Spring 2018

Evaluation of Conventional, Regenerative, and Alternative Therapies for Common Ailments in the Respiratory and Musculoskeletal Systems of the Performance Horse

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EVALUATION OF CONVENTIONAL, REGENERATIVE, AND ALTERNATIVE THERAPIES FOR COMMON AILMENTS IN THE RESPIRATORY AND MUSCULOSKELETAL SYSTEMS OF THE PERFORMANCE HORSE

By

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B.S., California Polytechnic State University, San Luis Obispo, 2016

A Research Paper

Submitted in Partial Fulfillment of the Requirements for the

Master of Science.

Department of Animal Science, Food and Nutrition

In the Graduate School

Southern Illinois University Carbondale

May 2018

RESEARCH PAPER APPROVAL

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February 9th, 2018

ACKNOWLEDGMENTS

I would like to acknowledge and thank my major professor, Dr. Rebecca Atkinson, for taking me on as her graduate student, and endless support throughout the completion of my Master's degree. Her knowledge, support, and patience throughout this entire experience is highly appreciated. I would also like to thank my committee members, Dr. Jennie Cook and Stephanie Speiser, M.S. for their extensive knowledge and support while obtaining my degree.

Most importantly, I would like to thank my family and close friends who have supported me every step of the way. This entire support system has meant the world to me and is the backbone of my academic and personal success.

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Acute Phase Protein (APP)

Acute Phase Response (APR)

Adenosine Triphosphate (ATP)

Aspartate Aminotransferase (AST)

Association of Racing Commissioners International (ARCI)

Autologous Conditioned Serum (ACS)

Bronchoalveolar Lavage (BAL)

Central Nervous System (CNS)

Chronic Exertional Rhabdomyolysis (CER)

C-Reactive Protein (CRP)

Creatine Kinase (CK)

Cyclooxygenase (COX)

Dental Pulp Stem Cell (DPSC)

Dihydropyridine Receptors (DHPR)

Exercise Induced Pulmonary Hemorrhage (EIPH)

Exertional Rhabdomyolysis (ER)

Exhausted Horse Syndrome (EHS)

Febrile Nonhemolytic Reaction (FNHTR)

Federation Equestre Internationale (FEI)

Food and Drug Authority (FDA)

Glycogen Branching Enzyme (GBE1)

Glycogen Branching Enzyme Deficiency (GBED)

Glycogen Synthase 1 Gene (GYS1)

Inflammatory Airway Disease (IAD)

Interleukin-1β (IL-1β)

Interleukin-1 Receptor Antagonist Protein (IRAP)

Interleukin-6 (IL-6)

Mesenchymal Stem Cell (MSC)

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Osteoarthritis (OA)

Platelet Rich Plasma (PRP)

Polysaccharide Storage Myopathy (PSSM)

Recurrent Airway Obstruction (RAO)

Recurrent Exertional Rhabdomyolysis (RER)

Serum Amyloid A (SAA)

Sporadic Exertional Rhabdomyolysis (SER)

Superficial Digital Flexor Tendon (SDFT)

Tumor Necrosis Factor-α (TNF-α)

United States Equestrian Federation (USEF)

White Blood Cells (WBC)

HEADING 1

INTRODUCTION

Many common ailments in equine related to performance or that reduce performance, originate in the respiratory and musculoskeletal systems. The respiratory system is responsible for proper ventilation and respiration, and the musculoskeletal system is responsible for muscle contraction and locomotor movement both at rest and during exercise. While these two systems appear to be highly developed, they are the source of common problems during performance that will be further discussed in this paper. Within the respiratory system, inflammation is extremely common, and can lead to more serious conditions such as recurrent airway obstruction and exercise induced pulmonary hemorrhage (Mair et al., 2013; Couëtil et al., 2016). Within the musculoskeletal system, there are varying levels of fatigue and exhaustion as well as lameness (Marlin and Nankervis, 2002) which is considered by veterinarians as the number one cause of issues in the performance horse. Since horses are considered “performance animals”, it is extremely important to address and treat any conditions that lead to a reduction or inhibition of performance.

Currently, there are conventional, regenerative, and alternative methods used to treat common exercise related ailments. Conventional treatments consist of commonly used drugs and medications but can generally not be used on a long-term basis due to well-known side-effects. Nonsteroidal anti-inflammatory drugs, for example, possess great relieving properties, but can also result in gastrointestinal ulcers due to their role in blocking gastrointestinal prostaglandins (Dowling, 2002; Bland, 2015). To combat the issues with conventional treatments, regenerative and alternative therapies have been developed in attempt to provide similar treatment of exercise related issues with fewer side-effects. Stem cell therapy, physical therapies, nervous system

stimulation, and supplementation of vitamins and minerals have all been evaluated and have begun to be utilized by many horse owners. Fundamentally, it is necessary to understand how exercise normally affects the equine respiratory and musculoskeletal systems to further comprehend how these various treatments work to resolve the potential ailments and abnormal responses.

HEADING 2

EXERCISE

Any form of movement or activity being performed by the horse is considered exercise; if they are moving from a resting position, work is being done (Marlin and Nankervis, 2002). For exercise and muscle contraction to occur, the generation of ATP is required. Within the muscle, there is a minimal store of ATP that can only maintain muscular contractions for up to a few seconds (Hodgson et al., 2013). At the initiation of exercise creatine phosphate is broken down into creatine and phosphate by the enzyme creatine kinase (CK) within the skeletal muscle, producing a very rapid, yet very small amount of ATP (Reed et al., 2010a). Beyond this limited time frame, there is an increased metabolic breakdown of fuel stores to meet the increased demand for ATP (Hodgson et al., 2013). Metabolism can be broken down into two forms: aerobic and anaerobic. Aerobic, or oxidative, metabolism primarily breaks down lipids in a slow, energy-requiring process known as oxidative phosphorylation. Anaerobic metabolism primarily breaks down carbohydrates, generally in the form of glucose, to lactic acid through the much more rapid process of glycolysis. One of the primary differences between these two pathways is that aerobic metabolism requires oxygen, whereas anaerobic metabolism can occur in the absence of oxygen or when the oxygen supply is insufficient (Marlin and Nankervis, 2002; Hodgson et al., 2013). The activation of each of these processes is dependent on the intensity and duration of the exercise, and fundamentally the demand for energy at the level of the muscles (Hodgson et al., 2013).

Intensity and Duration

Intensity is directly related to the speed of exercise (Hodgson et al., 2013) as well as the level of effort required to undergo an activity (CDC, 2015). Duration is the total length of time

the exercise is performed (Hodgson et al., 2013). Thoroughbred and quarter horse racing would be examples of high intensity, short duration exercise; a large amount of work is exerted over a short period of time utilizing anaerobic metabolism. Show training and competing in multiple events within a single day would be an example of a medium intensity, medium duration exercise; work is being performed but not necessarily to a level of fatigue, using a combination of anaerobic and aerobic metabolism. Lastly, endurance racing would be an example of low intensity, long duration exercise where aerobic metabolism is necessary to meet the high, long-term demand for energy (KER, 2002). High intensity exercises are necessary to improve strength and anaerobic capacity, whereas low to medium intensities will lead to improvement in aerobic capacity. The opposite is true for duration; long durations will lead to improvement in aerobic capacity, and short durations, coupled with higher intensity, can improve strength and anaerobic capacity (Hodgson et al., 2013). Comprehending theses different levels of performance that horses undergo is critical to understand not only how their bodies will change and adapt but also how physiological consequences can develop.

Responses to Exercise

During any increase in level of exercise and muscular activity, there is an increase in the presence of exercise responses which help meet the short-term, physiological demands before returning to normal levels (Marlin and Nankervis, 2002). Within the respiratory system, increases in respiratory rate and the total volume of air being ventilated would be considered exercise responses because they change during exercise and then return to normal levels once exercise has ceased (Hodgson et al., 2013). Within the musculoskeletal system, increases in lactic acid as a byproduct of metabolism would also be considered an exercise response (Marlin

and Nankervis, 2002). Any of these short-term exercise responses should return to their baseline levels once the work is done.

Alternatively, conditioned responses, sometimes referred to as training responses, are long-term adaptations to repeated bouts of muscular activity. As there is a gradual increase in the amount of work being performed, the physiological systems will adapt to meet the increasing demands (Marlin and Nankervis, 2002) and reduce the strain associated with increased amount of stress placed on the body (Hinchcliff et al., 2007). Within the respiratory system, a noticeable conditioned response is an increase the horse's maximum oxygen uptake, allowing for a greater delivery of oxygen to the muscles (Hodgson et al., 2013). Within the musculoskeletal system, training has been observed to lead to an increased number of capillaries supplying the muscle (Marlin and Nankervis, 2002), as well as more efficient removal of lactic acid produced from metabolism (Hodgson et al., 2013).

HEADING 3

RESPIRATORY SYSTEM

To fully understand physiological respiratory issues during exercise in equine, it is necessary to have a comprehensive knowledge of the basic anatomy and its correlating physiological responses perceived as normal. Some of these physiological responses include how the circulation of blood interacts with the respiratory tract, the process of respiration and ventilation, the normal amount of air inspired and expired, and frequency of breathing. In equine, the most noticeable physiological changes to exercise within the respiratory system are short-term physiological adaptations and adjustments the body makes during the stress of exercise but generally return to what is considered normal at rest once the stress is removed. The respiratory system of the horse has adapted tremendously for short term responses to exercise. Some of these adaptations include large airways that allow for reduced resistance, energetically efficient breathing, extreme lung capacity, large volumes of blood supplied to the lungs, as well as an elongated respiratory system that strongly influences the air as it is inspired and expired (Marlin and Nankervis, 2002). The primary exercise responses documented within the equine system revolve around ventilation, perfusion, the ventilation perfusion interaction, respiratory-locomotor coupling, thermoregulation, work of breathing, and intrathoracic pressures and forces. While there are numerous short-term responses, the equine respiratory system is neither suited for long-term nor conditioned exercises responses, thus leading to physiological performance issues.

Anatomy

The respiratory system is divided into two sections: the upper and lower airways of the respiratory tract. The primary function throughout the entire airway is to transport oxygen from the external environment down to the level of the muscle through the process of gas exchange,

where it is then utilized in the production of energy (Hodgson et al., 2013). The upper airway consists of the nares, nasal cavities, soft palate, pharynx, larynx, trachea, bronchi, and alveoli-free bronchioles (Hodgson et al., 2013). This portion of the airway is referred to as the conducting zone, where no gas exchange occurs and the primary function is transportation of gases from the external environment to the locations of gas exchange like water moving through a pipe; it also warms, humidifies, and filters the air (Marlin and Nankervis, 2002; Raff and Levitzky, 2011). The lower airway is comprised of the respiratory bronchioles, alveolar ducts and alveolar sacs located within the lung. This portion of the airway is referred to as the respiratory zone since its primary function is gas exchange.

Throughout the entire airway, nerves and muscles stimulate its functional capacities. At the level of the lungs, the pulmonary and bronchial circulations function to transport diffused oxygen to its necessary target locations (Raff and Levitzky, 2011; Hodgson et al., 2013).

The equine nares, or nostrils, are incredibly important features of the respiratory system. The horse is known as an obligate nose breather, meaning it lacks the ability to inspire and expire air through its mouth (Marlin and Nankervis, 2002; Hodgson et al., 2013). The horse relies solely on the capabilities of the nares to trap and funnel the air into the respiratory tract. The extremely large and mobile properties of the nostrils allow for expansion during the inspiratory process, leading to flaring of the nares and then the subsequent collapse of the nasal diverticulum (Hodgson et al., 2013). The large surface area of the nasal cavities not only provides a large source for airflow resistance, but aids in the conducting zone functions of heat and water exchange, warming and humidifying the air as it is transported (Hodgson et al., 2013). Increases and decreases to airflow resistance in this portion of the airway can be controlled by

vasoconstriction and vasodilation of the vasculature through both sympathetic and parasympathetic innervation of nerve fibers (Hodgson et al., 2013).

The pharynx is divided into the nasopharynx and the oropharynx by the soft palate. The opening of the soft palate is known as the intrapharyngeal ostium and articulates with the conriculate cartilages and epiglottis of the larynx to form an airtight seal during breathing (Hodgson et al., 2013). The function of the larynx is to channel the air flow from the nasal passages down to the trachea, also aiding in the protection of the airway by closing during food consumption (Hodgson et al., 2013; Shrauner, 2013). During ingestion, the laryngeal structures adduct to form a tight seal, preventing food from entering the airway. During times such as exercise, the laryngeal structures abduct and intrinsic muscles contract, creating maximal opening of the larynx and decreased resistance to air flow (Hodgson et al., 2013). This specific anatomy of the laryngeal structures creates a physiological limitation, rendering the horse incapable of switching from nose breathing to mouth breathing during intense exercise as other species are able to. The rima glottidis, the opening of the larynx into the trachea, is the narrowest portion of the entire airway and if even partially obstructed can have a huge impact on airflow and performance (Shrauner, 2013).

The equine trachea is a highly flexible but rigid tube approximately 70 to 80 centimeters long, that extends from the larynx down to the lungs, including numerous dorsally open hyaline cartilaginous rings that help to maintain its structure and prevent collapse in this portion of the airway (Mair and Lane, 2005; Hodgson et al., 2013). The dorsal portion of the trachea is lined with a layer of smooth muscle, the dorsal tracheal membrane, controlled via the autonomic nervous system, that actually decreases tracheal compliance through smooth muscle contraction (Mair and Lane, 2005; Hodgson et al., 2013). This decreased compliance unfortunately increases

the possibility of collapse in the extra-thoracic portions of the trachea during forced inspiration, such as during heavy exercise (Hodgson et al., 2013). Between the cartilaginous rings and smooth muscle contraction it is almost an anatomical balancing act to maintain the opening of this airway. As the trachea continues down towards the lungs, it branches into two principle bronchi; termed the tracheal bifurcation (Hodgson et al., 2013). From this point, a series of branching occurs from the principle bronchi into segmental bronchi, then bronchioles, and ending at the terminal bronchioles (Hodgson et al., 2013). With each level of branching, there is a decrease in individual radius, but an overall increase in surface area across the structure for optimal levels of gas exchange (Raff and Levitzky, 2011).

The highly developed lung of the horse accounts for approximately 1% of the horses' overall body weight; its large capacity plays an important factor in high intensity exercise (Marlin and Nankervis, 2002; Hodgson et al., 2013). The left side of the lung can be divided into the apical lobe, a diaphragmatic lobe, and an accessory lobe; the right side of the lung can be divided into an apical lobe and a diaphragmatic lobe (Hodgson et al., 2013). The equine lung is not separated by intra-lobular fissures such as in other mammals, but is conjoined via adhesion in the mediastinum at its base (Sande and Tucker, 2004). The incomplete connective tissue septa between the lobules of the equine lung permits the transfer of air from lobule to lobule via accessory pathways (Marlin and Nankervis, 2002; Hodgson et al., 2013). This small amount of collateral ventilation plays a compensatory role during the occurrence of obstructive diseases (Hodgson et al., 2013), which will be discussed later on. While the lobules combine to form the segments and lobes of the lung, they remain in a state of isolation, separated by their individual broncho-vascular components (Sande and Tucker, 2004). Specifically in equine, the respiratory bronchioles are not sufficient enough to participate in gas exchange, so primary gas exchange

actually occurs further down in the lung at the level of the alveolarcapillaries (Hodgson et al., 2013). The equine lung contains approximately 10 million alveoli with a large surface density yet thin septa, and capillary numbers of more than 1000 times that (Hodgson et al., 2013).

There are three categories of muscles involved in respiration: inspiratory muscles, expiratory muscles, and other muscles involved in respiration. The most well recognized muscle involved in inspiration is the diaphragm which separates the abdomen and the thorax, and is innervated by the phrenic nerve originating in the cervical spinal cord (Hinchcliff et al., 2007; Hodgson et al., 2013). The other muscles involved in inspiration primarily serve to open and close the rib cage in accordance with the expansion of the lungs (Hodgson et al., 2013). The primary expiratory muscles are the abdominal muscles and the internal intercostal muscles which contract to increase the pressure in the abdominal cavity and reduce thoracic volume as air is expired (Hodgson et al., 2013). While contraction of these muscles is important, expiration relies heavily on the relaxation of the diaphragm (Hinchcliff et al., 2007; Raff and Levitzky, 2011; Hodgson et al., 2013). Some of the other muscles involved in respiration, such as the intrinsic laryngeal muscles, aid in modification of the airway and are located further up at the level of the nares, pharynx, and larynx (Hinchcliff et al., 2007; Hodgson et al., 2013).

Normal Physiological Responses

The relaxed volume of the respiratory system is identified as the equilibrium between the inward recoil of the lung and the outward recoil of the chest wall. Most species, including humans, breathe above or from this relaxed volume; the horse, however, breathes around this relaxed volume. In humans, inhalation is an active process by which the diaphragm contracts, and exhalation is a primarily passive process where the diaphragm relaxes (Raff and Levitzky, 2011). The horse is unique in that the second part of its exhalation is active, not passive, so

energy is required for the contraction of the expiratory muscles. As the expiratory muscles contract, abdominal pressure increases, and the relaxing diaphragm is forced forward which reduces the thoracic volume (Hodgson et al., 2013). It has been hypothesized that the active portion of exhalation and related passive portion of inhalation in equine results in a reduced work of breathing, leading to a shared amount of work between the inspiratory and expiratory muscles (Hodgson et al., 2013).

Ventilation is described by air moving in and out of the lungs, and can be quantifiably measured (Marlin and Nankervis, 2002). The amount of air inspired and expired in each breath is measured as tidal volume; generally around 12 milliliters per kilogram of body weight within the resting horse (Hodgson et al., 2013). This measurement can be multiplied by the respiratory frequency, approximately 14 breaths per min at rest, to estimate the total amount of air moved in and out of the lung per minute; termed the minute ventilation averaging around 80 liters per minute at rest (Marlin and Nankervis, 2002; Hodgson et al., 2013). Changes in minute ventilation are subsequently due to changes either in tidal volume, respiratory frequency, or both. Within the lung, there are areas known as dead space where gas exchange does not occur; for example in the upper conducting airways (Marlin and Nankervis, 2002; Hodgson et al., 2013). This means that only a portion of the minute ventilation reaches the level of the perfused alveoli within the lung where gas exchange occurs. This amount of air actually participating in the process of gas exchange from the lungs to the blood, or perfusion, is known as the alveolar ventilation. The normal ventilation to perfusion ratio in equine is around 0.8 to 1 (Hodgson et al., 2013).

The interaction of the respiratory system and the circulatory system is dependent upon the pulmonary and bronchial circulations. Through the pulmonary circulation, the pulmonary artery carries deoxygenated venous blood from the right side of the heart to the capillaries in the

lung at the level of the alveoli (Raff and Levitzky, 2011; Hodgson et al., 2013). It is here that the oxygenated blood diffuses across the walls of the alveoli and is returned to the left side of the heart via the pulmonary veins where it is further pumped throughout the body. The bronchial circulation carries about 1-2% of the oxygenated blood that has just been brought to the left side of the heart, and further supplies it to the airways and other structures within the lung (Hodgson et al., 2013). In locations such as the trachea there is air moving through constantly, however, nothing is actually diffusing across the membrane so oxygen must be supplied through circulation (Raff and Levitzky, 2011).

Exercise Responses

Exercise has an extreme effect on respiratory frequency and tidal volume, subsequently affecting the minute ventilation. It has been noted that with increases in speed there is a dramatic increase in minute ventilation from the resting levels of 80-100 liters per minute to nearly 1800 liters per minute during strenuous exercise (Franklin et al., 2012; Hodgson et al., 2013). During levels of submaximal exercise the increase in minute ventilation is relatively linear to the increase in speed, relating to the demand for oxygen at the level of the muscles (Franklin et al., 2012). At lower intensities of exercise such as a walk or trot, both increases in respiratory frequency and tidal volume contribute to increased levels of minute ventilation, but at intensities of exercise such as a canter the increase in minute ventilation is primarily due to the high respiratory frequency (Hodgson et al., 2013). The increase in breathing frequency is also described by a linear trend up to the level of a canter in the horse. Beyond this speed, increases are still present but they prove to be minimal (Franklin et al., 2012). Once the horse reaches a level of exercise relative to that of a gallop, the increase in minute ventilation is reliant on independent increases in tidal volume. (Hodgson et al., 2013).

The horse exhibits what is known as respiratory-locomotor coupling, where its respiration and breathing frequency are correlated with its stride and movement (Marlin, 2015). At a gallop this coupling sits at a tight 1:1 ratio of breathing frequency to stride. There have been average reports of 110 to 130 step and respiratory frequencies per minute, with maximum reports of 148 step and respiratory frequencies per minute (Hodgson et al., 2013). Because the respiratory frequency is tightly coupled to stride frequency, its effect on minute ventilation at this intensity of exercise is extremely limited. In fast galloping horses, increases in tidal volume from 12 to 15 liters have been reported, and this response is associated with the increased metabolic as well as thermoregulatory demands (Jones, 1994; Hodgson et al., 2013). While the recovery time of ventilation is dependent upon the intensity and duration of the exercise being performed, it is possible for respiratory frequency to remain elevated for 30-60 minutes post exercise (Mair et al., 2013).

Work of breathing increases dramatically during exercise due to increased expiratory volume, inertial forces, elastic forces, and resistance. During exercise there is an increase in intensity and blood flow resulting in increased flow acceleration through the trachea and other airways (Franklin et al., 2012). Elastic forces are dependent on the recoil of the lung with the chest wall and increase equally with the trans-pulmonary pressure. During high levels of exercise, stiffness of the chest wall accompanied by a threefold increase in lung volume leads to a significant increase in elastic work. Compulsory nasal breathing during exercise increases total pulmonary resistance fundamentally increasing the resistive work. Increases in inertial, elastic, and resistive work during exercise creates overall limitation for further increases in ventilation (Hodgson et al., 2013).

Conditioned Responses

The long-term training or condition responses are extremely minimal in equine compared to the short-term exercise responses. The most noticeable training response in the horse is a rapid and significant improvement in VO2 max, or the horse's maximum oxygen uptake. This increase in aerobic capacity is related to increases in cardiac output and oxygen consumption at the level of the muscles (Hodgson et al., 2013). This increase in maximal oxygen uptake is relatively modest at about 10-15% because the horse possesses the unique physiological capability of splenic contraction, where it is able to actually contract its spleen during exercise to increase its total red blood cell volume from approximately 40% at rest to 60% by the end of the exercise period, fundamentally increasing the amount of oxygen carried to the muscles (Hodgson et al., 2013; Marlin, 2015). Increases in the number of red blood cells released into circulation from the spleen has been observed to be related to increases in sympathetic nervous system activity, resulting from increases in overall exercise intensity (Wickler and Anderson, 2000; Hodgson et al., 2013). So, the extent of this response may differ across disciplines. Red blood cells concentrations have been observed to return to baseline levels within 1 hour post exercise in reining Quarter Horses, concluding that splenic contraction itself is an exercise response (Vazzana et al., 2014).

Knowledge of ventilation responses in the horse is limited to short-term responses. Ventilation in the horse is only capable of very limited adaptations to training; there is very minimal evidence relating to the effects of training on the ventilation response to exercise. Previous studies observed that training did not significantly alter minute ventilation, respiratory frequency, and tidal volume (Roberts et al., 1999). It has been hypothesized that during exercise the relative energy cost of ventilation is reduced and the fatigue of the respiratory muscles is

delayed (Hodgson et al., 2013). Hiraga and Sugano (2016) observed a reduction in overall respiratory frequency as a result of training and suggested this might be the result of improvements in cardiovascular function (Hiraga and Sugano, 2016). Potential increases in inspiratory muscle strength from training could lead to increases in ventilation as a result of reduced dead space and increased tidal volume (Marlin, 2015), but these are all assumptions that have yet to be proven.

HEADING 4

MUSCULOSKELETAL SYSTEM

The structure and function of the equine musculoskeletal system is the backbone of locomotion in the exercising horse and is responsible for the horse's extreme capabilities of both high speed and endurance movement. Unlike the respiratory system, training responses are much more prevalent in the equine musculoskeletal system than short-term exercise responses. The extent of the skeletal muscle adaptations depends on the frequency, intensity, and duration of the exercise conditioning program (Hodgson et al., 2013). The primary adaptations occur with the proportion of fiber types, capillarization to the muscle, oxidative capacity, energy utilization and stores, and muscle enzymes. To understand these adaptations, it is important to be familiar with the individual components that comprise the gross anatomy of the muscle, and the stimulation and processes that lead to muscle contraction.

Anatomy

Muscle bundles are comprised of hundreds of elongated, spindle-shaped muscle fibers or cells (Marlin and Nankervis, 2002; Valberg and Macleay, 2015). It is within the muscle fiber that force is generated through contraction (Hodgson et al., 2013). Muscle fibers are divided into identifiable groups based upon their speed of contraction, oxidative capacities, energy utilization, and diameter (Marlin and Nankervis, 2002; Hodgson et al., 2013; Valberg and Macleay, 2015). An extensive network of capillaries and nerves branch throughout layers of thin sheets of connective tissue surrounding the muscle fibers, to nourish as well as communicate with the muscle fibers (Marlin and Nankervis, 2002; Valberg and Macleay, 2015). At the specialized location of the motor end plate on the muscle cell membrane, motor neurons innervate the cells (Hodgson et al., 2013). This system of nerves and muscle fibers is termed the motor unit

(Valberg and Macleay, 2015). The muscle fiber itself is a system comprised of contractile proteins such as actin and myosin, a basement membrane, and internal structures to which neuromuscular stimulation occurs. Muscle fibers are strategically arranged within the muscle for maximum power and efficiency during contraction and locomotion (Hodgson et al., 2013). Numerous myofibrils pack together to form the striated structure of the muscle fiber (Raff and Levitzky, 2011). It is within these myofibrils that the myofilaments, actin and myosin, are aligned in an overlapping parallel formation to form the smallest functional unit of the muscle cell, the sarcomere (Raff and Levitzky, 2011; Hodgson et al., 2013; Valberg and Macleay, 2015). The polygonal shape of the myofibril has been measured between 1 to 3 micrometers in diameter, with the total number of myofibrils present in each muscle fiber varying dependent upon the muscle fiber's overall contractile function and total cross-sectional area (Hodgson et al., 2013).

The sarcomere, comprised of the thick and thin myofilaments, serves as the fundamental unit of contraction (Hodgson et al., 2013). There is a parallel alignment of the thick filament, myosin, and the thin filament, actin, that slide over each other and contract the muscle when proper stimulation occurs (Raff and Levitzky, 2011; Hodgson et al., 2013). The Z-line of the sarcomere is the end of the unit acting as a border between one sarcomere and another (Raff and Levitzky, 2011). The I-band of the sarcomere is the portion of the thin acting filament where it does not overlap with myosin (Hodgson et al., 2013). It extends from the Z-line to the center of the sarcomere; the contractile proteins troponin and tropomyosin are also present within this area (Marlin and Nankervis, 2002; Raff and Levitzky, 2011). The A-band is centrally located within the sarcomere and is considered the full length of the myosin thick filament, including the overlap with the actin thin filament. The H-band is the central region of the A-band consisting of

only myosin thick filament; no overlap of myosin and actin (Marlin and Nankervis, 2002; Hodgson et al., 2013). During contraction, the Z-lines move closer together, as the actin filament is pulled over the myosin filament, causing the I-band to shorten while the A-band remains the same length. The degree to which the myofilaments overlap plays an important role in the force of contraction within each muscle fiber (Raff and Levitzky, 2011).

Thick myofilaments are primarily composed of myosin protein. Two heavy chains of myosin spiral to form a double helix with long stable tails at their ends (Amory et al., 2010; Hodgson et al., 2013). At the opposite end are globular heads containing both actin and ATP binding sites (Hodgson et al., 2013). Actin is the primary protein within the composition of the thin myofilament (Marlin and Nankervis, 2002). Two strands of actin twist into a double helix to form the thin myofilament complex, containing binding sites for the myosin globular heads (Hodgson et al., 2013). The protein tropomyosin spirals the actin helix, covering the myosin binding sites. Bound to tropomyosin is troponin which contains three subunits: TN-T, TN-I, and TN-C, each with their specific physiological function. TN-T attaches to tropomyosin, TN-I inhibits the interaction between actin and myosin, and TN-C is the calcium binding component which removes the inhibitory effects of TN-I in the presence of calcium (Raff and Levitzky, 2011; Hodgson et al., 2013). With a release of calcium, the inhibitory effects of the troponin protein is removed, uncovering the binding sites, allowing myosin and actin to bind, and muscle contraction to occur (Marlin and Nankervis, 2002).

The plasma membrane of muscle cell fibers, the sarcolemma, serves to maintain the skeletal muscle structure while actively transporting substrates within the muscle cell (Marlin and Nankervis, 2002; Campbell and Stull, 2003; Hodgson et al., 2013). It is also directly involved in the excitation-contraction coupling process, transmitting the neural excitatory pulses

involved with the physiological steps of muscle contraction (Campbell and Stull, 2003; Hodgson et al., 2013). A lipid bilayer allows for the regulation and conductance of ions such as sodium, potassium, and calcium through conducting pathways and ligand-gated ion channels that span the membrane (Hodgson et al., 2013). Tubular invaginations, t-tubules, traverse across the myofibrils and penetrate deep into the muscle fiber (Marlin and Nankervis, 2002; Hodgson et al., 2013). Voltage gated ion channels contained within the t-tubules transmit electrical impulses through the muscle fiber aiding in the contraction process (Hodgson et al., 2013). The basal lamina of the skeletal muscle basement membrane serves as a direct link to the sarcolemma. Protein structures within the basement membrane act as mechanical barriers, protecting the sarcolemma from damage during muscle contraction (Campbell and Stull, 2003; Hodgson et al., 2013).

In order for contraction to take place, there has to be a connection between the nervous system and the muscle where stimulation and signaling can occur. The neuromuscular junction is the axon terminal resting point on the motor neuron, and is the fundamental site of communication between the nerves and the muscle fibers they supply (Hong and Etherington, 2011; Hodgson et al., 2013). Skeletal muscle is voluntary, requiring stimulation from the central nervous system (CNS) for muscle contraction (Raff and Levitzky, 2011). Action potentials travel down the axon in the direction of the skeletal muscle fibers, triggering motor neurons to innervate the muscle at the neuromuscular junction (Raff and Levitzky, 2011; Hodgson et al., 2013). This depolarization stimulates the release of the chemical neurotransmitter acetylcholine from the presynaptic axon terminal, which then diffuses across the synaptic cleft where it binds to its receptor on the sarcolemma. An influx of sodium ions into the cell results in another

depolarization, an end plate potential, on the postsynaptic sarcolemma (Raff and Levitzky, 2011; Hodgson et al., 2013).

Activation of voltage-gated sodium channels extends yet another wave of depolarization, this time through the t-tubules into an intracellular membrane system of the muscle fiber, the sarcoplasmic reticulum (Raff and Levitzky, 2011; Hodgson et al., 2013). This convoluted membrane structure branches, forming a network surrounding each myofibril while remaining physically separate from the sarcolemma (Rossi et al., 2008; Hodgson et al., 2013). The primary role of the sarcoplasmic reticulum is feedback control regulating calcium homeostasis within the skeletal muscle (Rossi and Dirksen, 2006; Rossi et al., 2008). High concentrations of calcium binding proteins (i.e. calsequestrin), ryanodine receptors, and calcium ATPase are present in the membrane of the sarcoplasmic reticulum (Rossi and Dirksen, 2006; Hodgson et al., 2013). Activation of the dihydropyridine receptors (DHPR) on the muscle cell membrane cause the ryanodine receptors in the sarcoplasmic reticulum to open (Raff and Levitzky, 2011; Hodgson et al., 2013). The calcium required for skeletal muscle contraction diffuses solely from the sarcoplasmic reticulum through the ryanodine receptors (calcium release channels) and into the cytosol (Rossi and Dirksen, 2006; Raff and Levitzky, 2011). The release of calcium is monitored strictly through the DHPR voltage sensors; if the voltage sensors are no longer activated, release of calcium is rapidly stopped (Lamb, 2000).

Once calcium levels have reached a certain threshold, they are able to bind to the TN-C, removing the inhibitory effects of the TN-I subunit on the troponin protein. This series of events beginning with depolarization of the motor neuron ending with the mechanical shortening of the muscle fibers is a process described as excitation-contraction coupling; the excitation of the muscle followed by the contraction of the muscle (Marlin and Nankervis, 2002; Raff and

Levitzky, 2011; Hodgson et al., 2013). When the inhibitory effects of the TN-I subunit are removed, tropomyosin is pulled from the actin, exposing the myosin binding sites on the actin protein (Hodgson et al., 2013). The myosin cross bridges are physically able to bind, pulling the actin towards the center of the sarcomere (Marlin and Nankervis, 2002; Hodgson et al., 2013). The sarcomere is shortened through contraction in a ratchet like fashion (Marlin and Nankervis, 2002; Hodgson et al., 2013). Calcium is actively transported from the cytosol back into the sarcoplasmic reticulum via calcium ATPase. As calcium is removed from the cytosol and no longer bound to tropomyosin, tropomyosin returns to its original position covering the myosin binding sites on the actin thin filament, and relaxation of the myofibrils occurs (Marlin and Nankervis, 2002; Raff and Levitzky, 2011; Hodgson et al., 2013). Relaxation of the muscle requires energy in the form of ATP, making it an active process (Valberg and Macleay, 2015).

Fiber Types

There are three types of muscles fibers within equine skeletal muscle: type I, type IIA, and type IIB. These categories differentiate based on oxidative capacity, glycolytic capacity, size, energy utilization, speed of contraction, mitochondrial content, color, onset of fatigue, and force of contraction (Raff and Levitzky, 2011; Hodgson et al., 2013). The presence of each type of fiber varies between specific muscle groups, breeds, and individual horses (Valberg and Macleay, 2015). Currently in horses, the contractile properties as well as the oxidative capacity of the muscle fiber are primarily used for their classifications (Hodgson et al., 2013). The types of muscle fibers are recruited or stimulated to contract based on the speed, duration, and intensity of the exercise performed. During slow exercise, such as a walk or trot, type I fibers primarily along with some type IIA fibers are recruited (Valberg and Macleay, 2015). As the speed increases recruitment of type II fibers increases with type IIB fiber recruitment during near

maximal exercise, when extremely forceful contractions are required (Hodgson et al., 2013; Valberg and Macleay, 2015).

Type I are classified as red endurance or slow twitch-low oxidative fibers (Marlin and Nankervis, 2002; Hodgson et al., 2013). They possess the greatest oxidative, aerobic capacity of the three as well as the lowest glycolytic, anaerobic capacity (Raff and Levitzky, 2011). High oxidative capacity is due to abundant amounts of mitochondria which are essential for aerobic metabolism, and also give type I fibers their red color (Raff and Levitzky, 2011; Valberg and Macleay, 2015). Since their primary role is aerobic metabolism, they have the highest resistance to fatigue allowing them to contract the slowest and for the longest periods of time (Marlin and Nankervis, 2002; Hodgson et al., 2013; Valberg and Macleay, 2015). They have the greatest capillarization and blood flow, but the smallest diameter and weakest force of contraction of the three types because they are not used for rapid and quick movements (Raff and Levitzky, 2011; Hodgson et al., 2013). High lipid stores and low glycogen stores correlate with type I aerobic and anaerobic capacities (Valberg and Macleay, 2015). Higher percentages as well as larger diameters of type I fibers have been recorded in horses undergoing endurance performance training (Rivero et al., 1993).

Type IIA are classified as intermediate or fast twitch-high oxidative fibers (Raff and Levitzky, 2011; Hodgson et al., 2013). They possess moderate oxidative and glycolytic capacities, with a fast force and speed of contraction (Marlin and Nankervis, 2002). They have intermediate amounts of mitochondria, and are red in color similar to the type I fibers (Raff and Levitzky, 2011). While they have a high glycogen content, they have low levels of lipids correlating with their role in rapid movement (Marlin and Nankervis, 2002). Resistance to

fatigue, diameter, capillarization are at intermediate levels compared to type I and type IIB fibers (Raff and Levitzky, 2011; Valberg and Macleay, 2015).

Type IIB are classified as white sprint or fast twitch-low oxidative fibers (Marlin and Nankervis, 2002; Hodgson et al., 2013). They have high glycolytic capacity with low oxidative capacity, so they also have an abundance of glycogen and low lipid content for energy utilization in anaerobic metabolism. They have the largest diameter, force, and speed of contraction; diameter of the fiber is generally proportional to its force of contraction (Valberg and Macleay, 2015). They are white in color due to their extremely few number of mitochondria and have a very low level of capillarization as well as resistance to fatigue. Type IIB fibers are the power fibers of the muscle, primarily used for forceful rapid movement such as a sprint or race. Endurance performance horses showed lower percentages of type IIB fibers (Rivero et al., 1993), and racing horses such as Thoroughbreds showed extremely high percentages of type IIB fibers compared to type I and type IIA (Valberg and Macleay, 2015).

Exercise Responses

One of the primary responses to exercise within the musculoskeletal system is an increase in body temperature. Heat is a byproduct of metabolism, so as metabolism increases so does the amount of heat circulating within the body (Hodgson et al., 2013). It has been noted that the greatest increase in temperature during exercise compared to normal levels in equine occurs at the level of the muscle (Hodgson et al., 1993). Along with heat, lactate production increases with increased metabolism (Hodgson et al., 2013). Lactate accumulation occurs at all intensities of exercise, but is primarily a byproduct of anaerobic metabolism within type II muscle fibers at high intensity exercise (Hodgson et al., 2013; Valberg and Macleay, 2015) As lactic acid increases at a rate proportionally faster than it can be removed from muscle, it moves down and

out of the cell circulating throughout the body (Marlin and Nankervis, 2002). Increases in lactate leads to decreases in pH, causing increased acidity within the blood and muscle. Both dramatic increases in temperature and the onset of blood lactate accumulation at concentrations of 4 mmol/L are contributing factors to the onset of fatigue, which impairs exercise performance (Hodgson et al., 2013).

The occurrence of aerobic and anaerobic metabolism within the muscle is dependent on the intensity of the exercise. Aerobic metabolism will occur until the horse reaches its VO2max, maximum oxygen consumption; it then must resort to anaerobic metabolism to meet the energy demands of the muscle (Valberg and Macleay, 2015). During low intensity exercise when type I and type IIA muscle fibers are recruited, aerobic metabolism primarily takes place using stored fatty acids along with a small amount of anaerobic metabolism using some of the glycogen stores (Marlin and Nankervis, 2002). Moderate intensities of exercise utilize both fatty acid and glycogen stores for aerobic metabolism, and can lead to a depletion of glycogen stores (Valberg and Macleay, 2015); however, due to the large quantity of fatty acids stored, depletion is unlikely (Marlin and Nankervis, 2002). At high intensity exercise, duration is the primary determinant of whether metabolism is aerobic or anaerobic. There is a high chance of glycogen store depletion as well as an increase in plasma potassium concentrations with increasing exercise intensity (Marlin and Nankervis, 2002; Hodgson et al., 2013). Upon the completion of exercise, plasma potassium is rapidly taken back up into the muscle (Hodgson et al., 2013).

Conditioned Responses

There is an increase in the amount of type IIA and a decrease in type IIB muscle fibers after a conditioning program. Studies have shown that short-term training in racehorses elicits a hypertrophic response, so an increase in the size of the actual muscle fibers (Rivero et al., 2007),

whereas in a long-term conditioning program, the diameter of the type IIB fibers decreases. For both endurance and speed training, a decrease in the number and diameter of type IIB fibers was recorded (Valberg and Macleay, 2015). Along with an increase in muscle fibers, there is an increased number of capillaries supplying the skeletal muscle, increasing surface area at the muscle for greater perfusion of blood (Marlin and Nankervis, 2002). The increase in capillarization along with the decrease in the size of the muscle fibers results in a more direct delivery of oxygen to the muscle fibers, and aids in more efficient removal of metabolic byproducts such as heat and lactate (Hodgson et al., 2013). Studies have shown that duration rather than intensity of exercise produces the greatest effect on these factors (Rivero et al., 2007).

The gradual increase in type IIA muscle fibers and capillarization accompanied by an increase in mitochondrial density and oxidative enzymes increases the oxidative capacity of the muscle (Lindholm, 1986; Hodgson et al., 2013). Development of new mitochondria has been recorded as an adaptation primarily during endurance training (Rivero et al., 2007). The muscle now has an increased capacity to utilize oxygen for aerobic metabolism, so the utilization of anaerobic metabolism decreases. Since lactate is primarily a byproduct of anaerobic metabolism, the onset of blood lactate accumulation is delayed, fundamentally delaying the onset of fatigue at the level of the muscle (Lindholm, 1986; Marlin and Nankervis, 2002). With a shift from anaerobic to aerobic metabolism, there is also a transition in the utilization of fuel stores. The muscle gains an increased capacity to utilize fat as a fuel source with an increased aerobic capacity, leading to increased efficiency of energy production. There is also a notable increase in the glycogen content utilized for either type of metabolism, aiding in the prevention of glycogen store depletion, further delaying the onset of fatigue (Marlin and Nankervis, 2002). Overall,

these long-term adaptations allow the horse to perform at increased intensities for increased amounts of time.

HEADING 5

INFLAMMATORY PROCESS

Inflammation is the body's principle response to injuries including swelling, redness, fever and pain. There are short-term and long-term adaptive responses to inflammation that occur when a tissue signals cells to respond to damage (Hotamisligil, 2006). So, even incredibly minor tissue damage, for instance to the skeletal muscles during exercise, can trigger an inflammatory response; strenuous exercise has been observed to induce a pro-inflammatory state in equine (Donovan et al., 2007). While the acute inflammatory response during exercise is essential for the musculoskeletal healing and repair process, chronic inflammation can lead to muscle damage that drastically impairs the horse's performance (Horohov et al., 2012). As a direct result of any tissue damage or irritation, phospholipase A2 is activated and cleaves arachidonic acid from the damaged phospholipid cell wall (Dowling, 2002). Arachidonic acid further activates the cyclooxygenase (COX) I, COX II, and lipoxygenase enzymes. These enzymes are responsible for prostaglandin and leukotriene production, which directly lead to pain, inflammation, and bronchoconstriction (Kidd and Urban, 2001). Currently in medicine, treatments and therapies aim to disrupt this pathway in attempts to reduce pain and inflammation, which will be discussed later on.

One of the key components of the inflammatory process is the activation of inflammation-associated pain. Nociception is the "detection of noxious stimuli and the subsequent transmission of encoded information to the brain" (Kidd and Urban, 2001). So, nociceptors are activated by any stimuli that could potentially be harmful, poisonous, or unpleasant for the body and further signal the body to respond. When tissue becomes damaged, there is a triggered release of inflammatory mediators which act both directly and indirectly on

the nociceptors. Peripheral nociceptors are directly activated by some of the inflammatory mediators, such as the prostaglandins synthesized by the COX I and II enzymes, resulting in the spontaneous pain associated with inflammation. Other inflammatory mediators, however, stimulate release of pain-inducing agents, such as pro-inflammatory cytokines, activating the nociceptors indirectly (Kidd and Urban, 2001).

The fundamental goal of this pain and inflammation is to signal the body and immune system to respond and repair. Neutrophils, which make up approximately 50% of the total circulating white blood cells (WBC) (Delves, 2017) and 5% of bronchoalveolar lavage (BAL) fluid cells (Couëtil et al., 2016), are the first type of immune cells to travel to the target site and can produce lipids, cytokines, proteases, and other products that can all play a role in the inflammatory process (Sampson, 2000). Cytokines work to mediate cell to cell interactions and enhance the effects of immune cells (Kidd and Urban, 2001). Neutrophils eventually undergo apoptosis followed by phagocytosis; this causes macrophages to switch from producing pro-inflammatory cytokines to production of prostaglandins and proteins involved in post-inflammatory repair (Sampson, 2000).

Some of the primary pro-inflammatory cytokines observed in the equine inflammatory process are tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). TNF-α plays a key role in regulation of immune cells, IL-1β mediates responses to both infection and injury, and IL-6 works to increase acute phase protein production (Reed et al., 2010c). Horohov et al. (2012) observed that racing-type exercise is associated with a significant, intensity-dependent expression of TNF-α and IL-1β roughly two hours after the exercise period, with no significant changes in IL-6 expression. It was observed that training resulted in an increased expression of IL-6 with a reduction in the expression TNF-α and IL-1β compared to

pre-training levels; it was theorized that upregulation of IL-6 actually reduces pro-inflammatory TNF-α and IL-1β expression (Horohov et al., 2012).

Along with phagocytic neutrophils, eosinophils are a key WBC that primarily increase in concentration in response to hyperresponsive reactions (Mair et al., 2013), such as Inflammatory Airway Disease (IAD) (Mazan et al., 2014). Eosinophils aid in the inflammatory-immune process through degradation of inflammatory mediators, such as leukotrienes, and subsequently modulating immediate hypersensitivity (Mair et al., 2013; Liesveld and Reagan, 2016). Since eosinophils primarily target organisms that are too large to undergo phagocytosis, they secrete toxins and proteins responsible for organism degradation (Delves, 2017). Generally, eosinophils constitute approximately 5% of the total circulating WBCs (Delves, 2017), but these baseline concentrations have been observed to decline with increases in stress and plasma cortisol, as well as corticosteroid administration (Liesveld and Reagan, 2016). Neutrophilia and eosinophilia are both utilized in the diagnosis of airway inflammation (Sprayberry and Robinson, 2015), which will be discussed further on.

Along with increased concentrations of WBCs, another common marker of inflammation are acute phase proteins; proteins whose plasma concentrations increase or decrease by a minimum of twenty-five percent during an inflammatory response. When any type of tissue damage occurs, such as from oxidative stress during exercise, the system acute phase response (APR) is triggered in attempts to minimize the damage from possible pathogens and restore the body to homeostasis (Satue et al., 2013). Inflammatory cytokine production stimulates the production and release of acute phase proteins such as C-reactive protein (CRP) and serum amyloid A (SAA) (Gabay and Kushner, 1999).

C-reactive protein is considered the “golden marker” of inflammation primarily in canine, swine, and humans (Ramamoorthy et al., 2012). While CRP concentrations have been observed in horses to increase in response to induced inflammation, arthritis, enteritis, and laminitis (Cywińska et al., 2012), SAA is actually the major acute phase protein in the equine inflammatory process (Satue et al., 2013). When tissues become damaged and inflamed, cytokines are released from the site of injury and further synthesize SAA within the liver; when tissue damage has ceased or been repaired, SAA is rapidly degraded within the liver. The rapid synthesis of SAA, response to tissue damage, and subsequent degradation make it an accurate marker of the real-time extent of inflammation and injury. Serum amyloid A can increase up to 1000 times its baseline level during the APR in proportion to the level of tissue damage. For example, an increase from baseline concentrations of 1 mg/L APR to concentrations of 1000 mg/L can be indicative of poor conditioning in horses (Satue et al., 2013). A significant positive correlation has been observed between SAA concentrations and WBC concentrations (Turlo et al., 2015). A primary role of SAA is inhibition of neutrophil function (Reed et al., 2010c). As an animal becomes more conditioned to exercise, this inflammatory response decreases and changes in acute phase protein concentrations become less observable (Beckstett, 2012). It could be theorized that changes become less observable due to increases in baseline levels of SAA, and while neutrophil production may not be altered, there potentially is a greater inhibition of neutrophil function from this increase in SAA, resulting in a reduced overall inflammatory response.

HEADING 6

COMMON AILMENTS IN THE EXERCISED HORSE

Fatigue

Fatigue is reached when the horse is no longer able to continue performing at a certain intensity or level of exercise; the animal must either stop exercising, or continue at a lower intensity (Hodgson et al., 2013). The onset of fatigue is primarily dictated by duration and intensity of exercise, the horse's physical condition, as well as environmental factors such as temperature, humidity, and altitude. Causes of muscular fatigue include depletion and impairment of muscle fuel stores, altered fluid and ion balances, impaired muscle fiber recruitment, and hyperthermia. Fatigue occurs during both sub-maximal and maximal levels of exercise as well as during aerobic and anaerobic production (Marlin and Nankervis, 2002; Munsterman and Vaughan, 2015). There are two classifications of fatigue, central and peripheral. Peripheral fatigue occurs primarily at the level of the muscle and relates to depletion of glycogen and other fuel stores, decreased ATP production, decreased pH, increased blood lactate, and other intermediates and byproducts of metabolism. Central fatigue involves neurological and psychological processes at the level of the CNS primarily relating to frequency of action potentials needed for muscle contraction. Pain, exertion, hyperthermia, and decreased blood glucose are all probable causes of central fatigue and can result in decreased muscle coordination, muscle tiredness, and motivation to perform (Munsterman and Vaughan, 2015) .

High intensity, maximal exercise can lead to rapid onset of fatigue in the horse due to rapid depletion of high energy glycogen and phosphate stores and increase of anaerobic metabolic byproducts such as lactate and heat, primarily within type IIB muscle fibers (Marlin and Nankervis, 2002). Increases in lactate production cause decreases in muscle pH resulting in a

more acidic environment which can reduce the respiratory capacity of the skeletal muscle and impair the functional capacities of the sarcoplasmic reticulum (Hodgson et al., 2013). Impairment of the sarcoplasmic reticulum leads to imbalances of calcium and potassium within the muscle cell required for the contractile process (Munsterman and Vaughan, 2015).

The onset of fatigue, resulting from prolonged submaximal exercise, is relative to the intensity and duration of the exercise performed. Fatigue will occur more rapidly in a horse trotting for a thirty-minute period verses walking for a thirty-minute period. Submaximal exercise fatigue is primarily a result of altered fluid and ion balances, depleted muscle glycogen stores, and hyperthermia (Marlin and Nankervis, 2002; Hodgson et al., 2013). As previously discussed, the horse sweats a tremendous amount during exercise to aid in thermoregulation. This large amount of total body water loss, if not replaced, can result in dehydration as well as imbalances in concentrations of electrolytes required for muscle contraction (Marlin and Nankervis, 2002). Prolonged metabolism leads to depletion of muscle glycogen stores; it is likely that the onset of fatigue precedes the exhaustion of lipid stores utilized for aerobic metabolism (Munsterman and Vaughan, 2015).

Heat generation during prolonged exercise is elevated as a byproduct of increased aerobic metabolism. High body temperatures can impair mitochondrial function decreasing capacity of aerobic metabolism, resulting in greater thermoregulatory demands, and increased risk of hyperthermia especially under hot and humid conditions (Marlin and Nankervis, 2002; Hodgson et al., 2013). Blood flow is increased to aid in heat dissipation at the level of the skin, causing blood flow to diverge away from the working muscle decreasing the exchange of oxygen, carbon dioxide, and other metabolic byproducts (Hodgson et al., 2013).

Exhausted Horse Syndrome

The onset of fatigue during exercise is a normal process, and aids in the prevention of structural damage that could occur if the horse continues exercising at that exercise intensity; however, there are fatigue induced syndromes that require further attention and treatment. Occasionally during both maximal and submaximal exercise intensities, horses can develop a more severe form of fatigue known as exhausted horse syndrome (EHS) (Mair et al., 2013). Water loss, increased heat generation from metabolism, electrolyte imbalances, and depletion of energy stores like in normal fatigue are all causes of EHS but to a more severe extent (Mair et al., 2013; Munsterman and Vaughan, 2015). Horses presenting with EHS may experience water loss up to 10% of their total body weight primarily from high levels of sweating (Munsterman and Vaughan, 2015). With that said, thirst is generally suppressed due to pain from small intestinal distention caused by decreased intestinal mobility (ileus) (Mair et al., 2013). The combination of increased water loss and decreased desire to consume water only increases the severity of dehydration. Exhausted horse syndrome can also lead to further problems such as colic and laminitis (Munsterman and Vaughan, 2015).

Low intensity events such as endurance training can lead to a metabolic alkalosis, or increases in pH above normal due to losses of calcium (hypocalcemia) and magnesium (hypomagnesia) through sweating (Munsterman and Vaughan, 2015). Depletion of calcium and magnesium can also contribute to neuromuscular dysfunction and ileus (Valberg, 2016). When the interaction between the nervous system and intestinal muscle is impaired, muscle contraction in a portion of the gastrointestinal tract may stop but digestion itself continues. As microbes continue to digest, gases are produced and trapped, leading to small intestinal distention. Fluids will migrate from the blood to the intestine, further dehydrating the horse. A lack of intestinal

muscle contraction can also result in a buildup of digesta, obstructing the gastrointestinal tract, potentially inducing colic. If the obstruction occurs in the small intestine, distention and fluid buildup can occur in the stomach, causing it to expand and potentially rupture (Piscopo, 2008).

On the other hand, during high intensity events and training such as 3-day eventing there is risk for metabolic acidosis or decreases below normal values due to increased lactic acid as a byproduct of anaerobic metabolism. There is usually the presence of metabolic alkalosis once the acidosis subsides after exercise recovery (Munsterman and Vaughan, 2015). To compensate for a metabolic acidosis, there is an increase in the respiratory frequency in attempts to reduce carbon dioxide in the body (Mair et al., 2013). Overall, the acidosis will induce the onset of fatigue more rapidly, and the alkalosis will impair nervous system, musculoskeletal system, and gastrointestinal function.

Based on these physiological alterations, clinical signs include increased heart rate and respiratory rate above normal even at rest, severe dehydration and concentrated or absent urine due to excessive sweating, muscle spasms or cramps due to electrolyte imbalances, increased body temperatures above normal, and gastrointestinal problems (Mair et al., 2013; Munsterman and Vaughan, 2015). Common treatments for EHS include administration of isotonic fluids and electrolytes intravenously or orally to aid in dehydration and electrolyte imbalances; cooling the horse through cold hosing, shading, cold water sponge baths, and even cool-water enemas; nonsteroidal anti-inflammatory drugs for muscle spasms, cramps, and pain once the horse is rehydrated (Mair et al., 2013; Munsterman and Vaughan, 2015).

Heat Stroke

Horses possess a large body mass relative to body surface area limiting thermoregulation and heat dissipation during exercise; coupled with exercise and training in hot and humid

conditions, the risk for heat related illnesses is increased. Heat stroke clinically occurs when the horse's core body temperature increases above 40 degrees Celsius and is followed by abnormal function of the CNS and the motor controls of the brain (Bouchama and Knochel, 2002; Mair et al., 2013). Heat stroke can be classified either as classic, which occurs with hot and humid environmental conditions in the absence of physical activity, or as exertional, which occurs with strenuous exercise but not necessarily in hot and humid environments. Horses performing in hot and humid environments typically experience uncompensable heat stress, where the environment impairs their evaporative cooling capabilities, which further increases the risk for exertional heat stroke (Leon and Bouchama, 2015).

The brain's ability to aid in thermoregulation is partially impaired because of increases in brain temperature during exercise, resulting in CNS abnormalities which can lead to loss of motor coordination/control and impaired neuromuscular function; in severe cases convulsions, coma, and death can occur (Lindinger, 1999; Bouchama and Knochel, 2002). In a study by Bouchama and De Vol (2001) assessing the acid-base balance during heat stroke in humans, metabolic acidosis was prevalent in 81% of patients. It was observed that the metabolic acidosis was significantly associated with the presence of hyperthermia, and concluded that metabolic acidosis is a predominant response to heat stroke (Bouchama and De Vol, 2001). Clinical signs of heat stroke in the horse include increased heart and respiratory rates, increased body temperatures of greater than 40 degrees Celsius, hot skin, depression, convulsions, disorientation, collapsing, coma, and death (Bouchama and Knochel, 2002; Mair et al., 2013).

Treatments for heat stroke aid in reducing and controlling the core body temperature which can be done through cold water baths and increased ventilation as well as restoring hydration via intravenous fluid administration. Nonsteroidal anti-inflammatory drugs can be used

for inflammation and pain management (Mair et al., 2013) once the horse has been properly hydrated to avoid detrimental effects on kidney function (Orme, 1986). Paying attention and treating the initial and less severe symptoms is as wise and preventative measure for coma and death.

Exertional Rhabdomyolysis "Tying Up"

The most common exertional myopathy in equine is Exertional Rhabdomyolysis (ER), which can be divided into two primary forms: sporadic, most common during rest after exercise, and chronic, which is generally the result of genetic abnormalities and disorders (Anderson and Chesworth, 2016). The term "rhabdomyolysis" describes skeletal muscle necrosis, which in this case is a result of some level of exertion or exercise being performed by the horse (Valberg, 2016).

Susceptibility to Sporadic Exertional Rhabdomyolysis (SER) has been observed across all breed lines in equine (Valberg, 2016). Commonly, SER results when the horse undergoes a level of exercise that exceeds its current physiological state of training. Some of the contributing factors increasing the risk of SER include strenuous exercise beyond the training level of the horse, electrolyte imbalances, vitamin E and selenium deficiencies, high non-structural carbohydrate diets, as well as heat stress and exhaustion as previously discussed (Mair et al., 2013; Valberg, 2016). Non-structural carbohydrates consist of either simple sugars or possess the ability to be broken down by endogenous enzymes; high grain diets would contain high amounts of non-structural carbohydrates (Pagan, 1998). As previously discussed, lactic acid production primarily occurs as a result of anaerobic metabolism (Marlin and Nankervis, 2002). If the horse is exercising above its level of training, it is likely exceeding its VO2max, primarily generating ATP through anaerobic metabolism, and rapidly increasing lactic acid production. This overall

rapid build of lactic acid in the muscle causes the observed stiffness in gait, reluctance to move, and early onset of fatigue in SER horses (Lindholm, 1986; Mair et al., 2013).

Chronic Exertional Rhabdomyolysis (CER) is defined as recurring episodes of rhabdomyolysis that occur even with only light bouts of exercise; CER has been observed to be caused by Recurrent Exertional Rhabdomyolysis (RER), Polysaccharide Storage Myopathy (PSSM) Type I, and PSSM Type II (Valberg, 2016). Recurrent Exertional Rhabdomyolysis is thought to be a result of abnormal intracellular calcium concentrations and regulation leading to muscle spasms and cramping, with increases in incidences following certain breed lines of horses. This genetic susceptibility has been primarily observed and studied in Standardbred and Thoroughbred horses (Mair et al., 2013; Anderson and Chesworth, 2016). Polysaccharide Storage Myopathy Type I occurs do to an inherited mutation of the glycogen synthase 1 (GYS1) gene which causes a defect in glycogen metabolism; the underlying cause of PSSM Type II has yet to be determined but has been observed to result in the similar abnormalities of glycogen metabolism. In both type I and type II PSSM, glycogen is abnormally accumulated and stored. Quarter horses, Draft horses, Warmblood breeds, and Light breeds have been observed to be affected by PSSM (Mair et al., 2013; Valberg, 2016). During rest periods post exercise, generally one to two days, there is a build-up of intracellular glycogen stores resulting in damage to the muscle fibers during the following exercise period (Anderson and Chesworth, 2016). It has been theorized that the build-up of intracellular glycogen stores increases lactate production resulting in a skeletal muscle necrosis and subsequent metabolic acidosis. This acute metabolic acidosis increases the production of inflammatory interleukins, while suppressing WBC function; fundamentally it stimulates the inflammatory response, while simultaneously impairing the immune response (Kraut and Madias, 2010).

While PSSM is one type of glycogenosis, a disease characterized by abnormal storage of glycogen in tissues (DiMauro and Lamperti, 2001), which can result in rhabdomyolysis during both rest and exertion, Glycogen Branching Enzyme Deficiency (GBED) is another reported glycogenosis in equine (DiMauro and Lamperti, 2001; Ward et al., 2004). Glycogen Branching Enzyme Deficiency is a result of an inherited nonsense mutation in the equine Glycogen Branching Enzyme (GBE1) gene which subsequently causes accumulation of poorly branched glycogen within the tissues (Ward et al., 2004). In comparison to PSSM, GBED is classified as a non-exertional myopathy, so it has been observed to be associated with rhabdomyolysis but its effects are not exacerbated by exertion; this condition can be fatal in foals (Aleman, 2008). Araujo et al. (2018) observed that the prevalence of testing heterozygous for the GBE1 mutation in 742 Quarter Horses was 7.95%, and prevalence was highest in cutting horses with no carriers detected in racing horses (Araujo et al., 2018).

Clinical signs of ER include abnormal, stiff, or halted gait; noticeable pain in the hind end often within 20 minutes of exercise; reluctance to move; increased heart rate, respiratory rate, core body temperature, and sweating; immobility in severe cases. Symptoms are variable dependent on severity of the disorder. (Lindholm, 1986; Mair et al., 2013). In severe cases of ER, both sporadic and chronic, there is also an increased risk of renal failure. As the skeletal muscle is degraded, myoglobin, creatine kinase (CK), and aspartate aminotransferase (AST) are released from the muscle into the blood stream. These muscle proteins end up precipitating in the kidney and impairing renal function (Clarkson, 2007). Increases in myoglobin within the urine can cause pigmenturia, or the urine to appear dark red in color (Reed et al., 2010a). High concentrations of muscle proteins within the kidney can also lead to significant increases in concentrations of urea, which can be an indication of renal failure (el-Ashker, 2011). Observance of clinical signs can

aid in diagnosis, along with elevated post-exercise levels of CK, AST, and lactate dehydrogenase in serum compared to pre-exercise (Mair et al., 2013; Anderson and Chesworth, 2016). Dehydration along with electrolyte imbalances such as low chloride and calcium concentrations are also possible ER indicators (Mair et al., 2013).

Identifying the specific type of exertional myopathy occurring is important in implementation of a treatment program. General treatment of ER includes rest periods for a minimum of 48 hours with increases in time dependent on severity of disorder, sometimes for weeks; fluid and electrolyte therapy to correct imbalances; anti-inflammatories, corticosteroids, and muscle relaxants; adjustments to a low carbohydrate, high fat diet specifically for horses with PSSM and GBED-related ER. These adjustments in diet, fluid and electrolyte therapy, and implementing rest periods can be preventative measures as well (Mair et al., 2013; Anderson and Chesworth, 2016).

Inflammatory Airway Disease

This disorder is representative of inflammation primarily in the peripheral airway as a result of excessive accumulation of mucus and inflammatory WBCs (Sprayberry and Robinson, 2015); the specific cause of this excessive accumulation in IAD is not fully understood (Reed et al., 2010b). Inflammatory airway disease (IAD) is generally mild, but can increase the risk of more severe respiratory problems (Couëtil et al., 2016). General inflammation in the airway can be a response to primary issues such as bronchitis, airborne allergens, viral infections, as well as blood from hemorrhage in the lower airway (Reed et al., 2010b; Mair et al., 2013). Airway inflammation in IAD horses is usually a hyperresponsive reaction induced by environmental factors such as dusty hay and bedding, or even just being stabled verses pastured can increase the exposure to endotoxins resulting in neutrophilic inflammation; it has also been observed that low

temperatures of inspired air increases neutrophil concentrations leading to IAD (Reed et al., 2010b). Couëtil et al (2016) concluded in a revised consensus statement on IAD in horses that there currently is no evidence of genetic susceptibility among breed lines, and that horses from all ages, breeds, and disciplines can develop IAD (Couëtil et al., 2016). While IAD has been observed across all ages, it is most commonly reported in young performance horses (Reed et al., 2010b; Couëtil et al., 2016). Wood et al (2005) observed a significant decrease in prevalence of IAD in horses with increasing age from 2 to 4 years old, with an annual prevalence of 78% in 2-year-olds, 50 % in 3-year-olds, and 20% in 4-year-olds; a decrease in the mean duration of IAD episodes with increases in age was also observed (Wood et al., 2005).

Inflammatory airway disease is typically marked by coughing, nasal discharge, increased respiratory rate, exercise intolerance and overall poor performance during exercise. While there can be an increase in respiratory rate, there is no observed increase in expiratory effort, so no noticeable labored breathing, at rest. The clinical signs are generally mild, so observance of these symptoms to a more severe extent could be representative of a more severe musculoskeletal or respiratory disorder such as recurrent airway obstruction (RAO) or exercise-induced pulmonary hemorrhage (EIPH). Since IAD primarily affects young horses, they should theoretically “grow out” of the condition; it should not affect their performance career if properly managed. While some young horses with IAD develop RAO when they are older, this relationship has not been examined.

Diagnosis can be determined through observation of clinical signs; age of the horse; BAL to assess neutrophil, eosinophil, mast cell concentrations; endoscopy of the airway looking for increased mucus in the trachea; differential diagnostic measures should be taken to rule out other possible diseases of the airway (Mair et al., 2013; Couëtil et al., 2016; Leclere and Lavoie,

2016). It has been concluded that a BAL is the most reliable diagnosis technique in identification of inflammation in the peripheral airways (Sprayberry and Robinson, 2015), and is most commonly used in diagnosis of lower airway conditions to obtain secretions from for cytological analysis. Generally, it is performed endoscopically where a 120 cm endoscope is passed down to the level of the bronchi where a lavage is performed (Mair et al., 2013). Cytology of BAL fluid in IAD horses will typically show mild increases in neutrophils (> 10% BAL fluid), with potential increases in eosinophils (> 5%) and mast cells (> 5%) (Couëtil et al., 2016).

Environmental management (removal of hay and dusty bedding/soaking hay and feeding it on the ground), use of bronchodilators to expand the airway, as well as anti-inflammatory drugs have all been utilized in treatment of IAD (Mair et al., 2013; Couëtil et al., 2016). The primary goal of treatment is to minimize inhalation of dust and allergens (Sprayberry and Robinson, 2015). It has been observed that horses consuming hay have higher tracheal neutrophil concentrations than horses grazing freely out in pasture, so changing from a stable to pasture environment could aid in decreasing inflammation (Robinson et al., 2006). Bronchodilators and corticosteroids can be administered both systemically and as an aerosol treatment to reduce inflammation; however, systemic administration has been concluded to improve lung function more rapidly. Supplementing a low dust diet with omega-3 fatty acids has also been observed to modulate the inflammatory response of the disease (Couëtil et al., 2016).

Recurrent Airway Obstruction

Recurrent Airway Obstruction is one of the most common equine respiratory disorders resulting from obstruction of the small portions of the airway at the level of the bronchioles (Mair et al., 2013); RAO is commonly referred to as heaves, broken wind, and equine asthma (Niedzwiedz, 2014; Setlakwe et al., 2014). Characterization of this disorder includes smooth

muscle contraction in combination with mucus and neutrophil accumulation that subsequently results in small airway obstruction; this obstruction is reversible. While RAO has been referred to as equine COPD, it should be noted that the pathophysiology in RAO of horses is different than the pathophysiology of human COPD; they are not the same condition (Reed et al., 2010b). The disease is most prevalent around seven to nine years of age, with risk increasing as well as worsening of airway function as age increases (Reed et al., 2010c; Mair et al., 2013). There is currently no evidence of breed or gender predisposition since RAO has been observed across all breeds of horses, ponies, and donkeys; incidences have, however, been observed through specific breeding lines (Mair et al., 2013; Niedzwiedz, 2014).

Recurrent Airway Obstruction arises from an allergic reaction to pollutants and dust sourced from feeding hay, bedding, and other potential environmental conditions. This reaction stimulates an inflammatory response where cytokine production and neutrophil concentrations increase within the bronchi, leading to subsequent bronchoconstriction (Niedzwiedz, 2014). It has been observed that collagen content and dysfunction of an elastic fiber network increases in the airway of heaves-affected horses, which could result in further obstruction (Setlakwe et al., 2014). Since collagen plays an essential role in the structural component of organs, increases in collagen can lead to increases in stiffness and decreases in elasticity of the lung, making expansion more difficult (Muiznieks and Keeley, 2013).

Immediate clinical signs to allergen exposure are not observed in RAO horses; clinical signs generally present several hours post-exposure (Reed et al., 2010c). A key differentiation between IAD and RAO is that RAO horses will present with increases in respiratory effort and lung dysfunction in a resting state, and IAD horses will not (Couëtil et al., 2016). The severity of the clinical disease can be divided into subclinical, mild clinical, and severely clinical forms

dependent on the severity of this reaction and response (Mair et al., 2013). Subclinical signs are minimal, including mucus accumulation, occasional coughing, minimal inflammation, and exercise intolerance in performance horses. Mild clinical signs include occasional but sporadic cough, mucus accumulation, minimal nasal discharge, as well as exercise intolerance. Severe clinical signs are the most noticeable with a chronic cough especially during exercise, increased respiratory rate, labored breathing during exercise and at rest, dilation of the nostrils accompanied by discharge, exercise intolerance, and a prolonged recovery period (Mair et al., 2013).. Due to an increased expiratory effort, hypertrophy of the external abdominal oblique muscle can occur resulting in what is known as a heave-line. This can also be accompanied by a severe weight loss from difficulty eating in advanced cases; clinical signs can be used as a diagnostic measure (Mair et al., 2013).

The primary diagnostic technique for equine RAO include BAL to analyze neutrophil concentrations, which are generally dramatically increased with RAO (Mair et al., 2013). Cytology of BAL fluid in RAO horses will typically show severe increases in neutrophils > 25%; neutrophilic increases up to 80-90% have been observed (Sprayberry and Robinson, 2015; Couëtil et al., 2016). Other diagnostic techniques include endoscopy of the trachea and bronchi to look for accumulation of mucus, swelling of the mucosa, and collapse of the airway during expiration; auscultation of the airway and lungs to detect abnormal airway sounds such as crackling, wheezing, or even subtle changes in normal breathing pattern; observation of responses with changes to less dusty feed and environment; as well as responses to drug therapy (Mair et al., 2013; Niedzwiedz, 2014).

The ideal treatment for RAO horses would be permanent modifications to the environment and management of the horse such as moving the horse to a pasture environment,

soaking hay and feeding on ground, increasing ventilation, and removing or watering down dust-prone bedding in an attempt to decrease the number of airborne allergens that trigger inflammation (Reed et al., 2010b; Sprayberry and Robinson, 2015). When making modifications to feed, it is best to avoid feeding round bale hay, which is high in endotoxin and dust content (House, 2016). Bronchodilators and corticosteroids are commonly used as temporary solutions to decrease inflammation and dilate the lower airway to minimize obstruction of airflow (Thomson and McPherson, 1983; Mair et al., 2013). It has been concluded that inhaled and systemically administered corticosteroids are beneficial in improving lung function in RAO horses (Couëtil et al., 2016), and the instillation of platelet-rich plasma in the respiratory tract has been studied as a possible regenerative therapy to promote healing and reduce inflammation within the airway (Dzyekanski et al., 2012). A key point to be made is that even with proper treatment, a horse that had been diagnosed with RAO will always be susceptible to recurrence of the disorder and its hypersensitive reactions (Reed et al., 2010b).

Exercise-Induced Pulmonary Hemorrhage

Exercise-Induced Pulmonary Hemorrhage is an extremely common condition in racehorses, such as thoroughbreds, that is a direct result of exercise. Running at high speeds results in hemorrhage at the level of the lungs, usually in the dorso-caudal regions (Mair et al., 2013; Couëtil et al., 2016). Studies have concluded that there is an increased risk of EIPH as age increases as well as throughout the different seasons of the year being higher in the hot and humid months (Newton and Wood, 2002). It has been theorized that stress failure of the thin-walled pulmonary capillaries, presence of lower airway diseases such as RAO, increases in pressure at high speeds, and changes in blood viscosity such as during dehydration individually and cumulatively play a role in the occurrence of EIPH (Mair et al., 2013). It appears that EIPH

is fundamentally inevitable with the presence of these factors during exercise, so prevention is not an achievable goal (Marlin, 2015). The rupturing of the capillaries results in pulmonary hemorrhage which also stimulates an inflammatory response in the airways (Mair et al., 2013). It has been observed that horses presenting with both IAD and EIPH have a more pronounced degree of exercise-induced hypoxemia, and subsequently a greater impairment of lung function (Sanchez et al., 2005).

While clinical signs of exercise-induced pulmonary hemorrhage do exist, a majority of horses who experience pulmonary hemorrhage do not display noticeable external symptoms. During exercise, there can sometimes be a reduction in exercise tolerance, respiratory distress with coughing, as well as an increase in the frequency of swallowing in an attempt to clear blood from the trachea. While there has been no research to date confirming behavioral changes in EIPH horses, veterinarians have observed changes in clinical practice such as distress, uneasiness, reluctance to work, lost stride rhythm and other alterations in behavior. Epistaxis, presence of blood at the nostrils, can be present but is fairly uncommon and only observed in a small number of cases (Mair et al., 2013). In a study of 107 thoroughbred horses, 41 displayed evidence of EIPH, and only one horse had blood at its nostrils (Raphel and Soma, 1982). In severe cases such as during prolonged strenuous exercise in hot and humid climates, pulmonary hemorrhage can be fatal (Newton and Wood, 2002; Mair et al., 2013). It has been concluded that moderate to severe cases of EIPH is associated with increased likelihood of finishing inferior specifically in Thoroughbred racehorses; however, there is evidence that there is no association between EIPH grades 1-3 and a shorter racing career (Hinchcliff et al., 2015).

Endoscopy of the airway, tracheal aspiration, and BAL can be used as diagnostic techniques for EIPH. An endoscopy can be performed approximately one to two hours post

exercise to identify red blood cells in the large airways (Mair et al., 2013), and is the preferred method for EIPH diagnosis (Reed et al., 2010b). While a BAL can be used in diagnosing EIPH, the results should be analyzed cautiously due to its sensitivity red blood cell concentrations. This technique has been observed to classify EIPH in horses with extremely low grade bleeding that does not result in poor performance (Sprayberry and Robinson, 2015). It would be best to use in combination with other diagnostic techniques. Differential diagnostic measures should also be taken to identify any other possible airway diseases such as inflammation and infection, and antibiotics can be administered if necessary. Horses should be rested to allow healing in the airway if EIPH has affected performance or resulted in exercise intolerance (Mair et al., 2013). In strenuously exercising EIPH horses, Furosemide can be administered to reduce plasma volume and subsequently decrease blood pressure within the pulmonary capillaries; it does not prevent EIPH occurrence, but does lessen the severity (Reed et al., 2010b; Sprayberry and Robinson, 2015).

Lameness

Inflammation does not only occur within the respiratory tract, but also throughout the musculoskeletal system; inflammation can be both a cause and result of lameness in the horse. The Merck Veterinary Manual defines lameness as an abnormal stance or gait caused by either a structural or a functional disorder of the locomotor system which can include joints, cartilage, ligaments, tendons, as well as skeletal muscle. The general term of lameness can be used to describe a more extensive problem such as a metabolic disorder or previous trauma (Adams, 2016). Most of the 9.2 million horses in the US are expected to serve some type of athletic role in service to humans, so if their ability to perform is compromised so is their overall purpose (Beckstett, 2012). It has been stated that lameness is the primary factor of loss of training in

young Thoroughbred horses (Lindholm, 1986) and that pain, which can result from inflammation, is the primary cause of lameness (Adams, 2016). Repetitive stress applied to the joints and body from speed work, jumping, and extreme hindquarter thrust results in inflammatory changes to the articular surface and/or the joint capsule which can result in pain, swelling, and ultimately irreversible damage to the joint (Hotamisligil, 2006). A study examining barrel racing horses concluded that foot pain in the forelimbs as well as osteoarthritis in the distal tarsal joints were the most common issues resulting in lameness and decreased performance (Dabareiner et al., 2005). Veterinarians have observed lameness to be the primary issue in performance horses.

Pain in the forelimb can be associated with a head nod during active movement as a diagnostic technique, whereas pain in the hindlimb can be associated with a sacral or pelvic rise. Fatigue, inflammation, repetitive stress, and inadequate conditioning to exercise and performance are all potential factors in the onset of lameness. Lameness due to muscle straining during exercise or musculature abnormalities such as from ER can be addressed with rest and physiotherapy followed by a recovery period. History of training, age, shoeing, response to anti-inflammatory drugs, environment, nutrition, and onset of lameness are all beneficial in diagnosis of the underlying cause of the lameness. Physical and visual examination of the back, neck, and limbs during rest and light to moderate exercise are necessary for diagnosis. Hoof testers should be used for observance of sensitivity from abscesses or bruises. Radiographs can help identify both acute and chronic changes to bony tissues, and ultrasound can assess any damage to soft tissues structures such as tendons and ligaments. Neurologic examinations should be performed if painful or mechanical issues are not observed to examine the possibilities of a neuromuscular disorder (Adams, 2016). The treatment of lameness will be fully dependent on the underlying

cause. Some of the most observed lameness in equine includes forefoot lameness, tendonitis, and osteoarthritis (OA).

Tendonitis

Tendonitis refers to any damage to the tendon as a result of strain or injury. Strain consists of intrinsic factors such as degradation of the tendon as a result of chronic inflammation, whereas injury consists of external trauma such as experiencing a blunt force during jumping (Mair et al., 2013); fatigue has also been observed as a contributing factor to tendon injuries (O'Sullivan, 2007). Both intrinsic and extrinsic factors can lead to ultimate failure of the tendon (Sprayberry and Robinson, 2015).

Tendons are primarily composed of type 1 collagen. Tendon cells, tenocytes, form an extracellular matrix to maintain the alignment of these collagen fibers. Tendons connect muscle to bone, so they play a key role in maintaining the musculoskeletal relationship that is essential for movement (Patterson-Kane and Firth, 2009; Sprayberry and Robinson, 2015). Prevalence of tendon injuries increases with increases in age, as repetitive loading of the tendon causes the tendon matrix to breakdown faster than the cellular repair can occur (Patterson-Kane and Firth, 2009). An injured tendon undergoes the classic wound healing processes, and the remodeling phase of the tendon has been observed to last up to 18 months, with decreases in elasticity (Sprayberry and Robinson, 2015).

Clinical signs of tendon injury included slight increases in skin temperature at the affected area, swelling, and moderate to severe lameness (Mair et al., 2013). Diagnostic measures of tendon injuries include palpitation to reveal warmth, swelling, and thickness of the injured tendon as well as using ultrasonography to identify abnormalities, tears, enlargement, lesions, and tendinous edema (Mair et al., 2013). For mild tendonitis, ice packs, cold hosing, and

stall rest can be utilized to limit further damage to the tendon (Mair et al., 2013). For more severe tendonitis, non-steroidal anti-inflammatory drugs can be administered followed by controlled exercise, which can be implemented into the treatment program once the inflammation has subsided to prevent further damage (O'Sullivan, 2007). Regenerative therapies such as stem cells and platelet-rich plasma as well as physical therapies such as shockwave therapy can also be utilized to promote healing (Mair et al., 2013).

Osteoarthritis

Osteoarthritis is a progressive deterioration of articular cartilage characterized by inflammation, joint pain, and decreased range of motion. There can also be negative effects on the bones, soft tissues, and other structures associated with the joint (Mair et al., 2013; Sprayberry and Robinson, 2015). The synovial joint consists of a layer of articular cartilage that provides a smooth surface for joint mobility, and a layer of subchondral bone. The synovial membrane that surrounds the joint produces the synovial fluid that lubricates the joint; the joint capsule and the surrounding ligaments provide stability (Sprayberry and Robinson, 2015). Equine OA can result from repetitive trauma to the synovial joint structures, persistent inflammation, as well as other joint diseases (Mair et al., 2013). Lasarzik et al (2016) observed that IL-1β concentrations in the synovial fluid of horses with OA were significantly higher that horses without OA (Lasarzik et al., 2016). Increased serum concentrations of SAA have also been observed during inflammatory arthritis induced by intraarticular injection of amphotericin B in horses (Jacobsen et al., 2006). Both IL-1β and SAA concentrations could be used as markers of arthritic inflammation.

Clinical signs of OA in equine include observed lameness, pain, decreased movement and range of motion (Mair et al., 2013), and possible synovial effusion (Sprayberry and Robinson,

2015). Lameness and flexion exams, analysis of synovial fluid, and radiography are all effective diagnosis techniques in identifying OA. Synovial fluid is normally clear with a slight yellow hue with <500 WBCs/μL; horses with OA will present high WBC counts up to 1000/μL (Sprayberry and Robinson, 2015). Radiography can reveal new bone formation, narrowed joint space, subchondral sclerosis, and other abnormalities within the joint (Mair et al., 2013; Sprayberry and Robinson, 2015). The primary goal of OA treatment is to minimize any pain and further deterioration of the joint (Sprayberry and Robinson, 2015). Treatment for OA includes physical therapies such as controlled exercise, swimming, and shock wave therapy; administration of NSAIDs and nutraceuticals; intraarticular medications such as interleukin-1 receptor agonist protein (IRAP), PRP, stem cells, and corticosteroids; and surgical management in severe cases (Mair et al., 2013).

Forefoot lameness

Foot pain in the forelimbs is one of the most common causes of lameness in the horse (Maliye et al., 2015; Burlington Equine Veterinary Services, 2018). Forefoot lameness can be a result of anatomical injury or predisposition, physiological dysfunction, improper foot care, and even strain and injury from exercise. The equine forefoot is made up of numerous bones, soft tissue structures (tendons, ligaments, bursae, muscles, etc), and nerves. Forefoot bones include the long pastern bone, short pastern bone, coffin bone (distal phalanx), and navicular bone. These bones are all susceptible to fracture, infection, inflammation, as well as osteoarthritis. Forefoot supporting structures include the deep digital flexor tendon, deep digital cushion, collateral ligament, branches of the suspensory ligaments, sesamoidean ligaments, navicular ligaments, frog, sensitive and insensitive laminae, joint capsules, and bursae which prevent irritation of the tendon as it passes over bone. Soft tissue structures are susceptible to inflammation, infection,

bruising, strain, injury, and laceration (Burlington Equine Veterinary Services, 2018). Some of the most common conditions resulting in lameness of the forefoot include abscesses, navicular syndrome, laminitis, bone fractures, deep digital flexor tendonitis, bursitis, osteoarthritis, severe bruising, and side-bone (Adams, 2016). There are many more conditions that result in forefoot lameness, this is just naming a few. It has also been observed that a high proportion of horses with forefoot pain have a primary soft tissue injury (Dyson et al., 2005).

Forefoot lameness can often be accompanied by a compensatory lameness due to compensatory load redistribution. In a study conducted by Maliye et al (2015), 28 horses were utilized to quantify the compensatory response to naturally-occurring forelimb lameness. It was concluded that forelimb lameness does result in a significant compensatory load distribution. In this specific study the primary compensatory lameness observed was contralateral hindlimb lameness (Maliye et al., 2015). Other clinical signs of forefoot lameness are more so dependent on the underlying cause but can include hesitance to bear weight on affected foot, inflammation, swelling, sensitivity, and increases in digital pulse. Effective diagnostic measures include testing the hoof for sensitivity, anesthetic blocking, radiography, ultrasonography, and magnetic resonance imaging (Adams, 2016; Burlington Equine Veterinary Services, 2018). In order to identify the specific anatomical location causing the forefoot lameness, veterinarians will generally work “from the ground up” (Burlington Equine Veterinary Services, 2018). Diagnostic analgesia is utilized in various portions of the forefoot such as the nerves and joints to help specify and localize the lameness (Schumacher et al., 2004). Treatment protocol will depend on underlying cause and can consist of rest periods, minimizing mobility; modifications to trimming and shoeing; NSAID and supplement administration; physical therapies such as shockwave

therapy, laser therapy, and controlled exercise; and surgical intervention in severe disorders (Mair et al., 2013; Burlington Equine Veterinary Services, 2018).

HEADING 7

CONVENTIONAL TREATMENTS OF COMMON AILMENTS IN THE EXERCISED HORSE

To properly treat common ailments in equine such as inflammation, which severely reduces performance, there currently is administration of conventional drugs. An example of a conventional treatment would be Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), which are administered to reduce inflammation and can increase performance in both the musculoskeletal and respiratory systems (Kallings, 1993). Consequentially, conventional treatments have been observed to lead to ulcers and susceptibility to secondary illnesses (Dowling, 2002). This chapter will further discuss the function, benefits, and consequences of conventional treatments.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs are regularly used to alleviate pain from inflammation, used in musculoskeletal conditions, and for treatment of abdominal pain (Kallings,

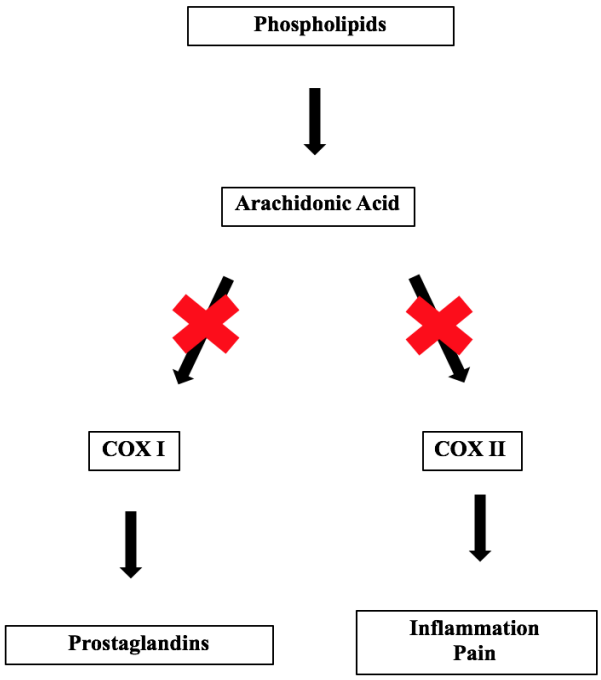

Figure 1. NSAID Pathway

1993; Kallings et al., 2010). These drugs work by blocking the cyclooxygenase (COX) I and II enzymes, which in return prevents the conversion of arachidonic acid to prostaglandins as well as the pain and clinical inflammatory responses (Figure 1). The COX-I enzymes are involved in prostaglandin production during both normal physiological function and injury; COX-II enzymes are primarily associated with pain and inflammation itself due to their low levels during normal physiological function and upregulation during injury (Marshall and Blikslager, 2011; Blikslager, 2015). While blockage of inflammatory prostaglandins aids in reducing pain and inflammation symptoms, it can also result in adverse side effects such as tissue damage, ulceration, and colic (Dowling, 2002; Bland, 2015) because the prostaglandins that are inhibited play a key role in maintaining the mucosal blood flow and barrier of the GI tract. Additionally, NSAIDs can damage epithelial cells and increase permeability to the GI trait (Mpofu et al., 2004). Other key prostaglandins include those involved in maintaining renal blood flow when there is an increased amount of vasoconstrictor substances, such as angiotensin II and anti-diuretic hormone, during a state of dehydration. If the production of these prostaglandins is inhibited when the horse is dehydrated, such as in Heat Stroke, renal blood flow becomes insufficient and can result in renal toxicity and necrosis (Orme, 1986).

Some of the most commonly used NSAIDs used in horses are phenylbutazone (bute), flunixin (banamine), and firocoxib (equiox). These drugs have an increased affinity for blood proteins, so they are well absorbed from the stomach when administered orally (Dowling, 2002). A study on the effects of flunixin on equine exercise responses reported increased heart rate specifically during submaximal exercise (8 m/s) with no significant changes in heart rate during maximal exertion (Kallings et al., 2010). It was theorized that the increase in heart rate during submaximal exercise was a result of changes in sensitivity of the SA node of the heart to

sympathetic nervous system stimulation. Kallings et al (2010) also reported increases in plasma lactate levels as well as stride length (Kallings et al., 2010). Firocoxib products are becoming extremely popular because they preferentially target the COX-II enzyme which reduces pain and inflammation, with minimal effects on the COX-I enzymes so prostaglandin production present during normal physiological function is not inhibited. However, with high doses of firocoxib, its ability to primarily target the COX-II enzyme is decreased (Blikslager, 2015). Breed, age, and high doses of NSAIDs predispose horse's to toxicity (Lees and Higgins, 1985). Since non-steroidal anti-inflammatory drugs are effective in reducing inflammation, but consequently increase the risk of ulcers and colic, they should only be administered on a short-term basis and cautiously to horses with history of gastrointestinal problems.

Corticosteroids (Steroidal Anti-Inflammatory Drugs)

Corticosteroids have powerful anti-inflammatory properties. They work by blocking both the lipoxygenase and cyclooxygenase enzymes which further prevents the production of

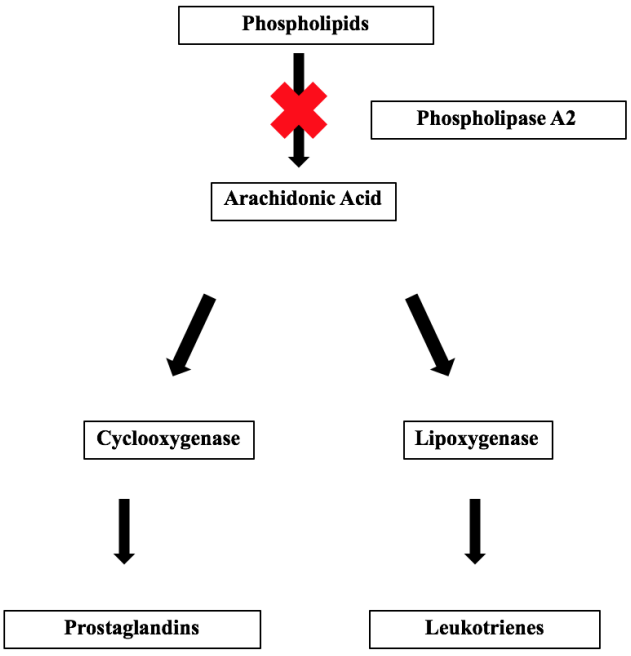

Figure 2. Corticosteroid Pathway

prostaglandins and inflammatory response (Figure 2). They also increase lipocortin production which prevents the cleavage of arachidonic acid from the damaged cell walls by inhibiting the production of phospholipase A2 (Dowling, 2002). Corticosteroids also have immunosuppressive effects by inhibiting the function of white blood cells as well as production of antibodies, which can increase the horse's susceptibility to bacterial, viral, and fungal infections; they have been associated with abscess formation (Dowling, 2002; Mpofu et al., 2004). Excessive water consumption and urination are not uncommon since corticosteroids tend to effect body water regulation. Commonly used corticosteroids in equine are hydrocortisone, methylprednisolone, prednisolone, and dexamethasone. They can be administered orally, intravenously, intramuscularly, or via inhalation depending on the underlying condition (Dowling, 2002).

The lipoxygenase enzyme is responsible for the production of leukotrienes D_4 and B_4, which have been observed to increase neutrophil production in the lungs and subsequently induce bronchoconstriction (Marr et al., 1998). Corticosteroids inhibit activity of the lipoxygenase enzyme, and are commonly used to relieve clinical signs of chronic obstructive pulmonary disease, acting to reduce inflammation as well as the number of inflammatory cells in the airway (Barnes and Adcock, 2003; Mair et al., 2013).

Inhalation of corticosteroids for airway inflammation is preferred since it delivers maximal concentrations more directly to the target site (Mair et al., 2013), it can also be injected locally to relieve pain in joints and tendons (Dowling, 2002). Systemic absorption of inhaled corticosteroids does result in suppression of the hypothalamic-pituitary-adrenal axis, adrenocorticotropic hormone, and subsequently naturally produced cortisol in the body (Rao Bondugulapati and Rees, 2016). Cortisol plays a key role in homeostasis during exercise; immune system modulation; the inflammatory process; and mobilization of substrates such as

fatty acids, amino acids and glucose (Hodgson et al., 2013). The extent of the impairment of these functions is relative to the quantity of corticosteroids administered (Rao Bondugulapati and Rees, 2016). There is also an increased risk for laminitis, ulcerations, and toxicity when administering corticosteroids (Dowling, 2002); however, it has been concluded that oral administration of prednisolone specifically does not increase the risk for laminitis (Jordan et al., 2017). Daily administration of dexamethasone been observed to significantly increase blood lactate concentrations within a 2-day period of administration in equine (Mizen et al., 2017), which could result in a more rapid onset of fatigue in the exercising horse.

Corticosteroids have been proven to be an effective anti-inflammatory and bronchodilator; however, their immunosuppressive effects increase susceptibility to infection and they can have adverse effects on the necessary physiological functions of the performance horse. Based on these properties, they should not be administered concurrently with immune disorders, or on a long-term basis to avoid negative effects on equine performance.

β-Agonistic Bronchodilators

While corticosteroids can be utilized in reducing airway inflammation, beta-agonists bronchodilators are the most effective and popular in treating horses with RAO (Mair et al., 2013; Arroyo et al., 2016). Bronchodilators aid in reducing obstruction by relaxing constricted smooth muscle portions of the airway, allowing them to dilate and expand. Innervation of beta-adrenergic receptors throughout the smooth muscle of the airway leads to relaxation; the most popular bronchodilators act as beta-adrenergic receptor agonists, targeting these receptors and stimulating relaxation. Commonly used bronchodilators in equine include epinephrine, isoproterenol, albuterol, and clenbuterol. Isoproterenol can be administered intravenously,

slowly, for emergency bronchoconnstriction relief or through inhalation or injection for more short-term use (Dowling, 2015).

Bronchodilators are used commonly as a short-term treatment of clinical signs of acute airway inflammation and recurrent airway obstruction (Mair et al., 2013; Arroyo et al., 2016); however, they do not possess long term effects or treat the underlying disorder. Administration of albuterol, via inhalation, immediately before exercise can aid in reducing the presence bronchoconstriction and coughing during the exercise period (Mair et al., 2013); Mazan et al (2014) observed no adverse effects in equine from albuterol inhalation twice daily for a ten day period (Mazan et al., 2014); however, a single dose of aerosolized albuterol has been observed to only last for 1-3 hours (House, 2016). Levalbuterol is a beta-agonist that has been used in the treatment of human asthma. In a study comparing the effects of levalbuterol compared to albuterol in the treatment of airway obstruction in horses, it was concluded that there was no significant difference in the magnitude of improvement in the airway. Doses of levalbuterol, however, lasted twice as long as those of albuterol suggesting it might be a beneficial alternative (Arroyo et al., 2016). Previous data has suggested that prolonged administration of clenbuterol along with training can result in decreased aerobic performance (Kearns and McKeever, 2002). Thus, long term administration of bronchodilators is not recommended.

Drug Regulations

Currently, Dressage, Jumping, Eventing, Endurance, Driving, Reining, Vaulting, and Para-Equestrian Dressage are all regulated by the Federation Equstre Internationale (FEI). The FEI maintains a Code of Conduct to which anyone involved in equestrian sport must adhere to. Part of this code of conduct is a list of banned substances that are considered to have no therapeutic use and controlled substances that are considered therapeutic and commonly used to

treat equine illness and injury (Sprayberry and Robinson, 2015). Of all the most commonly administered conventional medicines previously discussed, albuterol, clenbuterol, dexamethasone, flunixin, fibroxocib, hydrocortisone, methylprednisolone, phenylbutazone, prednisolone are all listed as controlled FEI substances, however, isoproterenol is listed as a banned FEI substance as of January 2018 (FEI, 2018a). Improper usage of controlled substances and any use of banned substances during involvement in these sports can result in fines and legal contributions up to $16,000 with multiple offenses as well as suspensions up to 2 years (FEI, 2018a).

The Association of Racing Commissioners International (ARCI), located in Lexington, Kentucky, functions as the regulating body for horse racing in the United States and updates a list of the Prohibited Substances in Racing in their Uniform Classification Guidelines for Foreign Substances. The list of Prohibited Substances in Racing categorizes the regulated drugs into 5 classes (1, 2, 3, 4, and 5) based on use of drug and if it would be accepted for use. The substances are further categorized into 4 penalty classes that differ in penalty severity (A, B, C, and D). Penalty categories outline the appropriate penalty for usage as well as increases in offenses (ARCI, 2018b). Epinephrine and isoproterenol are categorized as a class 2 drug with a class A penalty. Albuterol and clenbuterol are categorized as a class 3 drug with a class B penatly. Dexmethasone, flunixin, fibroxocib, hydrocortisone, methylprednisolone, phenylbutazone, and predinisolone are all categorized as a class 4 drug with a class C penalty. Penalties on flunixin and phenylbutazone are further classified by the specific concentrations identified in plasma. Along with the penalty classes, violations can also occur through the stacking of NSAIDs; where more than one NSAID is present in a biological sample (ARCI, 2018b).

Penalties are dependent upon the category and if it is the 1st, 2nd, or 3rd offense. Use of substances that are categorized as a class A penalty can result in a 1-year suspension and $10,000 fine for the 1st offense up to a 5-year suspension and $50,000 fine for the 3rd offense. Use of substances in categorized as a class B penalty can result in a 15-day suspension and $500 fine for the 1st offense up to a 60-day suspension along with a $2,500 fine for the 3rd offense. Use of substances categorized as a class C penalty can result in a written warning and $500 fine for the 1st offense up to disqualification and a $2,500 fine for the 3rd offense (ARCI, 2018b).

Along with FEI and ARCI drug regulations, in the United States drugs are also regulated by the United States Equestrian Federation (USEF). Albuterol, clenbuterol, and epinephrine are permitted with a properly submitted medication report form. Dexamethasone, fibroxocib, flunixin, phenylbutazone are permitted but in restricted doses as well as time recommendations relative to competition (USEF, 2018). Along with specific regulations on these individual drugs, it is also stated that no medication shall be administered to a horse within 12 hours prior to a competition, and that only one NSAID is permitted in plasma and/or urine samples that are collected for testing. Intra-articular administration of local anesthetics, such as those used in lameness diagnosis, as well as corticosteroid administration is not permitted within 24 hours of the competition (USEF, 2018). Penalties for improper usage of NSAIDs and dexamethasone can range from a $750 fine on a first offense to a $3000 fine and 1-month suspension for a third offense. Penalties for usage of USEF forbidden substances that are FDA approved and possess a therapeutic use can result in fines up to $12,000 as well as 12-month suspensions (USEF, 2018). Penalties for usage of USEF forbidden substances that are not FDA approved can result in fines up to $24,000 and suspensions greater than 2 years (USEF, 2018). The fundamental message is that equine drug regulations are very serious and should be adhered to.

Non-steroidal anti-inflammatory drugs, corticosteroids, and bronchodilators are all conventional treatments currently utilized for exercise related ailments in equine. While they have been determined to be efficient treatments, they possess proven side effects such as ulcers, susceptibility to secondary infections, as well as inefficiency or problems from long-term administration. They are also highly regulated and may not even be an option depending on the type of sport the horse is involved in. These consequences have led to an increased demand for regenerative and alternative methods to treat exercise related ailments and increase performance in equine.

HEADING 8

REGENERATIVE THERAPIES FOR COMMON AILMENTS IN THE EXERCISED HORSE

Consequences related to conventional treatments have increased the popularity of regenerative as well as alternative therapies in equine medicine. Regenerative therapies include autologous conditioned serum (ACS), platelet-rich plasma (PRP), autologous protein solution (APS), and stem cells which all work to naturally trigger the healing and repair process within the body for a variety of musculoskeletal and respiratory conditions.

Autologous Conditioned Serum

As previously discussed, IL-1β concentrations are significantly higher in the synovial fluid of horses with OA (Lasarzik et al., 2016). Since this protein has been observed to play a key role in the development of OA, the goal of treatment would be to block the cellular receptor for IL-1β (Sandoval et al., 2013). This is most commonly done through administration of Interleukin-1 Receptor Agonist Protein (IRAP). IRAP is primarily administered intra-articular as a component of autologous conditioned serum (ACS), which can be processed through commercially available kits; ACS can also be administered for tendon and ligament injuries (Sprayberry and Robinson, 2015).

It was observed that 4 ACS doses at 7-day intervals resulted in significant improvement in clinical lameness as well as decreased OA pathology within the synovial membrane in 16 horses with OA-affected joints; there were no observed negative side effects (Frisbie et al., 2007). A previous study conducted by Frisbie et al (2005) observed a greater degree of joint repair in OA-affected horses treated with ACS compared to control (Frisbie et al., 2005). Autologous conditioned serum is prepared by incubating blood filled with medical-grade glass

beads overnight which stimulates the release of endogenous substances such as IRAP (Bertone et al., 2014; Sprayberry and Robinson, 2015). Because the processing of ACS requires sterile kits, incubators, and a centrifuge, it can be a more expensive treatment option.

Platelet Rich Plasma

When injury occurs in the horse, it triggers the activation of platelets circulating in the blood. Activation stimulates the cellular process of degranulation within the platelets. which further releases bioactive substances important in healing and regeneration (Sprayberry and Robinson, 2015). Platelet Rich Plasma (PRP) is an autologous compound, prepared from the patient's own blood, that delivers a concentrated dose of platelets, white blood cells, and plasma directly to the site of injury via injection (Textor, 2011; Rinnovati et al.). A blood sample is collected from the patient, processed via centrifuge to separate red blood cells from plasma, and then this platelet rich plasma is injected at the site of injury for tissue regeneration (Textor, 2011). Platelets have been observed as reservoirs of growth factors and anti-inflammatory cytokines that play an important role in connective tissue healing and cellular regeneration in both humans and equine (Torricelli et al., 2011; Rinnovati et al., 2016). Activated platelets secrete numerous growth factors that stimulate angiogenesis and the proliferation of undifferentiated mesenchymal stem cells, downregulating the pro-inflammatory cytokines IL-1β and TNF-α, advancing the musculoskeletal tissue healing process (Torricelli et al., 2011; Carmona et al., 2018). The administration of PRP has been used as a technique to improve the quality and speed of healing in damaged areas such as tendons, ligaments, and even in the lungs (Torricelli et al., 2011).

Most research to date on PRP administration in equine observes the effects within the musculoskeletal system. Carmona et al (2007) observed significant improvement in joint

effusion scores after two injections of PRP, and no horses presented with any local or systemic side effects (Carmona et al., 2007). In a clinical study analyzing the therapeutic effects of PRP on horses with lameness due to overuse musculoskeletal injuries, specifically either suspensory ligament desmopathies or superficial flexor tendinopathies, all horses showed a highly significant mark of improvement in lameness and returned to their pre-injury level of performance within one year. This specific study concluded that the most effective dosage of platelet rich plasma should be at a concentrated level of at least 400% of the peripheral blood platelet count (Torricelli et al., 2011), which is in agreement with previous studies (Marx, 2000). Waselau et al (2008) has suggested that Standardbred horses with moderate to severe musculoskeletal disorders had an excellent prognosis for returning to racing when treated with a single dose of PRP followed by a controlled exercise program (Waselau et al., 2008). In a study by Zuffova et al (2013) observing PRP administration in Thoroughbred horses with superficial digital flexor tendiopathies, 100% of horses with mild injury, 56% of horses with moderate injury, and 64% of horses with severe injury returned to racing post-treatment. It was then concluded that administration of platelet rich plasma in combination with controlled exercise positively influenced the healing process of the superficial digital flexor tendinopathies in both acute and chronic cases (Zuffova et al., 2013) and early treatment of superficial digital flexor tendinopathies will enhance these effects (Geburek et al., 2016).

While there are veterinarians utilizing PRP in treatment of respiratory conditions such as EIPH, minimal research has been conducted. In mice, PRP has stimulated lung vascularization and alveolar regeneration, overall improving pulmonary efficiency and function. Researchers have concluded that PRP is potentially a beneficial technique in the regeneration of damaged lung tissue in humans (Mammoto et al., 2016). The limited research on PRP as a respiratory

therapy has implicated that intrabronchial administration of 20 mL of PRP containing 200% of whole blood platelet concentration effectively attenuates airway inflammation associated with IAD (Dzyekanski et al., 2012). As such, this therapy warrants further investigation.

Giraldo et al (2013) observed the effects of breed, sex and age on cellular content and growth factor release from equine pure-platelet rich plasma. There was an observed significant difference in whole blood platelet and WBC counts between Argentinean Creole horses and Colombian Creole horses; platelet and growth factor concentrations in whole blood as well as platelet rich plasma were also significantly higher in females compared to males (Giraldo et al., 2013). The same study suggested adapting centrifugation protocols for different breeds since the size and weight of platelets and WBCs may be different across equine breeds (Giraldo et al., 2013). When observing the effects of environmental factors such as time of day, hydration, NSAID therapy, and exercise on platelet concentrations in platelet rich plasma, horses being administered non-steroidal anti-inflammatory drugs have the most significantly altered concentrations of platelets; none of the other environmental factors mentioned have been associated with a significant change (Rinnovati et al., 2016).

Thus far, there has been no research conducted on PRP transfusion; however, there may be a risk of stimulating an immediate immune-mediated adverse reaction. Most commonly observed in horses is the febrile nonhemolytic reaction (FNHTR), a systemic inflammatory response triggered by inflammatory cytokines which can be released by platelets over time (Sprayberry and Robinson, 2015), such as during a period of storage for transfusion.

Autologous Protein Solution

Both ACS and PRP individually have demonstrated impressive healing qualities on musculoskeletal injuries in the performance horse. In recent years, autologous protein solution

(APS) has been introduced as a promising regenerative therapy because of its properties similar to PRP and ACS in combination. Specifically in humans with OA of the knee, APS has been observed to improve joint pain within a 1-year period (Kon et al., 2018). It contains a highly concentrated number of platelets along with the high concentrations of plasma proteins that produce anti-inflammatory growth factors and cytokines such as IRAP.

Autologous protein solution is created in a two-step process where the patient's blood is processed through the APS separator to concentrate the WBCs and platelets in a small amount of plasma, and then further processed through the APS concentrator (Bertone et al., 2014). The processing for APS has been observed to preferentially increase the concentration of anti-inflammatory cytokines (i.e. IRAP) compared to inflammatory cytokines (i.e. IL-1β); O'Shaughnessey et al (2014) observed a 6.6 fold increase in human APS IRAP concentrations compared to baseline concentrations (O'Shaughnessey et al., 2014). Similarly, in equine APS has been observed to have significantly greater concentrations of both IRAP and WBC compared with normal blood concentrations. In the same study, exercising horses with OA in high motion joints that received intra-articular injections of APS were observed to have synovial joint fluid concentrations of IL-1β, TNF-α, WBCs, and neutrophils all within normal range at 14 days post-treatment; there were no observed negative side-effects with APS injection (Bertone et al., 2014). To the author's knowledge, this is the only study analyzing the effects of APS in equine thus far. The commercial APS treatment in equine is known as "Pro-stride" and can range in price from $450-800 depending on the number of injections, with on-the-spot processing taking less than an hour (Hyman, 2015). While more research on the effects of APS in equine is recommended, it appears to be a suitable regenerative therapy option for joint related lameness.

Stem Cell Therapy

Stem cell therapy is currently used to promote healing for a variety of musculoskeletal injuries in equine such as tendinopathies and OA. Stem cells, or progenitor cells, can be defined as "cells that are capable of self-renewal and can differentiate into specific cell lineages and cell types" (Sutter, 2007). Primarily in equine medicine, stem cells used in therapy are derived from mesenchymal tissue and then applied to a site of injury where they differentiate into specific cells, such as tenocytes, chondrocytes, and osteocytes, and fundamentally produce a wound-healing matrix which is much more beneficial than a scar tissue which is poorly functional (Sutter, 2007). While there is a sub-population of cells located within growing tissues that is able to differentiate and replenish cells that are lost during injury, it has not been confirmed that this sub-population exists in adult tissues, such as the adult tendon. Transplantation of mesenchymal stem cells (MSC) that do possess these differentiation properties into the site of injury can aid in necessary regeneration and tissue healing (Richardson et al., 2007).

One of the most common stem cell collection procedures is a bone marrow aspirate, where bone marrow is collected from the sternum and then injected directly into the site of injury (Sutter, 2007). Renzi et al (2013) observed no negative side effects or inflammation associated with the implantation of bone marrow mesenchymal stem cells (Renzi et al., 2013). While MSCs can be collected from bone marrow, Murata et al (2014) observed synovial fluid to be a potential source of MSCs used for chondrocyte generation (Murata et al., 2014). The same study showed that the number of synovial fluid MSCs in horses with diseased joints was significantly increased compared to synovial fluid MSC concentrations from horses with healthy joints; suggesting that MSCs may play a role in the repair process of damaged cartilage (Murata et al., 2014). Interestingly, it has also been observed that dental pulp stem cells (DPSC) derived from wolf

teeth in Thoroughbred horses possess the potential for self-renewal and differentiation along with the expression of MSC surface markers. Since they meet the minimal criteria for classification of MSCs, Ishikawa et al (2017) concluded that eDPSCs may be a new source for stem cell therapy (Ishikawa et al., 2017).

Autologous conditioned serum, PRP, ACS, and stem cells are all promising regenerative therapies for both respiratory and musculoskeletal conditions effecting equine performance. At this time, there are no outlined regulations on the usage of ACS, PRP, APS, or Stem Cell Therapy (ARCI, 2018a; FEI, 2018b; USEF, 2018). While some regenerative therapies have been further researched compared to others, they all provide an autologous and natural therapy that can be used to address underlying conditions compared to the traditional treatments previously discussed that work to only mask the clinical signs.

HEADING 9

ALTERNATIVE THERAPIES FOR COMMON AILMENTS IN THE EXERCISED HORSE

Along with regenerative therapies, alternative therapies, such as physiotherapies and supplementation, are used to stimulate and enhance the body's natural physiological function. For example, different forms of physical therapy have been observed to stimulate the nervous system, increase flexibility and mobilization of muscles and joints, reduce inflammation, increase blood flow, and increase musculoskeletal function (Haussler, 2009; Andris, 2016). As more research is being conducted and published on these therapies, they are becoming more accepted in the field of equine veterinary medicine; however, there may still be side-effects from alternative therapies that have not been observed thus far.

Acupuncture

Acupuncture techniques are used in horses to detect sensitivity, aid in the diagnosis of lameness, as well as treatment of conditions such as musculoskeletal pain (Le Jeune and Jones, 2014). Acupuncture scanning can be accomplished via palpation of acupuncture points located along specific pathways and meridians; there are over 100 known acupoints in equine (Shmalberg and Huisheng, 2009; Le Jeune and Jones, 2014). In a study observing the correlation of positive acupuncture scans and lameness, 102 performance horses underwent a two-minute scan of acupuncture points to determine sensitivity and were further evaluated as either lame (51 horses) or sound (51 horses). Acupuncture scanning detected sensitivity in 82.4% of lame horses. It was concluded that acupuncture scans can be a beneficial component of an overall exam, but should be accompanied by other diagnostic lameness techniques (Le Jeune and Jones, 2014).

One of the primary effects of acupuncture techniques is stimulation of the nervous system, which can increase blood flow and improve physiological function (White and Medicine, 2009). Stimulation of acupuncture points has been observed to aid in inflammation and pain management through triggering of the pituitary gland to release the adrenocorticotropic hormone which further stimulates the release of the cortisol, a naturally produced steroidal anti-inflammatory, from the adrenal gland (Huisheng et al., 1996). In a study by Martin et al (1987) observing the effects of acupuncture on chronic back pain in equine, thirteen out of fifteen horses displayed pain improvement after a mean of eight weekly acupuncture treatments (Martin and Klide, 1987). Acupuncture techniques are a highly recommended treatment for chronic musculoskeletal back pain, a common cause of equine lameness (Tangjitjaroen et al., 2009).

In addition to lameness evaluation and treatment, acupuncture techniques can be used to reduce inflammation and induce a bronchodilator effect throughout the airway in horses suffering from RAO (Tangjitjaroen et al., 2009). Stimulation of specific acupoints can trigger the release of endogenous opioids within the CNS which has been observed to result in reduced expression of pro-inflammatory cytokines TNF-α and IL-1β (Yin et al., 2005). Single treatments of acupuncture in combination with proper environmental management for 10 horses resulted in improved pulmonary function and parameters such as tidal volume and minute ventilation in horses with airway inflammation; however, these improvements were not significant (Wilson et al., 2004). Acupuncture is not considered a long-term treatment of airway inflammation, but could be beneficial in managing the clinical signs along with proper environmental management (Tangjitjaroen et al., 2009).

It has also been observed that acupuncture treatment does significantly increase rectal and skin temperature in horses subjected to a combination of road transport and exercise (Rizzo et

al., 2017). Current FEI regulations require that equine acupuncture treatments must be carried out and supervised by a permitted treating veterinarian, and only allows the use of solid needles (FEI, 2018c), whereas USEF only recommends administration of acupuncture therapy be performed by a licensed veterinarian (USEF, 2018). More research should be conducted to confirm the effects of acupuncture stimulation on respiratory and thermoregulatory parameters in equine.

Chiropractic Therapy

Chiropractic therapy is defined as the application of high-velocity, low-amplitude thrusts to induce therapeutic effects within articular structures, muscle function, and neurological reflexes (Haussler, 2009). Fundamentally, these chiropractic manipulations work to realign the joints of the musculoskeletal system, optimizing nervous system stimulation, blood flow, and movement in the performance horse. This treatment can result in significant and beneficial alterations to the kinematics of the spine, increasing overall spinal mobility and flexibility (Haussler, 2009). The overall goal of chiropractic therapy is to restore normal joint function, stimulate neurological reflexes, reduce pain, and reduce muscle hypertonicity (Snow, 2013). Articular neurophysiology, biochemical alterations, pathologic changes within the joint capsule, and articular degeneration are all basic elements of dysfunction in the joint and/or spine (Haussler, 2009). Horse owners will seek out chiropractic therapy as a means to treat clinical signs of lameness including back pain, abnormal movement, stiffness, and loss of performance (Schultz et al., 2015).

Sullivan et al (2008) observed that out of 40 horses, the 8 horses randomly assigned to receive chiropractic treatment showed a significantly higher mechanical nociceptive threshold within the thoracic vertebrae of the spine within 7 days of treatment (Sullivan et al., 2008).

Chiropractic treatment has also been observed to increase stride length and improve collection in lesson horses, possibly as a result of increased flexibility and decreased pain in the spinal joints (Schultz et al., 2015). Significant improvement of symmetric pelvic rotation can also result from chiropractic joint manipulation, leading to improved gait and performance (Gomez Alvarez et al., 2008). Gomez Alvarez et al (2008) also observed that in 10 Warmblood horses range of motion increased directly after chiropractic treatment but decreased within the duration of one month.

While chiropractic therapy has been concluded to have a beneficial effect on performance in horses, the effects are generally temporary. In clinical practice, veterinarians have observed chiropractic therapy to be an extremely beneficial treatment for performance horses of various disciplines. This form of therapy can be used alone or accompanied by other methods; either way a valid treatment for joint related musculoskeletal related lameness (Gomez Alvarez et al., 2008). The United States Equestrian Federation recommendeds that chiropractic therapy be adiministered either by or under the direct supervision of a veterinarian (USEF, 2018), and FEI requires that chiropractic may only be carried out by either a permitted equine therapist or a permitted treating veterinarian (FEI, 2018c).

Massage Therapy

Massage therapy is the manipulation of the skin and underlying soft tissues either manually, with an instrument, or with a machine for therapeutic purposes; this includes rubbing, kneading, tapping, and introduction of mechanical vibrations (Haussler, 2009). Therapeutic massage can relieve muscle tightness, increase muscle compliance, increase joint mobility, and decrease stiffness within both muscle and joints (Scott and Swenson, 2009). Equine massage therapy focuses mainly on stress point therapy, trigger point therapy, and myofascial release

techniques. Trigger point therapy involves applying direct pressure to a hyperirritable area and is most commonly recognized in sport's massages in humans. It is common to combine massage therapy techniques with exercise and other physiotherapies to improve musculoskeletal function (Buchner and Schildboeck, 2006).

Equine studies focusing on the effects of post-exercise massage on range of motion observed increased range of motion, increased stride length, and decreased stride frequency implying a positive effect on the athletic performance horse (Scott and Swenson, 2009). Additionally, mechanical pressure from massage increases muscle temperature and arteriolar pressure, fundamentally increasing blood flow. Alterations in heart rate, blood pressure, and physiological hormones as a result of massage therapy can also induce a relaxation response during times of stress and anxiety (Scott and Swenson, 2009). A pilot study by Salter et al (2011) observed significant increases in cutaneous temperatures within five minutes of massage therapy, suggesting increases in blood flow and perfusion directed to the areas being massaged (Salter et al., 2011). Increases in blood flow could result in more efficient removal of metabolic byproducts such as heat, improving performance capabilities. Consistent massage therapy has also been observed to have positive effects on attempts to reduce stress levels in race horses, concluding that it is a beneficial technique for equine welfare and performance (Kedzierski et al., 2017). Some have observed increased manual lymph drainage corresponding with massage in equine, but there are currently no scientific studies to support this claim. Currently, massage therapy is considered a non-restricted supportive therapy that may be administered by any person on horses they are directly responsible for (FEI, 2018c). In general, more research should be conducted to prove the effects of this technique on equine physiology (Haussler, 2009).

Physical Therapy

Mobilization of joints and soft tissue by physical therapists is utilized in assessing quality and quantity of range of motion in joints for the treatment of musculoskeletal disorders. These techniques overall aid in rehabilitation of neuromotor control and help to activate spinal reflexes, increase flexibility, strengthen the musculoskeletal system, and stimulate proprioception. Physical therapy is primarily used in equine as a means to increase joint mobilization after removal of a cast or fixture that has caused immobilization (Haussler, 2009), and is the primary form of therapy for horses competing in competitions that prohibit the use of medications (Andris, 2016). Some common forms of physical therapy in equine rehabilitation include thermal therapy, hydrotherapy, rehabilitative exercise, shockwave therapy, laser therapy, and manipulative therapy.

Thermal therapy consists of both hot and cold techniques. Cold thermal therapy is utilized in reducing pain during acute injury through techniques such as ice water immersion, ice surface application, and cold packs. Cold therapy reduces local blood flow, pain, and inflammation. Heat thermal therapy is applied during chronic injury to enhance tissue stretching and the healing response primarily in the forms of warm water, hot packs, and therapeutic ultrasound. Heat therapy increases local blood flow, relaxes muscles, reduces pain, and can increase joint and tendon mobility (Andris, 2016). Cooling equipment and cooling or heating pads are considered non-restrictive supportive therapies that can be adniminstered by any personnel directly responsible for the horse; however, therapeutic ultrasound therapy must be administered by either a permitted equine therapist or permitted treating veterinarian (FEI, 2018c)

The use of hot and cold therapy can also be applied to equine hydrotherapy, where water is utilized to treat injuries in the muscles and joints. Hunt (2001) observed that three to four weeks of cold water hydrotherapy resolved the fluid component of superficial digital flexor tendon (SDFT) lesions in 27 horses (Hunt, 2001). All of the non-exercise physical therapy techniques can be accompanied with controlled exercise, such as hand-walking, treadmill exercise, and retraining to assist the horse in regaining musculoskeletal tissue strength and returning to normal performance abilities (Andris, 2016). For example, it has been observed that administration of hydrotherapy in combination with progressive exercise loading for 16-18 weeks allowed horses to successfully return to racing within a six to eight month period (Hunt, 2001).

Along with basic hydrotherapy, treadmill exercise, both conventional and water, is becoming increasingly popular in performance horse rehabilitation. It is recommended that conventional treadmill exercise be utilized for horses with acute SDFT, sacroiliac pain, suspensory desmitis, and upward fixation of the patella, while water treadmill exercise is recommended as a post treatment for horses with SDFT, sacroiliac pain, and proximal suspensory desmitis. Range of motion in pelvic flexion and axial rotation has been observed in significantly increase during water treadmill exercise at water depths at the level of the fetlock and higher compared to a control depth of the hoof (Mooij et al., 2013). Stride length was also observed to increase with increases in pelvic flexion. Mooij et al (2013) concluded that water depth during treadmill exercise does have an effect on back motion in equine (Mooij et al., 2013). Both conventional and water treadmill exercise are not recommended for horses with wounds and deep digital flexor tendinopathies (Nankervis et al., 2017).

Shockwave therapy has been used in horses with conditions such as tendinitis, osteoarthritis, and deep muscle pain. Shockwave impulses pass through tissue interfaces leading to tissue compression, stimulating the healing of bone and soft tissue. Following a two-day rest period, horses can undergo rehabilitation exercise. It has been observed to reduce inflammation, increase tissue healing, increase blood vessel proliferation, and reduce pain (Andris, 2016), and Chen et al (2013) observed that pro-inflammatory cytokine IL-1β was significantly down regulated in 11 Thoroughbred horses treated with shockwave therapy (Chen et al., 2013). It is currently recommend that shockwave therapy not be used within 5 days prior to USEF competition (USEF, 2018), prohibited to administer within 5 days of FEI events (FEI, 2018c), and ARCI does not permit racing for a minimum of 10 days following a shockwave treatment (ARCI, 2018a).

Low-level laser therapy is used for treatment of wounds and soft tissue injuries, osteoarthritis, and in attempts to reduce pain (Andris, 2016). Jann et al (2012) observed that superficial metacarpal wounds healed significantly faster with the administration of low level laser therapy compared to control wounds, concluding that low level laser therapy has a positive effect on wound healing (Jann et al., 2012). Along with wound healing properties, the combination of both laser therapy and chiropractic techniques has also been observed to reduce equine musculoskeletal pain more substantially than from the sole practice of one of these techniques (Andris, 2016). It has been suggested that laser therapy reduces pain and inflammation via inhibition of the COX II enzyme and reducing inflammatory prostaglandin production (Saayman et al., 2011). The FEI classifies laser therapy using lasers of classes I to III as a non-restricted therapy that can be administered by any personnel on horses they are directly responsible for (FEI, 2018c).

Manipulative therapy such as range of motion exercises and stretching are utilized to strengthen muscles, increase flexibility, and reduce pain and muscle discomfort (Andris, 2016). It has been observed in rats that manipulative therapy in the lumbar region in adult rats results in a short-term reduction in nociceptive behavior, so fundamentally, a short-term reduction in pain (Onifer et al., 2015). All in all, physical therapy techniques, used either individually or in combination, have a great potential for optimizing physiological function in the performance horse.

Nutraceuticals

Nutraceuticals are considered any natural dietary supplement, typically plant based, that provide beneficial precursors and catalysts aiding in the prevention and treatment of diseases; primarily lameness in the horse (Montgomery, 2011). One of the largest issues with nutraceuticals is that they are not FDA approved and have very little research to prove their efficacy. While they are being tested more and more, equine research is still very minimal; proper dosage, side effects, and health benefits for horses is still unknown (Brown, 2009; Bland, 2015). One of the most well-known nutraceutical supplements is Glucosamine, which is favorably absorbed across the epithelium of the small intestine (Wright, 2001). Glucosamine and chondroitin sulfate in combination have been studied for their effects in treating equine musculoskeletal related lameness (Montgomery, 2011). There are many claims that glucosamine can actually improve motor function in older horses, but a study analyzing the effects of three-month oral supplementation of glucosamine, at 9.96 g, and chondroitin suflate, at 2.0 g, in 24 horses with a maximum lameness score of 1, concluded there was no sufficient evidence that oral supplementation results in substantial improvement of locomotor performance in horses (Higler et al., 2014). Another study observing the effects of oral supplementation of GLC 5500, a

commercial glucosamine and chondroitin sulfate product, in 50 horses with chronic joint pain and a lameness score of 2 or greater concluded that oral supplementation of glucosamine and chondroitin sulfate did aid in reducing the pain associated with lameness, and was not accompanied by any adverse side effects (Montgomery, 2011). Even with the current research on glucosamine effects in equine, the quality of some glucosamine products available has been tested and observed to be substantially different from the claims on their product labels; a consequence due to the lack of FDA regulations (Oke et al., 2006).

Another up and coming nutraceutical that has been thought to aid in preventing joint diseases and gastrointestinal diseases associated with inflammation in horses is the herb turmeric. Curcumin, the active ingredient of turmeric, is known for having anti-inflammatory, wound healing, and anti-parasitic properties in other species (Oke et al., 2006; El-Bahy and Bazh, 2015); however, minimal research has been conducted on its efficacy in equine. While nutraceuticals are gaining popularity in veterinary medicine due to being relatively inexpensive and having minimal to no demonstrable side effects, the lack of FDA approval for these substances leads to very little scientific evidence proving or disproving their efficacy for the conditions that they claim to address. Glucosamine and curcumin have not been listed on the previously mentioned lists of substance regulation (ARCI, 2018b; FEI, 2018a; USEF, 2018). Due to the minimal amount of research that has been conducted observing effects of nutraceuticals specifically in equine, it is difficult to properly assess the benefits and consequences of this type of supplementation (Brown, 2009).

Other Supplements

A common supplement in equine feed is Vitamin E, which contains antioxidant properties that aid in the prevention of cellular damage and oxidative stress. Intense exercise can

lead to oxidative stress in the performance horse, meaning they might benefit from increased vitamin E supplementation. Studies have observed decreased levels of beta-carotene, necessary for Vitamin A synthesis, with increased levels of vitamin E (Williams and Carlucci, 2006). Vitamin A serves a key role in vision, immune system function, normal growth, as well as maintenance of epithelial cells (Hinchcliff et al., 2013). Supplementation of antioxidants such as sodium selenite and ascorbic acid have been observed to significantly improve the clinical signs of chronic lower airway disease. These improvements have been associated with subsequent increases of selenium, vitamin C, and vitamin E in the epithelial lining fluid of the airway, improvements in lung function, and reduction in oxidative damage. It has been suggested selenium supplementation could be an effective treatment of airway inflammation such as during chronic obstructive pulmonary disease and inflammatory airway disease (Youssef et al., 2013). Currently. Vitamins A, C, E are all categorized by ARCI as non-classified substances that only affect the physiology of the horse through improvement of nutrition or to treat/prevent infections; supplementation is not regulated (ARCI, 2018b).

Amino acid supplementation has been utilized in aging horses to minimize the quantity of muscle mass loss. In aging, lightly exercised horses it was observed that supplementation of the amino acids lysine and threonine improved the maintenance and reduced overall muscle mass loss (Graham-Thiers and Kronfeld, 2005). While these benefits have been recorded, however, the ideal requirement of amino acid supplementation has not been defined (Graham-Thiers and Kronfeld, 2005).

Ribose supplementation was hypothesized to decrease ammonia and lactic acid accumulation during exercise, prolonging the onset of fatigue and improving overall performance. In a study by Kavazis et al. (2004) analyzing the effects of ribose supplementation

8 horses were provided 0.15 g of either glucose or ribose supplement per kilogram of body weight twice daily prior to participating in a standard exercise test. After the standard exercise test, blood was collected, and ammonia, lactic acid, and glucose levels were analyzed. After a two week period, it was concluded that the ribose supplementation did not influence the above factors, and does not affect overall anaerobic exercise capacity (Kavazis et al., 2004). Betaine supplementation has also been observed for its effects associated with lactate concentrations (Warren et al., 1999). When supplementing 80 milligrams of betaine per kilogram of body weight for 14 days, there was no observed changes in lactate concentrations prior to exercise. However, lactate concentrations were observed to significantly decrease during the recovery period post exercise in untrained horses compared to baseline levels. It was concluded that betaine supplementation does influence post exercise lactate metabolism, specifically in the untrained horse (Warren et al., 1999). Amino acids, ribose, and betaine have not been listed on the previously mentioned lists of substance regulation (ARCI, 2018b; FEI, 2018a; USEF, 2018).

HEADING 10

CONCLUSION

As discussed, some of the primary exercise related ailments that reduce equine performance originate within the respiratory and musculoskeletal systems. Inflammation and fatigue alone and on a minimal basis are relatively inevitable, but without proper treatment can lead to more severe conditions such as Exhausted Horse Syndrome, Exertional Rhabdomyolysis, and Recurrent Airway Obstruction. Through the evaluation of these different traditional treatments and alternative and regenerative therapies for common exercise related ailments in the performance horse, it is evident that there is currently no perfect solution. Traditional treatments can be used effectively on a short-term basis for reducing inflammation and inducing bronchodilation. However, any individual that administers these medications must be aware of their potential consequences such as ulcers and increased susceptibility to secondary infections. Regenerative therapies are becoming more popular as more research is being conducted and are an autologous solution that stimulates natural healing processes. While the alternative treatments are still relatively new to the field of equine medicine, they have shown very promising results when used in conjunction and sometimes in place of the traditional treatments. These regenerative and alternative therapies both focus to enhance the horse's natural physiological processes, and so far, minimal side effects have been observed. Further research on the efficacy of some of these therapies and supplements would be extremely beneficial in the education of the equine industry.

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Research Paper Title:

Evaluation of Conventional, Regenerative, and Alternative Therapies for Common Ailments in the Respiratory and Musculoskeletal Systems of the Performance Horse

Major Professor: Rebecca Atkinson, Ph.D. Animal Science, Food, and Nutrition