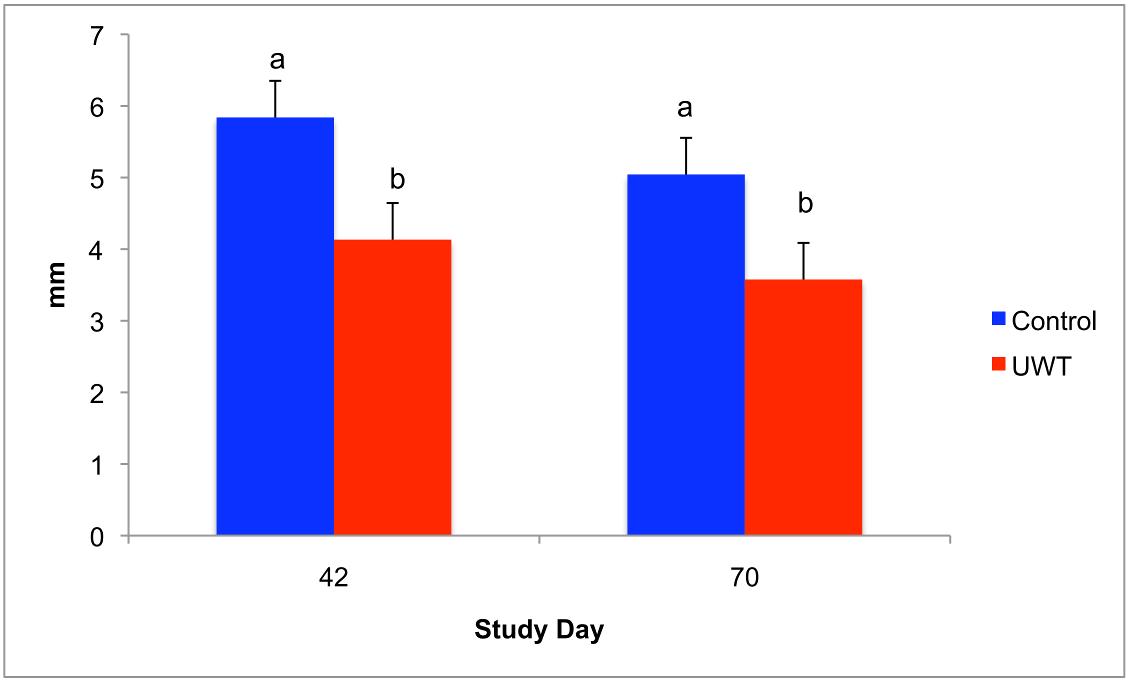

Figure 2.15: Graph of the COP radius during the blindfolded stance condition between the underwater treadmill exercise and control groups on days 42 and 70 (post-treatment analysis). Different letters indicate significant ($P < 0.05$) differences between the treatment groups.

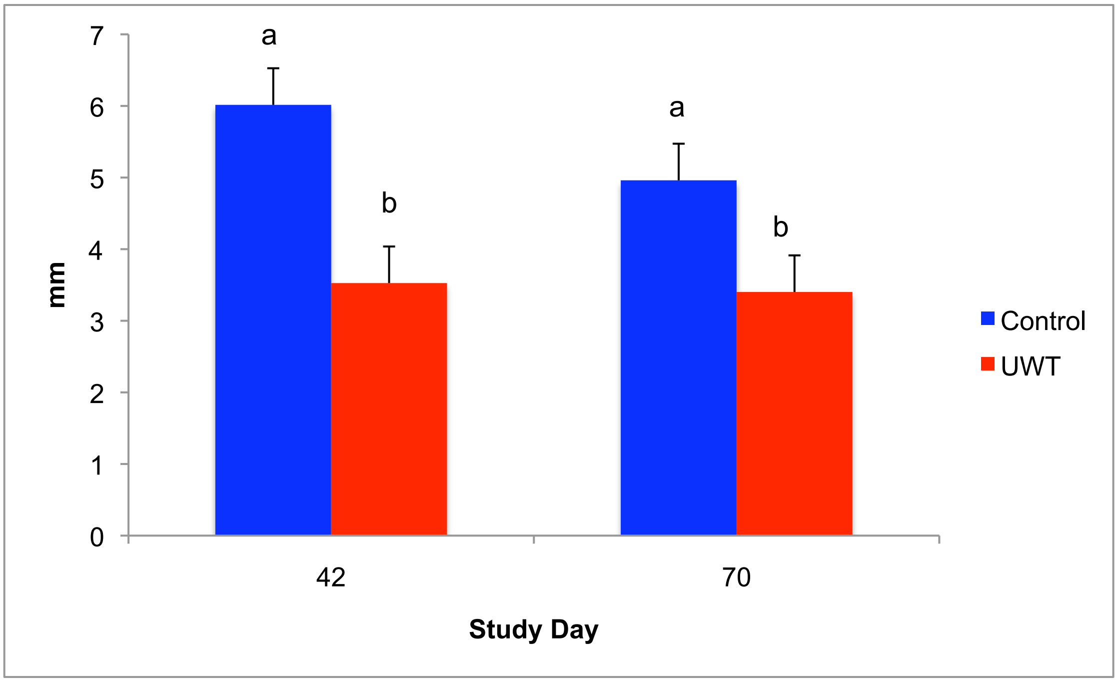

Figure 2.16: Graph of the COP radius during the base-narrow stance condition between the underwater treadmill exercise and control groups on days 42 and 70 (post-treatment analysis). Different letters indicate significant ($P < 0.05$) differences between the treatment groups.

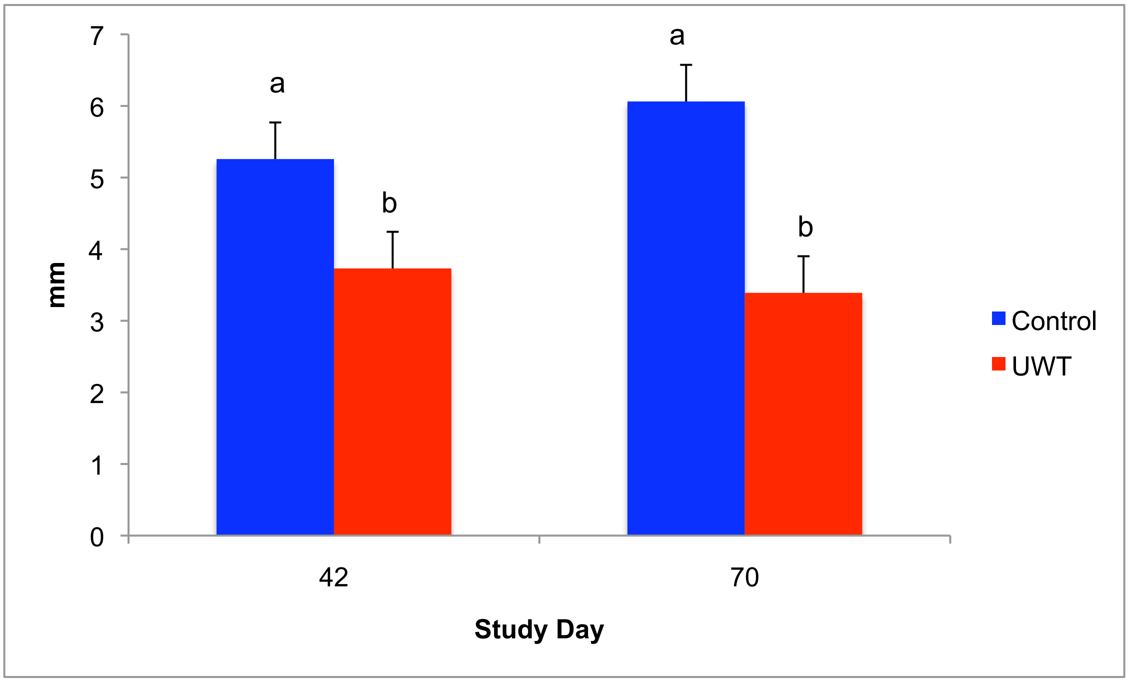

Figure 2.17: Graph of the COP radius during the normal stance condition between the underwater treadmill exercise and control groups on days 42 and 70 (post-treatment analysis). Different letters indicate significant (P < 0.05) differences between the treatment groups.

Table 2.1: The mean postural sway and kinematic values for the normal square stance condition prior to normalization.

COP variables	**Day -7**	**Day 14**	**Day 42**	**Day 70**
Craniocaudal Sway (mm)	14.9 ± 1.1	15.8 ± 1.1	16.7 ± 1.1	14.7 ± 1.1
Mediolateral Sway (mm)	12.6 ± 1.0	14.6 ± 1.0	13.9 ± 1.0	11.6 ± 1.0
COP Area (mm^2)	246.0 ± 35.6	270.0 ± 35.6	269.0 ± 35.6	199.0 ± 35.6
COP Velocity (mm/s)	14.8 ± 1.0	15.1 ± 1.0	14.0 ± 1.0	13.6 ± 1.0
COP Radius (mm)	4.4 ± 0.2	4.6 ± 0.2	4.7 ± 0.2	4.4 ± 0.2
Kinematic variables				
Craniocaudal length (m)	1.07 ± 0.01	1.07 ± 0.01	1.08 ± 0.01	1.07 ± 0.01
Mediolateral width (m)	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.23 ± 0.01
Base of support (m^2)	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.24 ± 0.01
Head height (m)	1.37 ± 0.27	1.36 ± 0.27	1.33 ± 0.27	1.30 ± 0.27

Table 2.2: The normalized postural sway variables for the normal square stance condition.

Normalized COP variables	**Day -7**	**Day 14**	**Day 42**	**Day 70**
Craniocaudal Sway Normalized %	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
Mediolateral Sway Normalized %	5.2 ± 0.1	6.1 ± 0.1	5.6 ± 0.1	5.0 ± 0.1
COP Area Normalized %	94.6 ± 3.6	103.8 ± 3.6	107.6 ± 3.6	82.9 ± 3.6
Head Height Normalized %	92.0 ± 2.3	91.0 ± 2.3	89.4 ± 2.3	88.0 ± 2.3

CHAPTER THREE

PHYSIOLOGIC OUTCOME VARIABLES USED TO ASSESS THE EFFICACY OF UNDERWATER TREADMILL EXERCISE FOR THE MANAGEMENT OF HORSES WITH EXPERIMENTALLY-INDUCED CARPAL OSTEOARTHRITIS

Introduction

Osteoarthritis (OA) is one of the most debilitating musculoskeletal disorders among equine athletes.[1] It is a common cause of poor performance, early retirement, and reduced life expectancy. Medical and surgical management of equine associated lameness disorders cost more than 700 million dollars within the United States alone in 1998.[2] Osteoarthritis is a progressive disease characterized by joint pain, inflammation, synovial effusion, limited range of motion, and a progressive deterioration of articular cartilage.[3] The disease process affects not only the articular cartilage but the entire joint, including subchondral bone, joint capsule, synovial membrane, and periarticular soft tissues.[3] As joint disease progresses characteristic pathologic changes occur including fibrosis and thickening of the joint capsule, articular cartilage fibrillation and erosion, and osteophyte formation, all leading to functional impairments.[3]

Joint disease has been defined as a complex imbalance in the homeostatic mechanism between degradation and repair, with the inflammatory cascade playing a crucial role in the progressive catabolic process.[4] Numerous conventional intra-articular joint therapies have been utilized to manage the pathologic manifestations of equine joint disease. These treatments are targeted towards decreasing the degree of inflammation within the damaged joints.[5-7] However, little attention has been focused on various forms of rehabilitation to aid in modifying the underlying disease process. Physical rehabilitation has become an effective treatment option for reducing or limiting harmful compensatory gait abnormalities in humans.[8,9] Rehabilitation programs that address OA and musculoskeletal injuries often incorporate some form of aquatic exercise. Exercising in water provides an effective medium for increasing joint mobility, promoting normal motor patterns, increasing muscle activation, diminishing limb edema, and reducing the incidence of secondary musculoskeletal injuries due to primary joint pathology.[10,11] The increased resistance and buoyancy inherent in aquatic exercise minimizes joint instability and weight bearing stresses applied to the limb.[12] Therefore, aquatic therapy has been an effective method of rehabilitation for individuals with OA that have difficulty with weight bearing associated land exercise.[13] Osteoarthritic human patients in aquatic therapy programs often demonstrate a significant reduction in pain, increased muscle strength, and improved motor control.[14] Additional studies in human patients with lower extremity OA demonstrated a significant reduction in impulse loading (i.e., abnormal loading rate) of the knee and improved joint range of motion following aquatic exercise.[15] Several studies on aquatic therapy have been conducted in dogs. Aquatic therapy in dogs following cranial cruciate ligament reconstruction produces significant increases in joint range of motion, not only in the operated stifle, but also in the non-operated stifle.[16] Clinical outcome measures of thigh circumference and stifle joint range of motion were assessed in cranial cruciate ligament deficient dogs after tibial plateau osteotomy.[17] Underwater treadmill exercise improved stifle passive joint range of motion and increased thigh circumference, compared to cage rest and controlled walking.[18] Underwater treadmill exercise has become an increasingly popular therapy for the rehabilitation of equine musculoskeletal injuries; unfortunately, there is no scientific evaluation of its effectiveness for the treatment of OA and its associated alterations in musculoskeletal function in horses.

Physiologic outcome variables

The ability to quantitatively assess the progression of OA is a necessary first step in understanding the factors that influence the disease process. Assessment of OA is multifactorial in nature and is based on clinical examination (e.g., gait and joint range of motion measurements), radiographic and MRI morphologic changes, local and referred pain quantification, intra-articular and synovial fluid parameters indicating the status of OA progression or regression. In addition, histologic and biochemical evaluation of the articular cartilage provides an assessment of the structural and functional disease process. The diagnostic tools used to detect and characterize the progression of OA will aid clinicians in choosing treatment modalities and assist in monitoring the efficacy of therapeutic interventions.

Goniometry

Horses with musculoskeletal disorders are often assessed subjectively prior to and following therapy. Evidence-based practice requires the use of valid, reliable, and sensitive tools to monitor treatment effectiveness. Clinical measurement of joint motion is a fundamental evaluation method in determining the influence of joint pain on passive joint range of motion. Goniometry is a reliable and objective method of determining joint range of motion and is often used to assess articular responses to physical therapy.[19] The repeatability and reliability of goniometry has been well documented in human, canine, and feline patients.[19-22] Equine studies have demonstrated that joint capsule compliance decreases with increased severity and progression of synovitis and OA, which leads to a significant reduction in joint range of motion.[23,24] Precise goniometric measurements of joint ranges of motion following induction of OA can provide an accurate assessment of the severity of joint disease and monitor responses to aquatic therapy.

Intra-articular Pressure

Synovial fluid is composed of an ultrafiltrate of blood plasma and products (e.g., hyaluronic acid) produced by the synovial membrane cells.[25,26] The volume of synovial fluid is maintained through a mechanical pumping action of joint motion that stimulates clearance of excess fluid through lymphatics and veins within the synovial membrane.[25,26] The constant synovial fluid volume maintained in a normal joint indicates a net balance of fluid transfer into and out of the joint. Intra-articular pressures (IAP) recorded from normal healthy human joints demonstrate that during both rest and exercise the joint maintains a subatmospheric (negative) or low atmospheric (slightly positive) intra-articular pressure.[27,28] However, many joint disorders manifest with a persistent synovitis, which increases the synovial fluid volume and increases the IAP both at rest and during exercise. The increased IAP during exercise may impede blood flow to the synovial lining causing intermittent hypoxic reperfusion injury, which contributes to the persistence of inflammation.[27-29] Doppler flow meters show that an increase in IAP of as little as 20 mm Hg is sufficient to significantly decrease blood flow to the synovium in human patients with knee OA.[30] Assessment of IAP in the stifle of healthy dogs indicates that during extreme flexion the IAP can increase significantly from baseline values (-17 mm Hg) to IAP values greater than 50 mm Hg.[25,26] A similar study was conducted in the middle carpal joint of anesthetized horses to assess IAP changes associated with infusion of saline at two different carpal flexion angles.[31] Joint compliance was significantly reduced at a 90 degree carpal flexion angle, resulting in significantly higher IAPs which was most likely due to increased tension on the joint capsule compared to carpal flexion angles maintained at 135 degrees.[31] Additional deleterious effects of increased IAP include: muscle inhibition, distortion of the joint capsule, increased capsule elastance (stiffness), and decreased joint range of motion.[27] IAP measurements in the horse can provide an assessment of the IAP dynamics associated with the onset and progression of OA and determine IAP responses to aquatic therapy.

Pressure Algometry

Pain is characteristically the primary compliant in human patients with OA. However, as the equine patient cannot verbally express their level of discomfort the assessment of pain is typically subjective based on clinical presentation and examination. Often the localization of the primary source of pain can be difficult due to increased nociceptive plasticity (i.e., behavioral and cellular modifications produced by activation of nociceptors) causing radiating and referred pain.[32] Human patients with OA often demonstrate increased pain intensity or tenderness over apparently normal tissues (allodynia) in remote sites from the injured joint.[32] Tissue and joint trauma result in the production of a regional inflammatory response that releases a wide range of inflammatory mediators. Nociceptive nerve fibers and joint mechanoreceptors (type IV pain receptors) are activated in the presence of varying inflammatory mediators, often responding to more than one mediator at relatively narrow ranges of stimulus intensity.[32,33] Prostaglandin E_2 (PGE_2), for example is released in response to inflammatory mediators produced within traumatized joints and tissues.[34] Prostaglandins have been shown to increase nociceptive fiber sensitivity, lowering the activation threshold and thus making the nerve respond to a lower noxious stimulus (hyperalgesia).[32] In addition, substance P, a neuropeptide released from sensory nerve endings and from many types of immune cells stimulates the production of PGE_2 and collagenase from synoviocytes, as well as has chemotactic properties for neutrophils, monocytes, and lymphocytes.[32] Initially, the activation of nociceptors in response to tissue inflammation and the production of acute pain is the body's adaptive mechanism to minimize further tissue damage and promote healing.[34] However, the persistence of pain and inflammation may progress to a pathologic condition that no longer serves as a protective mechanism but transitions into a maladaptive response characterized by persistent pain, progression in the underlying disease, and increased susceptibility to secondary musculoskeletal injuries.

Mechanical nociceptive threshold (MNT) assessment has been investigated in numerous human clinical studies[35] and more recently used to establish normal reference values[36] in the horse, as well as assessing equine axial and appendicular musculoskeletal pain.[37,38] Use of the pressure algometer provides a repeatable, objective quantification of pain, allowing changes in stimulus intensity to be compared.[35] Importantly, human studies have demonstrated that MNTs are not only decreased over the injured region, but that lower thresholds are often found over sites remote to the primary source of pain.[35] Similarly, experimentally-induced OA in the equine carpus resulted in lower MNT values recorded from sites tested directly in the region of the carpus, as well as decreased values in sites both distal and proximal to the osteoarthritic joint.[38] MNT testing can be used to quantify the degree of pain associated with the onset and progression of OA, to identify and localize referred musculoskeletal pain, and may assess nociceptive changes related to aquatic therapy.

Lameness Exam

Lameness exams are used routinely to aid in the diagnosis of musculoskeletal injuries. The subjective assessment of gait alterations are traditionally assigned a score (0-5) based on the severity of the movement disorder.[39] The grading scale provides the clinician with an initial baseline level of lameness that can be used to determine improvement based on diagnostic analgesia or to assess the effects of therapeutic interventions. Therefore, assessing the response the therapy requires consistent lameness scoring at varying time points. The repeatability of a numerical grading scale for scoring lameness has been found to be good to excellent when evaluating moderate to severe lamenesses.[40] Inter-observer agreement was 93% for detecting lameness with a score of 1.5 or higher, however lameness scores of less than

1.5 demonstrated only a 62% agreement.[41] Furthermore, intra-observer gait analysis studies demonstrates that changes in mild to moderate lameness can consistently be detected at multiple time points by a single assessor, indicating a high level of reliability.[42] Clinical scoring of lameness in research settings is optimally done by a blinded orthopaedic surgeon experienced in assessing limb pain and locomotor system function. Reliable, semiquantitative lameness grades provide a valid assessment of locomotor changes occurring over time in response to the progression of OA and in response to aquatic therapy.

Diagnostic Imaging

The progression of OA is characterized by articular cartilage damage, which ultimately results in cartilage loss with end-stage disease. Radiographs are often the primary diagnostic tool used to characterize osseous changes associated with joint disease, however radiography is inadequate for imaging early OA changes.[43] Radiographic imaging can readily detect changes associated with OA at later stages of the disease process once significant articular cartilage damage and osseous changes have occurred.[44] Positive radiographic findings include: osteophyte and enthesophyte formation, subchondral bone lysis and sclerosis, and loss of joint space. Unfortunately, there is often a discrepancy between the clinical signs of joint disease and radiographic changes, which has led to the use of magnetic resonance imaging (MRI) as an additional diagnostic imaging tool that may allow for the earlier detection of joint damage. Previous work in equine cadaver carpal joints demonstrate that MRI is able to detect subtle changes in the synovial membrane, articular cartilage, and subchondral bone due to visualization of changes in fluid distribution and accumulation.[45] Furthermore, a significant correlation was demonstrated between the degree of pathologic lesions visualized on MRI and the extent of the articular lesions characterized microscopically.[45] The combined use of radiographs and MRI helps to identify soft tissue and osseous changes associated with the development of OA, as well as, discriminate articular changes associated with aquatic therapy intervention.

Synovial Fluid Analysis

The initiation and progression of OA can originate from various causes, with trauma and synovitis being the most common etiologies. Cyclic overuse and repetitive trauma predispose the horse to inappropriate or excessive biomechanical stresses applied to the articular surface.[46] The ensuing inflammatory process can originate in the synovial membrane, joint capsule, articular cartilage, or subchondral bone.[3,7] In response to cellular injury, the primary tissue of insult releases inflammatory mediators that are responsible for up-regulation of the associated inflammatory pathways and for producing enzymatic degradation of hyaline articular cartilage.[3,7] The principle inflammatory mediators involved in equine OA are cytokines IL-1 and TNFα.[7] The binding of IL-1 to its receptor on the cell surface stimulates the up-regulation of several other inflammatory pathways including the increased production of aggrecanase, prostaglandins (PGE_2), and matrix metalloproteinases (MMPs).[47] PGE_2 is released from synoviocytes and chondrocytes in response to IL-1, causing proteoglycan degradation and inhibited synthesis, vasodilatation, enhanced sensitization of nerve endings, bone demineralization, and up-regulation of plasminogen.[47] Horses diagnosed with acute synovitis or early-onset OA often demonstrate higher synovial fluid concentrations of PGE_2, compared to horses with chronic joint disease.[47,48]

Synovial fluid analysis provides a minimally invasive assessment of chemical and cellular alterations occurring within the joint due to degenerative changes associated with OA. In particular, studies have demonstrated that elevations in synovial fluid total protein, PGE_2 and glycosaminoglycan (GAG) concentrations are associated with joint trauma, inflammation and OA.[5,6,43,46] Specifically, the dimethyl-methylene blue binding assay (DMMB) has been shown to be a repeatable, sensitive, and specific method for determining synovial fluid GAG concentration under both normal and osteoarthritic conditions.[49] High levels of GAG concentrations have been isolated from equine synovial fluid early in the articular cartilage degradative process as the rate of proteoglycan loss is higher in acute joint disease.[6,50] Similar DMMB assay results demonstrated that significantly increased synovial fluid concentrations of GAGs were detected in human patients with acute joint disease, but that decreased GAG concentrations were found in chronic conditions.[44]

Prostaglandin E_2 is principally used as an indicator of synovial inflammation, with elevations in PGE_2 frequently associated with the initiation of OA.[47,48] The extraction of PGE_2 from synovial fluid involves a highly sensitive immunoassay[a] that has demonstrated reliable and repeatable results.[5,43,51-56] Multiple studies have demonstrated that the varying concentrations of PGE_2 within the synovial fluid can greatly influence articular cartilage metabolism by depressing proteoglycan synthesis and accelerating cartilage destruction.[53]

Conventional synovial fluid analysis including total protein concentration and white blood cell differentials also provide an indication of the magnitude of synovial membrane inflammation. Although, neither indicate a specific diagnosis both offer a quantitative assessment of the degree of synovitis and articular cartilage destruction occurring within the joint. Synovial fluid analysis can be used to monitor the progression of joint disease, determine the severity of inflammation, and quantitate the efficacy of underwater treadmill exercise to diminish joint inflammation and cartilage breakdown.

Articular Cartilage Matrix Evaluation

The DMMB assay provides a reliable method for determining both synovial fluid and articular cartilage GAG content.[43,51-55,57] Analysis of equine articular cartilage explants removed from experimentally-induced osteoarthritic joints have demonstrated a decreased GAG content.[6,58] Depletion of GAGs within the articular cartilage reduces the compressive strength needed to withstand normal biomechanical loads. Reduced GAG content indicates a limited ability of the articular cartilage matrix to maintain a supporting role in joint health.

The assessment of chondrocyte GAG synthesis utilizes the measurement of articular cartilage precipitable radiolabelled GAG levels. Equine studies indicate that cartilage from osteoarthritic joints have higher GAG synthesis rates compared to healthy controls.[6,55,58] The elevation in articular cartilage matrix metabolism in the presence of decreased GAG concentrations may be a cellular metabolic attempt to return GAG content to a normal level.[6] The use of articular cartilage GAG content and GAG synthesis rates can provide a sensitive indication of cartilage metabolism and consequently the severity of damage between control and underwater treadmill treated joints.

Macroscopic Middle Carpal Joint Evaluation

In the equine osteochondral chip fragment-exercise OA model, osteochondral fragmentation of the distal radial carpal bone leads to mild degenerative changes in the articular cartilage of the middle carpal joint.[5,54,55] A macroscopic scoring system of the gross morphologic alterations includes grading full and partial thickness cartilage erosions, synovial membrane hemorrhage, fracture size, presence or absence of synovial adhesions, and corresponding lesions on the radial facet of the third carpal bone.[59] Degenerative changes after osteochondral fragmentation are predominately found on the radial facet of the third carpal bone and within the distal radial carpal bone. Macroscopic scoring provides a gross characterization of the articular cartilage surface changes, indicating the severity and chronicity of disease.

Histology

Traditionally, a modified-Mankin scoring system has been used in the histological evaluation of lesions associated with the equine osteochondral chip fragment-exercise OA model.[59] Validation of this model demonstrated an intra-class correlation agreement value of 0.82 (1 = perfect homogeneity) for trained evaluators and a intra-class correlation agreement of 0.92 for untrained evaluators.[59] One of the earliest detectable histologic changes associated with OA is fibrillation of the articular cartilage surface.[59,60] Histologic examination of equine articular cartilage has demonstrated that the severity of fibrillation correlates with alterations in staining characteristics and the degree of chondrocyte necrosis.[61] Safranin O-fast green (SOFG) histochemical stain intensity uptake is directly correlated to the amount of GAG present in the articular cartilage matrix.[61,62] Several equine studies have demonstrated that the intensity of staining is decreased within the various articular cartilage zones due to the presence of OA.[5,59,63-65] Additional histologic pathology typically indentified in equine osteoarthritic cartilage using haematoxylin and eosin (H & E) staining includes chondrocyte necrosis, chondrone formation, fibrillation, and focal cell loss. Articular cartilage and synovial membrane histology provides a semiquantitative assessment of the pathologic characteristics associated with OA and the potential disease-modifying effects allied with aquatic therapy.

Purpose of study

The potential therapeutic effects of aquatic therapy in horses are unproven despite its wide-spread use. The purpose of this study was to quantify the clinical signs and disease-modifying effects of underwater treadmill exercise, compared with effects of controlled hand walking in horses with experimentally-induced carpal OA by evaluation of clinical (i.e., lameness, joint range of motion, response to flexion, synovial effusion, and pain thresholds), radiographic, MRI, gross, biochemical, and histologic outcome measures. Our hypothesis was that underwater treadmill exercise would have a significant positive influence on both clinical signs and structural effects of OA in horses. Furthermore, this study could provide proof of principle that underwater treadmill exercise is a feasible management intervention for horses with carpal OA.

Materials and Methods

Animals - Sixteen skeletally mature horses, age 2 – 4 years (mean ± SD mass, 385 ± 40 kg; height at withers, 1.46 ± 0.04 m) were included in the study. Prior to inclusion in the study, all horses underwent a lameness examination, range of motion (flexion) testing of the carpal joints, evaluation for middle carpal joint effusion, body condition assessment, and radiography of the carpal joints; only horses without abnormal findings were permitted in the study. A minimum 14-day acclimatization period to new environmental factors and training to safely enter and use the high-speed and underwater treadmills occurred prior to baseline data collection. The Colorado State University Institutional Animal Care and Use Committee approved the study protocol.

Experimental induction of osteoarthritis – As described,[58] following general anesthesia and routine preparation for surgery, each horse underwent bilateral arthroscopic surgery of the middle carpal joints to ensure that there were no pre-existing abnormalities (termed day 0). During this procedure, an osteochondral fragment was created in one randomly selected middle carpal joint. An 8-mm-wide fragment was generated by use of an 8-mm curved osteotome directed perpendicular to the articular cartilage surface of the radial carpal bone at the level of the medial synovial plica. The dorsal surface of the fragment was allowed to remain adhered to the joint capsule proximally. A motorized burr was used to debride the exposed subchondral bone between the fragment and the parent bone. A 15-mm-wide defect bed was created and the debris was not actively lavaged from the joint, thereby adding in the induction of OA. This joint was designated as the osteochondral fragmented joint (OCF) and the sham-operated joint was used as the control joint. The arthroscopic portals were closed with 2-0 nylon suture in a simple interrupted pattern, and cyanoacrylate glue[b] was applied to the incision. The thoracic limbs were bandaged, and the horses were allowed to recover from anesthesia and surgery. All study horses received a single dose of ceftiofur[c] (4.0 mg/kg) IM just prior to surgery and 2 grams of phenylbutazone[d] orally once a day for 5 days after surgery. Bandages were changed every three to five days and maintained until suture removal at 10 days after surgery.

Exercise - Each horse was housed in a 3.7 m^2 stall. Beginning on day 15, all horses were exercised on a high-speed treadmill[e] 5 days each week, until the end of the study to promote the development of OA. Each day, the horses were trotted at 4.4 m/s for 2 minutes, galloped at 8.8 m/s for 2 minutes, and trotted again at 4.4 m/s to simulate the strenuous exercise associated with race training.

Treatment groups - One week after surgery (day 7), the horses were ranked by lameness score and randomly assigned to one of two groups with 8 horses in each group.[52] The intervention group was exposed to underwater treadmill exercise[f] (UWT) starting on day 15. The underwater treadmill exercise protocol consisted of horses exercising at a brisk walk (2.1 m/sec) for 5 minutes, once a day, for 5 days. The water level in the underwater treadmill was maintained at the point of the shoulder for the duration of the study, which provided approximately 60% reduction in weight bearing.[31] At weekly intervals the duration of underwater treadmill exercise was increased by 5 minutes, until a maximum of 20 minutes was reached. Horses then continued to be exercised for 20 minutes once a day, five days a week, for the remainder of the ten-week study (8 weeks total of exercise). The control group was exercised on a low-speed treadmill (without water) at the same speed, frequency and duration as the intervention group also starting at day 15. The low-speed exercise provided within the control group simulated hand walking and light exercise as prescribed in current rehabilitation protocols.

Assessment of clinical outcomes - For each horse, clinical examinations of both thoracic limbs were performed weekly from day -7 (baseline; before surgery) throughout the study period. Lameness was graded by an experienced equine orthopaedic surgeon that was unaware of the treatment group assignments on a standardized 0 to 5 scale (0 represented normal gait, and 5 represented severe lameness), with the horse trotting on hard ground.[39] Horses were visually assessed and videographed moving in a straight line both away from and toward the evaluator, with each side of the horse evaluated. After lameness grading, flexion of the carpal joint was performed followed by observation of the horse during trotting as an indication of increased lameness following manipulation and stress concentrated around the joint of interest (middle carpal joint). The response to flexion was graded on a scale of 0 to 4 (0 represented no response, and 4 represented a severe lameness response). As an indication of increased volume of synovial inflammation in the middle carpal joint, the extent of effusion was graded on a scale of 0 to 4 (0 represented a normal amount of fluid, and 4 represented a severe increase in fluid volume). A board-certified, large animal surgeon who was unaware of treatment assignments assessed the clinical lameness outcome parameters.

Diagnostic imaging - Radiographic evaluation of both carpi were performed prior to inclusion in the study (day –7) to confirm the absence of osseous pathologic changes prior to entry into the study, following induction of OA (fourteen days after osteochondral fragment creation) to assess acute osseous changes, and at termination of the study (day 70) to evaluate the progression of OA. A board-certified radiologist who was unaware of the treatment groups assessed the radiographic and MRI images. The radiographic images (Fig 3.1) were evaluated for bone proliferation at the joint capsule attachment along the dorsal margin of the radial carpal bone, subchondral bone lysis, sclerosis, and osteophyte formation on a scale on 0 to 4 (0 represented no detectable abnormality and 4 represented severe change).

At study conclusion, bilateral carpal MRI scans were completed, which included sagittal, transverse, and frontal plane images made by use of proton density and short T1 inversion recovery sequences. The MRI images (Fig 3.2) were evaluated for pathologic changes in joint tissues associated with OA by assessing the synovial fluid volume, the presence of synovial membrane proliferation, joint capsule thickness, joint capsule edema, joint capsule fibrosis, subchondral bone edema and sclerosis of the radial carpal and third carpal bones on a 0 to 4 scale (0 represented normal and 4 represented severe change).

Synovial fluid - Beginning on day 0 until the end of the study (day 70), synovial fluid was aseptically aspirated once per week from both middle carpal joints of each horse to monitor the degree of synovitis and articular cartilage destruction occurring within the joints. Synovial fluid (2 to 4 ml) was aspirated using a 20-gauge, 1.5-inch needle and a 6-ml syringe. Synovial fluid was placed in tubes containing EDTA for routine synovial fluid analysis and the remaining sampled stored at -80°C for PGE_2 and GAG concentration analysis.

Synovial fluid analysis included assessment of color, clarity, mucin clot formation, WBC count and differential, and total protein concentration. Synovial fluid color, clarity, mucin content, and blood contamination were evaluated subjectively. The color of the synovial fluid was graded on a scale of 1 to 4 (1 = straw, 2 = yellow, 3 = orange 4 = red). Clarity was graded on a scale of 1 to 3 (1= clear, 2 = hazy, and 3 = cloudy). A mucinous precipitate test was utilized to grade the quality of the synovial fluid clot formation in the presence of 2.5%

acetic acid. Mucin content was graded on a scale of 1 to 3 (1 = good, 2 = fair, and 3 = poor). Blood contamination of synovial fluid samples was graded as 1 or 2 (1 = samples with no blood contamination, and 2 = samples with blood contamination. Total protein and WBC concentrations were determined via refractometry and use of an automated cell counter. Synovial fluid was also examined cytologically to determine the differential WBC counts.

Two biomarker protein assays were performed on synovial fluid. A modified 1,9-dimethyl-methylene blue binding assay was used to determine GAG concentration (glycosaminoglycan/ml).[57] Synovial fluid concentration of PGE_2 was assessed by use of a commercially available, competitive, high-sensitivity enzyme immunoassay kit as directed by the manufacturer's instructions.[a] Samples were processed in duplicate and PGE_2 concentrations were expressed in picograms per milliliter.

Middle carpal joint intra–articular pressure - Beginning on day 0 until the end of the study (day 70), middle carpal joint IAP's were measured aseptically from both middle carpal joints of each horse in conjunction with synovial fluid collection. The pressure monitor system consists of a digital monitor that contains a transducer with an auto-zeroing device and a sterile diaphragm chamber that connects to a sterile saline filled syringe. After assembly, the system and the 20-gauge needle with attached 3-way stopcock were primed with saline, in order to ensure hydrodynamic communication with the joint lumen.[27] The intra–articular middle carpal joint pressures were assessed in the dorsal lateral pouches in a flexed joint position, prior to any prescribed exercise. A 1.5-inch, 20-gauge needle attached to a 3-way stopcock was inserted into the dorsal lateral joint pouch. The 3-way stopcock was initially placed in the off position to prevent synovial fluid from leaking out of the hub of the needle. Once the needle was inserted a hand-held, intra–compartmental pressure monitoring system[g] was attached directly to the 3-way stopcock and the valve was opened to the joint (Fig 3.3). The IAP was recorded when minimal movement from the horse occurred and the IAP reading had been allowed to stabilize over 5 seconds. Three consecutive measurements were obtained and the mean IAP value for each joint was determined.

Goniometric measurements – Joint range of motion data were collected weekly from both thoracic limbs starting at baseline (day -7) and continuing for the duration of the study. Hair was removed with a #40 clipper blade at the thoracic limb centers of joint rotation[66] and permanently marked with red lacquer (Table 3.1). Joints of interest included the scapulohumeral, humeroradial, carpal joint complex, and metacarpophalangeal joints. The center of a hand-held goniometer[h] with two stainless steel extending arms (ranging from 23 cm to 66 cm) was placed laterally over each joint axis of rotation (Fig 3.4). To limit variability, each arm of the goniometer extended to the marked proximal and distal centers of joint rotation, except the proximal arm of the goniometer was placed along the spine of the scapula to assess the scapulohumeral joint range of motion. Each joint was mobilized and held at end ranges of motion by an experienced veterinarian, while joint angles were recorded by the same assistant over the course of the study. Previous pilot work demonstrated a coefficient of variation of less than 6% across all thoracic limb articulations. Three consecutive measurements of each flexion and extension angle were recorded and mean values were reported.

Mechanical nociceptive threshold testing - Pressure algometry was used to establish MNT values at 10 topographical locations within each thoracic limb. The sites were chosen based on the ability to consistently identify and assess local bone or joint pain in the region of the osteochondral fragment and at proximal and distal bone and soft tissue landmarks to assess peripheral sensitization within the thoracic limbs (Table 3.2). A fixed-order protocol was used to reduce between-subject variability. All musculoskeletal landmarks were tested in a distal-to-proximal order, beginning with the left thoracic limb and then the right thoracic limb. Measurement sites within each limb included osseous (n = 5) and soft tissue landmarks (n = 5). MNT testing values were measured at baseline (1 week prior to osteochondral fragment induction, or day -7) and once every week for the duration of the study. Each measurement session lasted approximately 20 minutes. Sessions were scheduled on the day prior to middle carpal joint synovial fluid collection and prior to daily exercise protocols to limit variability and the effects of confounding variables. All measurements were conducted in the horse's stalls.

A pressure algometer[i] with a 1–cm^2 rubber plunger tip and a calibrated range from 0 – 30 kg/cm^2 was used to determine MNT values. Pressure was applied perpendicularly to predetermined anatomic landmarks at a rate of approximately 10 $kg/cm^2/s$ over 2 to 3 seconds until a local avoidance reaction was observed (Fig 3.5). Avoidance reactions included skin twitching, local muscle fasciculations, lifting of the thoracic limb, or moving away from the applied pressure. When a reaction was observed, the applied pressure was discontinued immediately and the corresponding MNT value was recorded. The examiner did not view the pressure gauge reading during application of pressure to limit potential bias. The instrument automatically records the highest applied pressure and resets to zero after each measurement. Three consecutive measurements at 3-to-4 second intervals were recorded at each site and the mean value for each site reported.

Gross observation of middle carpal joints - At study conclusion, all horses were euthanized by administration of pentobarbital sodium. For each horse, a necropsy was performed during which both middle carpal joints were specifically examined for the degree and location of articular cartilage fibrillation or erosion. A subjective grade (scale of 0 to 4) was assigned for partial and full–thickness cartilage erosion as well as synovial membrane hemorrhage. For each of theses parameters, grade 0 represented no pathologic change and 4 represented a severe change. A total erosion score also was assigned based on the degree of articular cartilage fibrillation and synovial membrane hemorrhage present in the entire joint (overall joint health) using a 0 to 4 scale.

Histologic examination - At necropsy, samples of synovial membrane and joint capsule were harvested from the region dorsal to the osteochondral fragment and placed in neutral–buffered 10% formalin for H & E staining. Five-micron sections of the tissue samples were prepared. An evaluator who was unaware of treatment assignment assessed the samples for cellular infiltration, synovial intimal hyperplasia, subintimal edema, subintimal fibrosis, and subintimal vascularity.[59] Each variable was graded on a scale of 0 to 4 (0 represented no abnormal change and 4 represented the most severe change). A cumulative histologic score was calculated for synovial membrane samples.

Articular cartilage samples (5 mm^2) were also obtained from each middle carpal joint. Collection locations were chosen to represent an area directly adjacent to the osteochondral fragment on the radial carpal bone, a site on the opposing articulating surface (third carpal bone), and a remote articular location (fourth carpal bone). Samples were stored in neutral–buffered 10% formalin for 7 days and then processed for histologic examination by an evaluator who was unaware of treatment assignment. The 5-μm sections were stained with both H & E and safranin - O fast green (SOFG). The H & E sections were evaluated for articular cartilage fibrillation, chondrocyte necrosis, chondrone formation (chondrocyte division within a lacuna), and focal cell loss.[59] Numeric values ranging from 0 to 4 were assigned to each variable (0 represented no abnormal change, and 4 represented the most severe change). A cumulative H & E histologic score for each articular cartilage sample was determined by addition of the scores for all above variables. Articular cartilage sections (sample taken from the radial, third and fourth carpal bones) stained with SOFG were evaluated for intensity of staining in the tangential, intermediate, radiate territorial, and radiate interterritorial zones of the third carpal, fourth carpal, and radial carpal bones.[59] Numeric values ranging from 0 to 4 were assigned to each variable (0 indicated no stain uptake and 4 indicated normal stain uptake), and a cumulative SOFG histologic score for each articular cartilage sample was calculated by summation of the four zonal scores.

Articular cartilage matrix evaluation - To estimate articular cartilage proteoglycan content, the total articular cartilage GAG content was measured by use of a 1,9-dimethyl-methylene blue technique.[57] Articular cartilage samples were obtained from the area directly adjacent to the osteochondral fragment on the radial carpal bone and a remote site from the intermediate carpal bone. Each piece was stored at -80° C prior to further processing and analysis. Cartilage was digested prior to analysis at a ratio of 10 mg of wet weight per ml of papain digest. Samples were processed in duplicate and results were reported in micrograms of GAG per milliliter.

For analysis of cartilage matrix metabolism, articular cartilage samples were aseptically collected from the weight bearing surface that was remote from the osteochondral fragment within each joint (intermediate carpal bone), and incorporation of sulfur 35 radiolabelled SO_4 was measured using reported methods.[58] Samples were processed in duplicate, and the results were reported as disintegrations per minute per µg of GAG.

Statistical analysis - Results were analyzed by use of a mixed model ANOVA.[j] The ANOVA tables were used to determine significant ($P < 0.05$) main effects and interactions between main effect variables. The following independent, main effect variables were considered: presence or absence of OA, day of sample collection, and treatment (aquatic therapy or controlled hand walking). When individual comparisons were made, least-square means were used and $P < 0.05$ was considered significant. Trends in the data were reported as $0.05 < P < 0.10$. Values are reported as mean ± SEM. The potential effect of horse on the outcome variables was controlled via the introduction of a random effect for horse. Residual plots were used to determine normality of distribution and an equal variance with a mean of zero. The residuals should ideally resemble a random sample from a normal distribution with mean zero and constant variance. All parameters had a normal distribution except the residual versus predicated values for the initial PGE_2 data had evidence of a non-normal distribution; the error variance increased with predicted values. A log transformation was used to achieve homogeneity of variance for the PGE_2 data only.

Results

Clinical examinations - Mild lameness was evident in all limbs of horses in which an osteochondral fragment was induced (mean ± SEM lameness score, 1.81 ± 0.05). This mean lameness score differed significantly ($P < 0.0001$) from the baseline value measured before OA was induced (0.03 ± 0.09), and the difference persisted throughout the study period. The mean lameness scores for the aquatic therapy OCF limbs (2.50 ± 0.18) were significantly higher ($P = 0.04$) at day 21 (7 days after the initiation of aquatic therapy), compared with the scores from the control group OCF limbs (2.00 ± 0.18) (Fig 3.6). However, the mean lameness scores for the aquatic therapy OCF limbs at day 42 and 49 (1.40 ± 0.18 and 1.90 ± 0.18, respectively), which was 28 and 35 days after the initiation of aquatic therapy, were significantly lower ($P = 0.04$) compared to the control groups OCF limbs (1.90 ± 0.18 and 2.40 ± 0.18, respectively). The aquatic therapy horses showed a trend for reduced lameness scores compared to the control horses on day 28, which was maintained for the remainder of the study, although significant improvements were only appreciated on days 42 and 49.

The mean score for response to carpal flexion was significantly ($P < 0.0001$) increased in OCF (1.80 ± 0.06) versus sham-operated (0.05 ± 0.06) joints in all horses after osteochondral fragmentation (Fig 3.7). The significant increased flexion response within the OCF joints persisted throughout the study period. In addition, a significant treatment effect ($P = 0.01$) was appreciated in flexion scores as the aquatic therapy OCF joints were significantly ($P = 0.01$) more responsive to carpal flexion (1.95 ± 0.08) than the control group OCF joints (1.66 ± 0.08). When least square mean comparisons were conducted between the treatment group's OCF joints over the entire study period the aquatic therapy OCF joints had a significantly greater flexion response on days 35, 56, and 63.

Significant ($P < 0.0001$) increases in mean synovial effusion scores were evident in OCF joints (1.53 ± 0.05), compared with those from the sham-operated joints (0.03 ± 0.05) during the period after OA was induced (Fig 3.8). No significant treatment effects were detected.

Diagnostic Imaging - A significant increase in radiographically detected middle carpal joint lesions were noted for all radiographic outcome variables after OCF surgery. Subjective radiographic scoring indicates that underwater treadmill exercise resulted in more pronounced radiographic changes following surgical induction of the osteochondral fragment, which is primarily due to the increased radiographic lytic changes appreciated in the radial carpal bone. At study conclusion, the OCF joints exercised in the underwater treadmill demonstrated a significantly higher subchondral bone lysis (2.62 ± 0.16; P < 0.0001) score, compared to the control group OCF joints (1.63 ± 0.16). Total radiographic scores before treatment for sham-operated (0.19 ± 0.31) versus OCF (3.44 ± 0.31) joints were significantly different (P < 0.0001). A significant difference was not detected between the treatment group OCF joints on day 14; however, a significant treatment effect was detected on day 70 (Fig 3.9). The radiographic total score was significantly higher (P = 0.001) in the aquatic therapy OCF joints (7.37 ± 0.44) compared to the control OCF joints (5.25 ± 0.44).

A significant (P < 0.0001) increase was detected in the total MRI score for OCF (22.00 ± 0.83) versus sham-operated joints (15.00 ± 0.83) after surgery in both groups. Induction of OA did not result in significant third carpal bone sclerosis. However, a significant increase in MRI changes was detected for the remaining outcome variables in the OCF joints, compared to sham-operated joints. There were no significant differences detected for the total MRI score or for any of the other MRI variables between horses that received aquatic therapy or controlled hand walking. Joint capsule fibrosis showed a trend (P = 0.08) for being higher in the control group OCF joint, compared to the aquatic therapy OCF joint.

Synovial Fluid Analysis – Results of routine synovial fluid analysis indicated that the WBC count was significantly (P < 0.0001) increased in OCF versus sham-operated joints throughout the study period (382.00 ± 65.00 cells/dl vs. 190.00 ± 65.00 cells/dl, respectively). Synovial fluid color, clarity, and mucin clot formation also differed significantly depending on the presence or absence of an osteochondral fragment. Induction of an osteochondral fragment resulted in a subjective mean fluid color score of 2.63 ± 0.14 and a fluid clarity score of 1.28 ± 0.09 compared to the sham-operated joints of 1.89 ± 0.14 and 0.77 ± 0.09, indicating fluid from the osteochondral fragmented joint was on average a hazy, yellow-orange color. Sham-operated joints had a mean mucin clot score of 1.60 ± 0.06 compared to a mean score of 1.73 ± 0.06 in OCF joints, indicating less mucin content in fragmented joints. Aquatic therapy intervention did not affect synovial fluid WBC counts, color, clarity, or mucin clot formation.

Osteochondral fragmentation induced a significant increase ($P < 0.0001$) in the synovial fluid total protein concentration (2.15 ± 0.09 g/dl) throughout the study period, compared to sham-operated joints (1.53 ± 0.09 g/dl) (Fig 3.10). However, synovial fluid total protein concentrations did not differ significantly according to whether the horse was in the aquatic therapy or control group. Interestingly, the only significant difference between the treatment groups occurred on day 21. The synovial fluid total protein concentration within the aquatic therapy OCF joints was significantly elevated (2.74 ± 0.22 g/dl; $P = 0.01$) compared to the control group OCF joints (1.95 ± 0.22 g/dl).

A significant increase ($P < 0.0001$) in mean synovial fluid GAG concentration was detected in OCF joints (92.0.0 ± 15.00 μg/ml of GAG/ml) compared with that of sham-operated joints (67.00 ± 15.00 μg/ml of GAG/ml). The presence or absence of aquatic therapy did not significantly influence the synovial fluid GAG concentrations.

Synovial fluid PGE_2 concentrations were significantly ($P < 0.0001$) increased with osteochondral fragmentation (1.87 ± 0.05 ln pg/ml), compared with sham-operated (1.67 ± 0.05 ln pg/ml) joints. The presence or absence of aquatic therapy did not significantly influence the synovial fluid PGE_2 concentrations.

Intra–articular pressure - Middle carpal joint IAP was significantly (P 0.0001) elevated in all OCF joints (33.80 ± 3.00 mm Hg) compared to sham-operated joints (7.90 ± 3.00 mm Hg). The mean IAPs differed significantly ($P < 0.0001$) from the baseline values measured before osteochondral fragment induction (-0.80 ± 4.30), and the difference persisted throughout the study period. Middle carpal joint IAPs did not differ significantly according to whether the horse was in the aquatic therapy or control group. When the IAP changes were analyzed between the OCF and sham-operated limbs in the aquatic therapy and control groups across the study days, the aquatic therapy OCF joint did demonstrate a significantly reduced IAP value (23.00 ± 7.60 mm Hg; $P = 0.002$), compared to the control group OCF joint (47.00 ± 7.60 mm Hg) on day 35 (Fig 3.11). Further significant reductions in the IAPs over time were demonstrated within the aquatic therapy OCF joints. Specifically, significant ($P = 0.02$) reductions in IAPs within the aquatic therapy OCF joints occurred between days

21 and 28 (60.30 ± 7.60 mm Hg decreasing to 38.50 ± 7.60 mm Hg), with a continued decrease between days 28 and 35 (38.50 ± 7.60 mm Hg improving to 23.00 ± 7.60 mm Hg). Unlike the aquatic therapy group, the control group OCF joints gradually improved over time but did not demonstrate the significant decline in IAPs within the OCF joints.

Goniometry – Osteochondral fragmentation significantly (P = 0.01) influenced the degree of passive carpal flexion angles measured throughout the study period. The OCF mean carpal flexion angle (33.0 ± 0.8 degrees) was significantly higher than the mean carpal flexion angle measured from the sham-operated joints (25.0 ± 0.8 degrees) (Fig 3.12). OCF passive carpal flexion angles were also significantly (P =0.02) influenced by the presence or absence of aquatic therapy. Specifically, the aquatic therapy OCF joints demonstrated a significantly increased carpal flexion angle (31.0 ± 1.0 degrees), compared to the control OCF carpus (34.0 ± 1.0 degrees). Significant improvement in carpal flexion angles for horses exercised in the underwater treadmill began at day 21 and continued through the remainder of the study (Fig 3.13). At study conclusion a significant difference was no longer present between the aquatic therapy OCF and sham-operated passive carpal flexion angles. Osteochondral fragmentation did not result in significant effects on the degree of carpal extension across the data collection time points, and no significant treatment effects were observed.

Osteochondral fragmentation did not produce significant changes in the degree of metacarpophalangeal (fetlock joint) flexion or extension, radiohumeral (elbow joint) extension, or scapulohumeral (shoulder joint) flexion (Table 3.3). Elbow joint flexion, and shoulder joint extension angles were both significantly influenced by osteochondral fragmentation. The degree of elbow flexion (62.0 ± 0.9 degrees) was reduced significantly ($P = 0.002$) in the OCF limb, compared with the angles measured from the sham-operated limb (60.0 ± 0.9 degrees). Conversely, the degree of shoulder extension measured from the OCF limb was significantly greater (134.0 ± 0.9 degrees; $P = 0.01$) than the sham-operated limb (133.0 ± 0.9 degrees). There were no significant treatment effects in the degree of fetlock flexion and extension, shoulder flexion and extension, or elbow flexion and extension. Furthermore, the degree of shoulder extension and elbow flexion did not significantly change over the study days. However, there was a significant pattern of influence on the degree of elbow extension and shoulder flexion across study days ($P < 0.0001$). The degree of elbow extension and shoulder flexion significantly improved over the study days and was not influenced by osteochondral fragmentation or treatment.

Pressure Algometry – At baseline, no significant differences in MNT values were found between sham-operated and OCF limbs. Osteochondral fragmentation significantly decreased the MNTs for three of the bony sites tested in the region of the carpus including the dorsal radial carpal bone, dorsal third carpal bone, and lateral carpal accessory bone (Table 3.4). The lateral suspensory branch proximal to the attachment on the lateral proximal sesamoid bone also had a lower MNT value within the OCF limb. MNT values recorded from the lateral suspensory ligament branch and the lateral carpal accessory bone were not significantly influenced by the presence or absence of aquatic therapy. Furthermore, all of the sites tested did demonstrate a significant single main effect of study day (P = 0.001) indicating that MNTs differ consistently across the testing days regardless of the effect of OA or treatment application.

The influence of aquatic therapy on the MNT values over the dorsal third carpal bone within the OCF limbs approached significance (P = 0.08), demonstrating a trend for the OCF limbs exercised in the underwater treadmill to have higher MNT values over the third carpal bone. Specifically, the OCF limbs exercised in the underwater treadmill demonstrated significantly (P = 0.01) higher MNT values at day 63 to 70, compared to the control group OCF limbs (Fig 3.14). In addition, within the control treated horses the third carpal bone MNT values were significantly lower in the OCF joints compared to the sham-operated joints at day 35 through 63 (Fig 3.15). Conversely, horses exercised in the underwater treadmill only demonstrated a significant difference between the OCF and sham-operated joints on day 7 and 49 (Fig 3.16).

Although a significant treatment effect was not detected, when individual comparisons were conducted the dorsal radial carpal bone MNT values recorded from the aquatic therapy OCF joints were significantly higher (P = 0.02) at day

70, compared to the control group OCF joints (Fig 3.17). The OCF limbs exercised in the underwater treadmill began at day 49 to have a trend for higher radial carpal bone MNTs, compared to the plateau of values that were present in the control group OCF joints. In addition, within the control group the MNTs were significantly (P = 0.04) lower in the OCF joints compared to the sham-operated joints at days 56 to 63 (Fig 3.18). Conversely, the horses exercised in the underwater treadmill did not demonstrate a significant difference between the OCF and sham-operated joints beyond day 21, with significant differences only present on day 7 and 21 (Fig 3.19).

There were no significant changes in the coronary band, dorsal third metacarpal bone, ulnaris lateralis, or sixth cervical vertebrae's MNT values following the induction of an osteochondral fragment, nor any evidence of a treatment effect. Furthermore, osteochondral fragmentation did not significantly change the MNT values recorded from the long head of the triceps brachii muscle, nor was there any evidence of a treatment or study day effect.

Osteochondral fragmentation did not have a significant effect on the extensor carpi radialis (ECR) muscle MNT values. However, the pattern of influence of study day on each horses ECR MNT values consistently changed depending on whether the horse was exercised in the underwater treadmill or not (study day x treatment interaction P = 0.002). The horses exercised in the underwater treadmill demonstrated significantly higher ECR MNT values at day

35, and days 49 through 70, with an improving trend beginning at day 28 (Fig 3.20).

Gross Examination - On postmortem examination, all OCF fragments appeared grossly similar with no significant differences in the fracture size between the control and underwater treadmill groups. The mean scores for articular cartilage fibrillation for full ($P = 0.004$) and partial thickness ($P = 0.001$) as well as synovial membrane hemorrhage ($P = 0.001$) were significantly increased in OCF joints, compared with sham-operated joints. The aquatic therapy OCF joints had an increased full thickness erosion score (1.0 ± 0.1) compared to control group OCF joints (0.5 ± 0.1), but this difference is only slightly significant ($P = 0.052$). No significant treatment effects were evident in any other gross pathologic scores or comparisons.

Histologic Examination – Evaluation of SOFG stained sections of articular cartilage revealed no significant loss of stain deposition in any of the four evaluated regions within the OCF or sham-operated joints. Evaluation of articular cartilage for SOFG staining scores did not differ significantly according to whether the horse was in the aquatic therapy or control group.

Histologic evaluation of the H & E stained articular cartilage sections revealed an increasing trend ($P = 0.07$) in the cumulative pathology score from the OCF (3.8 ± 0.3) versus sham-operated (3.1 ± 0.3) joints, when all locations were considered. Specifically, individual comparisons based on the presence of an osteochondral fragment and location revealed that within the third carpal bone the OCF joints had significantly increased numbers of chondrone formations (1.7 ± 0.2; P = 0.02) compared to their sham-operated counterparts (1.0 ± 0.2). Additional comparisons for chondrone formation were analyzed, as a trend (P = 0.09) was found within and between the two treatment groups. Specifically, the control group OCF third carpal bone demonstrated an increased number of chondrone formations (2.1 ± 0.3), compared to the OCF third carpal bone from horses exercised in the underwater treadmill (1.3 ± 0.3). In addition, a significant difference (P = 0.02) was demonstrated in the number of chondrones due to the induction of OA between the OCF and sham-operated third carpal bones within the control group (Fig 3.21). Underwater treadmill exercise demonstrated a potential treatment effect in the third carpal bone as having a reduced number of chondrone formations in the OCF joints compared to similarly affected control joints, as well as maintaining no significant difference in the degree of chondrone formation between the underwater treadmill exercised OCF and sham-operated joints.

The degree of fibrillation present between the OCF (0.4 ± 0.1) and sham-operated joints (0.1 ± 0.1) approached significance (P = 0.07). Histologic evaluation of the articular cartilage via H & E staining revealed a significant (P = 0.04) increase in the degree of fibrillation present in the articular cartilage removed from the OCF radial carpal bone. No significant improvements in the degree of fibrillation following underwater treadmill exercise were detected.

Morphologic analysis of articular cartilage focal cell loss and chondrocyte necrosis via H & E staining revealed no significant differences based on the induction of OA from any of the three carpal bone locations. Underwater treadmill exercise did not significantly influence the articular cartilage focal cell loss or chondrocyte necrosis scores.

Osteochondral fragmentation did not result in significant effects on degree of synovial membrane vascularity, subintimal fibrosis, or cellular infiltration, and no significant treatment effects were observed. Significantly higher synovial membrane intimal hyperplasia was seen in joints containing an OCF (score 1.5 ± 0.2; P = 0.009), compared to their respective contralateral sham-operated joints (0.6 ± 0.2). In addition, a significant (P = 0.05) treatment effect was demonstrated in those horses that exercised in the underwater had significantly less intimal hyperplasia in their sham-operated joints (0.1 ± 0.3) compared to the control horses sham-operated joints (1.1 ± 0.3).

Induction of an osteochondral fragment resulted in a significantly (P = 0.02) higher degree of synovial membrane subintimal edema in the OCF joints (1.06 ± 0.20) compared to the contralateral sham-operated joints (0.4 ± 0.2). The OCF joints exercised in the underwater treadmill also had a significantly (P =

0.02) higher degree of subintimal edema compared to the control OCF joints (1.5 ± 0.2 versus 0.6 ± 0.2, respectively).

Articular cartilage matrix evaluation – Evaluation of total articular cartilage GAG content did not reveal significant differences between OCF and sham-operated joints. In addition, no significant differences were detected in matrix metabolism with induction of OA. Underwater treadmill exercise did not significantly influence the total articular cartilage GAG content or the articular cartilage matrix metabolism.

Discussion

In the present study involving induction of OA via osteochondral fragmentation in the middle carpal joint of horses, underwater treadmill exercise demonstrated limited positive effects on clinical signs or disease-modifying outcome variables. It should be noted that the aquatic therapy OCF limb had a significantly higher lameness score one week after starting underwater treadmill exercise (day 21), but improvement in clinical lameness scores was detected beginning 2 weeks after initiation of therapy and continued that trend for the remainder of the study. These results indicate that the aquatic therapy horses had to overcome a greater degree of pain initially before beginning to show improvement in lameness scores. Early in the course of disease the osteochondral fragment is displaced from the parent bone but remains attached to the proximal joint capsule. The increased resistance of moving through water and the increased joint range of motion required to continue forward motion may have applied tensile stresses on the joint capsule and fragment, leading to an exaggerated inflammatory response within the aquatic therapy OCF joints initially. An elevated inflammatory response within the OCF joints following the first week of underwater treadmill exercise was further supported by the highest total protein concentration and IAPs occurring in conjunction with the elevated lameness score. Increased synovial fluid concentrations of total protein indicate the degree of inflammatory response occurring within the joint, with greater degrees of synovitis often associated with an elevation in pain and lameness.[6] The trend for improvement in clinical lameness scores from this study are similar to results reported from osteoarthritic human patients that demonstrate a reduction in joint pain and significant improvements in functional gait tasks following a course of aquatic therapy.[67]

The clinical significance of the response to flexion scores between OCF joints of the aquatic therapy and control group is equivocal. The intent of the carpal flexion test is to exert pressure and traction on joint structures to determine if an aggravated pain response can be elicited. Radiographically, two of the three horses with osteophytes (grade 2) at the dorsal margin of the distal radial carpal bone had exercised in the underwater treadmill. These osteophytes may have come in contact and irritated the synovial membrane and joint capsule during induced carpal flexion, and elicited an acute pain response. However, biomechanical differences during active carpal range of motion during lameness evaluation potentially caused the osteophyte to be asymptomatic. Furthermore, the angle at which the carpus is held for the flexion tests is based on the comfort level of the individual horse. Horses in the underwater treadmill demonstrated improved passive carpal flexion angles measured by goniometry and thus were able to achieve a smaller flexion angle during flexion range of motion measurements. The achieved smaller flexion angle in the aquatic therapy OCF joints would place increased pressure and soft tissue tension across the dorsal joint surfaces, inducing an increased pain response.

Horses within both treatment groups showed the greatest restriction in passive carpal flexion in the first week after surgery. Aquatic therapy horses began a significant increase in passive range of carpal flexion angles in OCF joints one week after the initiation of therapy and this trend continued throughout the remainder of the study. By the end of the study, the passive range of carpal flexion within the OCF joint had returned to baseline levels only in the aquatic therapy horses. This indicated that underwater treadmill exercise was able to re-establish baseline levels of carpal flexion, returning the carpal joint to full range of motion.

Continuous passive range of motion (CPM) has been advocated in human patients following arthroscopic surgery to aid in the healing of articular cartilage defects.[69-72] The primary goals of passive range of motion exercise are to prevent joint capsule fibrosis, maintain tissue mobility, improve vascular dynamics, allow for synovial fluid diffusion, and decrease pain.[30,69-72] Similarly, aquatic therapy in the horse may be utilized to improve the health of the joint through therapeutic mechanisms provided by water. It is possible that early aquatic therapy (following suture removal) may reduce edema, improve range of motion, and enhance fluid exchange within the injured joint.

The use of aquatic therapy may also aid in the reduction of synovial effusion and IAP by stimulating the physiologic pumping action that occurs during normal joint range of motion. Several studies using an intra-compartmental pressure monitoring device, similar to what was used in this study, demonstrated an increase in the IAP within clinically effusive OA human joints and within saline-infused knee, ankle, and wrist joints.[27,28] These studies have concluded that increased synovial fluid volume, fibrosis of the joint capsule (i.e., decreased compliance), and decreased joint range of motion all contribute to the elevation of IAP.[31] Both human and canine studies have demonstrated that afferent fibers arising from articular mechanoreceptors signal changes in articular pressures that in the presence of effusion result in alterations of normal motor patterns, which consequently disrupts locomotor function.[25,26] In our study significant increases in the IAPs within the middle carpal joints due to osteochondral fragmentation and synovitis resulted in further impairment of joint function and mobility. The IAP within the OA affected joint of horses exercised in the underwater treadmill peaked on day 21 (one week after starting aquatic therapy), which corresponds with the most severe lameness and greatest reduction in passive carpal flexion. After the first week of aquatic therapy, the IAPs within the OA affected joints improved significantly. At study conclusion, the IAPs measured in the aquatic therapy OCF joints were no longer statistically different from the sham-operated joints in either group. These results do not provide any direct explanations for the mechanism of action responsible for the improvements; however, reduced joint capsule fibrosis measured on MRI support improved joint capsule compliance and thus a decreased IAP.

Magnetic resonance imaging provided useful information regarding the status of the joint capsule in the OCF joints of the control and aquatic therapy groups. Osteochondral fragmentation resulted in significant changes within the majority of MRI outcome variables assessed, including stimulating joint capsule fibrosis. As the compliance of the joint capsule decreases, an elevation in the IAP occurs, which restricts joint range of motion and increases pain.[23,24] Reduced joint capsule fibrosis in the aquatic therapy group could result in the improved passive joint range of motion and a reduction in IAPs. MRI is often used as a sensitive imaging modality for characterizing soft tissue abnormalities. Previous studies using the osteochondral fragment model have demonstrated that MRI appears to be the best tool to identify significant changes that occur due to osteochondral fragmentation.[43] However, because the MRI's performed in this study were conducted at study conclusion, it was not possible to determine the effectiveness of aquatic therapy to modify the pathologic soft tissue changes over time, which is a limitation of this study.

Radiographic changes were also demonstrated within the aquatic therapy OCF joints, specifically increased radial carpal bone lysis. Radiographic evidence of lysis within the radial carpal bone is routinely documented in the osteochondral fragment model.[43] The increased lysis seen in the aquatic therapy horses could be due to the effect of buoyancy. A reduction in weight bearing mechanical stress applied at the region of the osteochondral fragment due to increased buoyancy during underwater treadmill exercise may have induced changes in the bone response. Similar reductions in bone density have been reported in rat swimming models.[68] A five-week long, daily swimming exercise protocol in normal rats demonstrated significant bone loss in the distal femur[68] due to the lack of ground reaction forces induced by water immersion. Similar results would be expected within the horse given that during periods of decreased compressive bone loading disruption of normal homeostasis between bone resorption and restoration occurs. The reduction in limb loading during aquatic exercise may have caused an increase in osteoclastic activity, while decreasing osteoblastic activity, which resulted in a greater degree of bone loss. Although radiography is valuable in characterizing structural changes within the OA affected joints, it was unable to differentiate adaptive from pathologic changes due to aquatic therapy in this model.

Induction of an osteochondral fragment significantly reduced the MNTs at sites tested within the carpal region. The use of the pressure algometer provided an assessment of acute pain immediately following surgery at known arthroscopic surgical sites. As expected, the MNT values over the OCF and sham-operated surgical sites had the lowest values within the first week after surgery. Following suture removal, MNT testing continued to differentiate the presence of an osteochondral fragment from the sham-operated limb by demonstrating lower MNT values in comparison. Furthermore, a treatment effect was demonstrated within the carpal regions as higher pain thresholds were recorded from the OCF radial carpal and third carpal bone of horses exercised in the underwater treadmill. The elevation in nociceptive thresholds within the aquatic therapy OCF carpal regions may provide a more objective assessment of the resolution of pain occurring within the region directly associated with the osteochondral fragment. It should also be noted that within the control group OCF and sham-operated limbs, the MNT values from the sham-operated limbs were consistently higher, especially within the third carpal bone. Unlike the control group, the aquatic therapy group did not demonstrate large differences between the OCF and sham-operated limbs. The physiologic effects of aquatic therapy on modulation of nociceptive thresholds was not evaluated in this study, but may have acted through several different systemic mechanisms, including providing therapeutic effects via activation of mechanosensitive afferents. Although the induction of OA did not influence the MNT values recorded from the extensor carpi radialis muscle, those horses exercised in the underwater treadmill demonstrated significantly higher MNT values compared to the control group. This may indicate the global influence of aquatic therapy to reduce radiating or referred pain within sites distant to local joint trauma.

Many MNT values demonstrated a significant main effect of study day, which can be described as the effect of this independent variable averaging over all levels of the other independent variables (presence or absence of OA and treatment group) in the experiment. When the main effect of study day is significant, then the null hypothesis that there is no difference in the MNT values between the study days is rejected. Simply, there is an effect of study day on the nociceptive threshold values, ignoring the effects of all the other independent variables. Similar to a previous equine study,[38] MNT values recorded in this study were lowest immediately following surgery, gradually increasing weekly and often superseding baseline values, which was indicative of continued adaptation over time. This may be a limitation in the application of threshold comparisons within the same subject over time as individual MNT values tend to increase with repeated measures over time.[69] However, in the current study pressure algometry was able to localize a known source of pain within the thoracic limb and demonstrate improvements in nociceptive thresholds following aquatic therapy.

Regarding disease-modifying effects, underwater treadmill exercise demonstrated a potential treatment effect in the third carpal bone as measured by a reduced number of chondrone formations in the OCF joints compared to control group OCF joints, as well as maintaining no significant difference in the degree of chondrone formation between the underwater treadmill exercised OCF and sham-operated joints. The presence of chondrones (clusters of chondrocytes within a single lacuna) occurs in association with articular cartilage loss of proteoglycans.[55] The greater the degree of articular cartilage erosion, the larger the increase in chondrone formations. Underwater treadmill exercise may have had a protective effect within the articular cartilage, diminishing proteoglycan loses, which resulted in less chondrone formations. In addition, horses exercised in the underwater treadmill had significantly less intimal hyperplasia of the synovial membrane in their sham-operated joints compared to similar joints within control horses. Previous equine studies have reported that both exercise and repeated arthrocentesis can produce low grade synovitis conditions within the joint.[70] The reduction in intimal hyperplasia demonstrated in the sham-operated joints exercised in the underwater treadmill indicates that aquatic therapy may aid in reducing the inflammatory response associated with low grade synovitis.

Conclusion

To our knowledge this is the first controlled study that has identified clinical improvement in OA affected joints of horses exercised in an underwater treadmill. The significant increases in lameness, total protein concentrations and

IAPs one week after initiation of aquatic therapy in the OCF joints indicates that specific to this OA model a greater inflammatory condition was initially induced. Therefore, the maximal effects of aquatic exercise on the treatment for the relief of OA-associated clinical signs may have been slightly masked as those horses had a greater inflammatory response to overcome. It may be more appropriate for the initial management of OA to exercise at a lower water level, promoting an increase in joint flexion and extension ranges of motion without the initial resistance of water at higher depths. Anecdotally, water at varying depths promotes joint specific increases in ranges of motion, therefore providing the ability to adapt therapeutic protocols to target certain joints. However, the increases in joint range of motion at lower water depths must be weighed against the importance of decreasing load-bearing stress across the joint surface provided by the effects of buoyancy and higher water levels. Regardless, the improvement in clinical signs of OA in the aquatic therapy group was further supported by evidence of disease-modifying effects at the histologic level. The precise mechanisms by which clinical signs and disease-modifying effects were induced are unclear. Nevertheless, results from this study indicate that underwater treadmill exercise is a potentially viable therapeutic option in managing OA in horses.

Material Sources

a. PGE_2 Kit, Assay Designs, Ann Arbor, MI.
b. Super glue (cyanoacrylate adhesive), Pacer Technology, Rancho Cucamonga, CA.
c. Naxcel, Pfizer Inc, New York, NY.
d. Equi-Phar, Schering-Plough Animal Health Corp, Union, NJ.
e. Equigym, Equigym LLC, Lexington, KY.
f. Equine underwater treadmill, Ferno Veterinary Systems, Wilmington, OH.
g. Intra-compartmental pressure monitor system, Stryker, UK
h. Extendable Goniometer, Lafayette Instrument Co, Inc., Lafayette, IN
i. Force gauge model FPK 60, Wagner Instruments Inc, Greenwich, CT.
j. Glimmix SAS Procedure, SAS Institute Inc, Cary, NC.

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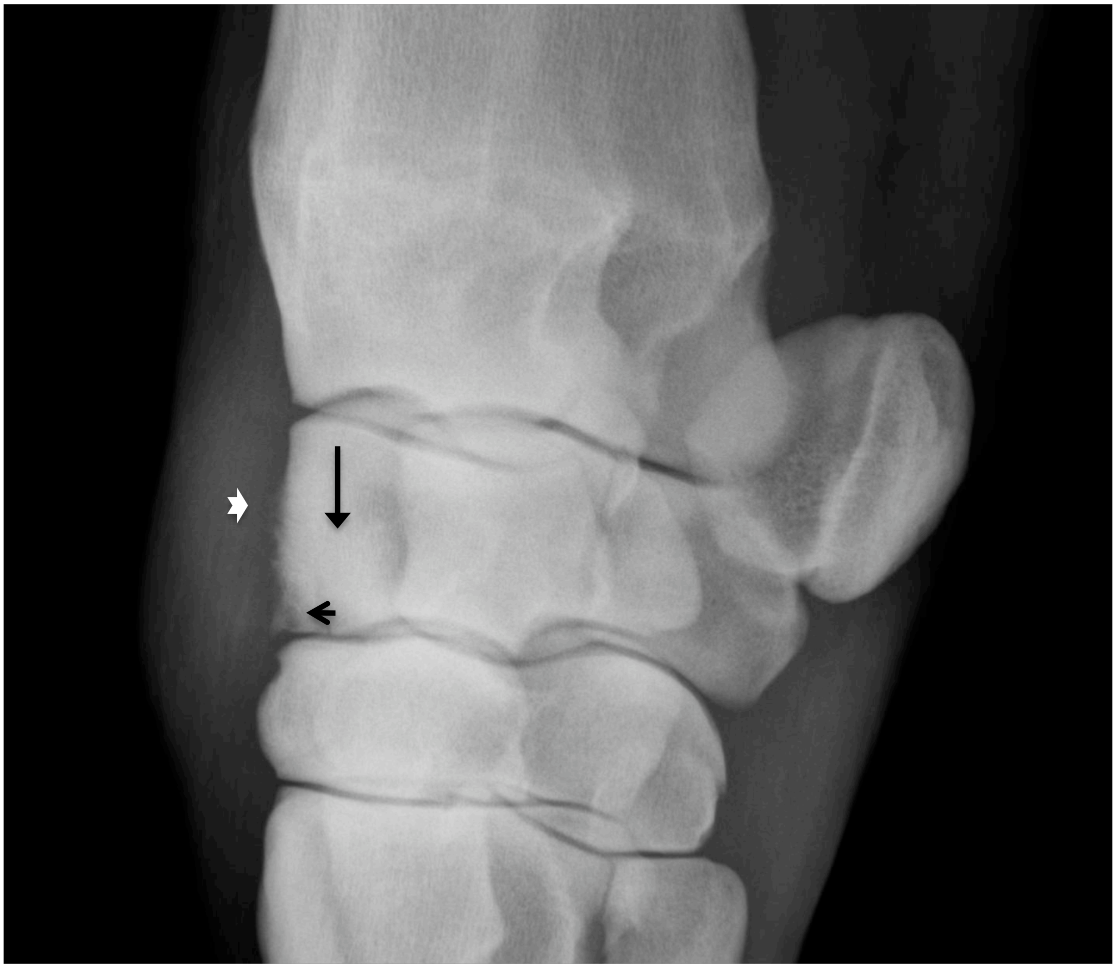

Figure 3.1: End of study (day 70) dorsal-lateral palmar-medial oblique radiograph of the left carpus, demonstrating bone proliferation along the dorsal margin of the radial carpal bone at the joint capsule attachment (white arrow head), subchondral bone lysis (black arrow head), and sclerosis (long black arrow) associated with the progression of OA after induction of an osteochondral fragment on the distal radial carpal bone.

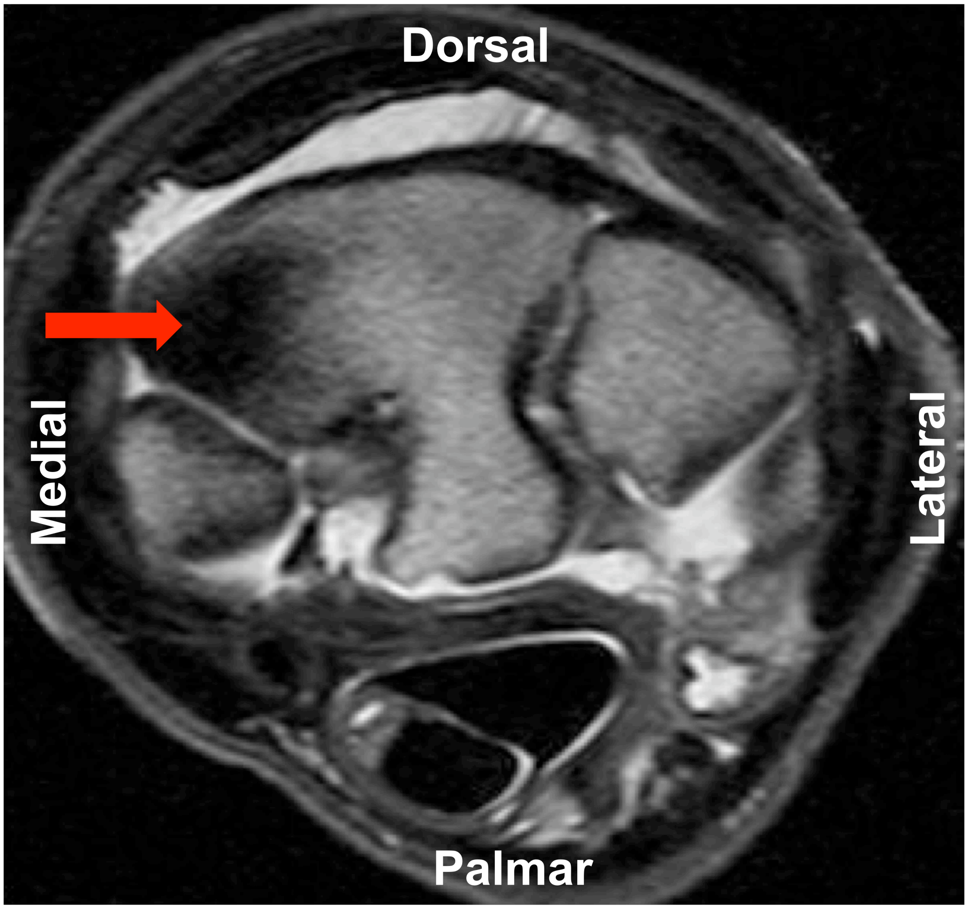

Figure 3.2A: MRI axial fast spin echo sequence at the level of the middle carpal joint demonstrating subchondral bone sclerosis within the third carpal bone (red arrow) opposite the distal radial carpal bone fragment.

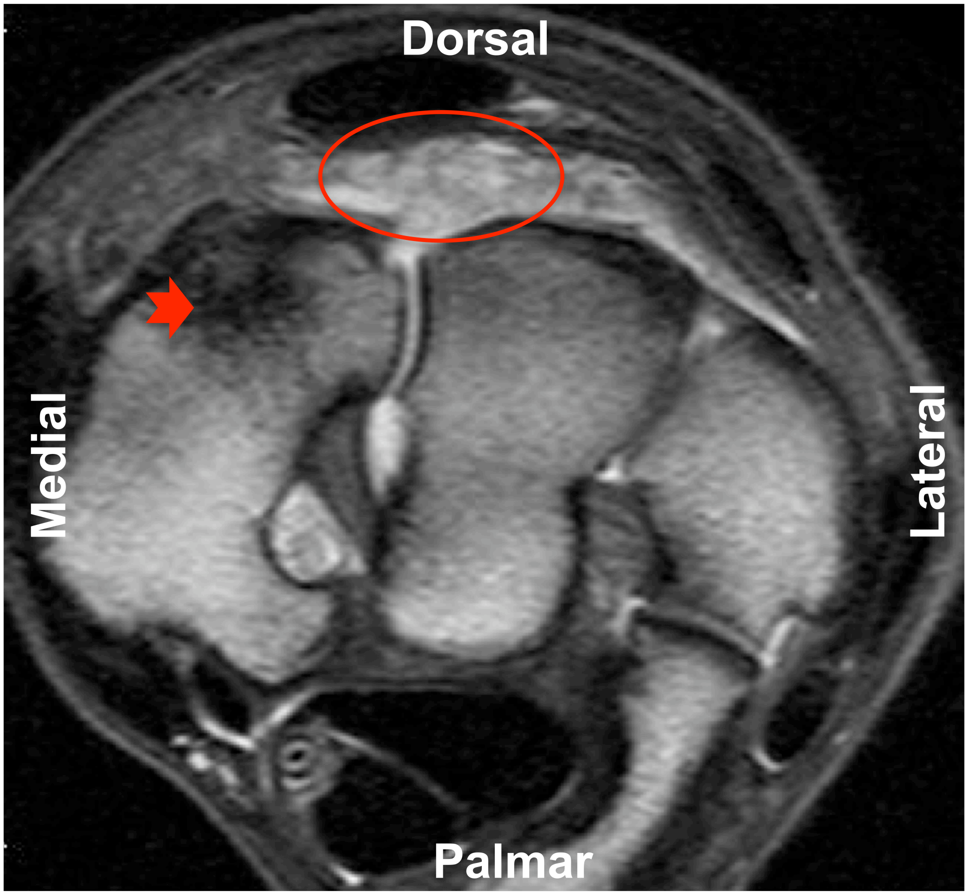

Figure 3.2B: MRI axial fast spin echo sequence demonstrating subchondral bone sclerosis within the distal radial carpal bone (red arrow head), along with increased synovial proliferation (red circle).

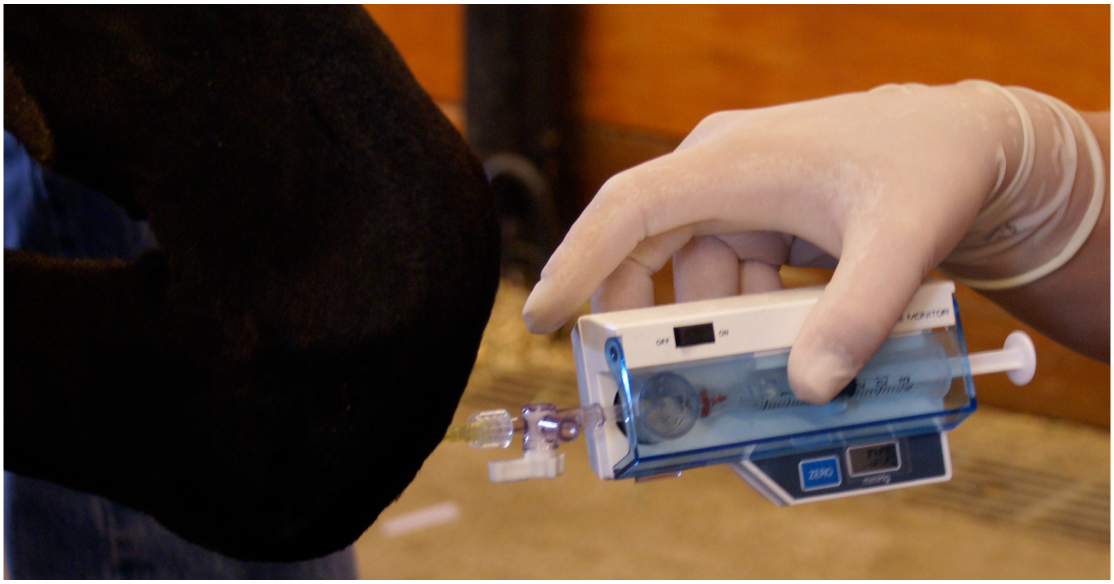

Figure 3.3: Photograph of aseptic needle placement into the middle carpal joint during limb flexion with the intra-compartmental pressure monitor system attached to the 3-way stopcock. With the stopcock open to the joint, continuous measurements of the intra-articular pressure were recorded.

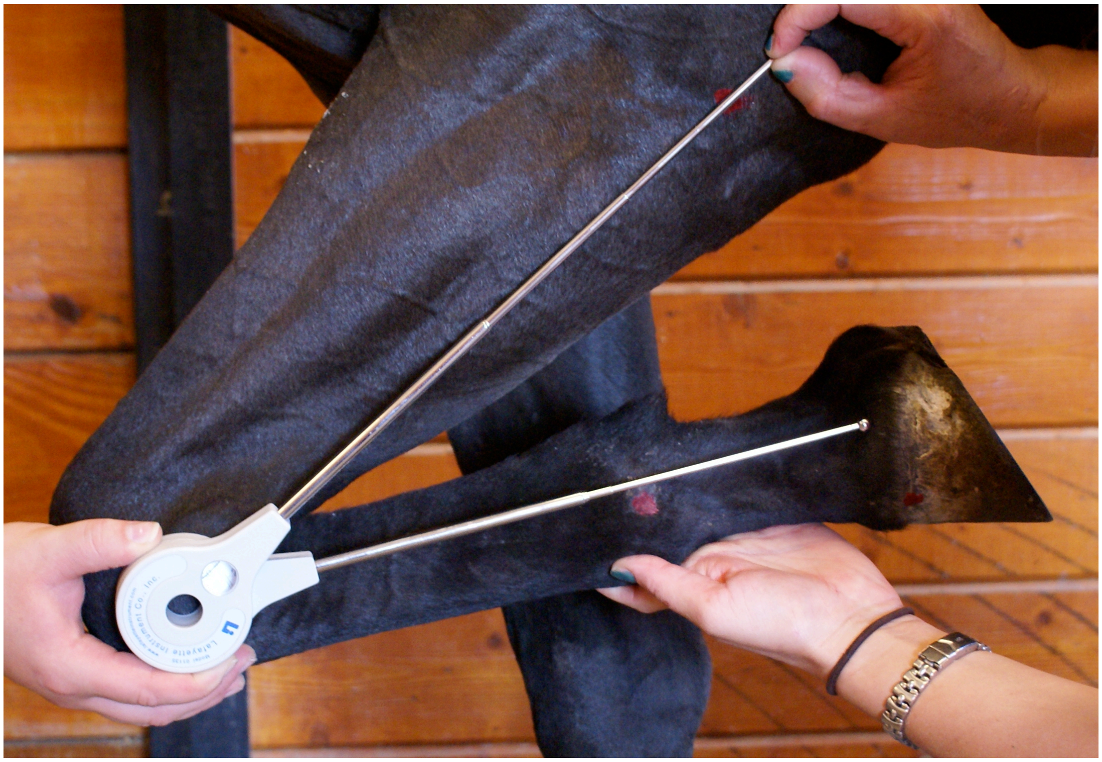

Figure 3.4: Photograph of goniometric measurement of the left carpal angle at flexion end range of motion. Note the placement of the goniometer over the center of joint rotation for the carpus, with the proximal arm extending to the center of joint rotation for the humeroradial joint and the distal arm extending to the center of joint rotation for the metacarpophalangeal joint.

Figure 3.5: Photograph of the application of the pressure algometer over a bony landmark along the left dorsal carpus used to quantifying local MNT values associated with osteochondral fragmentation.

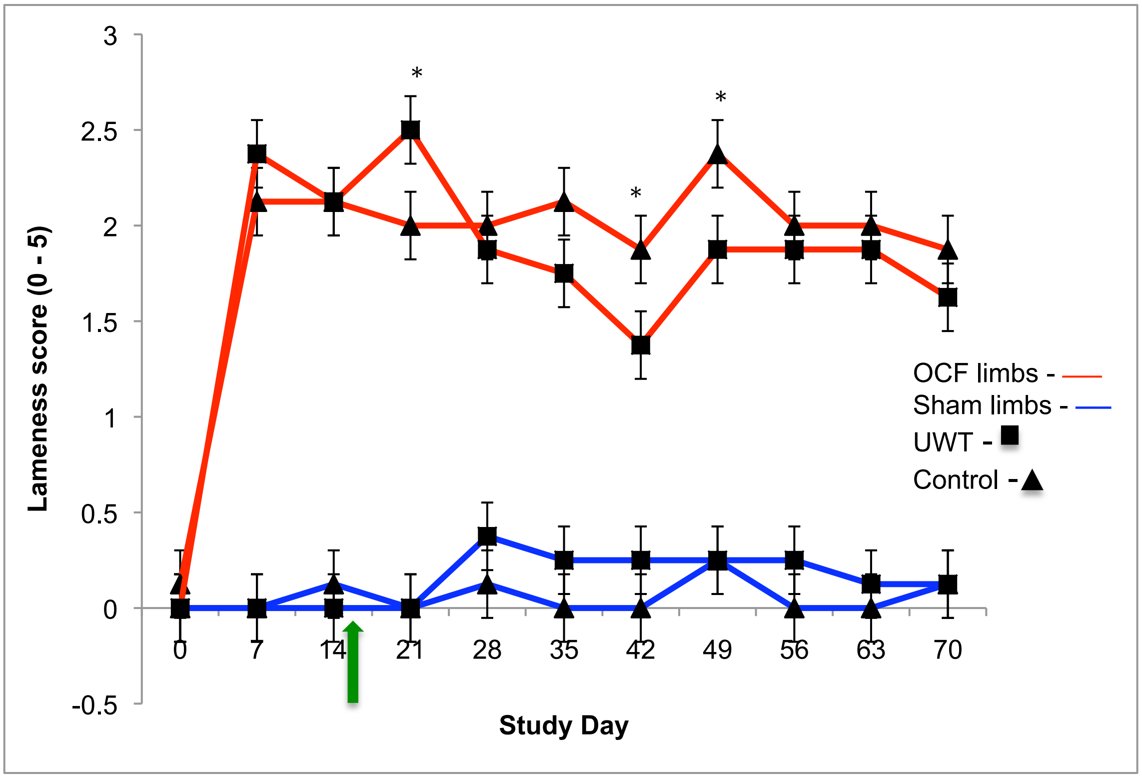

Figure 3.6: Mean ± SEM lameness scores between the OCF and sham-operated limbs within the control and underwater treadmill groups. There was a significant increase in lameness attributable to osteochondral fragmentation that differed from baseline and continued throughout the study period. An asterisk denotes significant differences in the OCF limbs between the treatment groups depending on study day. The green arrow represents the initiation of underwater treadmill exercise.

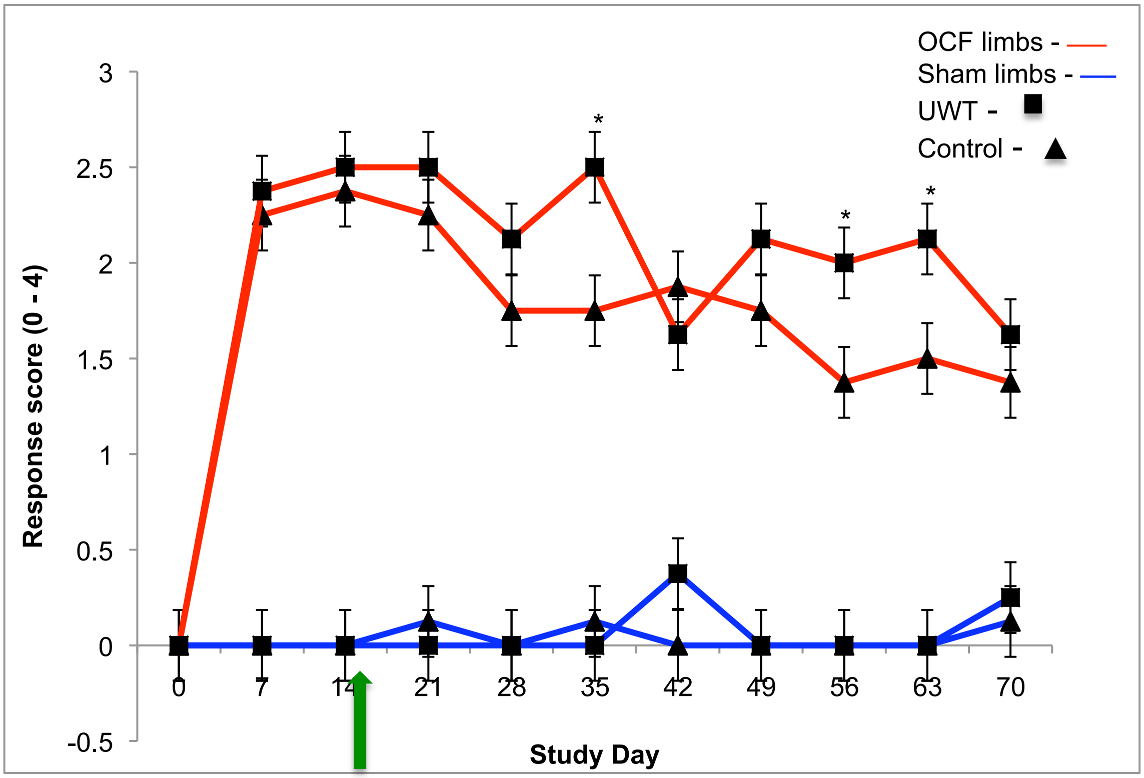

Figure 3.7: Mean ± SEM flexion response scores between the OCF and sham-operated joints within the control and underwater treadmill groups. There was a significant increase in flexion responses attributable to osteochondral fragmentation that differed from baseline and continued throughout the study period. An asterisk denotes significant differences in the OCF joints between the treatment groups depending on study day. The green arrow represents the initiation of underwater treadmill exercise.

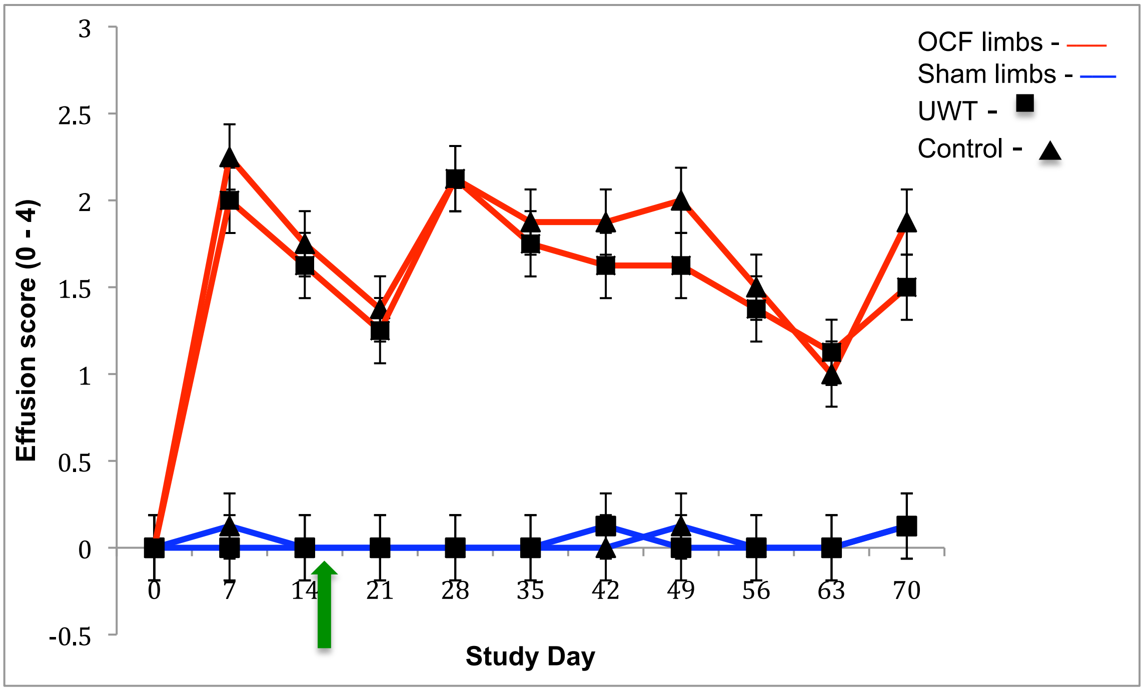

Figure 3.8: Middle carpal joint mean ± SEM effusions scores between the OCF and sham-operated joints within the control and underwater treadmill groups. There was a significant increase in effusion scores attributable to osteochondral fragmentation that differed from baseline and continued throughout the study period. The green arrow represents the initiation of underwater treadmill exercise.

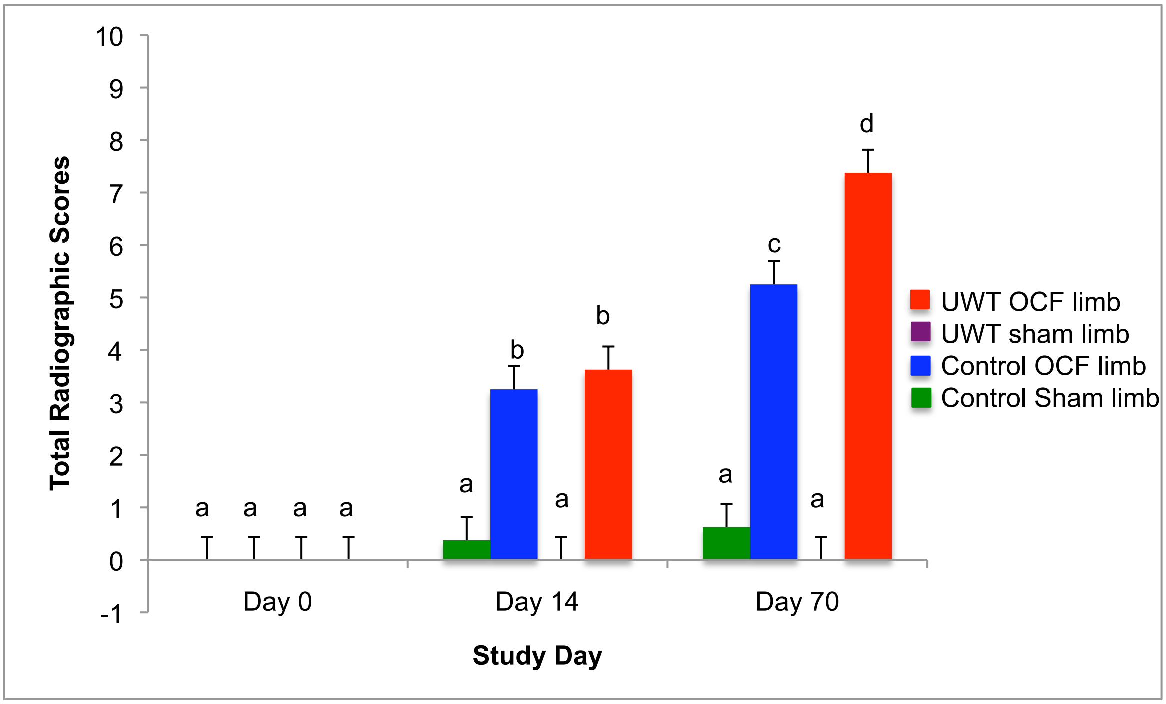

Figure 3.9: Mean ± SEM total radiographic scores for the middle carpal joints between the OCF and sham-operated joints within the control and underwater treadmill groups. Different letters reflect significant ($P < 0.05$) differences between the total radiographic scores.

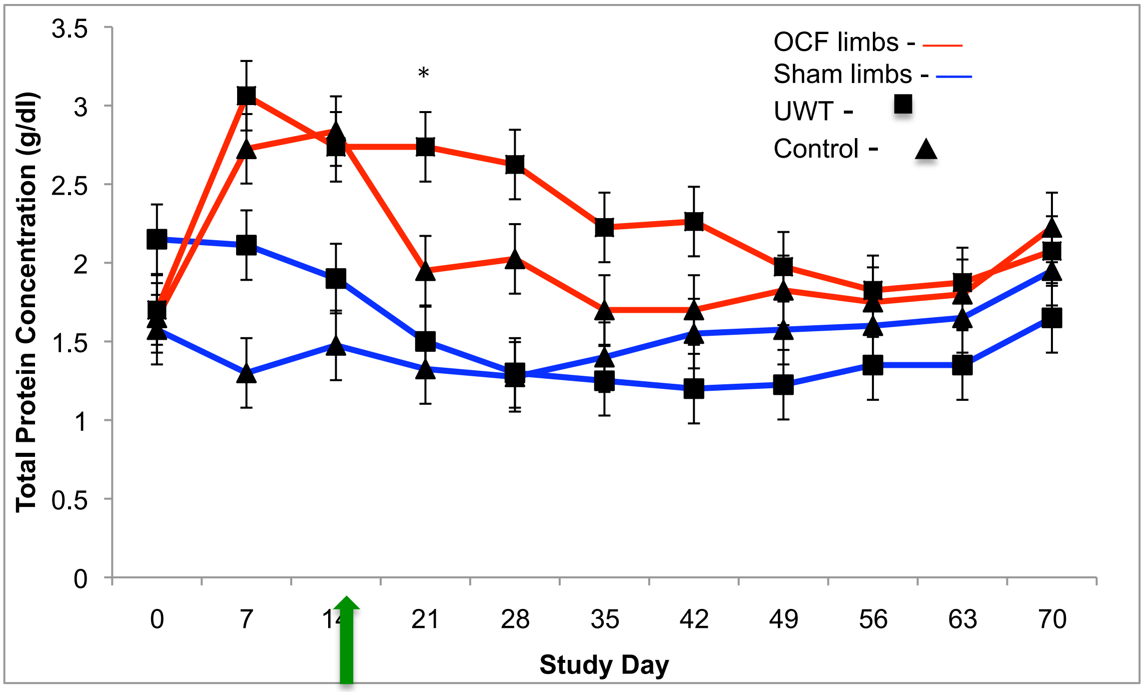

Figure 3.10: Mean ± SEM concentrations of synovial fluid total protein between the OCF and sham-operated joints within the control and underwater treadmill groups. There was a significant increase in synovial fluid total protein concentrations attributable to osteochondral fragmentation that differed from baseline and continued through day 49 of the study. An asterisk denotes significant differences in the OCF joints between the treatment groups depending on study day. The green arrow represents the initiation of underwater treadmill exercise.

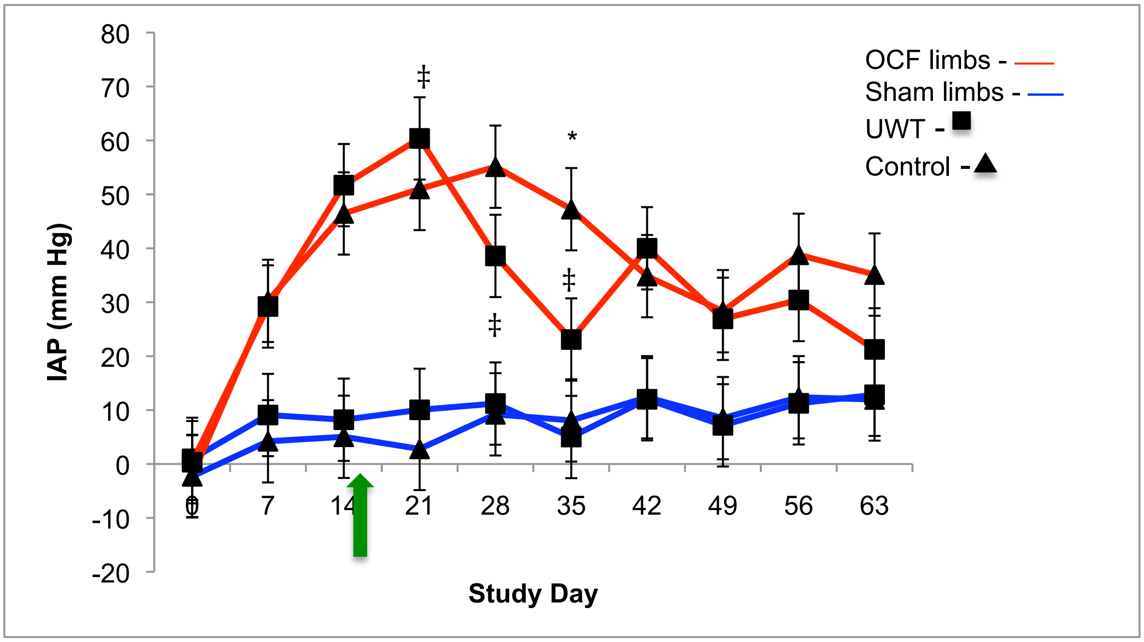

Figure 3.11: Mean ± SEM middle carpal joint IAPs between the OCF and sham-operated joints within the control and underwater treadmill groups. The IAPs measured within OCF joints differed significantly from the sham-operated joints beginning on study day 7 and continuing through day 56 of the study. An asterisk denotes significant differences in the OCF joints between the treatment groups depending on study day. The double dagger sign indicates significant improvements within the aquatic therapy OCF joints across the study days. The green arrow represents the initiation of underwater treadmill exercise.

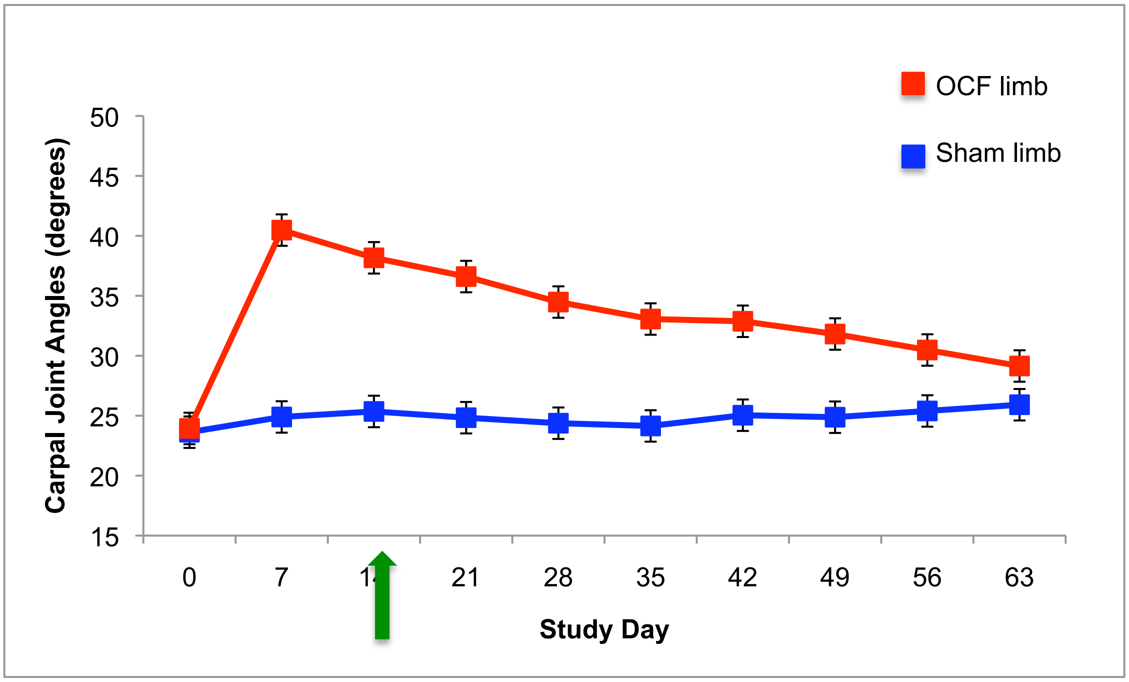

Figure 3.12: Mean ± SEM passive carpal flexion angles measured from OCF and sham-operated joints depending on study day. The OCF joint differed significantly from the sham-operated joint beginning on study day 7 and continuing through the remainder of study. The green arrow represents the initiation of underwater treadmill exercise.

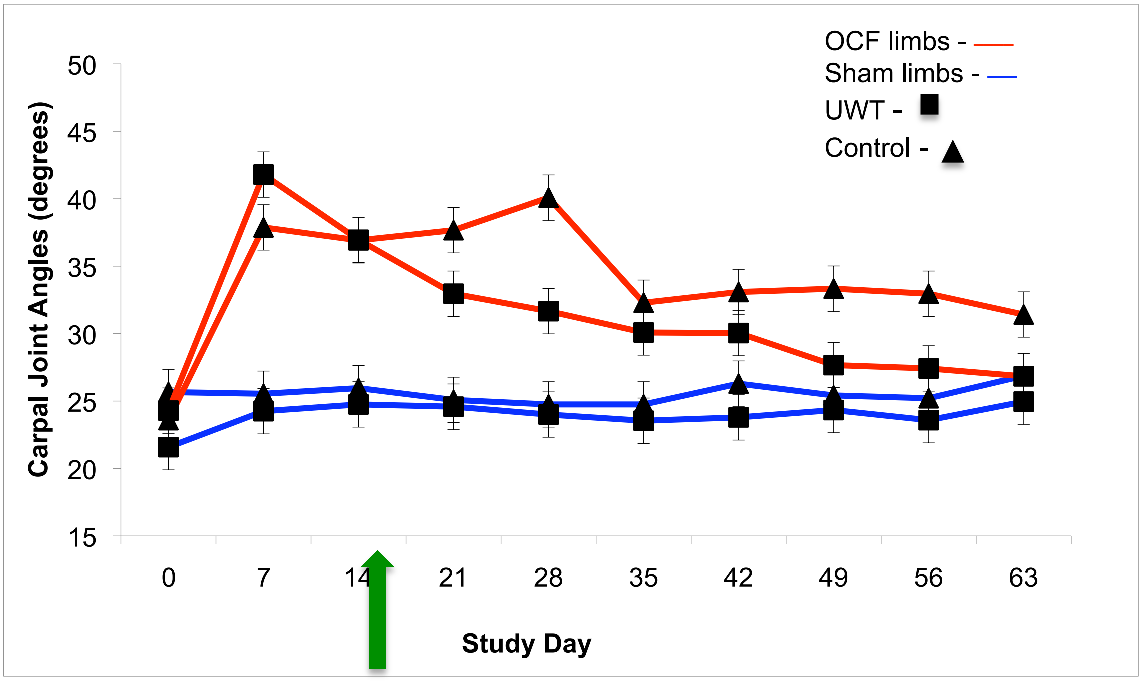

Figure 3.13: Mean ± SEM passive carpal flexion angles between the OCF and sham-operated joints within the control and underwater treadmill groups. The green arrow represents the initiation of underwater treadmill exercise.

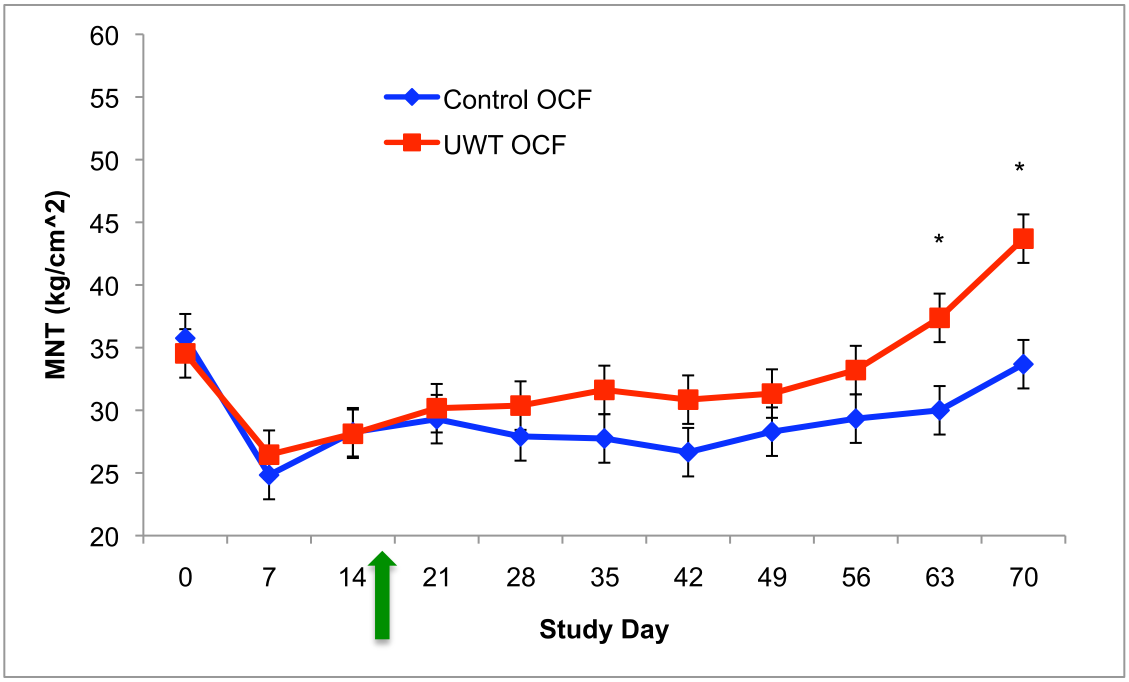

Figure 3.14: Mean ± SEM dorsal third carpal bone MNT values measured from OCF affected joints that were subsequently treated with aquatic therapy or controlled hand walking. An asterisk denotes significant differences between the treatment groups OCF limbs. The green arrow represents the initiation of underwater treadmill exercise.

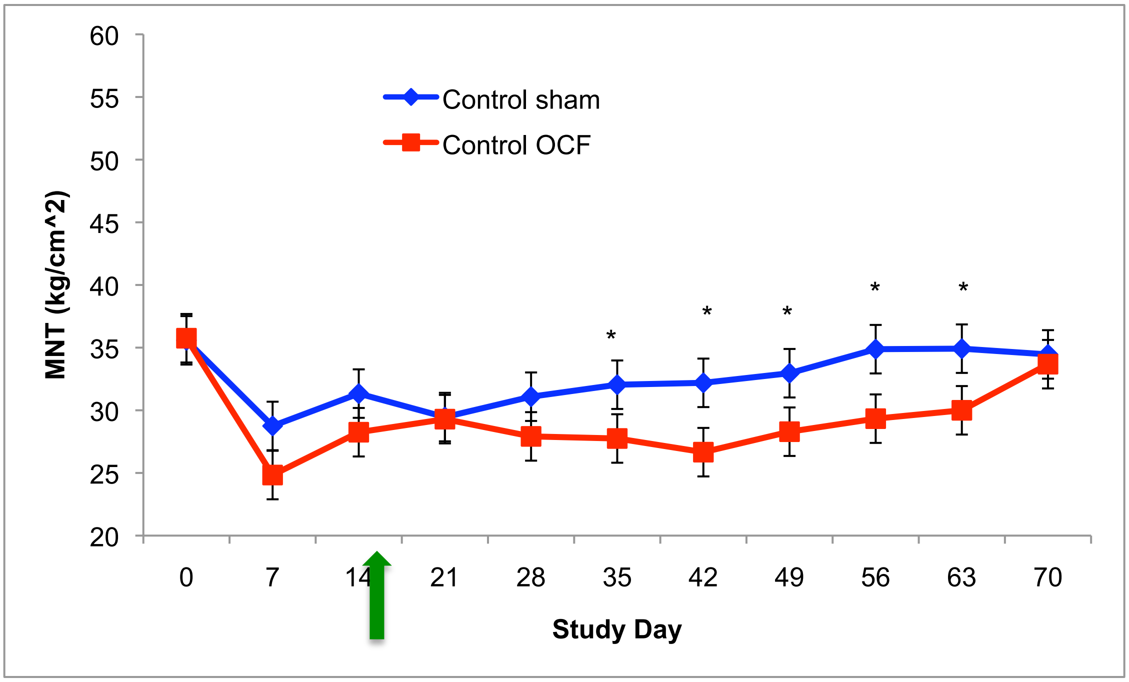

Figure 3.15: Mean ± SEM dorsal third carpal bone MNT values measured within the control group OCF and sham-operated joints. An asterisk denotes significant differences between the control group thoracic limbs. The green arrow represents the initiation of underwater treadmill exercise.

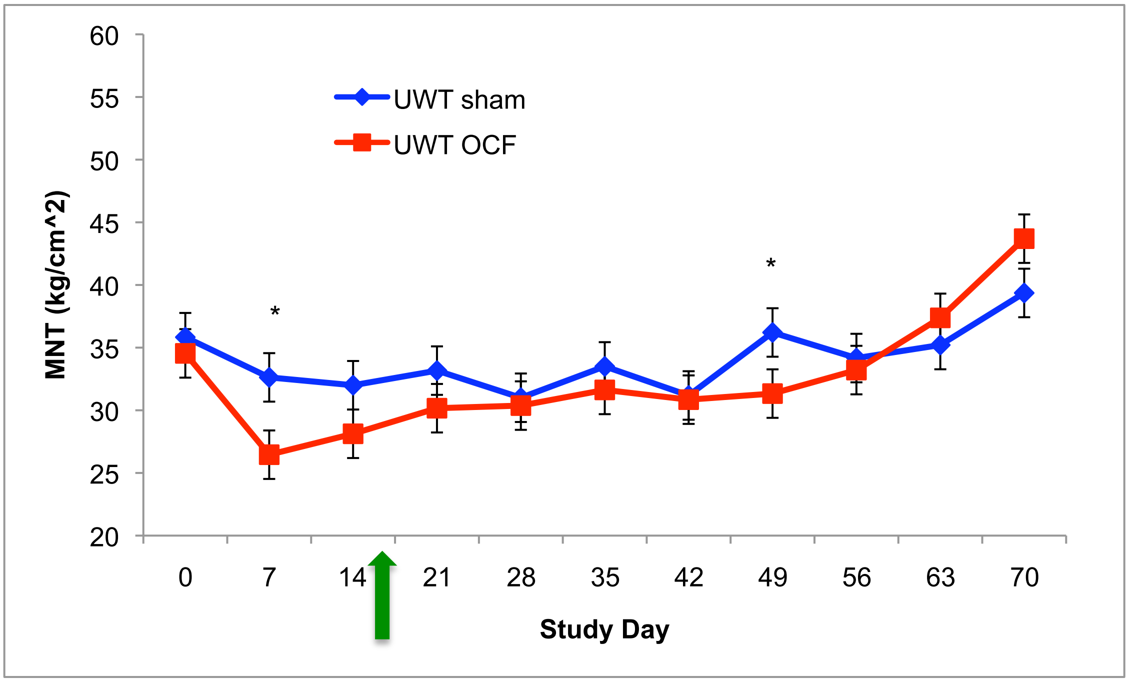

Figure 3.16: Mean ± SEM MNT values measured from the dorsal third carpal bone within the aquatic therapy OCF and sham-operated joints. An asterisk denotes significant differences between the thoracic limbs exercised in the underwater treadmill. The green arrow represents the initiation of underwater treadmill exercise.

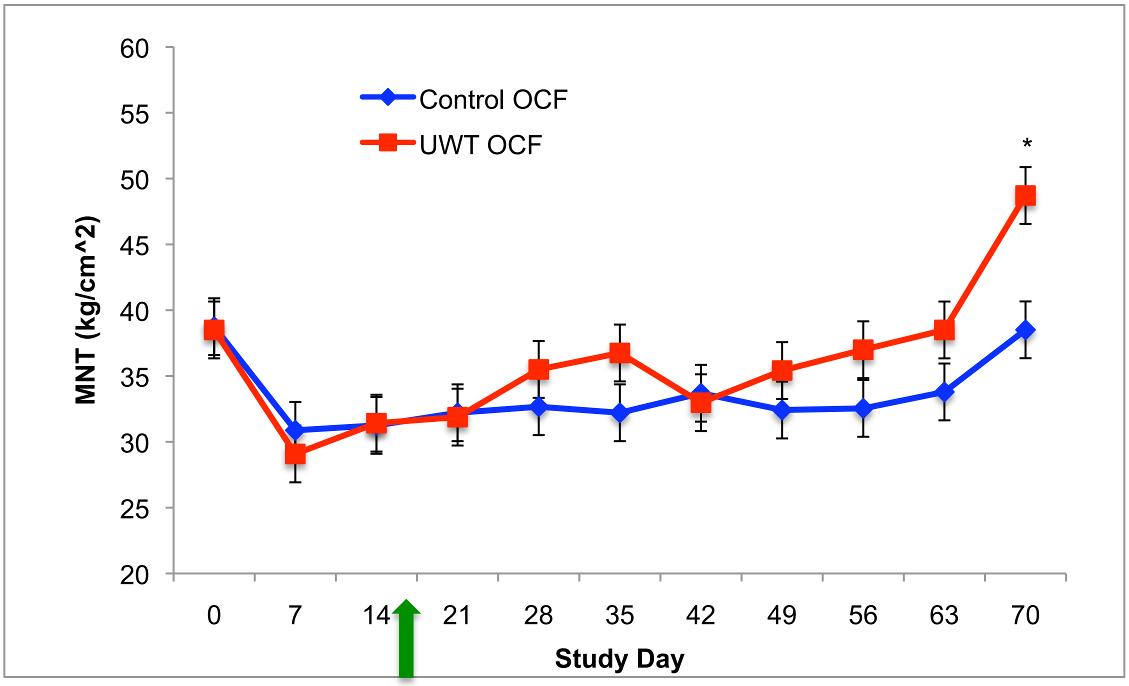

Figure 3.17: Mean ± SEM dorsal radial carpal bone MNT values measured from OCF affected joints that were subsequently treated with aquatic therapy or controlled hand walking. An asterisk denotes significant differences between the treatment groups OCF limbs. The green arrow represents the initiation of underwater treadmill exercise.

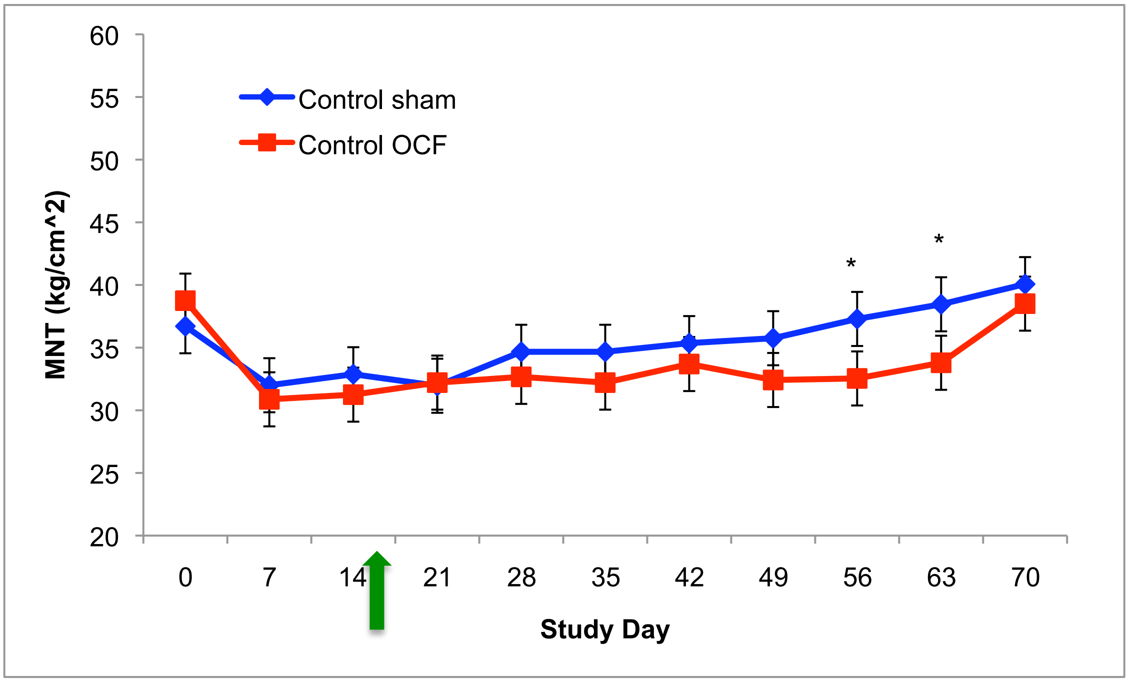

Figure 3.18: Mean ± SEM dorsal radial carpal bone MNT values measured within the control group OCF and sham-operated joints. An asterisk denotes significant differences between the control group thoracic limbs. The green arrow represents the initiation of underwater treadmill exercise.

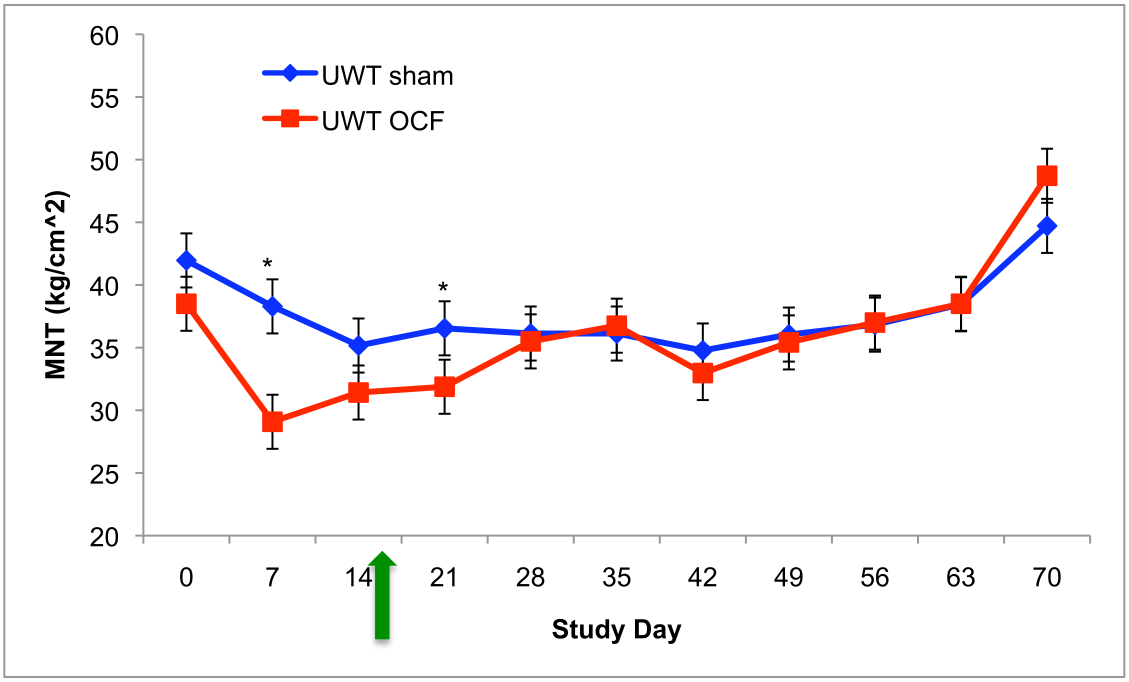

Figure 3.19: Mean ± SEM MNT values measured from the dorsal radial carpal bone within the aquatic therapy OCF and sham-operated joints. An asterisk denotes significant differences between the thoracic limbs exercised in the underwater treadmill. The green arrow represents the initiation of underwater treadmill exercise.

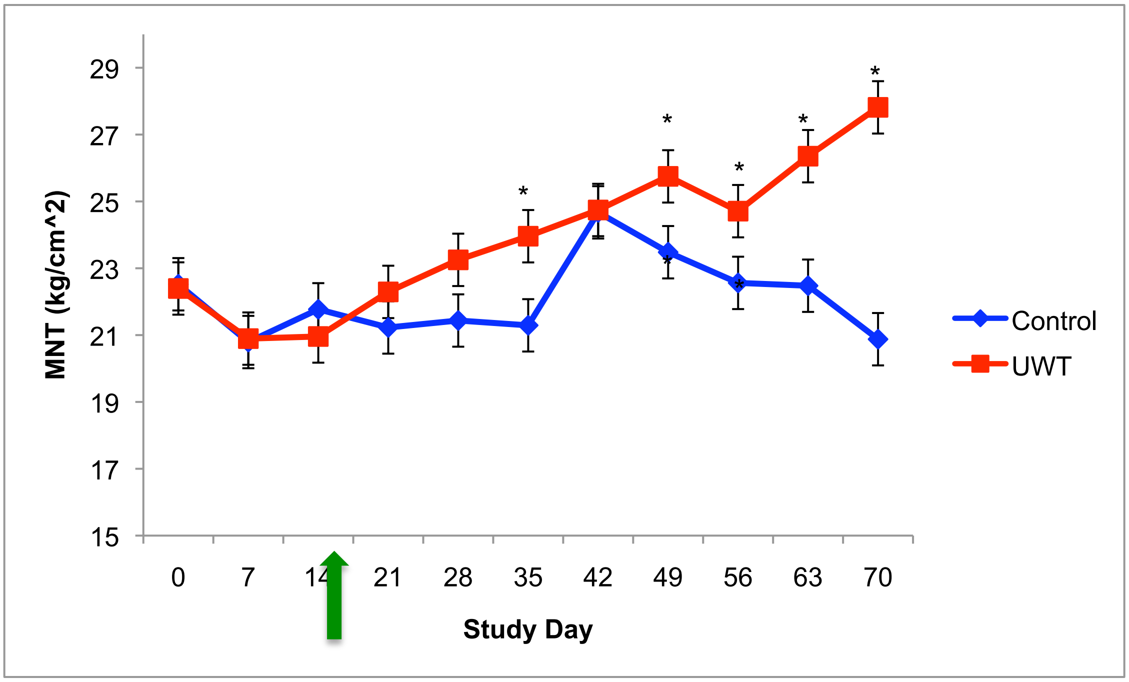

Figure 3.20: Mean ± SEM MNT values measured from ECR muscle within the control and aquatic therapy horses. An asterisk denotes significant differences between the groups depending on study day. The green arrow represents the initiation of underwater treadmill exercise.

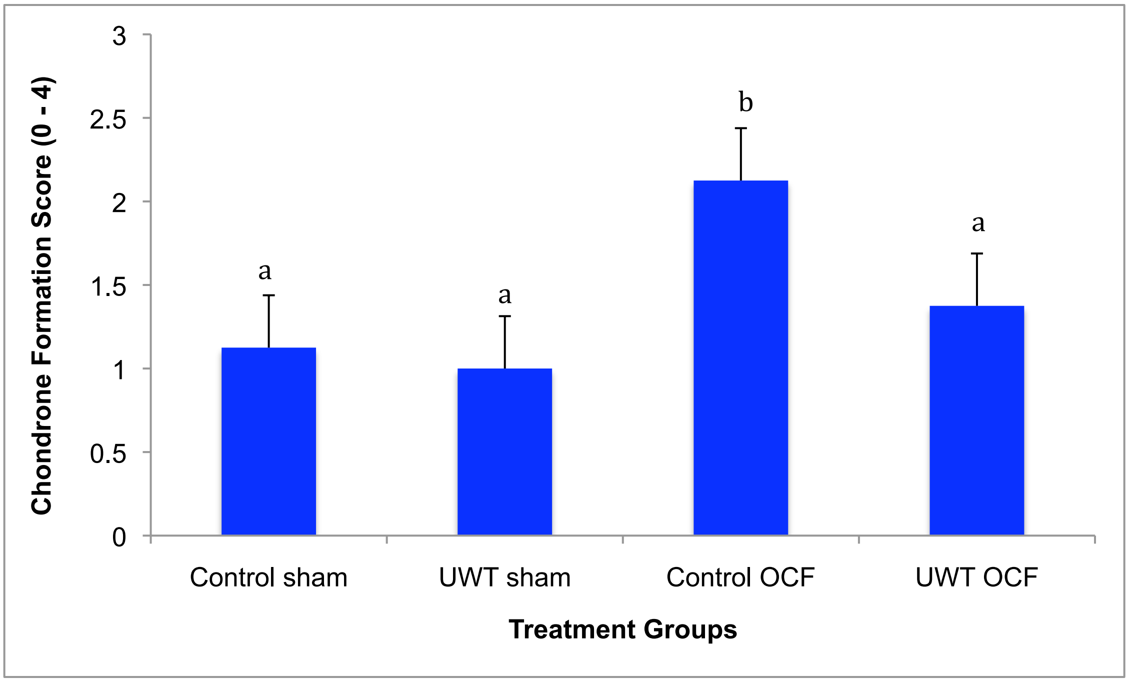

Figure 3.21: Mean ± SEM histologic grading scores for the degree of chondrone formation within the OCF and sham-operated third carpal bones from the aquatic therapy and control group horses. Different letters reflect significant differences in the data.

Table 3.1: Joint centers of rotation within the thoracic limb that were used to assess individual joint range of motion.[67]

Joint of Interest	Center of joint rotation
Scapulohumeral	Distal and caudal to the caudal aspect of the greater tubercle of the humerus
Humeroradial	Lateral collateral ligament attachment on the lateral epicondyle of the humerus
Carpus	Proximopalmar quadrant of the ulnar carpal bone
Metacarpophalangeal	Lateral collateral ligament attachment on the distal aspect of the third metacarpal bone

Table 3.2: Musculoskeletal landmarks used to assess mechanical nociceptive thresholds within the thoracic limb and adjacent cervical regions.

Landmark	Tissue	Anatomic Location
Dorsal coronary band	Bony	Midline coronary band just lateral to common digital extensor tendon
Lateral suspensory ligament branch	Soft tissue	Just proximal to the lateral suspensory branch insertion on the lateral proximal sesamoid bone
Carpal accessory bone	Bony	Lateral aspect of the carpal accessory bone, midbody near insertion of ulnaris lateralis tendon
Dorsal third metacarpal bone	Bony	Insertion of the extensor carpi radialis tendon on the dorsal proximal aspect of the third metacarpal bone
Dorsal radial carpal bone	Bony	Dorsal aspect of the radial carpal bone, medial to the tendon of the extensor carpi radialis muscle
Dorsal third carpal bone	Bony	Dorsal aspect of the third carpal bone, medial to the tendon of the extensor carpi radialis muscle
Extensor carpi radialis muscle (ECR)	Soft tissue	Cranial aspect of the radius, 15 cm distal from the point of the olecranon, over the mid-belly of the extensor carpi radialis muscle
Ulnaris lateralis muscle (UL)	Soft tissue	Lateral aspect of the radius, 12 cm distal from the point of the olecranon, over the mid-belly of the ulnaris lateralis muscle
Triceps brachii muscle, long head (TBL)	Soft tissue	Lateral aspect of the long head of the triceps brachii muscle at the level of the scapulohumeral joint
Sixth cervical vertebrae	Soft tissue	Over the cervical serratus ventralis muscle at the level of the sixth cervical vertebrae transverse process

Table 3.3: Passive joint range of motion angles within the thoracic limb associated with the presence or absence of an osteochondral fragment (mean ± SEM). An asterisk indicates significant (P < 0.05) differences between the OCF and sham-operated joint angle variables.

Joint	**Limb**	**Extension**	**Flexion**
Shoulder	OCF	134.0 ± 0.9 *	99.0 ± 0.9
	Sham	133.0 ± 0.9 *	98.0 ± 0.9
Elbow	OCF	135.0 ± 1.1	62.0 ± 0.9 *
	Sham	135.0 ± 1.1	60.0 ± 0.9 *
Carpus	OCF	175.0 ± 1.1	33.0 ± 0.8 *
	Sham	175.0 ± 1.1	25.0 ± 0.8 *
Fetlock	OCF	214.0 ± 1.2	122.0 ± 1.8
	Sham	215.0 ± 1.2	124.0 ± 1.8

Table 3.4: Mechanical nociceptive threshold values within thoracic limb landmarks that were associated with the presence or absence of an osteochondral fragment (mean ± SEM). An asterisk indicates significant ($P < 0.05$) differences between the OCF and sham-operated MNT values.

Landmark	Limb	MNT Value
Dorsal coronary band	OCF Sham	36.0 ± 0.7 36.0 ± 0.7
Lateral suspensory ligament branch	OCF Sham	30.0 ± 0.8 * 29.0 ± 0.8 *
Carpal accessory bone	OCF Sham	21.0 ± 0.6 * 20.0 ± 0.6 *
Dorsal MCIII	OCF Sham	22.0 ± 0.5 22.0 ± 0.5
Dorsal radial carpal bone	OCF Sham	35.0 ± 1.0 * 37.0 ± 1.0 *
Dorsal third carpal bone	OCF Sham	31.0 ± 0.8 * 33.0 ± 0.8 *
ECR	OCF Sham	23.0 ± 0.3 23.0 ± 0.3
UL	OCF Sham	25.0 ± 0.4 25.0 ± 0.4
TBL	OCF Sham	23.0 ± 0.3 23.0 ± 0.3
Sixth cervical vertebrae	OCF Sham	19.0 ± 0.4 19.0 ± 0.4

CHAPTER FOUR

BIOMECHANICAL RESPONSES TO THE INDUCTION AND PROGRESSION OF EXPERIMENTALLY-INDUCED EQUINE CARPAL OSTEOARTHRITIS

Introduction

Elite equine athletes undergo intense training and competition demands that often increase the risk for musculoskeletal injuries. Osteoarthritis and tendonitis are two of the most common musculoskeletal injuries that affect the equine forelimb.[1] Osteoarthritis is a progressive disease characterized by joint pain, inflammation, synovial effusion, limited range of motion, and a progressive deterioration of articular cartilage.[2] The disease process affects not only the articular cartilage but the entire joint, including subchondral bone, joint capsule, synovial membrane, and periarticular soft tissues.[2] As joint disease progresses characteristic pathologic changes occur including fibrosis and thickening of the joint capsule, articular cartilage fibrillation and erosion, and osteophyte formation, all leading to functional impairments.[2] Unremitting joint pain and inflammation often cause adaptive muscle guarding and altered weight bearing to protect the affected limb from further discomfort and injury.[3] Compensatory muscular adaptations are characterized by inefficient muscle activity leading to muscle weakness, joint instability and altered limb loading.[4] Maladaptive musculoskeletal responses may produce additional gait alterations and predispose other articulations to increased risk of injury (i.e., compensatory lameness).[5] In humans, compensatory changes in posture and movement exacerbate the initial joint injury, which cause further alterations in limb biomechanics and contribute to the progression of OA.[4] Similar compensatory mechanisms such as altered muscle activation patterns, muscle overuse or atrophy, increased joint stiffness, and a redistribution of limb loading are also likely to occur in horses.

Considerable equine research has been done to detect alterations in ground reaction forces associated with various degrees of lameness.[7-10] Compensatory load redistribution has been demonstrated in horses trotting on an instrumented treadmill with the use of a transient sole pressure lameness model.[6] This model demonstrated that a moderate degree of lameness decreased the peak vertical force on the affected thoracic and ipsilateral pelvic limbs, increased the peak vertical force slightly (by 2%) in the diagonal pelvic limb, and increased the stance duration in the affected and contralateral thoracic limbs.[6] A similar study in horses with experimentally-induced carpal lameness demonstrated similar peak vertical and time integrated force redistribution.[7] In addition, this study reported a reduction in peak braking force in the affected thoracic limb, increased peak braking force in the contralateral thoracic limb, increased peak propulsion force in the diagonal sound pelvic limb, and reduced peak propulsion force in the ipsilateral pelvic limb.[7] Ground reaction force parameters have also been used to evaluate the efficacy of various treatment protocols from medicinal therapies to shoeing regimes.[8,9] The use of force platform analysis allows for quantification of the changes in ground reaction force parameters over time due to the progression and pathology associated with OA. It also enables an evaluation of the changes in these forces due to the application of various therapies.

Modern gait analysis not only offers the ability to determine the forces that produce movements, but also provides insight into the changes in joint dynamics in response to musculoskeletal pain. Of particular importance are the kinematic abnormalities associated with musculoskeletal dysfunction believed to be compensatory strategies aimed at minimizing joint pain. Various equine studies have demonstrated the complex joint biomechanical alterations associated with orthopaedic disease.[10-14] Using intra-articular injections of *Escherichia coli* lipopolysaccharide into the radial carpal joint of ponies, a significantly decreased carpal flexion angle during swing phase was demonstrated.[10] In addition, the maximum fetlock joint extension angle during stance was reduced, along with the carpus maintaining a more flexed position during stance phase.[10] In contrast, a study inducing synovitis of the middle carpal joint using amphotericin-B concluded that active carpal range of motion did not significantly change between the lame and non-lame thoracic limbs.[14] However, a significantly decreased fetlock joint range of motion was present in the lame limb, but remained unchanged in the sound limb.[14] Similar changes in fetlock joint extension angles during stance phase have been demonstrated using the transient lameness model that applies pressure to the corium of the sole.[13] Pain over the sole region caused a decrease in fetlock extension angles within the lame thoracic limb, while an increased fetlock extension angle was measured from the sound thoracic limb.[13] Alterations in fetlock extension angles were evident in early stages of lameness (grade one out of five).[13] As lameness scores progress to a grade three, an increase in maximal flexion of the shoulder joint and a decrease in maximal extension of the elbow joint were demonstrated in the lame thoracic limb.[13] However, joint angles during locomotion did not change significantly within the pelvic limbs even as lameness grades increased.[13] Kinematic analysis of human patients with knee OA demonstrate an increased knee flexion at the beginning of stance phase.[15] The abnormal degree of knee flexion in the early phase of stance serves to attenuate ground reaction forces as weight is loaded on the limb after initial contact.[15] These kinematic compensatory strategies are aimed at minimizing joint pain and instability. However, these maladaptive responses lead to further gait alterations, contribute to the progression of OA, and predispose other joints to an increased risk of injury. The combination of kinetic, kinematic, and electromyography analysis can provide an increased understanding of locomotor disturbances and the corresponding motor control strategies associated with the onset and progression of OA.

Muscles play a complex role in joint biomechanics as they serve to control joint motion, attenuate joint loading, and provide forces required for joint stabilization.[5] Recent human EMG research has expanded our understanding of neuromuscular responses to joint pain.[16] Mechanoreceptors are characterized as sensory receptors within periarticular tissues that respond to changes in joint position and acceleration of joint movements, as well as playing an important role in regulating and maintaining neuromuscular control of joint stability.[17] Pain, inflammation and joint effusion associated with OA alters the normal sensory input from articular mechanoreceptors, which may result in decreased motor neuron excitability and reduced muscle activation.[18] A common outcome in human patients following experimentally-induced knee effusion with corresponding elevations in the intra-articular pressure is the presence of significant quadriceps muscle inhibition.[19-23] A linear relationship exits between increased intra-articular pressure and the level of quadriceps muscle inhibition.[19] Electromyography studies conducted on the quadriceps muscle of human patients with knee OA often indicates a decrease in muscle strength and altered muscle timing.[24] Typically there is a delay in the onset of quadriceps muscle activation, which results in the inability of the muscle to stabilize the joint and attenuate the loading forces properly during locomotion.[20] The increased joint instability further alters the distribution of weight bearing forces across joint surfaces and increases the recruitment of adjacent muscles to aid in joint stability.[25] The resulting functional imbalances in paired agonist–antagonist muscle groups contribute to increased joint instability and altered limb biomechanics, which leads to progressive OA and chronic maladaptive compensatory mechanisms.[26]

Biomechanical studies assessing muscle function alterations in association with joint pain in the horse are limited. The majority of research has focused on understanding muscle activity during locomotion[27-31] and validating muscle-actuated models for determining net muscle force applied across a joint.[32-34] However, equine EMG studies are still in their infancy stages, as several published studies have qualitatively assessed the changes in intensity of the raw EMG signal.[30,31] The raw EMG signal offers valuable information but cannot be quantitatively compared between subjects. Furthermore, in an attempt to provide quantitative EMG data the majority of these studies determine the on/off muscle timing manually, based on visible changes in muscle activity from either the processed or raw EMG signal.[29-31,33] However, in human EMG studies the timing of muscle activation and deactivation depends on appropriate threshold definitions. Thresholds for determining EMG activation are calculated as either a percentage of the maximum amplitude for a given signal (e.g., 5%), or from a multiplication factor of the mean and standard deviation of the EMG signal recorded during muscle inactivity. Estimation of muscle activation patterns using quantitative on/off threshold calculations has been performed in limited equine studies that used a threshold value ranging from 5 to 50% of the maximum signal amplitude.[27,35] The large variation in techniques used to establish muscle activation patterns contributes to the differences among the published equine

EMG studies. EMG analysis within this study proposes establishing the multiplication factor of the mean and standard deviation of a muscle during a non-weight bearing condition as an appropriate method for determining muscle timing thresholds.

Recent equine biomechanical research indicates that the distal limb muscles of the thoracic limb are primarily used to position and stabilize the limb for ground contact. Muscle activation occurs just prior to or at ground contact and remains active for only a portion of stance phase.[27,33] Several equine studies have assessed the effect of gait velocity on muscle activation profiles and have determined that changes in gait velocity does not alter the onset of muscle activity relative to the percent of stride.[28,33] Conversely, similar studies have demonstrated that an increase in gait velocity increases not only the duration but also the intensity of muscle activity, along with changing muscle timing patterns.[29,35,36] However, a direct comparison between these studies is not possible due to differences in the muscles investigated, electrode placement, and signal processing. Although differences exist, EMG studies have provided relevant contributions to the understanding of equine movement. The relationship between musculoskeletal injuries and muscle function within the intrinsic muscles of the thoracic limb remains unknown. The use of EMG in this study will provide details of the timing and magnitude of muscle activation for the intrinsic muscles of the thoracic limb during movement and will provide an understanding of the role of individual muscles in movement strategies adopted due to the induction of OA. Furthermore, EMG analysis will identify the neuromuscular function of the thoracic limb intrinsic muscles in response to underwater treadmill exercise.

Physical rehabilitation has become an effective treatment option for reducing harmful compensatory gait abnormalities in humans.[17] Rehabilitation programs that address OA and musculoskeletal injuries often incorporate some form of aquatic exercise. Exercising in water provides an effective medium for increasing joint mobility, promoting normal motor patterns, increasing muscle activation, and reducing the incidence of secondary musculoskeletal injuries due to primary joint pathology.[37] Human patients with lower extremity OA show a significant gain in limb-loading parameters and improved joint range of motion following aquatic exercise.[38] Underwater treadmill exercise has become an increasingly popular therapy for the rehabilitation of equine musculoskeletal injuries; unfortunately, there is no scientific evaluation of its effectiveness for the treatment of OA and its associated alterations in musculoskeletal function in horses.

Purpose of Study

The two primary purposes of this study were (1) to quantify muscle activation patterns, in conjunction with forelimb kinetics and kinematics associated with middle carpal joint osteochondral fragmentation, and (2) to evaluate the influence of underwater treadmill exercise on forelimb biomechanics in horses with carpal OA. This study is expected to increase our knowledge of the biomechanical effects associated with joint pain, inflammation and subsequent OA, provide insights into adaptive and maladaptive compensatory mechanisms adopted by skeletal muscles, and evaluate whether underwater treadmill exercise is an effective therapeutic procedure to decrease the consequences of these conditions. Our hypotheses are that joint pain and the progression of OA will induce alterations in thoracic limb biomechanics and muscle activation patterns, compared to the contralateral unaffected thoracic limb, and that aquatic therapy will enhance neuromuscular function and reduce thoracic limb gait abnormalities associated with carpal joint pain and inflammation.

Materials and Methods

Animals - Sixteen skeletally mature horses, age 2 – 4 years (mean ± SD mass, 385 ± 40 kg; height at withers, 1.46 ± 0.04 m) were included in the study. Prior to inclusion in the study, all horses underwent a lameness examination, range of motion (flexion) testing of the carpal joints, evaluation for middle carpal joint effusion, body condition assessment, and radiography of the carpal joints; only horses without abnormal findings were permitted in the study. A minimum 14-day acclimatization period to new environmental factors and training to safely enter and use the high-speed and underwater treadmills occurred prior to baseline data collection. The Colorado State University Institutional Animal Care and Use Committee approved the study protocol.

Experimental induction of osteoarthritis – As described,[39] following general anesthesia and routine preparation for surgery, each horse underwent bilateral arthroscopic surgery of the middle carpal joints to ensure that there were no pre-existing abnormalities (termed day 0). During this procedure, an osteochondral fragment was created in one randomly selected middle carpal joint. An 8-mm-wide fragment was generated by use of an 8-mm curved osteotome directed perpendicular to the articular cartilage surface of the radial carpal bone at the level of the medial synovial plica. The dorsal surface of the fragment was allowed to remain adhered to the joint capsule proximally. A motorized burr was used to debride the exposed subchondral bone between the fragment and the parent bone. A 15-mm-wide defect bed was created and the debris was not actively lavaged from the joint, thereby adding in the induction of OA. This joint was designated as the osteochondral fragmented joint (OCF) and the sham-operated joint was used as the control joint. The arthroscopic portals were closed with 2-0 nylon suture in a simple interrupted pattern, and cyanoacrylate glue[a] was applied to the incision. The thoracic limbs were bandaged, and the horses were allowed to recover from anesthesia and surgery. All study horses received a single dose of ceftiofur[b] (4.0 mg/kg) IM just prior to surgery and 2 grams of phenylbutazone[c] orally once a day for 5 days after surgery. Bandages were changed every three to five days and maintained until suture removal at 10 days after surgery.

Exercise - Each horse was housed in a 3.7 m^2 stall. Beginning on day 15, all horses were exercised on a high-speed treadmill[d] 5 days each week, until the end of the study to promote the development of OA. Each day, the horses were trotted at 4.4 m/s for 2 minutes, galloped at 8.8 m/s for 2 minutes, and trotted again at 4.4 m/s to simulate the strenuous exercise associated with race training.

Treatment groups - One week after surgery (day 7), the horses were ranked by lameness score and randomly assigned to one of two groups with 8 horses in each group.[40] The intervention group was exposed to underwater treadmill exercise[e] (UWT) starting on day 15. The underwater treadmill exercise protocol consisted of horses exercising at a brisk walk (2.1 m/sec) for 5 minutes, once a day, for 5 days. The water level in the underwater treadmill was maintained at the point of the shoulder for the duration of the study, which provided approximately 60% reduction in weight bearing.[31] At weekly intervals the duration of underwater treadmill exercise was increased by 5 minutes, until a maximum of 20 minutes was reached. Horses then continued to be exercised for 20 minutes once a day, five days a week, for the remainder of the ten-week study (8 weeks total of exercise). The control group was exercised on a low-speed treadmill (without water) at the same speed, frequency and duration as the intervention group also starting at day 15. The low-speed exercise provided within the control group simulated hand walking and light exercise as prescribed in current rehabilitation protocols.

Gait experiments - Ground reaction forces, kinematics, and electromyographic (EMG) activity were recorded simultaneously from each horse at four different time points during the study: prior to induction of the osteochondral fragment (day -7), after induction of osteochondral fragment and prior to initiating exercise therapies (day 14), 4 weeks after starting therapy (day 42), and at study conclusion (day 70). Horses were trotted in hand at a velocity maintained between 2.75 and 3.38 m/s and trials were not accepted unless the speed variation was less than 10% from the average baseline speed for each horse. Forward velocity was measured by using a series of five infrared emitters and corresponding reflective sensors[f] placed 2 meters apart. Initial ground contact and toe off for multiple strides per 10-second trial were determined using a biaxial accelerometer (vertical orientation) glued to the dorsal lateral hoof wall of both front hooves (Fig 4.1). Impact acceleration peaks were used to determine thoracic limb hoof contact, while toe off (end of stance phase) was determined by analysis of accelerometer (Fig 4.2) and corresponding force platform signals. The raw accelerometer signal was processed using a high-pass filter with a cut off frequency of 150 Hz, then full wave rectified, and lastly low-pass filtered using a fourth-order Butterworth filter with a cut off frequency of 12 Hz (Fig 4.3). The accelerometer was used to determine the EMG timing activity of selected thoracic limb muscles in relation to multiple stance and swing phases collected within a trial. For evaluation of ground reaction forces and changes in joint angles, the resulting data were averaged across three trials and then normalized to stride duration. For the EMG evaluation, a minimum of 2 stride durations were used per trial, averaging across three trials and normalizing to stride duration. Stride duration was defined as the period of time between two consecutive hoof contacts, which encompasses a single stance and swing phase. Kinetic, kinematic and EMG temporal variables were reported as a percentage of stride duration.

Kinetic gait analysis - Ground reaction forces were recorded using two strain gauge based force platforms[g] (60 x 90-cm) mounted sequentially in a concrete base at the center of a 25-m runway. The force platforms and runway were covered with a rubber mat (9-mm thick) to prevent horses from slipping during locomotion over the force platforms. The rubber mat was incised around the periphery of the force platforms and a 60 x 90-cm section was adhered to each force platform to provide a continuous visual field. Each horse was led by an experienced handler in a straight line over the examination runway at a consistent trotting velocity. A kinetic trial was considered to be successful when the ipsilateral thoracic and pelvic limb contacted the center of each force platform. Three valid trials were recorded at each data collection. Orthogonal ground reaction force data were sampled at 3000 Hz for all trials, however only the vertical and craniocaudal forces were included in this study. A threshold of

25 N was used to determine hoof contact and hoof off. The following parameters were calculated from each trial: stance duration, peak vertical, braking and propulsion forces, time to peak vertical, braking and propulsion forces, vertical loading rates, and vertical, braking, and propulsion impulses. For comparisons between and within subjects the ground reaction force variables were normalized to subject body mass and expressed as N/kg or Ns/kg.

Kinematic gait analysis - Cyanoacrylate glue was used to adhere the 2.5-cm spherical retro-reflective markers to the horse's skin overlying nine anatomic landmarks[41] and the corresponding centers of joint rotation within the thoracic limbs, which included the tuber spinae scapulae, the caudal distal aspect of the greater tubercle of the humerus, the lateral epicondyle of the humerus at the attachment of the lateral collateral ligament, the proximal palmar aspect of the ulnar carpal bone, the distal metacarpal attachment of the lateral collateral ligament of the metacarpophalangeal joint, the lateral portion of the hoof wall overlying the distal interphalangeal joint, and the mid-dorsum of each hoof, 3 cm distal to the coronary band (Fig 4.4). Additional markers where placed over the left and right transverse process of the first cervical vertebrae, on the midline of the frontal bone between the supraorbital foramen, the spinous process of the fifth thoracic vertebrae, and over the tuber sacrale. Joint angles were calculated based on the anatomical flexor surface of each joint, which was defined as the palmar aspect of the limb for all joints except the humeroradial joint whose flexor surface is along the dorsal aspect of the thoracic limb (Fig 4.4). Optical data was collected at 200 Hz using a motion analysis system[h] with eight, high-speed infrared cameras[i] positioned on either side of the force platforms. For each session, the capture volume was calibrated using a customized calibration frame and wand with an accuracy of 0.71-mm. Raw coordinate data was low-pass filtered with a fourth-order recursive Butterworth filter at 12 Hz. Kinematic variables calculated from each trial included; peak joint flexion angles during the swing phase of gait, peak joint extension angles during the stance phase of gait, and the timing characteristics of peak joint angles during a stride cycle.

Electromyographic gait analysis - Surface and fine-wire electromyography (EMG) signals were collected from seven muscles spanning the carpus responsible for flexion/extension and stabilization of the carpal joint using an EMG telemetric system (Table 4.1). Stainless steel, 7-strand, teflon coated fine-wire electrodes[j] were cut at pre determined lengths (10-cm), with 1 cm of the ends bared and fed through a 23-gauge, 1.5-inch needle. One cm of the wire ends were reflected back against the shaft of the needle and the wire/needle combinations were steamed autoclaved. Horses were sedated with intravenous detomidine hydrochloride[k] (0.01 mg/kg). Hair was removed using a #40 clipper blade and following a sterile surgical preparation, subcutaneous local anesthesia was injected over the sites for fine-wire electrode insertion using 1 ml of lidocaine HCl 2%[l] injected with a 25-gauge, 5/8-inch needle. Two, fine-wire electrodes were inserted 3 cm apart into the center of the SDF and DDF muscle bellies under ultrasound guidance. The ends of the fine-wire electrodes exterior to the skin were gathered together with a butterfly tab of medical tape and were further anchored to the skin using 3-0 monocryl[m] (Fig 4.5). In addition, the free ends of the fine-wires were connected by a spring to pre-amplified cables attached to a telemetry EMG transmitter[n] secured to a surcingle. A ground surface electrode was placed over the skin on the proximal radius. Self-adhesive, bipolar surface electrodes[o] were adhered with cyanoacrylate glue onto shaved, clean skin over the remaining 10 thoracic limb muscles of interest. Surface electrodes were positioned over the middle of the muscle belly and locations were precisely recorded using clear overhead projector sheets. Each surface electrode placement was traced over, along with identifying marks on the limb in order to ensure consistent, repeatable placement between data sessions. Pre-amplified cables connected the surface electrodes to the telemetry unit secured to a surcingle on the horse (Fig 4.6). Data collection began after horses had fully recovered from sedation (90-120 minutes following sedation). To minimize changes in fine-wire electrode placement during repeated data collection sessions, at the conclusion of baseline data the fine-wire electrode placement was recorded based on the distance proximal to the carpal accessory bone and the depth of insertion recorded based on ultrasound guidance. Both surface and fine-wire electrodes were removed after each data collection session.

Raw EMG data was collected at 3000 Hz, high-pass filtered (120 Hz), zero-meaned, full wave rectified, followed by low-pass filtering with a recursive fourth-order Butterworth filter (15 Hz). EMG parameters of interest include the maximum amplitudes of the EMG signal during stride duration, on/off timing of each muscle, and the duration of muscle activity. The peak EMG amplitudes for each muscle were determined for a minimum of two stride durations per trial. The data from three trials were averaged and the mean peak EMG amplitude for each muscle recorded. Average peak EMG amplitudes were normalized to the average peak EMG amplitude calculated at baseline, which allowed for the comparison in changes of muscle activation magnitude within and between-individuals across data collection time frames.

In order to accurately determine the timing of muscle activation, the EMG amplitude mean and standard deviation were captured when the muscle of interest was less active. EMG signals were recorded from the thoracic limb in a non-weight bearing position with the carpus slightly flexed, similar to the position adopted when cleaning out the hoof. The EMG mean and standard deviation were determined during a one-second quiet period and used to calculate the appropriate threshold values for determining muscle activation on/off times (Fig 4.7). The recommended timing protocol utilized to determine the threshold level that a muscle needs to reach in order to be defined as active (above threshold) or inactive (below threshold) is defined as a value equal to two to eight standard deviations plus the mean.[42] Furthermore, to minimize single spontaneous EMG spikes from triggering the onset or offset of muscle activity a minimum period duration was set at 50 milliseconds. The threshold value calculated per muscle at baseline was the threshold value that was utilized for the remainder of the data collection sessions.

Statistical Analysis – Data were evaluated by use of an ANOVA or ANCOVA framework with a software program[p] depending on the presence or absence of a covariate, respectively. The ANOVA tables were used to determine significant main effects and interactions between main effect variables. When individual comparisons were made, least square means were used and $P < 0.05$ was considered significant. Trends in the data were reported as $0.05 < P < 0.10$. The following main effects were considered: presence or absence of OA, treatment (underwater treadmill therapy or controlled hand walking), day of data collection, and all interactions between main effect variables. Values are reported as mean ± SEM.

Results

Velocity – The velocity at which horses trotted in hand during biomechanical gait analysis did not significantly differ based on treatment group ($P = 0.89$) or day of data collection ($P = 0.69$) (Table 4.2).

Kinetics - Ground reaction force data was statistically analyzed using baseline data (day -7) as a covariate. The initial repeated measures ANOVA analysis incorporating all 4 data collection time periods demonstrated a difference in the peak vertical force (PFz) values measured at baseline. PFz differed significantly (P = 0.04) within the OCF limb between the randomized treatment groups. However, OA had not yet been induced and horses were not randomized into treatment groups until day 7. The differences present at baseline were the result of variability that normally occurs in PFz data collection. The literature supports that PFz typically has a low inter-day variability (<10% of the mean).[43] The literature also supports the acceptable intra-limb variability to have a coefficient of variation of 4.0 – 5.5% for PFz.[44,45] The intra-limb variability on day 0 within each group for PFz was 3%, with a respective 6% difference in PFz between treatment groups. Although the coefficient of variation calculated for this study demonstrated similar values to what is reported in the literature, using day 0 as a covariate was significant (P = 0.04).

Stance Duration - The induction of OA did not significantly alter the stance duration within the thoracic or pelvic limbs at any time point during the study period. However, the stance duration between OCF and sham-operated limbs differed significantly (P = 0.002) according to whether the horse was in the aquatic therapy or control group (Fig 4.8). The control group OCF limb had a significantly longer stance duration (315.0 ± 3.0 ms; P = 0.008) when compared to the control group sham-operated limb (311.0 ± 3.0 ms). While the aquatic therapy OCF limb had a significantly shorter stance duration (306.0 ± 3.0 ms; P = 0.004) when compared to the contralateral sham-operated limb (310.0 ± 3.0 ms). A significant treatment effect for pelvic limb stance duration was not detected.

Peak Vertical Force - Compared with the sham-operated joints, surgery to induce OA demonstrated a trend for lower PFz values in the OCF limb (P = 0.08). In addition, PFz between OCF and sham-operated limbs differed significantly (P = 0.02) according to whether the horse had controlled hand walking or was exercised in the underwater treadmill. Individual comparisons were conducted and the control group OCF limbs had a significantly lower PFz (10.0 ± 0.2 N/kg; P = 0.007), compared to the control sham-operated limbs (11.0 ± 0.2 N/kg) (Fig 4.9). However, there were no significant differences between the aquatic therapy OCF and sham-operated limbs. A treatment effect was present as horses exercised in the underwater treadmill had mean PFz values in the OCF limb that were statistically identical to that of the contralateral normal limb.

Osteochondral fragmentation induced a significant decrease in the mean PFz measured from the pelvic limb ipsilateral to the OCF thoracic limb (8.9 ± 0.07 N/kg; P = 0.01), compared to the diagonal pelvic limb (9.1 ± 0.07 N/kg) (Fig 4.10). The compensatory decrease in PFz from the ipsilateral pelvic limb correlates with the decrease in PFz within the OCF thoracic limb. However, aquatic therapy intervention did not significantly influence the pelvic limb PFz.

Time to PFz - The time to peak vertical force between OCF and sham-operated limbs differed significantly (P = 0.01) according to whether the horse was in the aquatic therapy or control group. The aquatic therapy OCF limb demonstrated a significantly faster time to PFz (137.0 ± 2.0 ms; P = 0.04) when compared to the contralateral sham-operated limb (140.0 ± 2.0 ms). Induction of OA did not significantly change the time to PFz in the pelvic limbs, nor was a treatment effect detected.

Loading rates - The loading rates between the OCF and sham-operated limbs differed significantly (P = 0.008) depending on whether the horse was in the aquatic therapy or control group. The control group OCF limb had a significantly decreased loading rate (7.4 ± 0.02 N/ms; P = 0.02), compared to the control sham-operated limb (7.6 ± 0.02 N/ms). However, there was no significant difference between the aquatic therapy OCF (7.7 ± 0.02 N/ms) and sham-operated limbs (7.6 ± 0.02 N/ms). The influence of aquatic therapy demonstrated an overall ability to improve the rate of limb loading in a vertical force direction.

Impulse - Induction of OA approached a significant influence on the vertical impulse measured between OCF (1.8 ± 0.01 Ns/kg; P = 0.08) and sham-operated limbs (1.9 ± 0.01 Ns/kg). The pelvic limb vertical impulse did not demonstrate significant changes due to the presence of OA. There were no significant differences detected in the thoracic or pelvic limb vertical impulses between horses that received aquatic therapy or controlled hand walking.

Peak braking and propulsion forces, braking and propulsion impulses, and time to peak braking and propulsion forces recorded from the thoracic and pelvic limbs did not change significantly with the induction of OA, nor was a treatment effect detected.

Accelerometer – Osteochondral fragmentation did not result in significant effects on the stride, stance or swing duration, or on the percentage of swing and stance duration determined from the accelerometer signal. In addition, aquatic therapy intervention did not significantly influence any of the accelerometer measured variables (Table 4.3).

Kinematics - Joint angle data was analyzed using baseline data (day -7) as a covariate. The initial repeated measures ANOVA analysis incorporating all 4 data collection time periods demonstrated a significant difference in shoulder and elbow stance and swing angles at baseline. Shoulder and elbow peak stance and swing angles differed between the randomized treatment groups at baseline prior to fragmentation and initiation of treatment. No significant intra-individual differences between the thoracic limb angles were detected for any of the four joint angles measured at baseline. Covariate analysis resulted in day 0 having a significant influence on the shoulder ($P = 0.03$) and elbow ($P = 0.04$) peak angles during stance, as well as on the shoulder ($P < 0.001$) and elbow ($P = 0.03$) peak angles during swing phase.

Stance Phase – Osteochondral fragmentation induced significantly greater peak shoulder extension angles during the stance phase of stride (128.0 ± 0.7

degrees; P = 0.002), compared to the sham-operated limb (125.0 ± 0.7 degrees) (Table 4.4). The presence or absence of aquatic therapy did not significantly influence the peak shoulder extension angles during stance phase.

Induction of an osteochondral fragmentation approached a significant (P = 0.06) influence of the degree of peak elbow joint extension angles during the stance phase of gait. The OCF limb maintained a slightly more extended peak elbow joint angle (149.0 ± 0.7 degrees), compared to the sham-operated limb (148.0 ± 0.7 degrees). The peak elbow extension angle during stance phase between the OCF and sham-operated limb was significantly (P = 0.01) influenced according to whether the horse was exercised in the underwater treadmill or hand walked (Fig 4.11). Specifically, the peak elbow joint angle during stance phase for the aquatic therapy OCF limb was in a more extended position (150.0 ± 1.0 degrees), compared to the control group OCF limb (148.0 ± 1.0 degrees). A significant (P = 0.003) difference was also demonstrated between the OCF and sham-operated limbs within the aquatic therapy group. The sham-operated limb exercised in the underwater treadmill had less peak extension in the elbow joint during stance phase (148.0 ± 1.0 degrees), compared to the contralateral OCF limb (150.0 ± 1.0 degrees).

Induction of OA and the presence or absence of aquatic therapy did not significantly influence the peak carpal or fetlock extension angles during stance phase. Furthermore, the percent of stride in which the shoulder, elbow, and fetlock joints reached maximum extension were not significantly influenced by osteochondral fragmentation or the application of aquatic exercise.

Depending on the study day, the percentage of stride duration that peak carpal extension occurred within was significantly ($P = 0.01$) influenced by the presence or absence of an osteochondral fragment (Fig 4.12). Peak carpal extension was reached significantly ($P = 0.01$) earlier during stride in the OCF joint on day 42 (25.0 ± 1.7%), compared to the similarly affected joint on day 14 (31.0 ± 1.7%). Study day 42 also demonstrated peak carpal extension occurring significantly earlier in the OCF joint (25.0 ± 1.7%; $P = 0.004$), compared to the sham-operated joint on the same day (31.0 ± 1.7%) (Fig 4.13).

Swing phase - Induction of an osteochondral fragment caused significantly less shoulder flexion during the swing phase of stride (120.0 ± 0.5 degrees; $P = 0.002$), compared to the sham-operated limb (117.0 ± 0.5 degrees) (Fig 4.14). The presence or absence of aquatic therapy did not significantly influence the degree of shoulder flexion during swing phase of stride.

The elbow swing angle was significantly influenced by osteochondral fragmentation, specifically the OCF limb had a larger elbow flexion angle (99.0 ± 0.5 degrees; $P = 0.002$) compared to the sham-operated limb (97.0 ± 0.5 degrees) (Fig 4.15). Similarly, osteochondral fragmentation induced significantly less carpal flexion during the swing phase of stride (107.0 ± 1.0 degrees; P =

0.003), compared to the sham-operated joint (104.0 ± 1.0 degrees) (Fig 4.16). The application of aquatic therapy did not significantly influence the elbow or carpal swing angles.

Osteochondral fragmentation did not result in significant effects on the degree of fetlock flexion, and no significant treatment effects were observed. In addition, the percent of stride in which the shoulder (55.0 ± 1.4%), elbow (89.0 ± 1.2%), carpus (75.0 ± 0.8%), and fetlock joint (72.0 ± 1.4%) reached maximum flexion were not significantly influenced by the induction of OA or the application of aquatic exercise.

Electromyography – *Extensor Carpi radialis (ECR)* - EMG activity began during 63% of stride duration (early swing phase) and continued until 83% of stride duration (end of swing phase), with an activity duration of 20% of stride (Table 4.5). The normalized peak EMG amplitude, onset of muscle activity, duration of activation, and deactivation of ECR muscle activity were not significantly influenced by the induction of OA, or the application of aquatic therapy.

Ulnaris lateralis (UL) - EMG activity began just prior to ground contact (90% of stride duration) and continued through the majority of stance phase (35% of stride duration), with an activity duration of 45% of stride. The induction of an osteochondral fragment significantly influenced the UL normalized peak

EMG amplitude depending on the time period of data collection (Fig 4.17). The OCF limb had a significantly (P = 0.006) higher normalized UL peak EMG amplitude at day 70, compared to the OCF limb at day 42 and at baseline. Furthermore, on day 70 the sham-operated limb had a significantly lower normalized peak UL amplitude (107.0 ± 19.3 normalized %; P = 0.005), compared to the OCF limb (149.0 ± 19.3 normalized %). Osteochondral fragmentation results in a reduced intensity of muscle activity within five weeks, followed by an elevation above baseline at study conclusion. UL peak EMG amplitude was not significantly influenced by the presence or absence of aquatic therapy.

In addition, the UL onset of muscle activity was significantly (P = 0.05) influenced by the presence or absence of an osteochondral fragment (Fig 4.18). The UL onset of muscle activity occurred significantly later in stride within the OCF limb (91.0 ± 0.8 %), compared to the sham-operated limb (89.0 ± 0.8 %). UL onset of muscle activity was also significantly (P = 0.02) influenced by the induction of an osteochondral fragment and the applied treatment (Fig 4.19). Within the control group the OCF limb had a significantly later onset of activity (91.0 ± 1.2%; P = 0.004), compared to the contralateral sham-operated UL muscle (88.0 ± 1.2%). However, there were no significant differences between the OCF and sham-operated limbs exercised in the underwater treadmill. Furthermore, osteochondral fragmentation significantly (P = 0.04) influenced the duration of muscle activation within the UL muscle depending on the day of data collection. When study day x OA least square mean comparisons were analyzed the duration of activation within the OCF UL muscle was significantly shorter on day 42 (295.0 ± 12.5 ms; P = 0.04) compared to days 14 (320.0 ± 12.5 ms) and 70 (319.0 ± 12.5 milliseconds). Similarly, the induction of OA demonstrated a trend (P = 0.06) for influencing the UL percent of stride activation and the percent of stride deactivation depending on the day of data collection. The percent activation during stride within the UL from the OCF limb on day 42 (42.0 ± 1.7%; P = 0.02) was significantly less than the percent of activation on both days 14 (47.0 ± 1.7%) and 70 (47.0 ± 1.7%). The UL within the OCF limb also inactivates significantly earlier on day 42 (34.0 ± 1.7%; P = 0.04) than on day 70 (38.0 ± 1.7%). However, no treatment effects were detected within the UL duration of activation, percentage of activation during stride, or percent of stride deactivation.

Flexor Carpi Radialis (FCR) - EMG activity of the FCR consisted of two bursts of similar amplitude, the first one started at the beginning of stance and ended in the middle of stance phase. While the second FCR burst of activity began early in swing phase and ended just prior to hoof contact (Table 4.5). Osteochondral fragmentation and the presence or absence of aquatic therapy did not significantly influence any of the EMG variables measured within the FCR.

Flexor Carpi Ulnaris (FCU) – EMG activity began just prior to ground contact and remained active through the majority of stance phase (Table 4.5). The main effect of OA approaches a significant influence (P = 0.06) on the duration of FCU activation. The FCU duration of muscle activation within the OCF limb was active longer (288.0 ± 10.4 ms), compared to the same muscle within the sham-operated limb (277.0 ± 10.4 ms). The remainder of the EMG variables were not significantly influenced by osteochondral fragmentation, nor the application of aquatic therapy.

Triceps Brachii Long Head (TBL) - Onset of muscle activity began at the end of swing phase and deactivated within the first 20% of stride duration (early stance phase). Osteochondral fragmentation significantly (P = 0.002) influenced the long head of the triceps brachii normalized peak EMG muscle amplitude. The normalized peak EMG amplitude recorded from the TBL within the OCF limb was significantly higher (213.0 ± 22.4 normalized %), compared to the same muscle within the sham-operated limb (126.0 ± 20.9 normalized %). However, the percent onset of TBL activity, duration of TBL muscle activity, percent of TBL activation, and the percent deactivation were not significantly influenced by osteochondral fragmentation, nor were significant differences detected in TBL EMG activity between horses that received aquatic exercise or controlled hand walking.

Superficial Digital Flexor (SDF) – EMG activity began just prior to ground contact, remaining active through a portion of stance phase (Table 4.5). The induction of an osteochondral fragment did not significantly influence any of the

EMG variables measured within the SDF. The presence or absence of aquatic therapy also did not significantly influence the SDF muscle activation profiles.

Deep Digital Flexor (DDF) – EMG activity is very similar to the SDF, in that muscle activity begins at the end of swing phase and continues through the majority of stance phase. The DDF duration of activation was longer and the percentage of stride that the muscle deactivates occurred later in comparison to the SDF. The induction of an osteochondral fragment significantly ($P = 0.02$) influenced the onset of DDF muscle activation. The DDF onset of muscle activity occurred significantly earlier in stride within the OCF limb (86.0 ± 1.4%), compared to the sham-operated limb (91.0 ± 1.4%). The percent of stride duration that the DDF muscle remains active was significantly ($P = 0.005$) influenced by the presence or absence of an osteochondral fragment, along with the application of aquatic therapy (Fig 4.20). Percent of DDF stride duration activity was significantly longer within the control OCF limb (49.0 ± 2.5%; $P = 0.04$), compared to the control sham-operated limb (44.0 ± 2.4%). Conversely, the aquatic therapy OCF limb percent DDF stride duration activity was significantly shorter (41.0 ± 2.4%; $P = 0.05$), compared to the contralateral thoracic limb DDF muscle (45.0 ± 2.4%). Furthermore, the percent DDF stride duration activity within the control OCF limb was significantly longer (49.0 ± 2.5%; $P = 0.02$) than the aquatic therapy OCF DDF muscle (41.0 ± 2.4%). The percentage of stride that the DDF muscle deactivates was also significantly ($P = 0.01$) influenced by the induction of an osteochondral fragment and the application of aquatic therapy (Fig 4.21). When individual comparisons were conducted the DDF muscle within the aquatic therapy OCF limb discontinued muscle activity (34.0 ± 1.8%; P = 0.02) significantly earlier than the contralateral thoracic limb DDF muscle (38.0 ± 1.9%).

Discussion

The results of the present study demonstrate that osteochondral fragmentation within the middle carpal joint leads to limited changes in the magnitude of ground reaction force variables, influences kinematic temporal and angular variables, along with considerably affecting EMG activity. Treatment with underwater treadmill exercise after osteochondral fragmentation yielded significant improvements for a small number of the variables in affected limbs, compared with values for respective variables in control OCF limbs. However, the method of osteochondral fragmentation used in this study has been shown to induce clinical signs consistent with low-grade lameness[46-48]; average lameness scores within this study for the OCF limb were 1.8 out of 5. Therefore, the degree of pain and inflammation associated with the onset and progression of carpal OA may have been minimal enough that large alterations in thoracic and pelvic limb biomechanics were not detected. Consequently, biomechanical modification attributable to treatment with aquatic therapy would be minimized.

Equine research has demonstrated compensatory gait patterns associated with varying severity of equine lameness. Typically, a lame horse will modify its gait to reduce loads on the lame limb and compensate by redistributing the load to the other limbs. In addition, to provide the momentum needed for propulsion the duration of time that the lame limb spends in contact with the ground will increase as a means of maintaining the impulse with a lower peak vertical force.[49] Mild lameness conditions often result in decreased peak vertical ground reaction forces in the lame limb, whereas compensatory changes in the remaining limbs are not observed.[49] Additional studies inducing subtle lameness using a transient sole pressure model demonstrate significant changes in peak vertical force and stance duration only within the lame thoracic limb.[6] However, induction of a moderate degree of lameness (using a similar model that applied additional pressure to the sole) did influence the ground reaction force parameters not only within the affected thoracic limb but within the contralateral thoracic and bilateral pelvic limbs as well.[6,49] A unilateral carpal joint synovitis model induced with amphotericin-B concluded that stance phase measurements were not significantly influenced by the induction of a moderate degree of lameness (scores ranging from 2.8 to 3.5).[14] Conversely, creating a 1-cm diameter, full thickness articular cartilage defect on the dorsal lateral aspect of the distal radial carpal bone induced significant alterations in both vertical and craniocaudal force components.[7] Lameness induced with the full thickness cartilage defect model was described as easily observable, however no subjective lameness grades were given.[7] The reliability of force platform analysis to detect compensatory load redistribution patterns appears to be influenced by the severity of experimentally-induced lameness.

In the present study, the influence of aquatic therapy demonstrates an overall ability to improve load bearing distribution as the peak vertical forces between the OCF and contralateral thoracic limbs were not statistically different. Conversely, peak vertical forces within the control OCF limbs were significantly lower compared to the contralateral sham-operated limbs. The compensatory reduced loading of the control OCF limb was further supported by the prolonged stance duration demonstrated within the same limb. Prolonged stance duration is a compensatory mechanism adopted to reduce the loading rate and peak forces by distributing the forces applied to the limb over an extended period of time.[6] Similar to the peak vertical forces, horses exercised in the underwater treadmill maintained similar vertical loading rates between the OCF and sham-operated limbs. While the OCF limb in the control group demonstrated a significantly decreased loading rate, meaning that the OCF limb was adopting a protective mechanism to reduce the strain rates applied to the limb during weight bearing. Results of force platform analysis demonstrated that the effects of aquatic therapy observed in this study were clinically significant. Horses exercised in the underwater treadmill demonstrated symmetrical limb loading between the OCF and contralateral normal thoracic limb, which indicates that axial loads were not shifted to unequally increase the load on different structures.

Kinematic analysis of the joint characteristics of horses with osteochondral fragmentation of the radial carpal bone revealed asymmetries involved in stance, as well as differences in the swing phase of gait. Asymmetrical joint angles described in horses with both natural-occurring and experimentally-induced carpal OA often demonstrate asymmetries during swing phase of gait, with few alterations occurring in stance.[10,14,50] Osteochondral fragmentation within the middle carpal joint in this study resulted in an overall reduction in peak flexion angles of the shoulder, elbow and carpal joints during the swing phase of gait. This results in a characteristic rigid, circumducting gait pattern in an attempt to minimize pain resulting from flexion of the carpus.[51] Similarly, the proximal joints within the OCF limb also maintained greater extension angles during stance, while the sham-operated limb shoulder and elbow joints demonstrated greater flexion angles during the corresponding weight bearing phase of stride. A study that induced lesions in the superficial digital flexor tendons of a single thoracic limb also demonstrated a more extended position of the shoulder and elbow joint during stance.[52] The more extended position of the proximal joints may be explained by the position of the trunk during loading of the lame limb. Typically, during loading of the lame thoracic limb, the head and neck are elevated, which decreases the normal downward acceleration leaving the trunk in a higher position during stance phase.[11] Conversely, considerable downward acceleration of the trunk occurs as the sound thoracic limb is loaded during which the proximal joints will act to attenuate weight bearing loads through active increases in flexion of the shoulder joint.[13]

Although no changes in fetlock joint extension during stance phase were demonstrated in this study, this is not a unique finding. Middle carpal joint synovitis induced with amphotericin-b concluded that joint range of motion data calculated from the carpus and fetlock were inadequate variables to quantify changes in locomotion due to carpal lameness.[14] However, multiple studies using models inducing supporting limb lameness (i.e., transient sole pressure models) describe the asymmetrical degree of fetlock extension as a sensitive variable representing the shifting weight bearing component of the lameness as it directly correlates with the vertical ground reaction force patterns between the lame and non lame thoracic limbs.[53] As the majority of kinematic changes in this study occurred during swing phase it can be concluded that changes in joint flexion angles indicate a mechanical alteration of thoracic limb movement and contribute to a swinging limb lameness. There were however, some asymmetries in proximal limb stance angles, which may indicate that the carpal osteochondral fragment OA model produces both a supporting and swinging type of lameness.

The present study also investigated whether osteochondral fragmentation would lead to changes in the EMG signal magnitude and timing characteristics for each of the muscles investigated. The results indicate that significant changes were noted within the OCF limb muscles responsible for extension of the elbow joint and stabilization of the carpus (UL, FCU, and TBL), as well as subtle changes within the deep digital flexor muscle. It should be noted that the standard error of the mean for the SDF and DDF normalized peak EMG amplitude were relatively large in comparison to the mean. The large variability present in the EMG signal recorded from the fine-wire electrodes may be due to the inability to sample from the same motor units at each data collection time frame. Wire migration, cable motion artifact, and weak electrical connection between the wires and spring cables all might be contributing to a greater degree of variation in the EMG signal.

The clinical application of EMG studies has been limited due to the inherent variability obtained in the results. EMG analysis commonly reveals large intra and inter-individual variation even among individuals performing the same task.[54] Variability in surface EMG recordings are often due to movement of the skin, changes in skin impedance (conductivity of skin), differences in thickness and electrical properties of the tissue layers between the surface electrodes and muscles, location of electrodes, cross talk, as well as morphometric variability in muscle mass and fiber type. The vast degree of variability is not surprising as the volume of muscle is large relative to the region sampled by the surface electrodes. Surface EMG electrodes only record activity from the superficial portion of the muscle belly, such that the EMG signal recording is sensitive to compartmentalization of recruitment patterns within the muscle.[55] The intrinsic muscles of the thoracic limbs have previously been reported to have anatomical subregions that differ in fiber type and orientation.[56,57] These anatomical subregions are known to function independently, resulting in an inability to characterize the activity of the entire muscle within the limited recording volume of the EMG electrodes.[58] Future studies may want to investigate the changes in muscle activity within a muscle's anatomical subregions using a combination of surface and fine-wire EMG electrodes.

In order to reduce the large variation in EMG recordings the EMG signals are often normalized to either a maximal voluntary contraction (MVC) or to the average peak EMG amplitude obtained during a standardized task.[59] Normalization to the mean peak EMG amplitude has been reported as an accepted method of normalization in human dynamic gait analysis.[60] In general, normalization to the mean peak EMG amplitude reduces the inter-subject variability and produces a general gait EMG profile; a template for comparison against pathologic gait.[60] This form of normalization indicates the average maximum level of activity displayed by a muscle throughout the gait cycle in relation to the average maximum activity recorded before osteochondral fragmentation. It does not however indicate the degree of muscle activation required during gait (i.e., to perform a given task), or how active a muscle is in relation to it maximal static activation.

The amplitude of an EMG signal can be used as an indirect measure of muscle activity, as the summation of active motor units at any particular time of contraction contribute to the amplitude of the signal.[55,59] The increased amplitude of the signal associated with the long head of the triceps brachii following induction of OA may indicate that increased activation of the muscle was required to maintain the greater extension angle of the elbow during stance phase. A similar increase in amplitude was detected in the UL of the OCF limb, which may be a compensatory response to increase joint stiffness, yielding greater joint stability. Findings in human EMG studies demonstrate an increase in muscle amplitude intensity following a period of muscle fatigue, due to the need of the muscle to recruit additional motor units to compensate for the decrease in force of contraction occurring in fatigued muscle.[59] Similar EMG studies in human patients with experimentally induced knee effusion demonstrated significant quadricep muscle inhibition, along with simultaneous increases in muscle activity within the hamstrings and posterior distal limb muscles.[20] The facilitation of the hamstrings and posterior distal limb muscles during knee effusion is likely to be a compensatory response to quadriceps inhibition in order to attenuate axial loads during initial ground contact.[20] Unlike the knee effusion model, injection of saline into the ankle did not induce neuromuscular inhibition. After injection of 10 ml of saline into the ankle joint an increase in muscle activity of the primary dorsiflexor and the plantar flexor muscles of the ankle was observed.[23] These results suggest that in cases where joint stability is crucial it is advantageous to limit the effects of reciprocal inhibition and allow for simultaneous activation of the agonist and antagonist muscles.[23] In the present study the resulting increased EMG signal intensity within the TBL and UL may have functioned not only to produce elbow extension, but also to modify joint loading and provide joint stability. Specifically, the greater muscle activity within the OCF affected UL may have been required to counteract axial loads across the OCF joint surface.

Until recently the majority of EMG research in the horse has focused on defining muscle activity during normal locomotion. Numerous equine studies have demonstrated the activation patterns of the thoracic, pelvic, and trunk muscles, however, few studies have demonstrated the change in activity due to joint pain and inflammation. EMG timing activity reported in this study is similar to previously published studies.[27,33] The majority of muscles (all but ECR) are activated prior to ground contact or at hoof strike in preparation for limb loading, and become inactivated just prior to swing phase once attenuation of ground reaction forces has been completed. The induction of middle carpal joint OA in the current study influenced the timing of muscle activity within the UL and the DDF muscles. Onset of muscle activity within the OCF UL occurred later in swing phase compared to the contralateral UL. Conversely, within the OCF limb onset of DDF muscle activity occurred earlier in swing phase compared to the contralateral thoracic limb DDF muscle. The changes in muscle activation between the two muscles are likely an individual response to the discharging afferents from the surrounding carpal joint mechanoreceptors. In addition, the application of aquatic therapy demonstrated an ability to maintain symmetrical timing of the UL muscles as horses exercised in the underwater treadmill did not demonstrate significant differences between the UL onset of muscle activity in the OCF and contralateral normal thoracic limbs. Conversely, UL muscle activation within the control OCF limbs occurred significantly later in the stride compared to the contralateral sham-operated limbs.

Limitations

One of the primary goals of this study was to improve our understanding of the interaction between alterations in thoracic limb movement adaptations and muscle activation strategies associated with the onset and progression of OA. However, as the main effect of OA was not significant for a majority of the kinetic and EMG variables, the null hypothesis that there was no difference between the sham-operated and OCF limb cannot be rejected. Similarly, within this study the influence of kinetic, kinematic, and EMG variables did not consistently change due to the presence or absence of an osteochondral fragment depending on the day of data collection. Therefore, the progressive changes in the biomechanical alterations in association with the onset and progression of OA could not be assessed. The OA model used in this study produces a reliable and repeatable low grade carpal osteoarthritis, typical of naturally-occurring disease in horses. The subtle clinical signs associated with osteochondral fragmentation and the short duration of the model may have limited the comparison of biomechanical alterations in the thoracic limbs over time.

In the study reported here, large variations in shoulder and elbow peak stance and swing angles were detected between the randomized treatment groups at baseline, prior to fragmentation and initiation of aquatic therapy. The variability in shoulder and elbow joint angles may be related to the fact that no correction was made for marker skin displacement, as the only algorithms available were devised for 11-14 year old Dutch Warmblood horses, with an average height of 166 cm, trotting at a speed of 3.1 m/sec.[61] Therefore, it was determined that these were not suitable for the present study population. Consequently, kinematic joint angle interpretation from the proximal thoracic limbs should be evaluated with caution as displacement of the skin during locomotion may influence the calculated measurements.

A further limitation to this study was that the extent of movement of the head and pelvic markers were not assessed. It has been previously reported that displacement of the head and pelvis are sensitive variables when attempting to detect compensatory movements.[62] Beyond ground reaction force data no additional biomechanical assessments were conducted on the pelvic limbs to discern potential compensatory adjustments existing within the pelvic limbs.

The inability of this study to evaluate individual motor unit action potentials with needle EMG may have contributed to the lack of significant differences appreciated within EMG activity measured from the majority of investigated thoracic limb musculature. Human EMG studies have reported that acute muscle timing and activity changes due to joint pain and inflammation are recognized only at the level of the motor unit initially.[63] However, surface EMG analysis does have important clinical implications in providing targeted therapies and monitoring the restoration of normal activation patterns. Further work is required to establish activation patterns associated with various orthopaedic conditions in order to understand the pathophysiology associated with musculoskeletal gait disturbances.

Conclusion

In summary, this research introduced the importance of considering the inter-relationships between gait and neuromuscular factors in the study of carpal OA disease progression. The author believes that this is the first controlled study to assess biomechanical alterations of the thoracic limbs following the management of OA with underwater treadmill exercise. Substantial biomechanical improvements following aquatic therapy programs have been reported in human patients with osteoarthritis.[16,38,64,65] Similar biomechanical modifications were demonstrated in the present study as two of the most encouraging findings from horses exercised in the underwater treadmill included, evenly distributed thoracic limb axial loading and symmetrical ulnaris lateralis muscle timing between the affected and unaffected limbs. Conversely, the control horses demonstrated limited axial loading of the OCF affected limb, which may have been an attempt to decrease mechanical stresses applied medially over the injured region. As a result of unequal attenuation of load bearing across the middle carpal joint, asynchronous neuromuscular activation of the lateral carpal joint stabilizers occurred. Insights from this study help us gain a better understanding of the important biomechanical aspects of musculoskeletal dysfunction due to joint pain and provides evidence based support for equine aquatic therapy as a viable therapeutic option in managing OA in horses.

Material Sources

a. Super glue (cyanoacrylate adhesive), Pacer Technology, Rancho Cucamonga, CA.
b. Naxcel, Pfizer Inc, New York, NY.
c. Equi-Phar, Schering-Plough Animal Health Corp, Union, NJ.
d. Equigym, Equigym LLC, Lexington, KY.
e. Equine underwater treadmill, Ferno Veterinary Systems, Wilmington, OH.
f. MEK 92-PAD photoelectric control Mekontrol Inc, Northboro, MA.
g. Bertec Force Platforms model FP6090-15, Bertec Corporations, Columbus, OH.
h. Motus Software version 9.1, Vicon, Centennial, CO.
i. Volant 200 Hz cameras, Vicon, Centennial, CO.
j. Stainless steel, 7 strand, teflon coated fine-wire electrode spool, A-M Systems, Inc, Sequim, WA
k. Dormosedan, Pfizer, Inc, New York, NY.
l. Lidocaine HCl 2%, Agri Labs, St. Joseph, MO
m. 3-0 Monocryl, Ethicon, San Angelo, TX
n. Noraxon Telemyo 2400 G2, Scottsdale, AZ
o. Blue Sensor Ambu, Inc, Glen Burnie, MD
p. Glimmix SAS Procedure, SAS Institute Inc, Cary, NC.

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Figure 4.1: Photograph of a biaxial accelerometer adhered to the dorsal hoof wall of the right front hoof. Accelerometer signals were used to identify hoof contacts necessary to define stride durations. Changes in the accelerometer signal were only measured in the vertical (Y) direction.

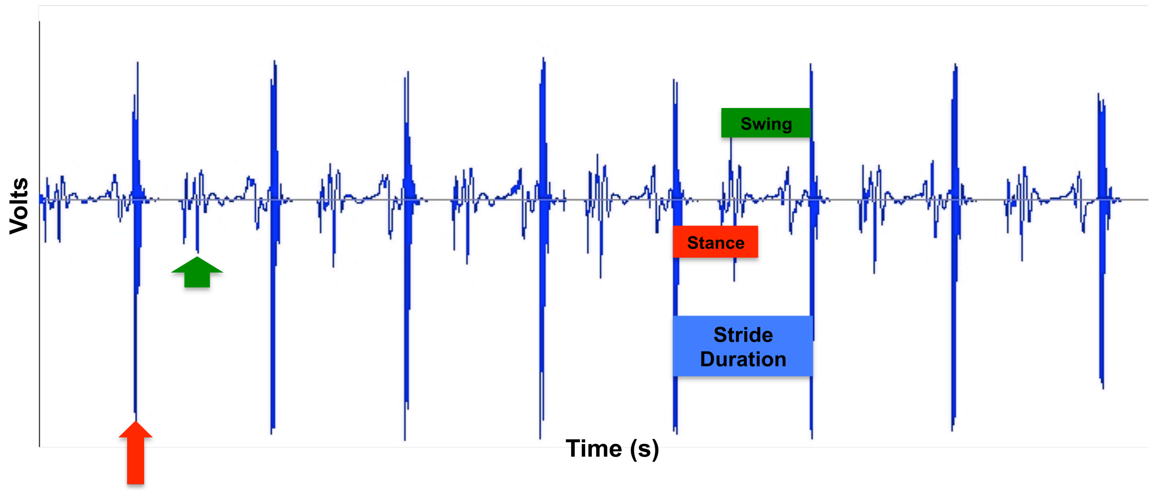

Figure 4.2: Raw accelerometer signal indicating initial ground contact (red arrow) and hoof off (green arrow head).

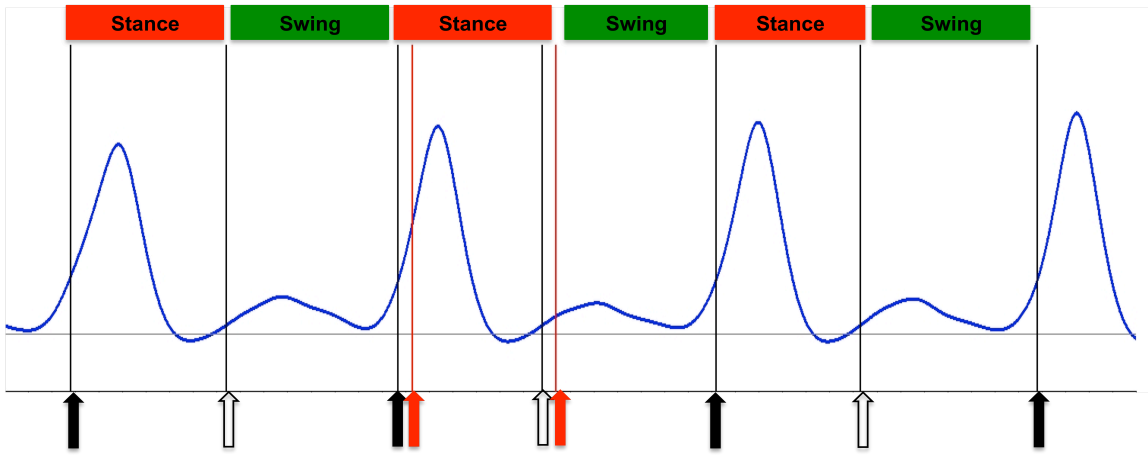

Figure 4.3: Graph of the processed accelerometer signal. Hoof contact event detections indicated by the solid black arrows in association with the onset of the peak accelerometer signal. Hoof off event detections indicated by the open arrows in association with the smaller accelerometer signal. Solid red arrows indicate stance phase determined by the ground reaction vertical force recorded from the force platform. The difference in hoof contact determined by the force platform versus the accelerometer was 30 ms and the difference in hoof off timing was 29 milliseconds. Total stance duration between the accelerometer and the force platform differed by 1 millisecond.

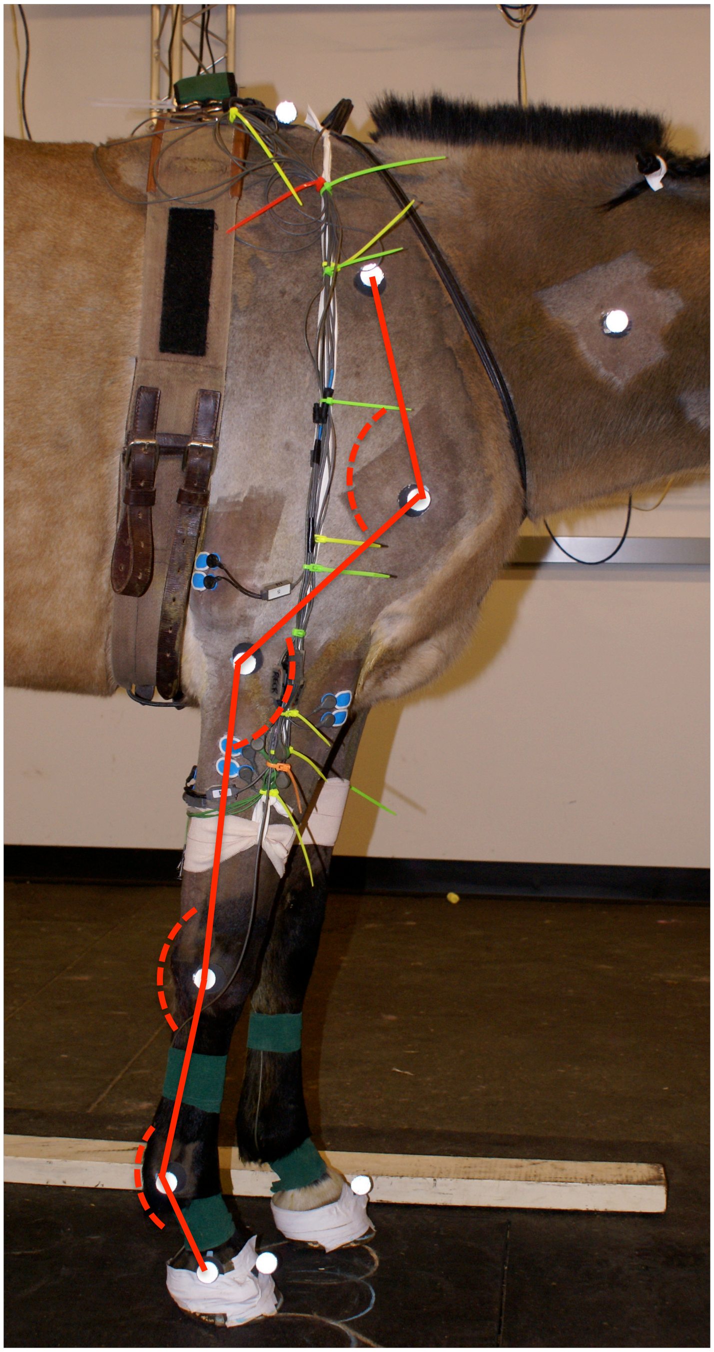

Figure 4.4: Photograph of placement of the retro-reflective markers over the centers of joint rotation for the right thoracic limb. The marker proximal to the scapulohumeral joint is placed over the upper 1/3 of the spine of the scapulae. A solid red line connects centers of joint rotation and the dashed red arc indicates the flexor surface for each joint.

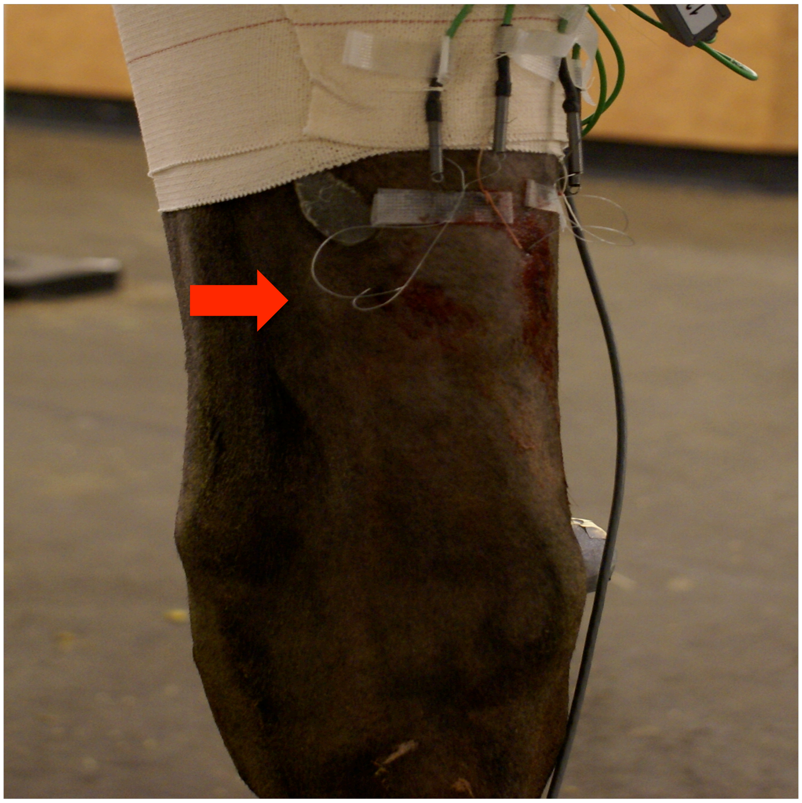

Figure 4.5: Photograph of fine-wire electrode placement into the superficial digital flexor muscle of the right thoracic limb (red arrow). The bared ends of the fine-wire electrodes exiting the skin are inserted into the spring connectors that are attached to the pre-amplified EMG cables. Notice the wires are not secured tightly to the skin so that some movement can occur during normal trotting gait.

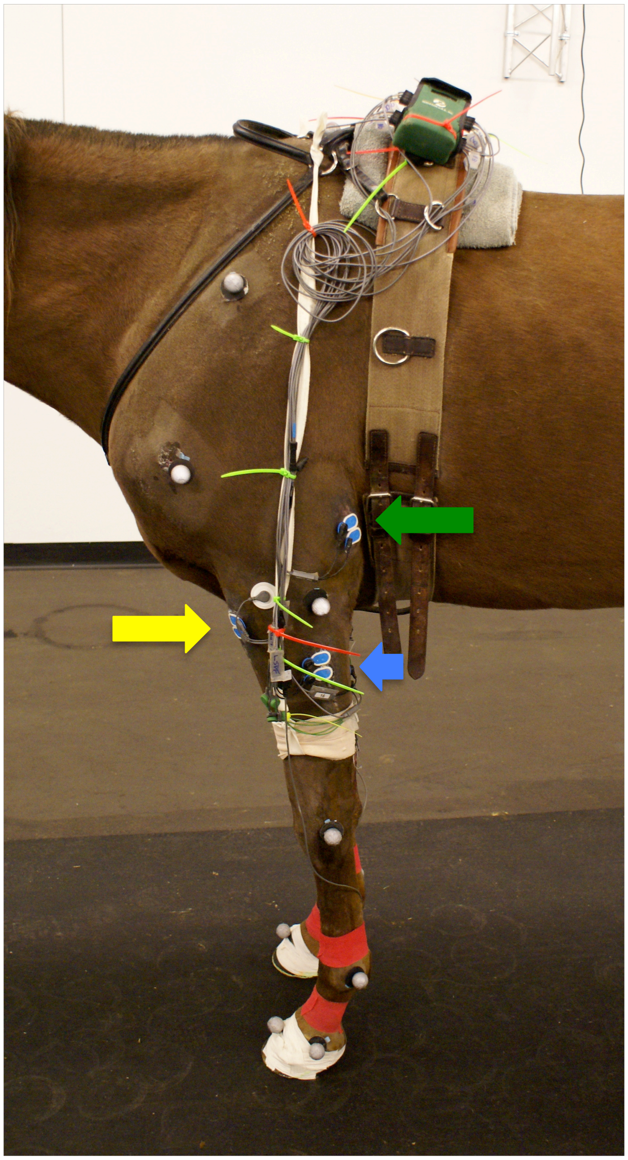

Figure 4.6: Photograph of surface EMG electrodes placed over the extensor carpi radialis (yellow arrow), the ulnaris lateralis (blue arrow head), and the long head of the triceps brachii (green arrow) within the left thoracic limb. Note the pre-amplified cables attached to the surface electrodes are secured with zip ties to a ribbon of tape coursing down the center of limb, this was used to minimize cable motion prior to reaching the telemetry unit (green box) secured to the surcingle.

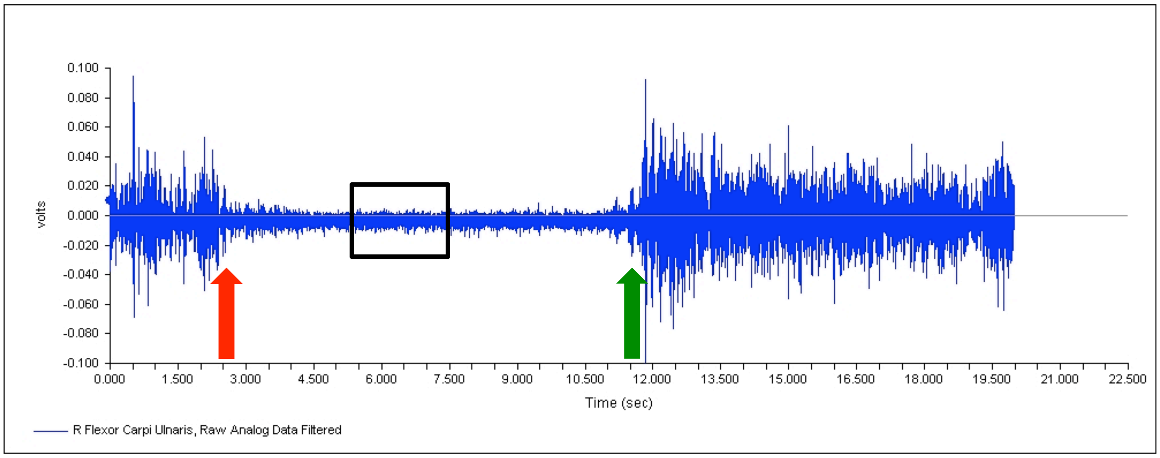

Figure 4.7: Raw EMG signal recorded from the right flexor carpi ulnaris muscle during quiet stance in full weight bearing of the limb (initial 3 seconds), followed by a period of inactivity as the limb is picked up similar to the position adopted when cleaning out the hoof, activation of muscle activity is demonstrated once the hoof is placed back on the ground and the limb is weight bearing again. The red arrow indicates when the limb is no longer weight bearing and the muscle activity is reduced. The green arrow indicates when the limb has been placed on the ground and the initiation of muscle activity during quiet stance. The black square indicates the time period that the un-weighted mean and standard deviation are determined for the flexor carpi ulnaris muscle and used to calculate the appropriate threshold values for determining muscle activation on/off times.

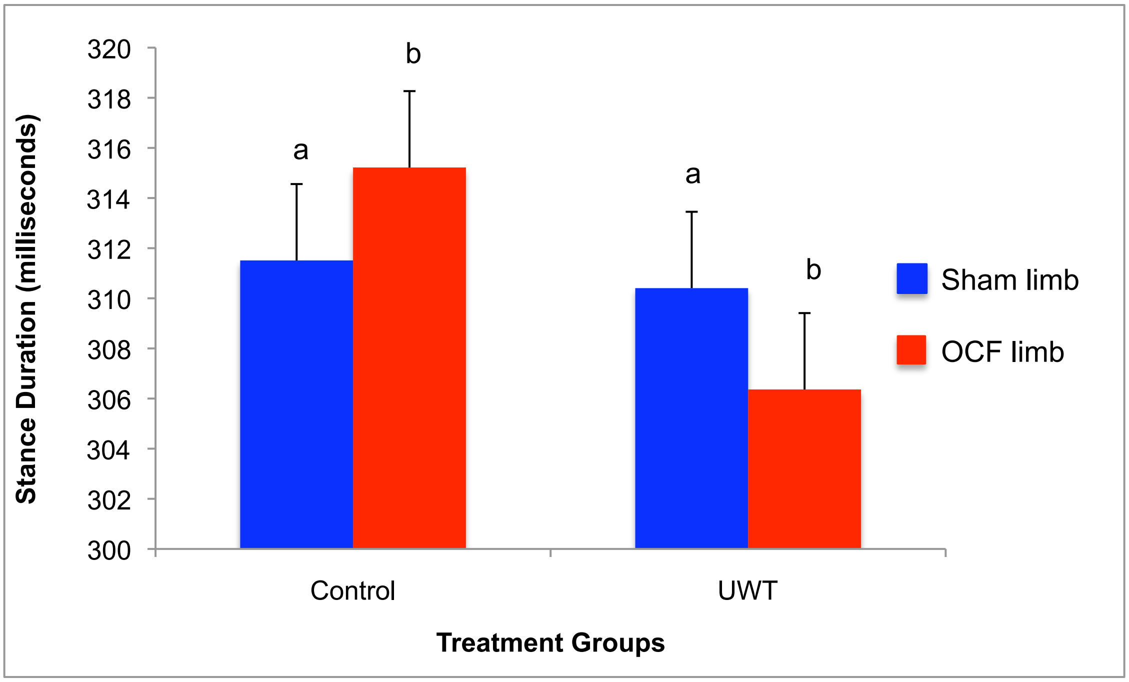

Figure 4.8: Thoracic limb stance duration measured during force platform analysis between the OCF and sham-operated limbs within the control and underwater treadmill groups (mean ± SEM). Different letters indicate significant ($P < 0.05$) differences between the stance durations.

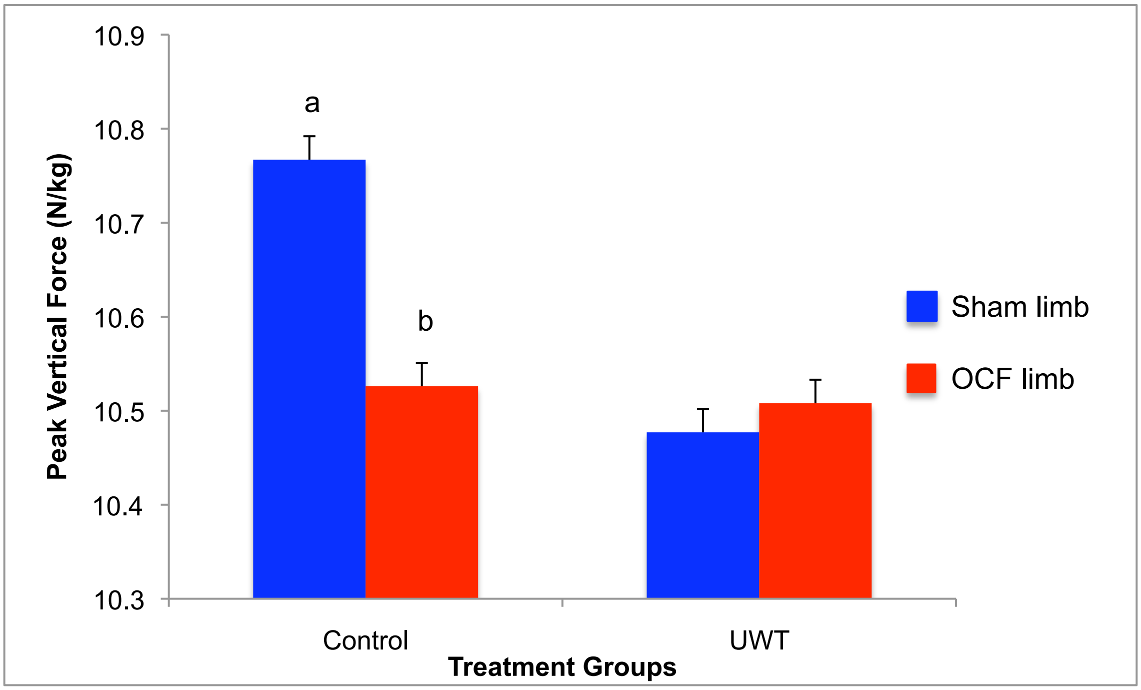

Figure 4.9: Thoracic limb peak vertical force measured during force platform analysis between the OCF and sham-operated limbs within the control and underwater treadmill groups (mean ± SEM). Different letters indicate significant ($P < 0.05$) differences between the thoracic limb peak vertical force values.

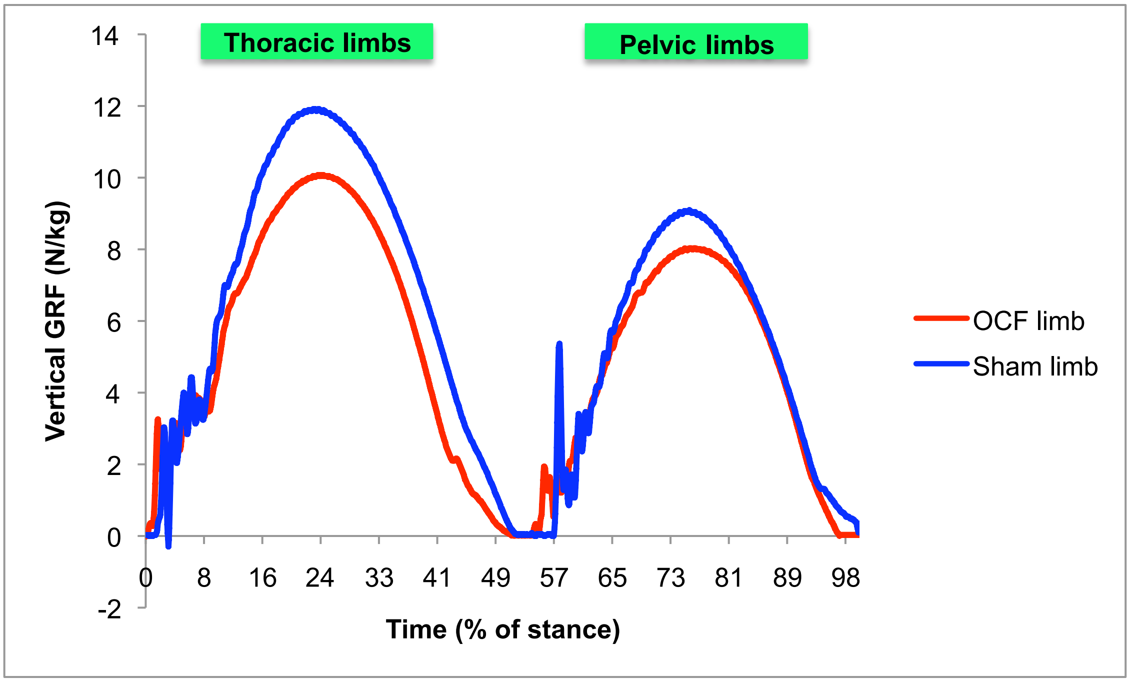

Figure 4.10: Representative vertical ground reaction forces during the stance phase of the OCF thoracic and ipsilateral pelvic limb (red line), compared to the sham-operated thoracic and diagonal pelvic limb (blue line). Note the decrease in the peak vertical ground reaction forces recorded from the OCF compared to the sham-operated thoracic limb, and the significant differences in peak vertical forces between the ipsilateral and diagonal pelvic limbs.

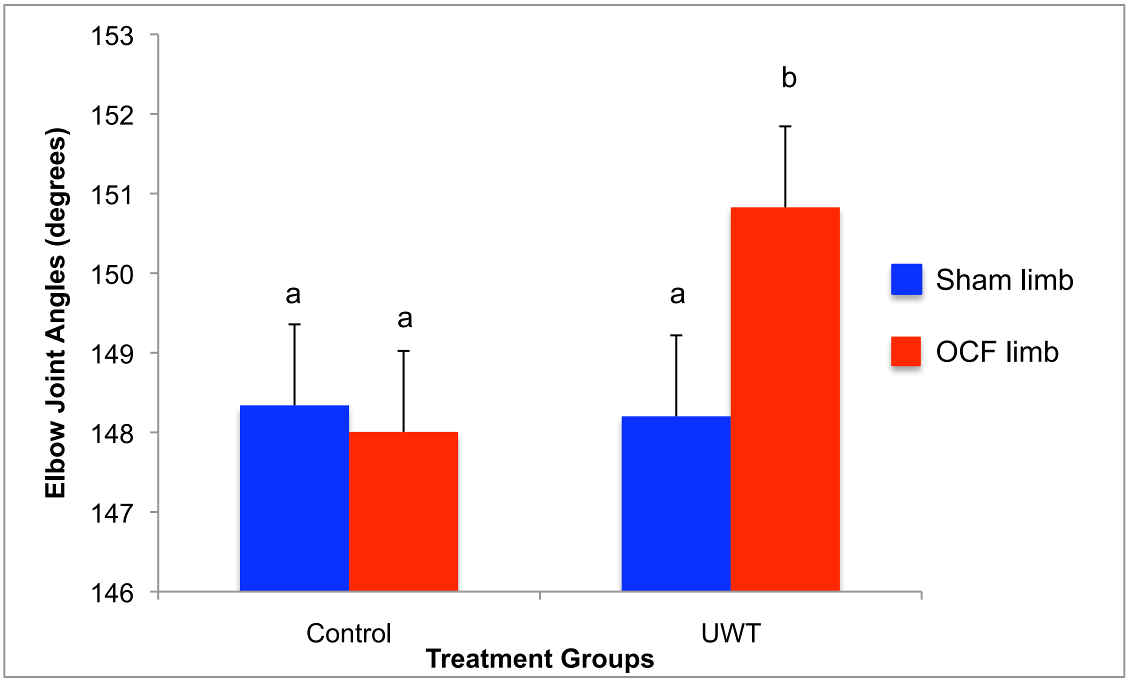

Figure 4.11: Peak elbow joint angles reached during stance phase of gait between the OCF and sham-operated limbs within the control and underwater treadmill groups (mean ± SEM). Different letters indicate significant ($P < 0.05$) differences between the elbow joint peak stance angles.

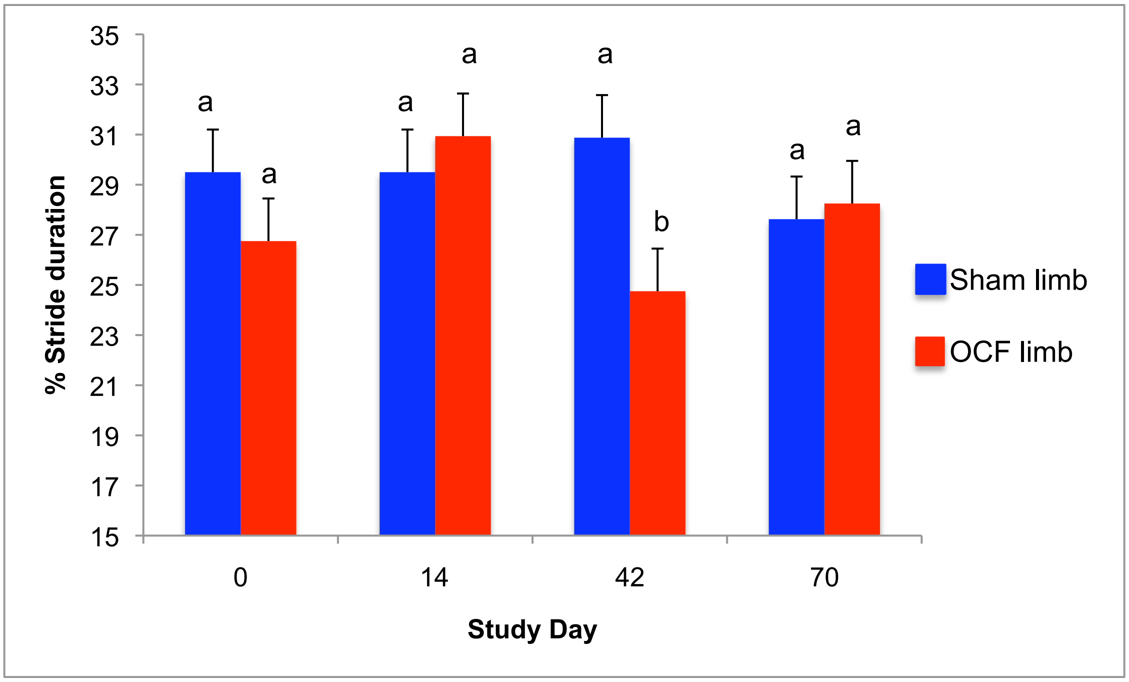

Figure 4.12: Percentage of stride that peak carpal extension was reached between the OCF and sham-operated joints depending on study day (mean ± SEM). Baseline values were included in the graph to demonstrate that no significant difference was present between the thoracic limbs. Different letters indicate significant ($P < 0.05$) differences between the percentages of stride that peak carpal extension was reached.

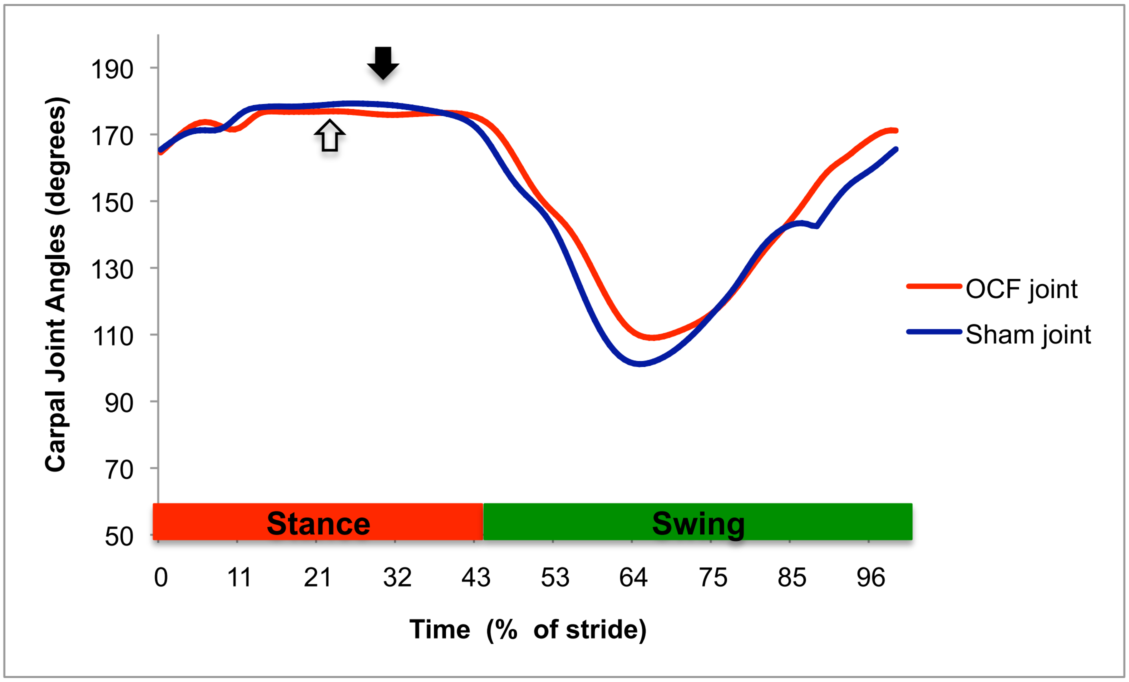

Figure 4.13: Mean carpal joint angle patterns during a single stride cycle recorded on day 42 between the OCF and sham-operated joints. The open arrow indicates timing of peak carpal extension in the OCF joint. The solid black arrow indicates timing of peak carpal extension in the sham-operated joint.

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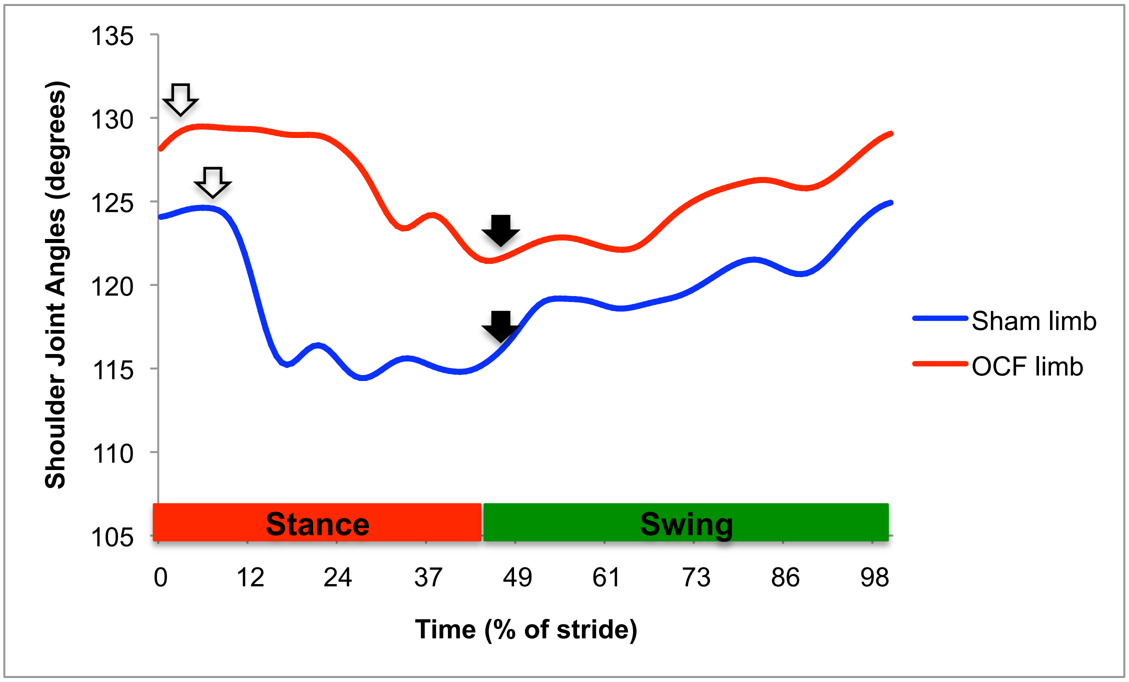

Figure 4.14: Mean shoulder joint angle patterns during a single stride cycle between OCF and sham-operated limbs. The open arrow demonstrates the significant differences in timing of peak extension angles during stance phase. The solid arrows indicates the significant differences in timing of peak flexion angles during swing phase

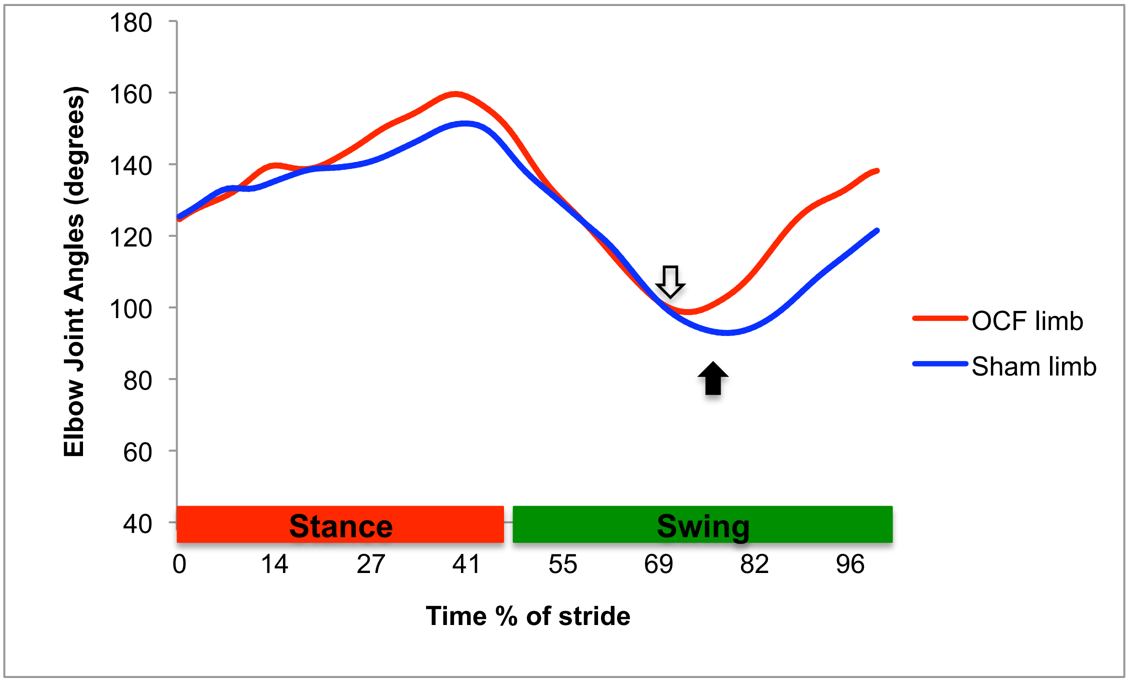

Figure 4.15: Mean elbow joint angle patterns between the OCF and sham-operated limbs normalized to 100% stride duration. The arrows demonstrate significant differences between peak flexion angles reached during swing phase.

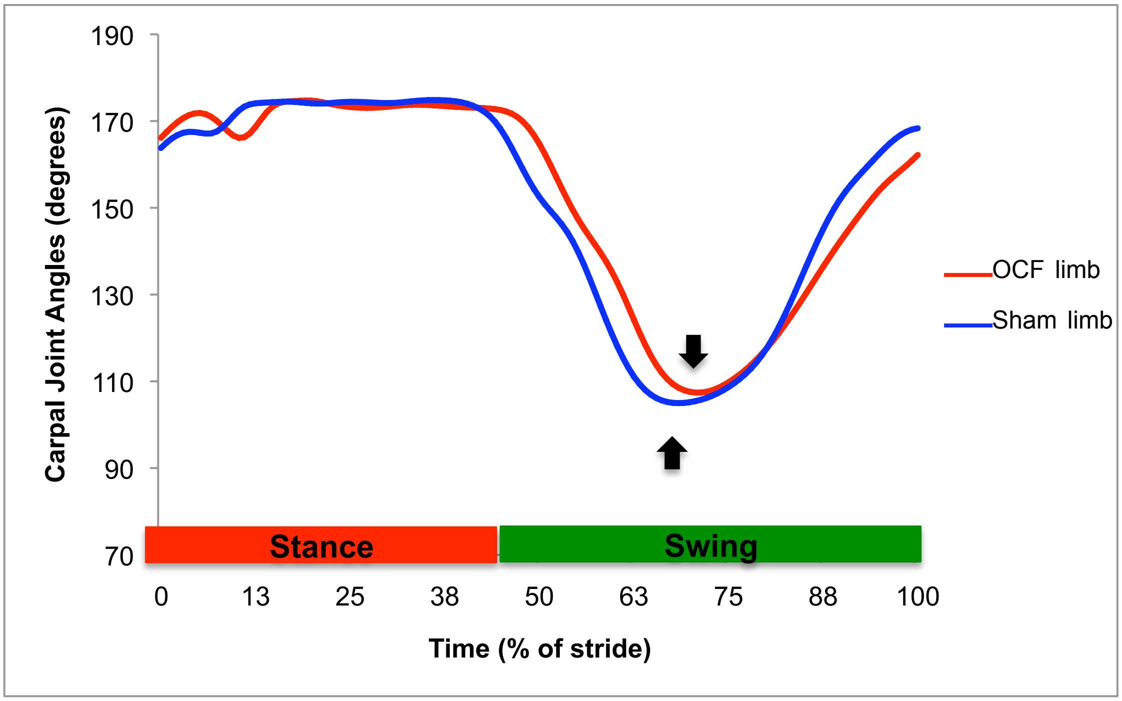

Figure 4.16: Mean carpal joint angle patterns between the OCF and sham-operated joints normalized to 100% stride duration. The arrows demonstrate significant differences between peak flexion angles reached during swing phase.

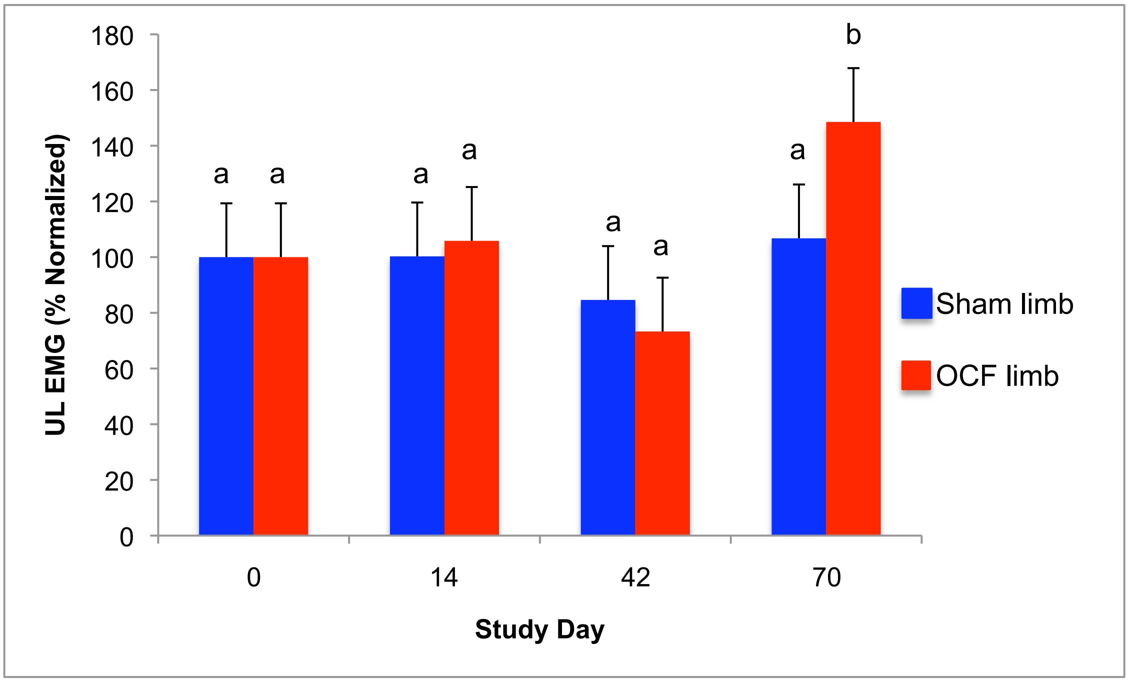

Figure 4.17: Normalized peak EMG amplitude for the UL muscle over the four data collection time points between the OCF and sham-operated limbs (mean ± SEM). Different letters indicate significant ($P < 0.05$) differences between the normalized UL peak EMG amplitudes.

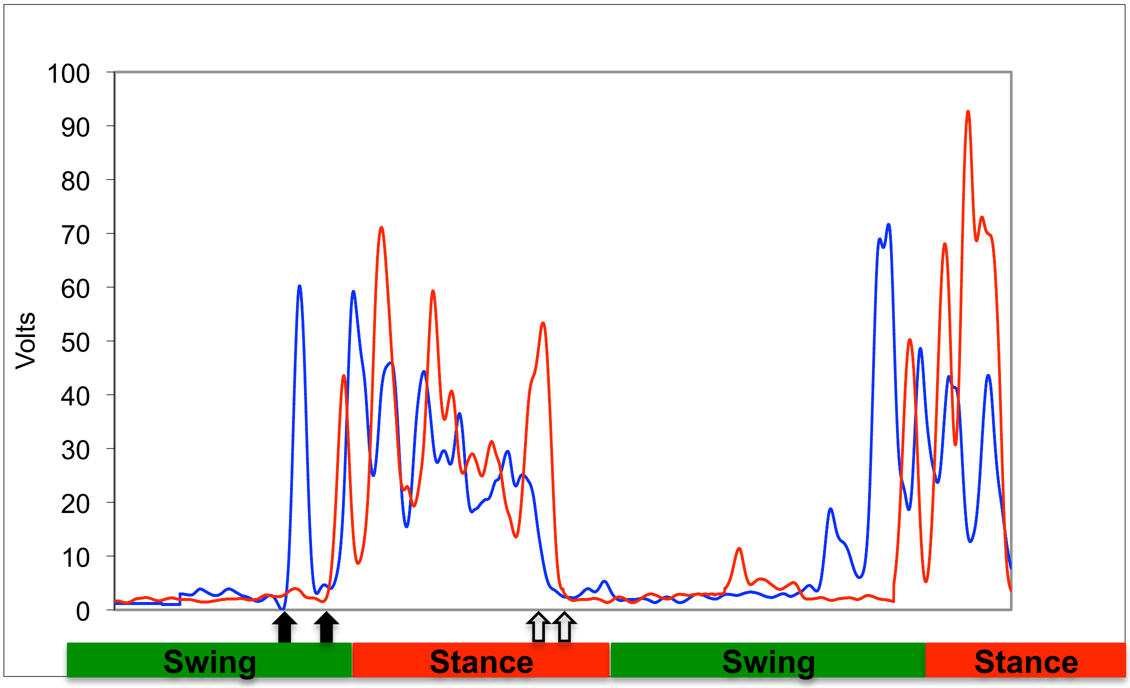

Figure 4.18: Representative ulnaris lateralis muscle activation profile from the OCF (red line) and sham-operated limbs (blue line). The solid black arrows demonstrate the significant difference in the onset of muscle activity within the OCF ulnaris lateralis compared to the ulnaris lateralis muscle onset of activity within the sham-operated limb. The open arrows demonstrate termination of muscle activity within the ulnaris lateralis.

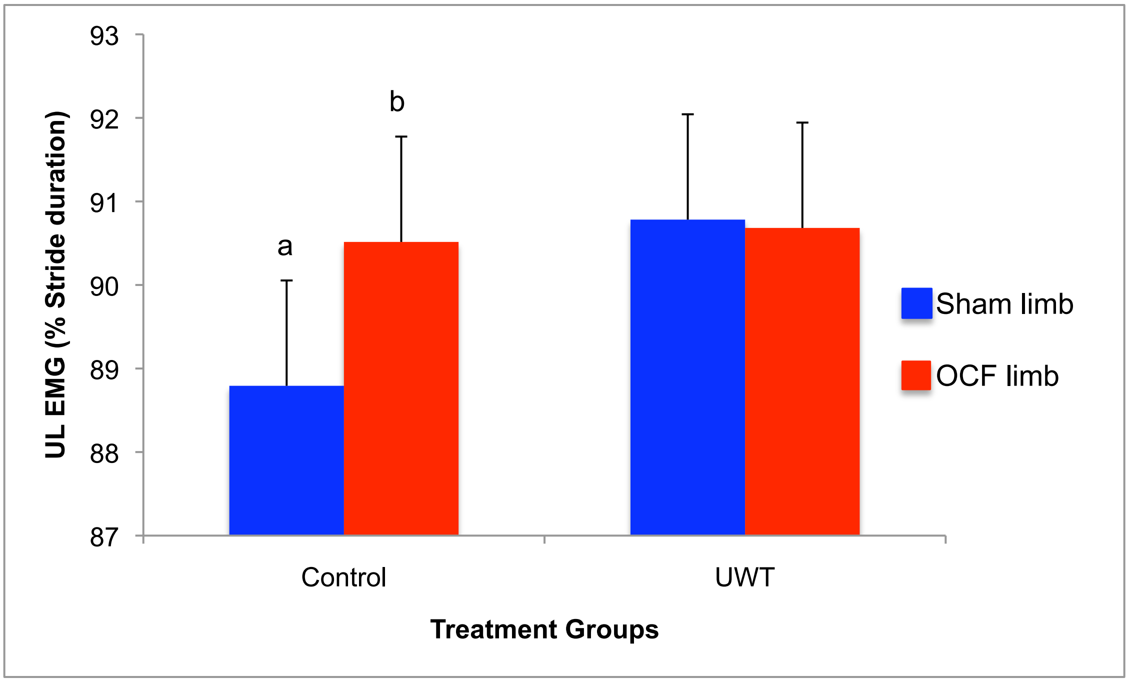

Figure 4.19: Percent of stride duration that the ulnaris lateralis muscle activates (mean ± SEM). Different letters indicate significant ($P < 0.05$) differences between percent onset of UL activity between the OCF and sham-operated limbs within each treatment group.

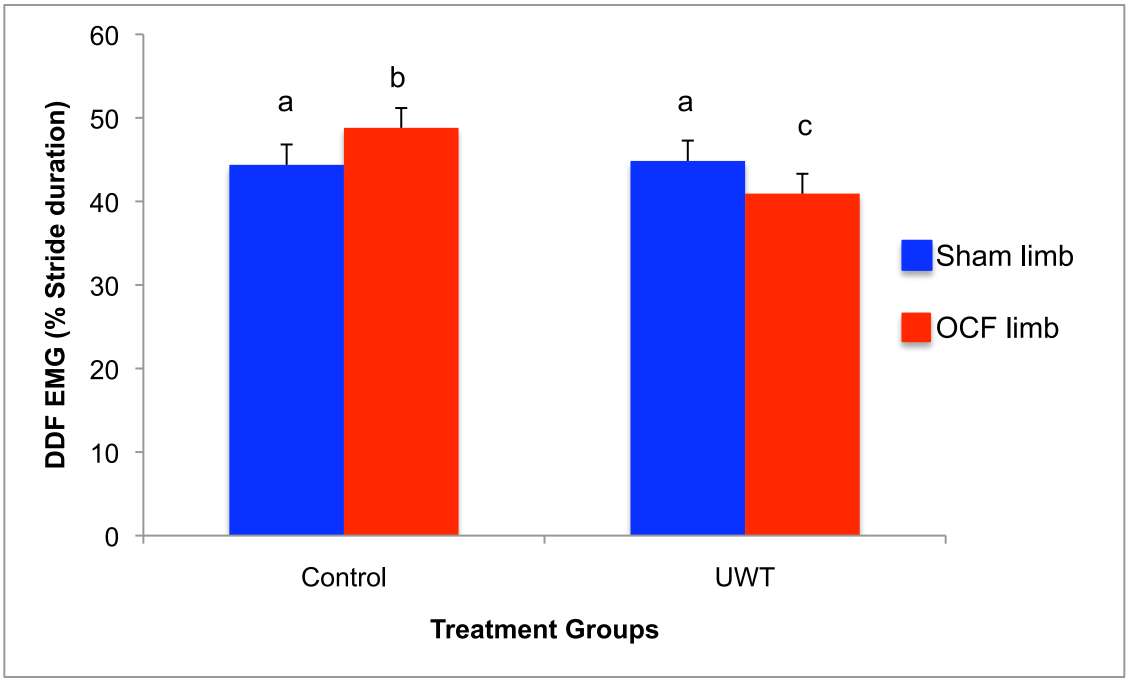

Figure 4.20: Percent of stride duration that the deep digital flexor muscle remains active between the OCF and sham-operated limbs within the control and underwater treadmill groups (mean ± SEM). Different letters indicate significant ($P < 0.05$) differences between percent of stride duration DDF activity.

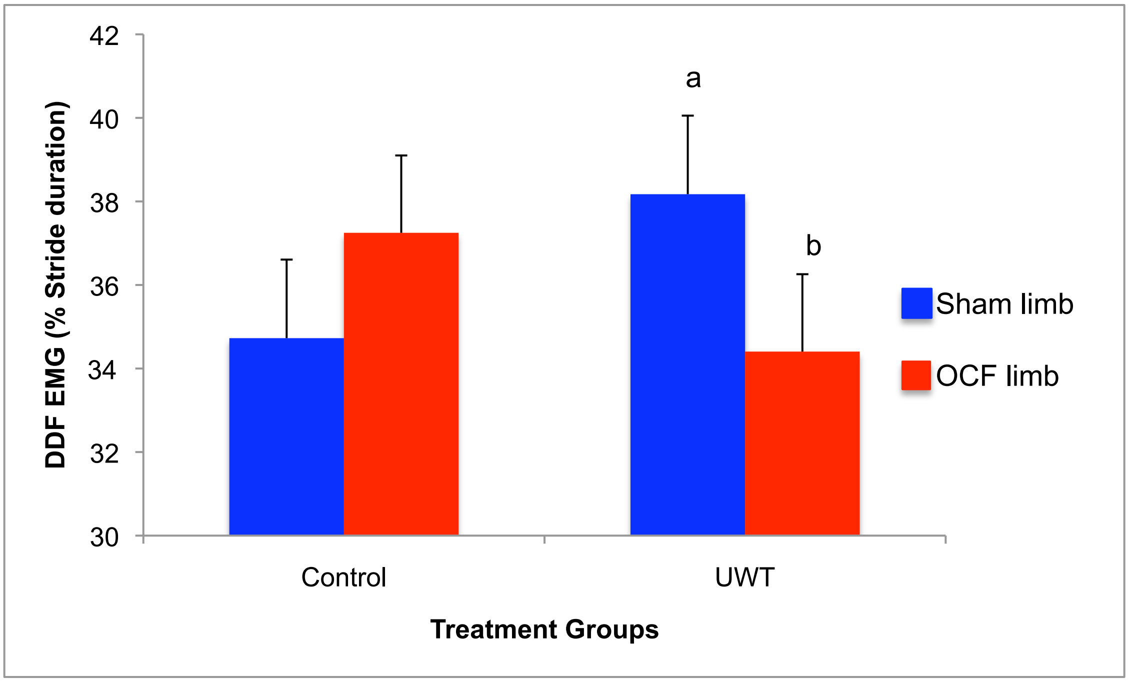

Figure 4.21: Percent of stride duration that the deep digital flexor muscle discontinues activity between the OCF and sham-operated limbs within the control and underwater treadmill groups (mean ± SEM). Different letters indicate significant ($P < 0.05$) differences between the percentages of DDF deactivation.

Table 4.1: Thoracic limb muscles, type of electrode used to record EMG activity, and approximate location of electrode placement.

Thoracic Limb Musculature	Electrode	Location
Extensor Carpi Radialis (ECR)	Surface	Cranial aspect of the radius, 8 cm distal to the level of the olecranon
Ulnaris Lateralis (UL)	Surface	Lateral aspect of the radius, 12 - 15 cm distal to the level of the olecranon
Flexor Carpi Radialis (FCR)	Surface	Medial aspect of the radius, 10 cm distal to the level of the olecranon
Flexor Carpi Ulnaris (FCU)	Surface	Medial aspect of the radius, 10 cm distal to the level of the olecranon
Triceps Brachii – Long Head (TBL)	Surface	25 cm caudal to the point of shoulder, along a horizontal line
Superficial Digital Flexor (SDF)	Fine-wire	Palmar aspect of radius, 9 – 12 cm proximal to the carpal accessory bone, 1 – 1.5 cm depth of electrode insertion
Deep Digital Flexor – Humeral Head (DDF)	Fine -wire	Palmar aspect of radius, 9 – 12 cm proximal to the carpal accessory bone, 2 - 2.5 cm depth of electrode insertion

Table 4.2: Velocity at which horses travelled during each data collection time period from the aquatic therapy and control groups (mean ± SEM).

Gait components	Study Day	Treatment Groups	
		Control	UWT
Velocity (m/s)	0	3.13 ± 0.05	3.17 ± 0.05
	14	3.10 ± 0.05	3.14 ± 0.05
	42	3.12 ± 0.05	3.10 ± 0.05
	70	3.10 ± 0.05	3.15 ± 0.05

Table 4.3: Thoracic limb stride characteristics recorded from the accelerometer signals during a single stride cycle (mean ± SEM).

Gait components	Study Day	Limb	Treatment	
			Control	UWT
Stride Duration	0	OCF	693.0 ± 11.1	685.0 ± 11.1
		Sham	690.0 ± 11.1	687.0 ± 11.1
	14	OCF	694.0 ± 11.1	685.0 ± 11.1
		Sham	690.0 ± 11.1	686.0 ± 11.1
	42	OCF	700.0 ± 11.1	695.0 ± 11.1
		Sham	700.0 ± 11.1	694.0 ± 11.1
	70	OCF	696.0 ± 11.1	686.0 ± 11.1
		Sham	697.0 ± 11.1	683.0 ± 11.1
Stance Duration	0	OCF	325 .0± 11.1	327.0 ± 11.1
		Sham	321.0 ± 11.1	326.0 ± 11.1
	14	OCF	322 .0± 11.1	328.0 ± 11.1
		Sham	319.0 ± 11.1	325.0 ± 11.1
	42	OCF	330.0 ± 11.1	328.0 ± 11.1
		Sham	323.0 ± 11.1	330.0 ± 11.1
	70	OCF	324.0 ± 11.1	319.0 ± 11.1
		Sham	323.0 ± 11.1	322.0 ± 11.1
Swing Duration	0	OCF	368.0 ± 10.5	358.0 ± 10.5
		Sham	368.0 ± 10.5	360.0 ± 10.5
	14	OCF	373.0 ± 10.5	359.0 ± 10.5
		Sham	371.0 ± 10.5	360.0 ± 10.5
	42	OCF	371.0 ± 10.5	367.0 ± 10.5
		Sham	376.0 ± 10.5	364.0 ± 10.5
	70	OCF	372.0 ± 10.5	367.0 ± 10.5
		Sham	374.0 ± 10.5	361.0 ± 10.5
% Stance Duration	0	OCF	47.0 ± 1.3	47.0 ± 1.3
		Sham	47.0 ± 1.3	47.0 ± 1.3
	14	OCF	46.0 ± 1.3	48.0 ± 1.3
		Sham	46.0 ± 1.3	47.0 ± 1.3
	42	OCF	47.0 ± 1.3	47.0 ± 1.3
		Sham	46.0 ± 1.3	48.0 ± 1.3
	70	OCF	47.0 ± 1.3	46.0 ± 1.3
		Sham	46.0 ± 1.3	47.0 ± 1.3

Table 4.3 continued:

Gait components	Study Day	Limb	Treatment Control	UWT
% Swing Duration	0	OCF	53.0 ± 1.3	52.0 ± 1.3
		Sham	53.0 ± 1.3	53.0 ± 1.3
	14	OCF	54.0 ± 1.3	52.0 ± 1.3
		Sham	54.0 ± 1.3	53.0 ± 1.3
	42	OCF	53.0 ± 1.3	53.0 ± 1.3
		Sham	54.0 ± 1.3	53.0 ± 1.3
	70	OCF	53.0 ± 1.3	54.0 ± 1.3
		Sham	54.0 ± 1.3	53.0 ± 1.3

Table 4.4: Thoracic limb joint angle characteristics during a stride cycle associated with the presence or absence of an osteochondral fragment (mean ± SEM). An asterisk indicates significant ($P < 0.05$) differences between the OCF and sham-operated joint angle variables.

Joint	Limb	Peak Stance Angle	Peak Swing Angle	% Stride Peak Extension	% Stride Peak Flexion
Shoulder	OCF	128.0 ± 0.7 *	120.0 ± 0.5 *	2.85 ± 0.4	53.0 ± 1.2
	Sham	125.0 ± 0.7 *	117.0 ± 0.5 *	2.44 ± 0.4	53.0 ± 1.2
Elbow	OCF	149.0 ± 0.7	99.0 ± 0.5 *	46.0 ± 0.8	88.0 ± 0.7
	Sham	148.0 ± 0.7	97.0 ± 0.5 *	47.0 ± 0.8	89.0 ± 0.7
Carpus	OCF	177.0 ± 0.3	107.0 ± 1.0 *	28.0 ± 0.9	75.0 ± 1.0
	Sham	176.0 ± 0.3	104.0 ± 1.0 *	29.0 ± 0.9	75.0 ± 1.0
Fetlock	OCF	249.0 ± 0.9	184.0 ± 0.2	30.0 ± 0.7	73.0 ± 1.2
	Sham	249.0 ± 0.9	184.0 ± 0.2	31.0 ± 0.7	75.0 ± 1.2

Table 4.5: Thoracic limb electromyography variables recorded from select thoracic limb muscles during a stride cycle associated with the presence or absence of an osteochondral fragment (mean ± SEM). An asterisk indicates significant ($P < 0.05$) differences between the OCF and sham-operated EMG variables.

Muscle groups	Limb	Normalized Peak EMG Amplitude	% Onset Muscle Activation	Duration Activation (ms)	% Duration Activation	% Muscle Deactivation
ECR	OCF	93.0 ± 10.5	64.0 ± 1.1	133.0 ± 7.4	20.0 ± 1.1	83.0 ± 1.0
	Sham	99.0 ± 10.5	64.0 ± 1.1	134.0 ± 7.4	19.0 ± 1.1	83.0 ± 1.0
UL	OCF	107.0 ± 12.7	91.0 ± 0.8*	307.0 ± 10.1	45.0 ± 1.3	35.0 ± 1.3
	Sham	98.0 ± 12.7	89.0 ± 0.8*	314.0 ± 10.1	46.0 ± 1.3	36.0 ± 1.3
FCR 1st Activation	OCF	136.0 ± 15.5	10.0 ± 0.94	126.0 ± 10.2	19.0 ± 1.5	29.0 ± 1.7
	Sham	138.0 ± 15.5	9.0 ± 0.94	125.0 ± 10.2	18.0 ± 1.5	28.0 ± 1.7
FCR 2nd Activation	OCF	136.0 ± 15.5	54.0 ± 0.8	149.0 ± 8.1	21.0 ± 0.9	75.0 ± 0.9
	Sham	138.0 ± 15.5	54.0 ± 0.8	147.0 ± 8.1	21.0 ± 0.9	76.0 ± 0.9
FCU	OCF	116.0 ± 5.8	93.0 ± 0.9	288.0 ± 10.4	42.0 ± 1.5	34.0 ± 1.2
	Sham	106.0 ± 5.8	93.0 ± 0.9	277.0 ± 10.4	40.0 ± 1.5	33.0 ± 1.2
TBL	OCF	214.0 ± 22.5 *	82.0 ± 6.2	254.0 ± 11.1	37.0 ± 1.5	18.0 ± 6.3
	Sham	126.0 ± 20.9 *	80.0 ± 6.2	247.0 ± 11.0	36.0 ± 1.5	16.0 ± 6.2
SDF	OCF	269.0 ± 71.7	90.0 ± 2.2	280.0 ± 11.6	41.0 ± 1.6	34.0 ± 1.6
	Sham	74.0 ± 69.1	89.0 ± 2.3	285.0 ± 12.1	42.0 ± 1.7	33.0 ± 1.7
DDF	OCF	80.0 ± 38.0	86.0 ± 1.4*	306.0 ± 17.9	45.0 ± 1.7	35.0 ± 1.3
	Sham	129.0 ± 66.0	91.0 ± 1.4*	334.0 ± 17.7	45.0 ± 1.7	36.0 ± 1.3

SUMMARY AND CONCLUSIONS TO DISSERTATION

In summary, this study supported the goal of the project by evaluating the effectiveness of underwater treadmill exercise to reduce morbidities associated with carpal OA. Aquatic therapy for the management of carpal OA demonstrated enhanced static postural control under varying stance conditions, both clinical sign and disease-modifying improvements, evenly distributed thoracic limb axial loading and symmetrical timing of select thoracic limb musculature. This study also raised several key points that are crucial to future studies identifying the effects of aquatic therapy for the treatment of musculoskeletal injuries. In addition, this study identified that future work is required to establish the biomechanical gait disturbances associated with various orthopaedic conditions. Understanding the neuromuscular pathophysiology involved in static and dynamic joint stability and limb function is crucial for developing targeted therapies aimed at diminishing the deleterious effects of OA.

The first key point recognizes that the ability to maintain postural control should also be studied under more dynamic conditions, including disturbances generated by external sources such as force platform oscillations or alterations in ground surfaces and textures. Alterations in postural control during quiet standing may influence performance on balance required tasks. Dynamic balance control involves proprioceptive perturbations and the ability to transform the afferent information into a proper motor response. An understanding of the different strategies adopted in order to maintain equilibrium depending on the nature of the task, static or dynamic will aid in indentifying patterns that may be indicative of potential increased risk for further injury.

The current study used a model of OA that effectively induced significant clinical, gross, histologic, and biochemical changes indicative of OA. Horses exercised in the underwater treadmill demonstrated elevated clinical lameness, intra-articular pressure, and total protein concentrations one week after aquatic therapy had been initiated. The elevated inflammatory and pain response may have been due to increased tension placed over the region of the osteochondral fragment during aquatic therapy. It may be more appropriate for the initial management of OA to exercise at a lower water level, promoting an increase in joint flexion and extension ranges of motion without the initial resistance of water at higher depths. Anecdotally, water at varying depths promotes joint specific increases in ranges of motion, therefore providing the ability to adapt therapeutic protocols to target certain joints. However, the increases in joint range of motion at lower depths must be weighed against the importance of decreasing load-bearing stress across the joint surface provided by the effects of buoyancy. Therefore, additional research is needed to establish changes in joint biomechanics in association with varying depths of water during aquatic therapy in order to provide validated treatment protocols.

Lastly, EMG was used to detect the amplitude, timing, and duration of muscle activity crucial for understanding the role of individual muscles in movement strategies adopted following the induction of an osteochondral fragment. The low grade OA produced with this model may have limited the ability to discern normal and pathologic neuromuscular responses to carpal joint pain and inflammation. Furthermore, the inherent variability associated with EMG data collection and the intrinsic anatomical differences within muscles compound the difficulty in assessing EMG changes over time. However, it is essential that future studies address the issue of distinguishing normal and pathologic neuromuscular changes associated with joint pathology. A better understanding of these defense mechanisms is expected to provide the equine practitioner with improved treatment and rehabilitation protocols that are focused not only on treating the primary injury, but also preventing the development of OA and other compensatory musculoskeletal injuries.

Overall, improvements in a number of outcome parameters investigated contribute to the authors enthusiasm for the continued use of aquatic therapy for the management of OA in the horse.