THE EFFECTS OF CIRCULAR EXERCISE ON MARKERS OF BONE AND JOINT HEALTH IN JUVENILE SHEEP AS A MODEL FOR YOUNG HORSES

By

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ABSTRACT

Though circular exercise is a common practice across many disciplines during both ridden and non-ridden exercise, it may be at the detriment of the bone and joint health of horses. In this study, 42 sheep were striated by sex and weight then randomly assigned to treatment groups determining various exercise protocols. Exercise was conducted on either a straight line, small circle (12 m diameter) or large circle (18 m diameter) at either a slow (1.3 m/s) or fast (2.0 m/s) speed. This study also utilized a non-exercised control. Sheep were exercised 4 d/wk for 12 wk. At the end of the study, all animals were humanely euthanized. Synovial fluid was collected from the carpus. All joints of the carpus and metacarpophalangeal joint were opened, photographed, and lesions were assessed. Cartilage samples were collected from the proximal surface of the fused third and fourth metacarpal. All forelimbs were collected for computed tomography scan analysis and biomechanical testing. Statistical analysis was performed in SAS 9.4. A total of 411 lesions were found, though there was no significant effect of treatment or leg. Bone mineral density was found to be lower in the lateral and medial cortices of the left leg of sheep in the large slow treatment group ($p < 0.05$). Fracture force tended to be higher in the right leg of sheep in the large, fast treatment group ($p = 0.08$). Serum osteocalcin and crosslinked c-telopeptides of type-I collagen were both found to have day ($p < 0.05$) and treatment ($p < 0.05$) effects. No effects of treatment or leg were found regarding markers of cartilage or synovial fluid quality. These results indicate that circular exercise may influence bone and joint health and that sheep are a valuable model species for musculoskeletal research.

This thesis is dedicated to all my fellow BR girls. For the love of horses, work hard.

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LIST OF ABBREVIATIONS

BMD	Bone Mineral Density
CT	Computed Tomography
CTX-1	Carboxy-Telopeptide of Type I Collagen Cross-Links
E	Young's Modulus or Modulus of Elasticity
ECM	Extracellular Matrix
EI	Flexural Rigidity
FLS	Fibroblast-like Synoviocyte
GAG	Glycosaminoglycan
HA	Hyaluronic Acid
ITM	Interterritorial Matrix
MCIII and IV	Fused third and fourth metacarpal
MLS	Macrophage-like Synoviocyte
MMP	Matrix Metalloproteinase
MOI	Moment of Inertia
MSC	Mesenchymal Stem Cell
OA	Osteoarthritis
OC	Osteochondrosis
OCN	Osteocalcin
PCM	Pericellular Matrix
PGE_2	Prostaglandin E_2
SF	Synovial Fluid
TM	Territorial Matrix

CHAPTER 1: INTRODUCTION

Both bone and joint health may be heavily influenced by training practices. It is well understood that exercise is critical in developing and maintaining bone quality [1]. This is particularly well documented in the growing animals, in which training while young has shown improved bone strength and reduced risk of injury [2-4]. This has also been demonstrated for joint health. Not only has exercise been proven beneficial for aiding in fluid exchange, improved mobility, and strengthening of supporting joint structures [5]. but it has also been shown to be necessary for the development of joints in young animals [6]. However, not all forms of exercise are beneficial, as injuries may develop relating to overuse or overloading, potentially leading to lameness, chronic mobility issues, or catastrophic failures requiring euthanasia [7-11]. Thus, in equine training programs, it is important to consider closely how an exercise regimen may influence the health of the musculoskeletal system.

Circular exercise is common practice across equestrian disciplines and may take many forms. Circular and curved exercise are frequently performed under saddle for sports such as racing, dressage, reining, show jumping, and barrel racing. It is also commonly used by owners and trainers for non-ridden exercise in the form of lunging or placing horses on a mechanical walker. Though used often, circular exercise—particularly when performed on a small turn radius or at fast speeds—may be at the detriment of bone and joint health. As horses (as well as humans and other animals) exercise on a curve, they lean into the center of the turn to offset new centripetal forces and maintain balance [12]. This lean angle increases with faster speeds and smaller turns. This angle also introduces asymmetric loading between inside and outside limbs due to changes in vertical ground reaction forces, hoof loading area, and inclination of the distal forelimb [12-14]. Thus, the forces acting on bones and joints have changed, potentially leading to disproportionate adaptations between limbs and increased risk of injury.

While there is notable literature on kinematic changes that occur during circular exercise, relatively few studies have investigated the effects on bone and joint quality. Thus, to build on the studies that do exist, lambs were used as a model for juvenile horses and subjected to circular exercise at various combinations of circle diameter and speeds. The results of this study will help aid our understanding of how exercising on a curve influences musculoskeletal characteristics, allowing owners and trainers to make informed decisions regarding training practices and improve the welfare and career longevity of equine athletes.

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CHAPTER 2: REVIEW OF THE LITERATURE

HISTORY OF THE HORSE AND MODERN EQUINE ATHLETES

Evolution and Use of Horses Today

The earliest recognized ancestor of the horse, *Eohippus* (meaning “Dawn Horse”), evolved in North America during the Eocene era [1]. However, this small ungulate has notable contrast to today’s modern horse; it isn’t until the Miocene era and evolution of species such as *Pliohippus* (meaning “More Horse”) and *Dinohippus* (meaning “Terrible Horse”) do we begin to see more recognizable traits that are found in the modern domestic horse (*Equus caballus*) [2,3]. These Miocene era Equidae evolved to be better suited to open plains environments, noted by dental characteristics suited to grazing, development of the stay apparatus, and skeletal structures indicating quick, agile animals [1,4].

With early horses being suited for speed to evade predators, it is clear how their descendants were destined for athleticism, which was further honed through selective breeding practices. While earliest records of domestication indicate that horses were used as a food source, they quickly became important work animals, often used for farming, travel, and war [5]. Though work-related use of horses has declined in recent decades, the number of horses used for racing, showing, and leisure have remained strong. The American Horse Council estimates a total of 7.2 million horses in the United States, with a total of 5.6 million involved in recreation, showing, or racing [6].

Musculoskeletal Demands and Injuries

For our modern equine athletes to perform well, it is important that they remain strong and sound to withstand the forces their jobs demand of them. Racing Quarter Horses and Thoroughbreds may reach speeds of 92.6 km/h and 61.2 km/h, respectively [7]. While other disciplines are performed at notably slower speeds, there is still significant demand on the body. For instance, elite show jumping horses have been estimated to produce 59,000 W of power from the hind limb during a jump [8] and dressage horses performing a collected canter may experience ground reaction forces approximately 1.5 times their body weight [9].

Preserving the skeletal and joint health of these athletes is necessary for ensuring equine welfare and career longevity. Racing horses often have careers lasting 2 to 3 years [10]. Horses competing in dressage, show jumping, or eventing only have slightly longer career prospects – approximately 3 to 6 years [11-13]. Across all disciplines, musculoskeletal injury is the leading cause for horses to be lost, accounting for one-third of horse wastage in each of the aforementioned sports [10]. Furthermore, equine lameness is known to be the leading cause of lost training days [14-17]. Thus, issues of musculoskeletal integrity that limit a horse's ability to train, require them to retire from their discipline, or result in euthanasia are not only welfare concerns for the animal but are major economic concerns for the industry.

Among racehorses, musculoskeletal injuries tend to be predominantly related to bone. Stress fractures of long bones and bone fatigue may result in catastrophic failures, requiring retirement or euthanasia [18]. The New York Racing Association reported a range of 0.99 to 1.1 catastrophic injuries per 1,000 starts for the years 1984 to 2002. Of catastrophic breakdowns that occurred from 1993 to 2002, 39.8% of fractures involved the third metacarpal and another 11.2% involved the carpus [19]. While national catastrophic injury rates have declined in recent years for Thoroughbred racing, The Jockey Club reported 1.25 catastrophic failures per 1,000 starts for 2022 [20].

Among general purpose and elite horses in non-racing disciplines, musculoskeletal injuries are more frequently noted to be related to soft tissue rather than bone, primarily the suspensory ligament and deep digital and superficial flexor tendons [21]. This may be associated with concurrent or future joint injuries due to the compromise of supporting joint structures resulting in osteochondral damage, subsequently predisposing horses to the development of chronic debilitating diseases such as osteoarthritis (OA) [22-24]. OA is a major concern for horse owners and trainers, as it is the most frequently reported chronic disease and estimated to cause 60% of all lameness [25-28].

Public Perceptions of Equine Sports

Not only are musculoskeletal issues a concern for animal welfare and cause of financial stress on owners and trainers, but they may also cast a negative light onto equine sports. A recent welfare survey conducted in 2022 by the Fédération Equestre Internationale (FEI) found that 65% of the public and 75% of equestrians had concerns regarding the welfare of competition horses [29,30]. Of public respondents, the second highest concern was related to risk of horse injury during competition or training (32% of respondents), preceded only by concern regarding treatment of the animals (34% of respondents) [29]. Training practices and injuries were also among the top five concerns reported by equestrians [30]. Positive perceptions of equestrian sports are necessary to maintain the industry's "social license", which describes the public trust and support that allows industry activities to continue [31,32]. Thus, protecting horses against musculoskeletal injury may also increase public support of equine sports.

BONE PHYSIOLOGY

Function and Composition of Bone

The skeleton, composed of bone, is responsible for many integral functions of animals. Foremost, the skeleton provides the structural integrity required to support the rest of the animal's body and is necessary for locomotion and protection of organs. Bone also functions as a major mineral reservoir for the body, contains bone marrow as the site for hematopoiesis, and houses cytokines and growth factors. Thus, bone is critical in maintaining homeostasis [33,34].

Bone tissue itself composes the majority of the body's connective tissue [35]. In contrast to other connective tissues, bone is approximately 50-70% mineral. This mineral component is primarily composed of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and forms a crystalline structure within the bone that provides the tissue's compressive strength [33,35]. Another 20-40% of bone is composed of the organic matrix (or osteoid), which is composed primarily of type-I collagen, along with some noncollagenous proteins and is nonmineralized [33,35,36]. The organic matrix of bone provides the tissue's tensile strength. The remainder of bone is composed of approximately 5-10% water and a small percentage of fat [33].

One vital characteristic of bone is that it is a dynamic tissue capable of responding to environmental stimuli [37]. This dynamic quality allows bone to adapt to loading, enabling it to withstand the forces required for support and movement in its current environment. These loading forces are known as strain [37-41]. Wolff's Law (in its modern, generalized interpretation) describes that increased strain will promote the strengthening of bone, and that disuse and reduced strain will result in the weakening of bone [41,42]. Through this characteristic, bone is able to change as necessary to meet the needs of the animal during growth and through adulthood.

Cellular Components of Bone

There are four key cells related to bone function: osteoblasts, osteocytes, bone lining cells, and osteoclasts. Osteoclasts are the resorptive cells responsible for the removal of bone matrix and are derived from hematopoietic stem cells in the bone marrow [34,43]. These stem cells will form osteoclast precursors, which circulate in the bloodstream until they are recruited back to the bone during resorptive events to differentiate into mature osteoclasts [43]. Mature osteoclasts adhere directly to the bone surface through the actin ring that the cell possesses along with the assistance of podosomes; within this sealing zone, an active domain site known as the ruffled border is found [44]. Osteoclasts contain many vesicles full of lysosomal enzymes that are secreted within the sealing zone. This allows for breakdown of bone through acidification and proteolysis; collagen fibers, along with their attachments to hydroxyapatite crystals, are digested and the crystals themselves are dissolved [44,45]. This removal of old bone is necessary to maintain structural integrity, and the freeing of minerals is key in calcium and phosphate homeostasis [46]. At the end of resorptive activity, most osteoclasts undergo apoptosis [44].

Osteoblasts are the formative cells, responsible for laying down matrix for new bone, and look characteristically like other cells responsible for protein synthesis. Osteoblasts have surface receptors for parathyroid hormone, secrete osteoid, and aid in matrix mineralization [34]. Osteoblasts arise from mesenchymal stem cells (MSC). These first develop as osteoblast precursor cells that later differentiate into fully functional osteoblasts. This requires MSCs to first be osteoblastic precursors based on specific gene expression. These may then form into preosteoblasts after a proliferation phase and once cells begin to express alkaline phosphatase. Eventually, these preosteoblasts will become mature osteoblasts, marked by the production of proteins such as osteocalcin and type-I collagen [34]. This allows for the deposition of osteoid and its subsequent mineralization through release of hydroxyapatite crystals formed within osteoblast matrix vesicles [34,47]. Mature osteoblasts are not terminally differentiated, however. After the bone formation process is complete, approximately 20-50% of cells will remain on the surface of bone and differentiate into osteocytes or bone lining cells, with the remaining 50-70% suspected to undergo apoptosis [34,48]. What determines the particular fate of an osteoblast is still an area of ongoing research, but it is suspected to be the result of complex cell signaling pathways rather than passive transformation [49,50].

Osteocytes, derived from osteoblasts, compose approximately 90% of bone cells and lie within the mineralized matrix [46]. The importance of osteocytes has become increasingly appreciated in recent decades. The primary role described of osteocytes is to be mechanosensing cells. Osteocytes have been shown to respond to and translate mechanic loading, likely received by the cell through increases of shear stress on fluid flowing though the lacunocanalicular system, which is the microscopic fluid system within bone that carries nutrients, signaling factors, and allows for inter-osteocyte communication [49,51,52]. Osteocytes also have the capability to remove and replace its perilacunocanalicular matrix, which may aid in calcium homeostasis [52], as well as function as an endocrine cell by secreting hormones targeting kidney function and maintaining phosphate homeostasis [49,52,53]. Further, osteocytes are known to modulate both osteoblast and osteoclast activity through secretion of biochemical factors that can regulate cell differentiation and mature cell activity [36,52,53].

Bone lining cells are also a terminal differentiation of osteoblasts. These are flat cells that cover the surface of bone, and do not exhibit any formative or resorptive activity [54]. Because most bone exists in a “resting” state, bone lining cells compose a significant portion of the total cell population in bone [55]. Bone lining cells form gap junctions with each other and with osteocytes within the bone matrix; as such, even though bone lining cells do not participate in changing the bone directly, they may be important regulators of osteoclast and osteoblast activity [34]. Bone lining cells have been suspected to play this regulatory role in three main ways: induction of the signaling cascade to activate and/or recruit osteoclasts and osteoblasts, re-differentiation into osteoblasts themselves, and restriction of access to the mineralized bone surface [55]. Bone lining cells are also thought to be an important component of the functional bone membrane, maintaining balance within the bone environment as a whole [55].

In summary, the maintenance of bone does not simply rely on one cell type, nor is one cell type more important than another. Osteoclasts, osteoblasts, osteocytes, and bone lining cells are all vital constituents in creating bone's ability to respond and adapt to its strain environment and maintenance of circulating mineral levels. Further, these cells do not function independently from one another. The maintenance of bone relies heavily on intercellular communication between cell types, regulating the action of each other [56]. Maintaining balance in the relationships between these cells is necessary to ensure bone health and homeostasis [54].

Modeling and Remodeling of Bone

Bone's ability to act as a dynamic tissue and respond to loading stimulus occurs through two distinct (though similar) processes: modeling and remodeling. Bone modeling occurs primarily in the growing animal, with the purpose to increase bone mass and alter bone shape [57]. Formative modeling involves the addition of new bone, utilizing osteoblasts, whereas resorptive modeling involves the removal of bone by osteoclasts. Both formative and resorptive modeling occur naturally to alter bone shape at genetic predilection sites [58]. This process is necessary for both longitudinal and radial growth as an animal matures. Modeling is also heavily influenced by local strain, such that increased loading promotes formative modeling and decreased strain will promote resorptive modeling [57]. This strain also serves as a signal for bone drift, which allows bone to alter its position around the central axis and appropriately respond to the strain environment beyond simply increasing bone density [57]. Because bone modeling is predominantly seen in growing individuals, their activity during this period can heavily influence their skeletal morphology and strength. Moderate exercise has been shown to have positive effects such as increased cortical area and increased bone strength [59,60]. This improved bone quality through exercise during bone modeling years may also offer lifelong benefits of increased strength and reduced fracture risk [61]. As such, it is generally recognized that the greatest skeletal benefits occur with exercise during growth [62].

Bone remodeling, in contrast, primarily occurs in adults. Remodeling is utilized primarily for the purpose of moderating bone damage and regular maintenance of the bone, rather than changing bone size or shape [58]. This is performed sequentially by resorption or removal of old bone by osteoclasts and the formation of new bone by osteoblasts at the same discrete site [57]. In contrast to modeling where resorption and formation occur independently, osteoclasts and osteoblasts work together sequentially within a bone (or sometimes referred to as basic) multi-cellular unit (BMU) that aids in ensuring minimal net change to total bone [33,46,58]. These remodeling events may be targeted or stochastic; targeted events are generally triggered by signals from osteocytes that indicate microdamage or apoptosis has occurred, and stochastic events are believed to be related calcium homeostasis [34,57]. A remodeling cycle of bone may take a total of 4-6 months, with about 2-6 wk dedicated to resorption and the remaining time dedicated to formation [33,57]. This cycle is divided into four steps: activation, resorption, reversal, and formation [33,57]. The activation phase is characterized by the recruitment of osteoclasts. Next, in the resorption phase, these osteoclasts will dissolve the mineral and free collagen fragments around which the mineral matrix forms. Once resorption is complete, reversal begins where mononuclear cells and specialized bone lining cells prepare the surface for formation. The final stage is characterized by osteoblasts laying down osteoid. This osteoid is then mineralized over the following months. When remodeling is not actively taking place, the bone is in quiescence, which represents most of the bone surface [57]. While bone remodeling is a necessary process to maintain bone strength, the remodeling period itself may result in bone weakness while bone resorption is occurring and/or osteoid mineralization is not yet complete [63,64]. There is evidence to support that many fractures in horses are often related to overuse, or in other words, when there is not sufficient time for bone remodeling to address the microdamage that occurs because of repetitive loading [64-66].

ASSESSING BONE QUALITY AND ACTIVITY

Biomarkers

Direct assessment of bone activity can prove difficult to perform in the live animal, but multiple non-invasive methods have been developed that allow for monitoring of bone metabolism [67]. Two of the most used serum or plasma bone markers in horses are osteocalcin (OCN) and crosslinked carboxy-telopeptides of type I collagen (CTX-1) [67]. Each of these allows for insight regarding bone formation and resorption, respectively.

Osteocalcin is a protein produced by osteoblasts during bone formation [68]. A majority of OCN is deposited into bone and contributes to the majority of its non-collagenous protein content. Osteocalcin levels detected in serum are representative of a smaller proportion of the protein that does not become incorporated into hydroxyapatite [69]. Recent studies have shown OCN to act within a negative feedback loop to inhibit excess bone formation; however, serum concentrations reflect newly synthesized OCN and thus activity of osteoblasts indicating formation of new bone [69,70]. Osteocalcin levels decrease with age, corresponding with decreased bone formation, though acute increases can be observed in response to exercise [71,72]. Serum OCN also demonstrates biphasic changes with levels being generally constant during the day and varying through the night, generating a need for consistent time of sampling [67]. Osteocalcin also requires γ-carboxylation for proper function. Carboxylated OCN is the protein form incorporated into bone, whereas undercarboxylated OCN demonstrates lower affinity for bone mineral and may indicate issues with bone formation [34,68]. Generally, for the purpose of assessing bone formation, commercial assays specifically target carboxylated OCN to avoid overestimation of osteoblast activity [68].

Crosslinked carboxy-telopeptides of type I collagen are indicative of osteoclast activity [67]. Type-I collagen is the most prominent protein in bone's organic matrix, and during resorption, is broken down by osteoclasts [44]. Following breakdown, fragments of type-1 collagen such as CTX-1 are released and enter circulation [73]. Similar to OCN, serum CTX-1 is biphasic, and levels decrease into adulthood – though levels increase again with senescence [74]. Exercise in adults has been shown to decrease CTX-1 levels, indicating a reduction in bone resorption [75].

Measuring both a biomarker of formation such as OCN and degradation such as CTX-1 is necessary to capture the overall state of bone, as evaluation of a single marker may over- or underestimate a particular aspect of bone activity [67]. Bone remodeling requires a balance between the two opposite processes of formation and degradation, and thus, complementary biomarkers of each should be evaluated and considered.

Computed Tomography Scans

Imaging techniques are another useful, non-invasive tool for evaluating quality of bone. Computed tomography (CT) scans are generally regarded as the best imaging option for assessment of bone [76]. Computed tomography scans allow for evaluation of whole bone as well as detailed assessment of morphology and density within a cross-sectional area. These scans utilize 360-degrees of x-ray as the animal or specimen moves through the gantry as predetermined intervals; this allows for cross-sectional or slice images as well as assembly of these images into a whole bone depiction. Each image pixel represents a volume (voxel) of tissue that correlates with the selected slice density settings of the machine [76]. These pixels are measured in Hounsfield units (HU), which are objective measurements of the x-ray's attenuation [76]. Through use of a phantom with various known mineral concentrations, HU can be converted to mg Ca hydroxyapatite/cm^3 to measure bone density. Further, CT provides greater ease of discerning between superimposed images of various structures [77]. While still a 2-dimensional image, these qualities give CT scans an advantage over other imaging techniques such as radiographs [76].

The use of CT for large animals, particularly horses, can prove challenging due to costs and facility limitations. This is primarily due to the need for adequate gantry size and transportation of anesthetized animals [77]. However, post-mortem evaluation of bones in the distal forelimb of sheep, calves, chickens, and horses has proven to be an accessible option for researchers interested in bone quality [78-82]. Through this technique bone cortical area, medullary area, cortical thicknesses, and bone density can all be readily assessed which provides a valuable depiction of the state of the bone.

Mechanical Testing

While strength and integrity of bone can be estimated from measurements such as thickness or density as achieved through CT scans or by evaluating the balance between formation and resorption through biomarker analysis, direct mechanical testing provides the best assessment [83]. By placing bone harvested post-mortem on a loading apparatus (typically performed as a three-point or four-point bending test), a load-deformation curve can be created that directly depicts the extrinsic stiffness of the bone [83]. This can also be depicted as a stress-strain curve, which reveals the bone's elastic and plastic deformation regions which define the loads at which bone demonstrates reversible and irreversible deformation, respectively [83]. As bone progresses through its plastic deformation, it will inevitably break entirely. This is referred to as the bone's ultimate or breaking strength [83]. Through mechanical testing, both the bone's ability to resist fracture or damage (estimated by Young's modulus or modulus of elasticity) and the maximum force bone is able to withstand (ultimate strength) can be assessed [83].

To generate these curves from a three- or four-point bending test, a bone is placed on two lower posts holding it in position. Loads are then applied from above by one or two posts, dependent on the type of bending test. As bone bends away from the neutral axis, or deforms, shear, stress, Young's modulus, and ultimate strength can be calculated based on the force applied and bone displacement [83]. These qualities of bone vary between bone types, but ultimately rely on primary factors such as degree of mineralization; organization, size, and composition of hydroxyapatite crystals; and size, orientation, and degree of crosslinking of collagen [84].

By evaluating the bone's true mechanical properties, the living implications of findings from biomarker or imaging analysis can be determined. This makes mechanical testing extremely valuable in assessing impacts of various factors, such as exercise, on bone quality. However, mechanical testing does have drawbacks: compared to non-invasive techniques discussed previously, bone must be tested *ex vivo* and determining both the yield point and ultimate strength of bones renders them unable to be used in any other form of evaluation.

JOINT AND CARTILAGE PHYSIOLOGY

Function of Joints and Cartilage

While the skeleton is necessary for locomotion, this locomotion would not be possible without joints. Joints describe the area in which two or more bones meet one another and can fall into three functional classifications: diarthrosis (free moving), amphiarthrosis (partially moving), or synarthrosis (nonmoving) [85]. In relation to the equine athlete (as well as athletes of all other species), diarthrodial, or synovial, joints are of the most interest as these are the joints predominately affected by OA. This disease is thought to affect approximately 50% of horses over the age of 15 and may be responsible for a majority of lameness cases in horses [25-28,86]. Thus, maintaining joint health is critical in decreasing the risk of developing chronic joint disease.

Diarthroses allow for osteokinematics due to the smooth articular cartilage and synovial fluid that the joint possesses, allowing the surfaces of bone to slide over one another [87]. This movement is tightly regulated by the surrounding muscle, ligaments, and joint capsule [85]. Further, the cartilage within joints is responsible for dissipating the stress associated with loading, which is primarily granted to the tissue by its unique extracellular matrix (ECM) that allows for resistance to compressive forces [88,89].

Structure of Joints

Diarthrotic joints occur where two or more bone surfaces meet one another. The ends of the bones within these junctions are covered with articular cartilage, which lies over the subchondral bone [90]. This gap between bones is spanned by ligaments, which primarily guide and restrict movement. Support may be further offered by tendons that pass through, around, or fuse with the joint capsule [85,90]. Surrounding the joint itself is the joint capsule. This capsule also provides support to the joint and may aid in its articulation [91]. The joint capsule is composed of two layers: the outer fibrous layer, composed of collagen, that attaches to the periosteum of the adjoining bones, and the inner synovial membrane that aids in the maintenance of joint fluid homeostasis [87]. The outer fibrous capsule is highly innervated, with some nerves likely being related to proprioception. This proprioception, along with fibers that also sense environmental stimuli such as pain, are necessary for joint protection and appropriate reflexive movement of the surrounding muscles [91]. The inner synovial membrane tends to be less innervated, but is more highly vascularized, allowing for the influx of water and solutes that generate synovial fluid [90,92]. This inner membrane is divided into two sub-layers: the intima and the subintima [90]. The subintima is deep to the fibrous layer of the capsule. The subintima may be composed of various tissue types (specifically adipose, areolar, or fibrous) depending on the particular joint and the amount of movement required [90]. The intima is the innermost lining of the joint capsule composed of synoviocytes, which are responsible for secretion of synovial fluid components [90,91]. The intima itself is designed for fluid exchange between the joint space and the vascular supply. Similar to the subintima, the intima also possesses variable three-dimensional structures that depends on the nature of the joint [93].

Synoviocytes and Synovial Fluid

Synoviocytes are split into two cell groups, though it is possible these are the same cell type with different phenotypes [87]. Type-A, or macrophage-like synoviocytes (MLS), possess phagocytic properties, aiding in degradation and immune defense [94-96]. Type B, or fibroblast-like synoviocytes (FLS), tend to be more prominent than MLS [94]. FLS are responsible for the production of compounds—particularly hyaluronic acid and lubricin—that lubricate and nourish the joint [95-97]. Further, FLS influence cartilage ECM composition through the secretion of matrix molecules and aid in their three-dimensional assembly [95-97]. FLS also aid in ECM maintenance through production of catabolic enzymes [97,98]. These roles of FLS are thought to be active responses to changes in the joint environment rather than passive processes [95]. FLS are further known to be mechanosensitive, as their activity is modulated by mechanical load and fluid shear stress [99-102]. Both MLS and FLS have the ability to release proinflammatory factors in response to joint damage [103]. Thus, synoviocytes are a key regulatory cell for proper joint homeostasis and play a major role in the characteristic changes seen in OA, such as synovitis and cartilage degradation [103].

Synovial fluid (SF) fills the joint capsule and is an ultrafiltrate of blood plasma which is regulated by the synovial membrane [92]. As such, SF tends to be quite similar to plasma, though there are notably fewer cells (few to no erythrocytes and less than 200 white blood cells) and approximately 25% of the protein content [92,104]. In addition to plasma content, SF also contains the secretions of synoviocytes (primarily FLS) and chondrocytes [104]. These contents of SF, particularly nutritive molecules and compounds for viscosity and lubrication, are indicative of the fluid's two main roles: nourishing the joint structures and reducing friction of articulating structures. SF allows for influx of necessary nutrients for the joint such as glucose and outflux of metabolic waste products, though the synovial membrane ensures large, necessary molecules of the SF remain within the joint capsule [104]. The presence of friction-reducing macromolecules in SF such as hyaluronic acid and lubricin work synergistically to increase the fluid's water content (and thus viscoelasticity of the fluid) and prevent surface adherence by decreasing the boundary friction coefficient of the surface of articular cartilage [105-108]. Generally, healthy SF displays thixotropic, non-Newtonian qualities; the fluid is more viscous at lower stress rates and more elastic at higher stress rates [109]. In states of joint disease, the rhetological properties and composition of synovial fluid is altered such that it no longer adequately aids in reducing friction, which may exacerbate disease progression [109,110].

Composition of Cartilage

The articular cartilage of synovial joints covers the epiphyses of long bones. Along with synovial fluid, cartilage aids in low-friction movement of the joint but lacks a perichondrium, which contrasts with other types of hyaline cartilage [111]. Articular cartilage also lacks both blood and lymphatic vessels, hence why it relies on SF for nutrient transport and waste removal [111]. Beyond contributing to the low-friction joint environment, articular cartilage also serves the important function of reducing stress on the joint by compressing and distributing the loads applied to it [112,113]. This property is due to it being a quasi-elastic material [111,113-115].

The cellular component of cartilage are chondrocytes, though these cells only compose approximately 1-2% of the wet weight; these cells produce and are surrounded by a complex ECM which composes the remaining 98-99% of the cartilage [111]. The primary components of the ECM are water (65-80%), collagen (10-20%), proteoglycans (10-20%), and a small percentage of non-collagenous proteins [112,116].

Water

Water within the ECM is responsible for cartilage's ability to withstand loads by increasing the pressurization and flow resistance [116]. The water content of cartilage also aids in delivery of nutrients to chondrocytes and aids in the maintenance of ionic balance of the matrix via dissolved electrolytes [116]. Water composes 65-80% of the ECM in normal cartilage, but in disease states such as OA, water content may be increased to upwards of 90%. Altered water content of the cartilage negatively affects the cartilage's elasticity, which decreases its ability resist mechanical loading [112].

Collagen

The collagen within the ECM is comprised primarily of Type II collagen (approximately 90-95%) [116]. Collagen within the ECM is composed of pro-collagen fibers, which are covalently cross-linked and adopt a helical formation [116,117]. These collagen fibrils provide the framework for cartilage and are key to the tissue's tensile strength [116]. These collagen fibrils are particularly susceptible to matrix metalloproteinases (MMP) that results in collagen degradation and may lead to compromised cartilage integrity [117,118]. Increased MMP production can occur as part of the senescent secretory phenotype of aged cartilage, a response to physical damage, or a response to oxidative stress [118, 119].

Proteoglycans

Proteoglycans within the ECM are responsible for both structural support and maintenance of matrix fluid pressure, which ensures compressive strength [116]. Each proteoglycan type has a unique structure and function, though they all share the general form of a base protein with side chains composed of glycosaminoglycans (GAG) [88,116]. The most abundant proteoglycan in articular cartilage is aggrecan which has sulfated GAG side chains--namely chondroitin sulfate and keratin sulfate [116]. Each individual aggrecan molecule contributes to the formation of a large, complex aggregate structure [116,120]. This aggregate is composed of multiple aggrecan subunits bound to hyaluronic acid (HA), a non-sulfated GAG, by a link protein [120]. Aggrecan has a notable negative charge; thus, a large aggregate molecule results in a strong influx of cations, and subsequently, water [121]. This results in a swelling of the cartilage. This swelling is counter-balanced by tension being placed on the collagen framework, and the cartilage reaches a state of equilibrium [120]. The water held within cartilage is displaced upon mechanical loading, bringing aggrecan molecules closer together, and re-establishes the charge gradient for water to be drawn in [120]. Thus, proteoglycans are key for cartilage's ability to resist compression. Other non-aggregating proteoglycans do not bind to HA, but aid in additional structural support through interactions with collagen [122]. In disease states, proteoglycan depletion is directly related to decreased number of functional chondrocytes that remain after joint injury, trauma, malalignment, or immobilization [112].

Non-Collagenous Proteins

The remainder of the ECM is composed of non-collagenous proteins. These proteins can vary widely in their structure and function, but this category broadly refers to structural proteins, regulating proteins, proteinases, and proteinase inhibitors [123]. Thus, though these are minor groups in comparison to collagenous proteins or proteoglycans, these proteins are essential for cartilage integrity and matrix homeostasis. Similarly to collagen proteins, non-collagenous proteins are also affected by MMP production [111]. This degradation can result in further structural and regulatory failure of damaged or diseased cartilage.

The organization of chondrocytes along with the composition of the extracellular matrix varies depending on the depth from the joint surface. First is the superficial zone, which is characterized by flat chondrocytes, tightly packed collagen fibrils, and minimal proteoglycan [116]. Due to this arrangement, this zone has the greatest tensile strength, which is necessary to withstand direct mechanical loading [112]. The next layer is the transitional or middle zone; in this zone, chondrocytes are spheroidal but found in a lower density than in the superficial zone [112]. The transitional zone has notably more ECM with thicker collagen fibrils and greater proteoglycan content; as such, this zone is when we begin to see characteristics that provide cartilage's compressive strength [116]. The deep zone lies directly above calcified cartilage and has the greatest ability to resist compression [116]. This zone has the greatest amount of proteoglycan and thick cartilage fibrils that are arranged perpendicular to the surface with columns of chondrocytes that are found parallel to collagen [116]. Between the deep zone and the subchondral bone is the calcified cartilage zone; here, chondrocytes are hypertrophic and imbedded in a calcified matrix [112,116]. This zone offers some additional compressive protection but primarily functions to anchor non-calcified cartilage to the subchondral bone [112,116].

Within each zone, cartilage can be further organized based on the ECM makeup. The three ECM regions within cartilage are the pericellular matrix (PCM), the territorial matrix (TM), and the interterritorial matrix (ITM) [116]. The PCM immediately surrounds the chondrocyte; this PCM-chondrocyte unit is known as a chondron [124]. In this region, type IV and IX collagen, aggrecan monomers, HA, and minor proteoglycans are found in greater or exclusive concentrations than other portions of the matrix [124]. Though the particular function of the PCM is not fully elucidated, is it suspected that it plays a key role in transducing biomechanical and biochemical information to the chondrocyte [124]. The TM surrounds a chondrocyte or group of chondrocytes and their PCM [125]. Collagen fibrils in this region tend to be arranged in a woven fashion that creates a basket-like structure around the chondron and protects it from the direct stress of loading [116,124,125]. Furthest from the chondrocyte is the ITM, which is the largest of the three regions. The ITM has the greatest proportion of Type II collagen and aggrecan and is the greatest contributor to articular cartilage's mechanical properties [116,126].

To summarize, cartilage is a highly specialized tissue that works to dissipate strain and protect underlying bone from the force of mechanical loading. Though each zone and region varies in composition and functional role, each segment is vital for proper joint function. In response to damage or disease, chondrocyte function and matrix constituents may be altered resulting in a negative impact on the tissue's tensile and compressive strength.

ASSESSING CARTILAGE AND JOINT QUALITY

Lesions

One macroscopic measure of cartilage quality is lesion number and area. Articular cartilage should possess a smooth surface to allow for the gliding quality it provides to diarthrodial joints. Lesions on the joint surface indicate loss or degradation of articular cartilage and may first appear as wear lines in the cartilage before progressing to partial or full thickness loss [127]. Development of some lesions may be directly mechanical, whereas other lesions may be the result of endochondral ossification failure as seen with developmental orthopedic diseases such as osteochondrosis [127-129]. Cartilage lesions are also a characteristic of OA, which may be induced traumatically through exercise or injury or as a consequence of chondrocyte senescence [111]. Once cartilage lesions develop, they generally lead to further degeneration of the joint due to compromised cartilage integrity [130].

Mechanical changes to articular cartilage and fibrillation have been noted at common clinical lesion sites in exercised horses [131]. Degenerative OA in horses has been long defined with lesions as a major diagnostic criterion. Simply put, these morphological changes are attributed to either abnormal joint stresses on otherwise healthy cartilage or normal joint stresses on abnormal cartilage [26]. In athletic horses, it is considered common for OA to develop and may be linked to a history of injury and physical overload throughout their careers [86].

A study by Vernon et al. in 2004 assessed lesion development in response to different forced exercise styles in sheep. This group found more severe development in circularly exercised sheep when compared to non-exercised or straight-line exercised sheep, demonstrating the influence of joint loading style in lesion development [132]. Thus, the assessment of cartilage lesions – their presence, number, and size – offers a view into the health of the cartilage and overall joint quality.

DNA and Glycosaminoglycans

Assessing DNA content of cartilage aids in assessment of cellularity of the tissue. Chondrocyte loss due to damage or aging is an indicative quality of joint disease such as OA and has been associated with increased cartilage fibrillation and thus lesion development [133]. This results in a disruption of joint homeostasis, leading to further degeneration [133]. In the case of the athletic horse, joint damage from injury or physical overload of a joint surface may result in stress-induced cell death, either through direct cell damage or as part of an inflammatory response [26,86]. Evaluating DNA content offers an estimation of cartilage cellularity and have been shown to correspond closely [134].

Glycosaminoglycan content is also a useful measure indicative of cartilage quality and is commonly used to assess ECM degradation [135]. As discussed previously, proteoglycans such as aggrecan play a critical role in cartilage's resistance to compression by maintaining structural integrity and water content [116]. Total GAG content of cartilage has been shown to decrease in osteoarthritic cartilage and is associated with a reduced compressive stiffness [136]. In a comparison of healthy and diseased metacarpophalangeal joints of horses, diseased joints were found to have reduced total GAG content [137]. Similarly with cell number, GAG content has been noted to decrease in association with damage to cartilage incurred from injury or physical overload [138].

Together, assessment of DNA and GAG content of cartilage offers insight as to the composition and quality of cartilage tissue which adds value beyond macroscopic evaluations such as cartilage lesions. With damaged cartilage characteristically showing decreased cellularity and GAG content, these measures allow for determination of negative effects on cartilage homeostasis.

Synovial Fluid

When assessing joint quality, it is important to keep in mind that the joint is not composed of cartilage alone. Changes in SF may also have notable implications on joint homeostasis. In diseased joints, SF is found to be less viscous and may possess increased protein, white blood cell, and hyaluronic acid content [139]. These changes contribute to the altered rhetological properties of SF seen in disease states. Thus, the fluid's capability of reducing friction for the joint surface is compromised. These changes are often associated with synovitis as the synovial membrane is an integral component in regulation SF composition; when the permeability of the synovial membrane is altered, it follows naturally that the SF is altered as well [139].

In addition to the aforementioned factors, SF prostaglandin E_2 (PGE_2) concentrations are a useful indicator for assessing joint damage. Prostaglandin E_2 is an eicosanoid associated with inflammatory processes, and inflammation is strong indicator of damage or disease within a joint. Though inflammation is a normal response to injury, acute and chronic inflammation can both result in pain, lameness, and effusion [24]. When inflammation persists, further damage to the joint may occur [140,141]. Particularly when looking at equine joint disease, synovitis and capsulitis are critical in the pathology and progression of joint deterioration [24]. Increased concentrations of PGE_2 are commonly found in the joints of horses with osteoarthritis as a result of the inflammatory process [24,142]. Similarly, increased concentrations of PGE_2 have been observed in synovial fluid when inflammation is experimentally induced by synoviocyte lipopolysaccharide challenge [143]. Thus, SF PGE_2 concentrations can directly assess inflammation of the joint capsule and synovial membrane that commonly characterize injury or disease.

PREVENTATIVE TRAINING AND MANAGEMENT

With musculoskeletal injuries being the leading cause for horse wastage and 60% of lameness cases being attributed to OA, preventative training and management techniques are key in protecting the welfare and career longevity of equine athletes [10,25-28]. For bone, it has been demonstrated that disuse or decreased loading results in bone loss [144]. This is due to the body's aim at maintaining bone mass as a minimum such that only enough bone is maintained for the skeleton to function without failure from loading [144]. For the horse, this concept is particularly important when management strategies are considered. Often, horses are stalled with limited time spent in turnout or on pasture due to fear of injury or space restrictions [145,146]. A study by Hoekstra et al. in 1999 found that stall-reared horses had lower radiographic bone aluminum equivalences compared to pasture-reared horses, indicating lower bone mass [147]. This concept of lower bone mass in confined horses also parallels bone loss found following bed rest or immobilization, highlighting the "use it or lose it" property of bone [148-150]. Additionally, horses with reduced turnout time demonstrate more active behaviors such as trotting, cantering, and bucking compared to horses with greater time in turnout resulting in increased chance of injury [151]. Thus, housing management of horses can play a major role in predisposition to musculoskeletal injury.

Another management practice that may influence skeletal integrity of horses is exercise during growth. It has been demonstrated that exercise during bone modeling years provides life-long benefits of increased bone strength and reduced fracture risk [61,62]. In Thoroughbred racehorses, it has been shown that risk of musculoskeletal injury and catastrophic breakdown increases the later a horse begins training [152]. Further, a greater proportion of racehorses remain sound when training begins before closure of the epiphyseal plates compared to horses that begin training after closure [153]. Not only does this data support starting training while young, but also reiterates the value of turnout allowance. Young horses may frequently be stalled upon commencement of training or during sale preparation, but voluntary exercise in turnout may provide additional strain necessary to improve bone integrity [147]. A 2019 study by Logan et al. demonstrated that even just one 71-m sprint per week for juvenile animals was sufficient in increasing bone strength by 20% compared to a non-exercised control [79]. Despite the evidence, many management systems still restrict exercise of young horses [10].

Management techniques allowing for exercise are not only valuable for bone but are critical in the maintenance of joint health as well. Motion of the joint is necessary for homeostasis; as blood flow is increased to the synovium, health of synovial villi is maintained [93]. Movement of joints also results in greater fluid exchange, aiding in turnover and replenishment of SF [154]. Further, chondrocytes appear to have mechanosensitive qualities, and mechanical loading may provide beneficial effects to ECM composition [131,154]. Similar findings have been demonstrated in horses, with GAG content of cartilage increased in horse undergoing high-intensity training compared to horses that had walk-only exercise [155]. The effects of exercise on juvenile horses are also of particular interest. Foals have homogenous cartilage at birth, and heterogeneity of cartilage is suspected to develop in response to loading [10]. Box-rested foals showed notable underdevelopment of joints compared to their exercised counterparts indicated by decreased GAG content in both their cartilage and tendons. These foals also demonstrated altered gait patterns in comparison to the exercised group [156]. Even in adult animals, the benefits of movement on joint health are well noted. For humans and horses, joint mobilization is a key point in managing OA, as movement has been shown to improve range of motion and cartilage nutrition while decreasing pain, effusion, and stiffness [157]. Exercise may also indirectly support joint health through the strengthening of supporting structures, allowing for improved joint stability and decreased risk of injury [157].

However, it is also important to note that the converse effects of exercise promotion exist. For bone, strenuous training without proper rest may lead to bone fatigue and subsequent failure [18]. This is related to repetitive damage incurred on the bone without adequate time or capability of repair [18,152]. Bone-related injuries may also be incurred when the onset and intensity of training exceeds bone's ability respond to the new strain environment [152]. Similarly for cartilage, overloading of the joint may result in loss of both chondrocytes and GAG along with a thickening of the calcified zone and overall softening on the cartilage due to compromise of the collagen network [119]. Further, injury to the joint and abnormal loading have been implicated in the development of OA [86]. How cartilage adapts to exercise is site-specific and highly dependent on the exercise protocol [155]. This is primarily due to uneven distribution of loads within and between joints. These loading patterns further vary by gait and type of physical activity performed [158]. While effects of exercise on joint health are less clearly elucidated than they are in bone, it is clear both components of the musculoskeletal system require care in management and training technique to maintain the balance between benefit and detriment.

EFFECTS OF CIRCULAR EXERCISE

When considering exercise protocol in horse training and management, it is important to recognize that varying types of exercise vary in their impacts on bone and joint quality. Frequently, circular exercise is used within a horse's exercise regimen. For instance, many owners lunge their horses with the aim of improving fitness which involves the horse moving in a circle on a line around the handler [159]. The use of mechanical walkers is also regularly used in the training regime of horses. These similarly involve a horse moving in a circle while the machine leads them forward by a line attached to a halter or encourages them forward by a moving gate or panel from behind [160]. While formal assessments of lunging frequency in training regimes is lacking, a survey of Australian Thoroughbred racehorse trainers reported horses spending up to 40 min/d on a walker as a primary form of non-ridden exercise [161]. Both lunging and horse walkers are also regularly included with equine rehabilitation protocols [162]. Circular exercise is not only included as a common aspect of a horse's fitness regimen but is also a major aspect of ridden work in a variety of disciplines including dressage, hunter/jumpers, reining, and barrel racing. These circles are often performed in small diameters at relatively high speeds. Though in contrast to the small circles seen in other disciplines during competition and training, Thoroughbred racehorses also experience the effect of circular exercise as they train or race through the curve of oval tracks [163].

As a horse exercises on a curve or circle, horses will lean in toward the center of the circle to maintain balance. This allows for adaptation to centripetal forces and better maintenance of regular ground reaction forces [164]. However, even when horses lean into a turn, loading of the limbs is still altered in comparison to straight-line exercise as the centripetal force of the turn cannot be fully eliminated [164]. A horse's lean angle increases with increasing speed and decreasing circle radius such that lean becomes greater with each gait (walk, trot, canter) and increasing centripetal force [163,164]. In addition to increased lean angle, limb abduction and inclination of the third metacarpal (MCIII) also increases [165]. While this affects both legs, inclination is found to be greater on the inside limb. These kinematic adaptions are noted to be greater when circles are performed on a flat surface rather than a banked one, as a banked surface allows maintenance of balance and limb posture more similar to when the horse is exercising on a straight line [165].

Another notable change to gait pattern includes greater peak vertical ground reaction forces of the outside limb compared to the inside limb, associated with the inside limb having increased duty factor [164]. Similar compensation strategies have been noted in humans, and both species also demonstrate decreased maximum speed on a curve related to increased foot contact time or stride duration [163]. In animals that do not appear to increase foot contact time, such as racing greyhounds, forces on the limbs increase on average by 65% when compared to running on a straight line [166]. This may be an advantage of quadruped animals in comparison to bipedal animals such as humans due to more efficient weight redistribution across limbs. This is also supported by decreased stride duration of horses galloping on a curve with training, indicating that there is some room for adaptation to curved running [167]. The increased force observed during curved running also correlates with increased strain on the outside forelimb that has been observed in Thoroughbred racehorses [168]. In Standardbred racehorses, though racing at much slower speeds than Thoroughbreds, strain on the outside limb was found to increase when tracks were underbanked [168]. The environment of the inside leg of runners is found to also be altered. Humans running on a sharp curve (5 m radius) experienced greater tibial torsion of the inside leg than when exercising on a slighter curve (15 m radius) [169]. It has also been documented that hoof loading area at the canter is reduced for the inside leg compared to the outside leg [170]. Thus, circular exercise not only alters overall kinematic patterns from straight-line travel, but also induces asymmetry between inside and outside limbs [171].

The effects of circular exercise pose a risk to both horse and rider safety. In a study evaluating injuries of racing Thoroughbreds at Midwestern tracks in the United States catastrophic injury was most likely to occur in the left (inside) forelimb [172]. However, a broader study looking at fatal injuries in racing Thoroughbreds found that amongst tracks with the smallest turn radii, the right (outside) forelimb had the greatest risk of fracture [173]. Beyond injury to bone, the resulting forces during circular exercise may also have a negative effect on joint health. OA is the leading cause of lameness in horses and has been associated with abnormal loading on normal cartilage [26]. While connections between circular exercise and joint health are lacking, Logan et al. in 2022 found that cartilage GAG content varied between right and left limbs of calves exercised on a 12-diameter circle [78]. This emphasizes the possible implications of circular exercise in the frequency of equine degenerative joint disease, but significant work is left to be done. In terms of risks to riders, a 2021 study investigating risk of jockey fall during Thoroughbred racing in Japan found that there was an increased risk with smaller track sizes [174]. It has also been reported that a change in limb kinematics may influence a jockey's sense of balance and overall perception of their safety [174].

In summary, there is a notable amount of literature regarding gait adaptions to circular or curved exercise. However, links of circular exercise to musculoskeletal injury and development of chronic joint diseases such as OA are lacking. This demonstrates a critical paucity in knowledge. It is known that exercise is critical for maintaining bone integrity and joint homeostasis. However, it is relatively unknown whether circular exercise—which is prominent across all disciplines—is of benefit or detriment to the horse.

SHEEP AS A MODEL FOR HORSES

The use of a model species in research is common practice. For instance, eighteen different model species have been used to date for *in vivo* OA research translational to humans [175]. Similarly, model species can be used as an effective means for equine research. Sheep in particularly have been successfully used to model the horse.

Vergara-Hernandez et al. used sheep as a model for horses to investigate the effects of bisphosphonate administration on bone in an exercised, juvenile model as administration of bisphosphonates is commonly used to treat bone disorders such as navicular syndrome in horses [82]. This group found sheep to be a useful preclinical model for horses, as they demonstrate similar kinematics and weight distribution during exercise [176].

Sheep have also been as an equine model to study the effects of circular exercise on joint health. Vernon et al. in 2010 assigned twenty sheep to varying exercise protocols: circular exercise, treadmill exercise, or no exercise. Exercise was performed 6 d/wk at 1.3 m/s for 6 to 8 wk. This study found that macroscopic and histological changes were observed in cartilage, but no local or systemic effects on cartilage matrix metabolism [177]. This research group also reported sheep as an effective model for equine research but advised that further research with varying exercise protocols and a greater number of animals is warranted before definitive conclusions on effects of circular exercise could be made [177].

Another model species used to investigate the effects of circular exercise on bone and joint health in horses is Holstein calves. A 2022 study by Logan et al. assigned 24 Holstein bull calves to four exercise regimens: no exercise, small circle (12 m diameter), large circle (18 m diameter), or treadmill exercise. Animals were exercised at 5 d/wk for 7 wk at approximately 1.3 m/s. This study found that dorsopalmar diameter of the fused third and fourth metacarpal was significantly greater on the inside leg of calves exercised on a small circle. Similarly, this treatment group also saw significantly greater mediolateral diameter of the medial proximal phalanx compared to the lateral proximal phalanx. Additionally, calves exercised on the small circle demonstrated increased cartilage GAG content on the outside leg [78]. This group similarly found that calves may be a good model species for horses and was able to detect potential effects of circular exercise on bone and joint parameters.

The use of model species for equine research, particularly sheep, has many potential benefits. For instance, sheep have demonstrated ease of exercise acclimation and docile behavior during sampling [176]. Additionally, sheep tend to be more readily available from a single farm, allowing for increased sample sizes for research and decreased variation among subjects due to similar genetics. Sheep also offer practical benefits within a research setting, as flock behavior allows for ease of group exercise which improves efficiency as well as safety for both animals and researchers. Further, sheep are generally better accepted for use in a terminal study over horses, allowing for *ex vivo* assessment of bone and cartilage.

OBJECTIVES AND HYPOTHESES

The objective of this study was to build upon the previous research of Vernon et al. in 2010 and Logan et al. in 2022 to further elucidate the effects of circle diameter and speed on bone and joint health in horses utilizing an ovine model. The objective and hypothesis of this study are as follows:

1. Objective: determine the effects that a combination of varying speeds (1.3 m/s or 2.0 m/s) and circle diameter (12 m or 18 m) have on measures of bone, cartilage, and synovial fluid quality when compared to animals exercised on a straight line or non-exercised control animals.
2. Hypothesis: Faster speeds and smaller circle diameters will result in greater detrimental effects on measures of bone, cartilage, and synovial fluid quality when compared to animals exercised at slower speeds, on a larger circle or straight line, or not exercised at all.

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CHAPTER 3: INCIDENCE RATE OF DEVELOPMENTAL LESIONS IN THE DISTAL FORELIMB OF EXERCISED LAMBS: CONSIDERATIONS AS A POTENTIAL MODEL FOR OSTEOCHONDROSIS

SIMPLE SUMMARY

Circular exercise is commonly used in equine training programs and as a method of exercise. However, this circular exercise may alter how a joint surface is loaded, resulting in damage to the joint and subsequent cartilage degeneration. This degeneration is a key sign of osteoarthritis, which may result in pain and decreased performance capacity. The aim of this study was to investigate how circular exercise affected joint health, with one vital indicator being cartilage lesions. Young, growing lambs were used as a model for young, growing horses. A total of 411 cartilage lesions were found in the distal forelimb joints of the lambs with lesions being significantly larger in the carpus. No differences were found among exercise treatments or right and left legs. This suggests that the lesions discovered in these lambs were likely a result of a developmental orthopedic disease and prompted further investigation as to their cause. Based on these findings, it is proposed that sheep may serve as a useful model species to investigate the pathology of osteochondrosis in species such as horses.

ABSTRACT

Circular exercise is prominent in the equine industry, potentially resulting in joint damage characterized by cartilage lesions. Cartilage degeneration is a feature of osteoarthritis, resulting in pain, decreased performance, and lameness. To evaluate the effects of circular exercise on joint health, 42 lambs were used as a model for young horses. Animals were striated by sex and weight, then randomly assigned to a non-exercised control, straight-line, small circle, or large circle exercise regime at a slow (1.3 m/s) or fast (2.0 m/s) speed. Upon study completion, animals were humanely euthanized. Carpal and metacarpophalangeal (MCP) joints

were analyzed for number of lesions and lesion surface area. Within the 42 sheep, 411 lesions were observed (99% in the carpal joint and 1% in the MCP joint). Average lesion area in the carpus was 22 ± 1 mm^2 and 1.6 ± 0.3 mm^2 in the MCP joint ($p < 0.05$). Of carpal lesions, 48% were found in the carpometacarpal, 41% in the intercarpal, and 11% in the radiocarpal. No differences were found among treatment groups or limbs. These data suggest lesions were likely due to osteochondrosis, emphasizing that growth rate, diet, and genetic components should be considered when evaluating cause of cartilage lesions.

INTRODUCTION

Maintaining joint health of equine athletes is an ongoing challenge for the industry. In racing Thoroughbreds, racing Standardbreds, elite show-jumping horses, and dressage horses, lameness has been identified as a leading cause of lost training days [1–4]. Lost training days can be extrapolated to lost income through costs of care, time spent not training or competing, and compromised performance. Approximately 60% of lameness cases have been associated with osteoarthritis (OA) [7,8].

Osteoarthritis results from a breakdown of articular cartilage linked to the senescence and death of chondrocytes leading to further compromise of the joint extracellular matrix [9]. In the horse, OA is one of the most commonly reported chronic diseases [10,11]. Osteoarthritis is a progressive disorder [12], which decreases a horse's ability for athletic performance [13]. Lost training days due to lameness, and decreased performance in tandem with costs for maintenance care of the condition can pose significant economic hardships to owners and trainers. Further, pain and gait alteration due to restricted joint mobility compromises equine welfare.

Joint health is a prominent economic and welfare challenge for the horse industry. An important consideration in preventing joint damage is proper training and exercise, as excessive and abnormal loading of the joint can increase the rate of joint degradation [14,15]. Circular exercise is of particular interest due to asymmetrical loading between inside and outside forelimbs when exercising on a turn. With decreased circle diameter and increased speed, animals increase lean angle and use the outside leg to propel themselves through the curve [16,17]. Changes in movement symmetry affecting gait kinematics and joint loading [18] could increase risks of injury or chronic damage and disease, such as osteoarthritis. Cartilage lesions are a key macroscopic indicator of joint damage and are useful in identifying alterations to the joint due to mechanical stress, capsule debris, or synovial adhesions with lesion size often being directly related to their severity [19].

The use of model species to investigate musculoskeletal issues in the horse has proven beneficial and has been used previously in this lab [20,21]. This project utilized an ovine model which is advantageous due to uniformity among individuals, similar genetic makeup, acceptability for use in a terminal study, and feasibility of group exercise. Though there are differences between the sheep and the horse, the information gathered from one species may provide new insights to direct and inspire research in the other.

The objective of this study was to evaluate markers of joint health, including lesion number, area, and location in an ovine model exercised in either a straight line or circle and at different speeds. It was hypothesized that decreasing circle diameter and increasing exercise speed would increase the number of articular cartilage lesions and cartilage area affected. However, upon examination, a striking number of lesions were found across all treatment groups. Though not an original objective of the study, this inspired further consideration as to their cause and potential implications for future research aims.

MATERIALS AND METHODS

Animals and Housing

All animal work was approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC PROTO202200234; approved September 16, 2022). Forty-two Polypay-Dorset cross lambs (21 ewes and 21 wethers), aged approximately four months with an average weight of 40 ± 1 kg, were obtained from the Michigan State University Sheep Teaching and Research Center. Lambs were group-housed in two pens (35 m^2 and 33 m^2) with straw bedding and had *ad libitum* access to hay and water. Sheep were group fed a grain-mineral mix each d at a rate of 0.65 kg/hd/d; diet formulation was designed by the Michigan State University Sheep Teaching and Research Center according to the 2007 Nutrient Requirements of Small Ruminants guidelines [22]. Lambs were allowed a 1-wk acclimation period to their housing before exercise began.

Feed Samples

Samples of hay and grain-mineral mix were collected at the beginning and end of the study and stored at -20℃ until nutritional analysis was performed by Equi-Analytical (New York, United States) (Table 3.1).

Table 3.1: Composition of the dietary ingredients fed to sheep during the study on a dry matter basis.

	Grass Hay	Grain-Mineral Mix
Digestible Energy (Mcal/kg)	2.09	3.18
Crude protein (%)	11.4	13.4
Acid detergent fiber (%)	43.3	19.1
Neutral detergent fiber (%)	61.4	27.9
Water soluble carbohydrates (%)	10.6	5.8
Ethanol soluble (%) carbohydrates (%)	7.2	4.2
Starch (%)	1.9	36.7
Non-fiber carbohydrates (%)	18.0	48.7
Calcium (%)	0.75	1.24
Phosphorus (%)	0.23	0.36
Magnesium (%)	0.29	0.22
Potassium (%)	1.75	0.99
Sodium (%)	0.04	0.34
Iron (ppm)	134	283
Zinc (ppm)	21.5	64.5
Copper (ppm)	8	5
Manganese (ppm)	69	39
Molybdenum (ppm)	3.2	1.2

Treatment Groups and Exercise Protocol

On d-0, weight was recorded for each lamb along with a blood sample collected via jugular venipuncture. Lambs were striated by sex and weight then randomly assigned treatment groups. Treatments groups consisted of a non-exercised control and straight line, small circle, and large circle groups exercising at either a slow or fast speed (Figure 1). The exercise protocol was based on previous investigations within this lab [20,21]. All treatments had 3 ewes and 3 wethers for a total of 6 sheep per treatment. Each treatment group was assigned to a pen with control sheep evenly split between both pens, and sheep remained in their treatment groups for 12 wk. Lambs randomized to circular or straight-line exercise groups were exercised 4 d/wk at a speed of 1.3 m/s for the entire duration of exercise (slow treatments) or at a speed of 1.3 m/s followed by an increase to 2.0 m/s (fast treatments). Exercise began at 390 m/d (slow treatments:

5.0 min/d at 1.3 m/s; fast treatments: 4 min/d at 1.3 m/s and 0.7 min/d at 2.0 m/s) and increased by 390 m each wk until animals reached 2,340 m/d (slow treatments: 30 min/d at 1.3 m/s; fast treatments: 24 min/d at 1.3 m/s and 3.9 min/d at 2.0 m/s) (Table 2). Circular treatments were exercised tracking right (clockwise) within a mechanical walker (Q-line Horse Exerciser) with large (18 m) or small (12 m) diameter tracks. Thus, the right leg was consistently the inside leg, and the left leg was consistently the outside leg for the duration of the exercise. Straight line treatments were exercised on an equine treadmill (Class Champion Model 940, Queensland, Australia). Control sheep remained in their housing pens except during days when blood samples and weight measurements were recorded. Samples and measurements were taken in a chute system near housing pens, ensuring free movement was confined to their pen area.

Figure 3.1: Treatment group assignments based on exercise style (straight line, large circle, small circle, or no exercise) and speed (slow (1.3 m/s) or fast (2.0 m/s)). Figure was created with BioRender.com.

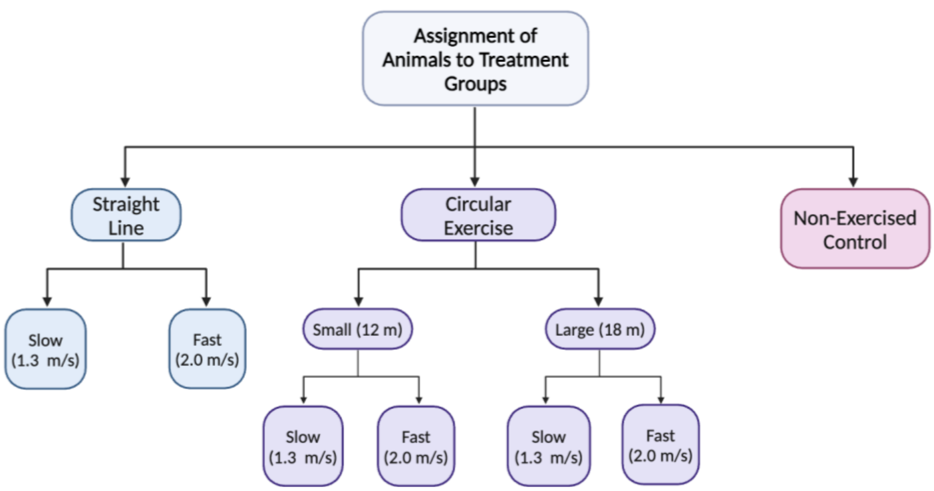


Table 3.2: Exercise protocol showing time and distance for all slow and fast treatment groups at their respective speeds.

Week of Treatment	1	2	3	4	5	6-12
Slow Treatments						
Time (min) at 1.3 m/sec	5	10	15	20	25	30
Distance (m) at 1.3 m/sec	390	780	1,170	1,560	1,950	2,340
Total Distance (m)	390	780	1,170	1,560	1,950	2,340
Fast Treatments						
Time (min) at 1.3 m/sec	4	8	12	16	20	24
Distance (m) at 1.3 m/sec	312	624	936	1,248	1,560	1,872
Time (min) at 2.0 m/sec	0.7	1.3	1.95	2.6	3.25	3.9
Distance (m) at 2.0 m/sec	78	156	234	312	390	468
Total Distance (m)	390	780	1,170	1,560	1,950	2,340

Sample Collection

Lamb weight was recorded every two wk beginning on d-0. Weight was measured using an electronic weight scale (Tru-Test; Model EziWeigh7i) affixed to a chute system. Between d-83 and 85, all lambs were humanely euthanized in the Michigan State University Meat Laboratory. The right and left distal forelimbs, removed at the middle of the radius, were collected and evaluated. All carpal joints (radiocarpal, intercarpal, and carpometacarpal) and the metacarpophalangeal (MCP) joint (fetlock) were opened and photographed for assessment of lesions.

Lesion Measurement

Joint surface photographs were obtained with an iPad Pro (MQDW2LL/A, Apple Inc., Cupertino, CA) with a 3.3 mm focal length in a consistent lighting environment. Photograph dimensions were 2448 x 3264 pixels and were taken perpendicular to the joint surface. All photographs of lesions were analyzed using ImageJ [23]. Photograph scale was standardized for each image by calibrating the software using a measuring tool included in each photo. The number of lesions per joint surface and two-dimensional area of each lesion were recorded. For a single joint, lesion area was totaled if more than one lesion was present.

Statistical Analysis

Statistical analysis was performed in SAS 9.4 (SAS Institute Inc., Cary, NC). Animal weight was evaluated using Proc Mixed with fixed effects of treatment and day. Results are reported as the LSmean ± SEM. Linear contrasts were run to ensure continual linear growth throughout the study. Number and location of lesions were evaluated using the frequency procedure to evaluate the incidence rate for each joint surface in the distal forelimb. Lesion area was evaluated using Proc Mixed with the fixed effect of joint, leg, and treatment. Correlation analyses were performed using Proc Corr to assess correlations between ADG and lesion number and lesion area. Both correlations were tested on the entire data set and separated by sex. Results are reported as Pearson's Correlation Coefficient (r). Values below 0.4 were considered a weak correlation, 0.4 to 0.6 a moderate correlation, and greater than 0.6 a strong correlation. Significance was set at $p < 0.05$.

RESULTS

Sheep had reached 70% of expected mature weight (54 ± 1 kg) at the time of humane euthanasia. Their size increased by 35% over 12 wk with an average daily gain (ADG) of 0.2 kg/d. They grew in a linear fashion ($p < 0.0001$) with a significant effect of day ($p < 0.0001$) on sheep weight, but no significant interaction of treatment by day ($p = 0.88$) confirming animals grew similarly regardless of treatment.

A total of 411 lesions were observed across all 84 limbs (42 sheep); 99% of lesions (406) occurred in the carpus and 1% (5) occurred in the MCP joint (Figure 2). Of the carpal lesions, 11% (45) were observed in the radiocarpal joint (Figure 3), 40% (165) were observed in the intercarpal joint (Figure 4), and 48% (196) occurred in the carpometacarpal joint (Figure 5).

Average lesion area was significantly greater in the carpus than in the MCP joint *($p < 0.05$)*, with carpal lesions having an average area of 22.0 ± 1.0 mm^2 and MCP lesions having an average area of 1.6 ± 0.3 mm^2. There were no differences among treatment groups ($p = 0.20$) or between limbs ($p = 0.16$) for the carpal joint. There were no differences based on limbs for the fetlock joint ($p = 0.82$) but there were insufficient data to calculate differences by treatment due to only 5 lesions recorded in the MCP across all animals. No animals demonstrated forelimb lameness or chronic mobility issues. One sheep had acute lameness of the hind right limb that resolved after 4 d of rest.

Across all animals, no correlation was found between ADG and lesion number ($r = 0.05$, $p = 0.73$) or ADG and lesion area ($r = 0.05$, $p = 0.74$). Similarly, when separated by sex, no correlation was found between ADG and lesion number or ADG and lesion area for wethers ($r = -0.17$, $p = 0.45$ and $r = -0.22$, $p = 0.34$, respectively) or ewes ($r = 0.12$, $p = 0.61$ and $r = 0.25$ $p = 0.28$, respectively).

Figure 3.2: Lesion found on the distal surface of the left MCP. Letters “M” and “L” indicate medial and lateral sides of image, respectively.

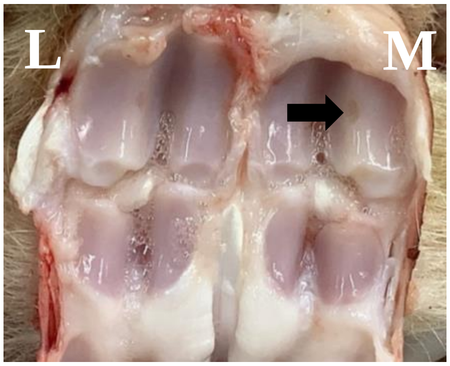


Figure 3.3: Lesion found on the proximal surface of the left radiocarpal joint. Letters "M" and "L" indicate medial and lateral sides of image, respectively.

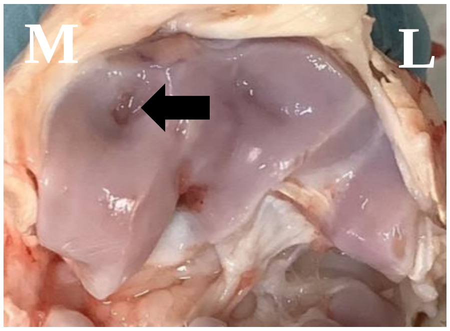


Figure 3.4: Lesion found on the distal surface of the right intercarpal joint. Letters "M" and "L" indicate medial and lateral sides of image, respectively.

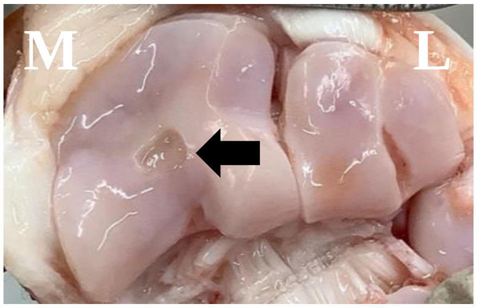
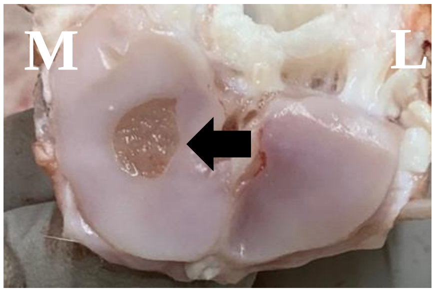


Figure 3.5: Lesion found on the distal surface of the left carpometacarpal joint. Letters "M" and "L" indicate medial and lateral sides of image, respectively.



DISCUSSION

The original objective of this study was to determine the effect of exercise on joint damage, with a key macroscopic marker being cartilage lesions. However, the large number of lesions found was unexpected, and their similar size and gross appearance across all treatment groups ($p = 0.20$) and limbs ($p = 0.16$) suggests these cartilage lesions developed independent of exercise. Of note, the high incidence rate of these lesions may have masked any treatment effect, though other factors were analyzed to evaluate joint health to evaluate the study's original objectives. However, the striking frequency of the lesions was a compelling discovery in and of itself, which prompted further considerations regarding their cause and future research directions that could be inspired from this incidental finding.

Though histological and radiographical analysis was outside the scope for the project's original aims, the gross appearance of these cartilage lesions is consistent with osseous cyst-like lesions (OCLL), which often appear centrally as a result of ossification failure and cartilage retention [24,25]. Previous hypotheses regarding the pathogenesis of these lesions have proposed them to be caused by an influx of synovial fluid through an osteochondrosis (OC) fissure [26,27] or an infolding of articular cartilage as pressure is applied at loading sites [25]. However, recent research has suggested vascularization failure and potential subsequent abnormal dilation of other blood vessels in epiphyseal growth cartilage may lead to the development of pseudocysts or true cysts [28]. Often, these lesions are found incidentally [25,29], as with the animals in this study, though they may result in lameness in some cases [26,27,28]. There is limited literature investigating the development of cuboidal bone OCLL in the horse. However, one study by Jaquet et al. investigating the progression of radiologic findings in 321 horses from 6 to 18 mo of age documented a total of 54 developmental OCLL in the carpus [30]. Developmental OCLL have also been documented in other species, such as dogs, pigs, and humans [31,32,33]. Developmental OCLL are thought to be a manifestation of OC [25,34,35], with some researchers proposing OCLL may predispose horses to subsequent osteochondritis dissecans [28].

Osteochondrosis is a developmental orthopedic disease (DOD) that results from ischemic chondronecrosis of growth cartilage present at the metaphyseal growth plate or beneath articular cartilage in the joints of juvenile animals, resulting in the formation of a necrotic lesion [36–39]. While OC is commonly also referred to as osteochondritis dissecans or osteochondrosis dissecans, each term describes various stages or manifestations of the disease. OC has been noted as the overall disease, with osteochondritis indicating an inflammatory response, and the word dissecans indicates a cartilage or osteochondral flap or free body has formed [40,41].

The development of these lesions is thought to be multifactorial; factors that may lead to ischemic chondronecrosis and subsequent lesion development via vascularization failure include nutritional deficiencies, genetic predispositions, and/or a rapid growth rate [38,42]. Work investigating OC in species such as pigs has prompted reevaluation and new considerations of disease pathogenesis in the horse [28]. Therefore, future work to investigate the etiology and pathology of the lesions found here may reveal new insights for the incidence of OC in sheep and other species already known to be affected by the disease. The following discussion outlines some considerations and hypotheses regarding the development of these lesions with supporting data in horses and other species.

Nutritional Deficiencies

Copper (Cu) deficiency is linked to OC in horses, particularly when dietary levels are below ten ppm [42–45]. Copper is necessary for proper joint development and maintenance. It is associated with the action of glycyl-L-histidyl-L-lysine (GHK) and N-lysyl-L-glycyl-L-histidyl-L-lysine-OH (KGHK), that aid in angiogenesis, collagen formation, and stimulate increased glycosaminoglycan (GAG) concentrations [46–48]. Copper is also necessary for lysyl oxidase function, an enzyme that forms collagen and elastin cross-linkages in the extracellular matrix which supports cartilage integrity [50–53]. Thus, these cuproenzymes are integral to proper joint development and maintenance.

Hay provided to the sheep had an average Cu concentration of 8 ppm, and the grain-mineral mix had an average Cu concentration of 5 ppm. These concentrations do meet the 2007 Small Ruminant NRC Cu requirement for sheep diets of 5 ppm, which is generally low due to their susceptibility to chronic copper toxicity [54–57]; however, these values are below the lower threshold of intake recommendations for the horse and would be suspected causative levels of OC or DOD [58]. Further, bioavailability may have been impacted by molybdenum concentrations provided at 3.2 ppm in the hay and 1.15 ppm in the grain-mineral mix [55,59,60]. While ossification of the carpal bones (where a majority of lesions were found) was likely complete at the start of study [61], their diet remained consistent from weaning through the end of the study. Thus, the copper and molybdenum levels discussed from this analysis of feed provided during the study should also be reflective of dietary levels since weaning. It is known that low dietary copper levels in sheep may lead to bone and connective tissue disorders [55,62]; personal communication with Dr. R. Ehrhardt [63] stated that incidences of angular limb deformities on large Polypay-Dorset farms decreased with Cu supplementation. Thus, it is possible that low dietary Cu levels—though meeting or exceeding minimum 2007 Small Ruminant NRC requirements—were a contributing factor to the development of these lesions.

Genetic Predisposition

In the horse, multiple quantitative trait loci have been identified across various breeds, specifically Dutch and Hanoverian Warmbloods [64–68], Norwegian and American Standardbreds [69,70], and French Trotters [71]. While the loci identified across breeds varied, the genetic basis for OC in horses has been well established, along with estimates of prevalence and heritability [68].

In sheep, there is minimal research published on OC, and sheep are not one of the six major species in which OC has been described and well-documented [38]. However, there have been a few case reports of OC occurring in Suffolks [72,73]. These observations indicate that OC does appear to be present in sheep, but current understanding is limited and thus the knowledge of genetic predispositions in sheep breeds is unknown. This paucity of information in combination with these incidental findings provides an opportunity in which OC may be further described in sheep. Not only may this benefit ovine welfare, but they may prove to be a useful model to advance understanding of the genetic contributions of disease development in other species such as the horse.

Rapid Growth Rate

Rapid growth rate and large body size has been implicated in the development of OC and other DODs in the horse as well as other species [42,68,74–78]. However, other studies have failed to provide evidence that growth rate alone is sufficient to result in the development of lesions [79]. Thus, current research suggests that the conditions surrounding growth rate may be more critical in the development of OC than the growth itself such as excessive mechanical loading, improper dietary nutrient ratios, and hormonal dysregulation [32,80,81]. This is further supported by data from the animals in this study, as no correlation was found between ADG and lesion number or ADG and lesion area indicating that growth rate was unlikely to be a sole causative factor.

In a review by Nakano et al. discussing the pathophysiology of OC in swine, it was proposed that increased weight at a younger age may contribute to OC as multiple studies have shown the ability to induce lesions when the joint is overloaded [82]. As such, it is possible that increasing an animal's weight through selection for rapid growth could cause more mechanical stress than the developing joint is able to readily withstand.

In growing animals, nutrient to energy ratios are higher than for slow growing or mature animals [83]. With this, it is imperative to ensure that the diet provided has nutrient concentrations sufficient to support the animals' growth rate. If nutrient content of the diet is not appropriate, deficiencies can lead to improper bone and joint development as seen in the pathology of many DODs, including OC [84].

Metabolic profiles associated with increased growth rates have also been suspected to play a role in the development of OC. In pigs, rapid growth has been associated with increased serum concentrations of somatomedin and growth hormone when compared to animals with a slower rate of growth [85]. Both hormones are known to play a role in connective tissue development and are suspected to effect cartilage development in rapidly growing animals. However, research directly investigating the effects of varying levels of these hormones on OC development is limited. Another suspected hormonal influence is insulin. In horses fed high energy diets, with notable contribution from non-structural carbohydrates, growing animals that developed OC were found to have increased postprandial serum glucose and insulin compared to horses that did not develop OC [86]. As such, it is suspected that dietary composition for growing horses may play a critical role in OC development [84].

The sheep in this study are of lines genetically selected for meat production predisposing them to a rapid growth rate. Over the course of the 12-wk study, sheep increased their average weight from 40 ± 1 kg to 54 ± 1 kg. This weight gain indicates an ADG of 0.2 kg/d and body size increase of 35%, which is similar to other growth rates documented for sheep on limit-fed diets [87], but notably less than ADG reported in horses at a similar stage of development. The ADG of long-yearling Thoroughbreds reared in the United States has been estimated between 0.6 and 0.8 kg/d, and ADG of yearling stock horses in the United States was reported at 0.7 kg/d

[88,89]. However, the lambs in this study gained more as a percentage of body weight than horses at this stage of growth. Sheep increased in size by 35%, whereas the reported ADG of horses at a similar growth stage would result in a body size increase of approximately 12% to 14%. While ADG of the sheep in this study did not correlate with lesion size or area, it is still necessary to consider growth rate in coordination with exercise regimen, diet formulation, and feed provision to be a contributing factor to the development of these lesions.

Impacts on the Equine Industry

In horses, OC is an important economic and welfare concern as the disease affects thousands of horses globally, with estimates of OC affecting between 20,000 and 25,000 horses in northwestern Europe alone [90]. The prognosis of horses diagnosed with OC depends heavily on the stage or severity of the disease; minor lesions may resolve with time and rest, whereas a dissecting lesion that creates a bone or cartilage flap or a free-floating loose body within the joint capsule may need to be treated via arthroscopic surgery [41]. While prognoses are generally positive for osteochondral fragments with appropriate removal, OCCL in the stifle can carry a lesser prognosis for performance [30,91]. Beyond the cost of veterinary care and potential performance impacts, there has been discussion in recent decades regarding selection against breeding stock that were diagnosed with OC or have tendencies to produce offspring with OC [92]. With the varying degrees of disease presentation and prognoses, a wide range of heritability and prevalence estimates, and the fact that OC has been documented in nearly every joint in the equine body [93], this is not a condition that will be easily eliminated from the industry, and it can be expected there will continue to be significant costs to owners and compromised equine welfare as a result. Thus, understanding the pathology of OC by studying its manifestations, such as OCLL, to develop sound preventative approaches can avoid the negative consequences of disease development.

CONCLUSIONS

Though the hypothesis that exercise performed at faster speeds and smaller circle diameters would result in greater macroscopic evidence of damage was not supported by these data (likely due to developmental lesions masking any potential treatment effect), the discovery of these lesions led to new considerations for future research directions. Despite decades of research on equine osteochondrosis, there are still questions regarding pathogenesis and management that need to be answered to aid in reducing disease prevalence in horses. The incidental findings from this study suggest that sheep may also be affected by OC, though additional work will be required to confirm the etiology of these lesions. We suggest that sheep could provide an ideal model system to study the disease pathology of OC considering the high prevalence of lesions, the relatively genetically uniform population, shorter intergenerational periods, shorter maturation periods, and feasibility of large group management and exercise. As such, sheep could prove to be advantageous in the efforts to deepen our understanding of the disease and improve the welfare of species affected by OC.

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CHAPTER 4: EFFECTS OF EXERCISE SPEED AND CIRCLE DIAMETER ON MARKERS OF BONE AND JOINT HEALTH IN JUVENILE ANIMALS

SIMPLE SUMMARY

Circular exercise is common practice in many disciplines, whether it be in the form of lunging, a mechanical walker, or ridden exercise. However, how horses adapt to circular exercise may put their bone and joint health at risk. To evaluate how the musculoskeletal system responds to circular exercise, juvenile sheep were used as a model for young horses. Circular exercise was found to influence traits of bone quality, such as density and fracture force. Various speeds and circle diameters also influenced markers of bone formation and bone resorption. Though no evidence of damage to joint was found in the evaluated parameters, it is possible that the same forces resulting in changes to bone may likely result in changes to joints when animals exercise on tighter or faster circles than those used in this study. As such, it is important for one to consider factors such as speed and circle diameter in their exercise programs to help ensure the musculoskeletal health of equine athletes.

ABSTRACT

Though circular exercise is commonly used in equestrian disciplines during both training and competition, this practice may be at the detriment of horses' musculoskeletal system. To investigate the effects of circular exercise on bone and joint health, 42 lambs were used as a model for young horses. Animals were randomly assigned to a non-exercised control, straight-line, small circle, or large circle exercise regime at a slow (1.3 m/s) or fast (2.0 m/s) speed. Blood samples were taken biweekly. Animals were humanely euthanized at the end of the study. Synovial fluid was collected from the carpus. Fused third and fourth metacarpal bones were collected and underwent biomechanical testing. Sheep in the large slow treatment group were found to have lower bone mineral density of the medial and lateral cortices of the left leg (p <

0.05). Fracture force of the metacarpal tended to be greater in the left leg for the large fast treatment group ($p = 0.08$). Serum osteocalcin and crosslinked C-telopeptides of type-I collagen were both found to have day and treatment effects ($p < 0.05$). These results indicate that even at relatively slow exercise speeds, circular exercise may elicit musculoskeletal changes.

INTRODUCTION

Preventing musculoskeletal injuries in horses is a major concern for the equine industry. Musculoskeletal injuries are the leading cause for horse wastage across many disciplines [1]. As such, these injuries may occur in a wide variety of ways. For instance, these may be catastrophic failure of bone that require a horse to be euthanized or more mild injuries that result in unsoundness. However, even in a mild case, lameness is the leading cost of lost training days. Thus, these injuries are not only glaring welfare concerns for equine athletes but are a major economic burden for owners and trainers.

Additionally, damage to the musculoskeletal system can lead to long-term debilitating conditions such as osteoarthritis (OA). Osteoarthritis is one of the most frequently reported chronic conditions in horses and accounts for up to 60% of all lameness cases [2-5]. This is often considered a common consequence following many equine athletes' careers and may be due to several influencing factors [6]. Generally, this condition develops when abnormal or unhealthy cartilage of the joint experiences normal loading events or when abnormal loads are applied to healthy cartilage [7]. Thus, these events encompass both overloading of the joints and injuries that affect the joint directly or compromise supporting joint structures like bone, tendon, ligaments, or muscles.

In racehorses, musculoskeletal injuries are predominately related to bone. Frequently, these injuries may be related to fatigue fractures which occur when there is not sufficient time for bone to heal from microdamage [8-11]. This damage is typically resolved by the bone's remodeling process, but when the damage accumulates at a rate that surpasses bone's ability to repair, it is weakened [8,12,13]. Additionally, bone's ability to resist fracture is heavily influenced by its strain environment, as the skeleton is a mechanosensitive system that responds to the forces placed on it [14]. This is especially important for growing animals, as loading during bone modeling has been shown to have life-long protective effects against fracture risk [15]. Thus, bone-related injuries are not only influenced by the frequency of training (which may result in overuse or fatigue) but also training and management techniques that define the bone's strain environment. For instance, amongst two-year-old Thoroughbred racehorses, those that began training before epiphyseal closure had decreased risk of injury later in their careers compared to horses that began training after closure [16]. Similarly, horses housed on pasture have been shown to have increased bone density compared to their stall-housed counterparts; this is likely due to increased exercise that in turn correlates with increased bone loading [17]. To further exemplify this point, just one short (71 m) sprint per wk demonstrated the ability to increase bone density of juvenile animals compared to those that were not exercised [18].

In horses competing in non-racing disciplines, musculoskeletal injuries are more often related to soft tissue rather than bone [19]. These injuries may compromise the joint environment, leading to instability, additional injury, and osteochondral damage [20-22]. Similarly to bone, these injuries are not only caused by overloading or overuse but may be heavily impacted by training method and management style. Exercise has been implicated in the development of joints as it has been with bone. Like bone, chondrocytes have been found to have mechanosensitive qualities, and loading of the joint has shown to be critical in proper joint development [1,23-25]. Movement of joints also promotes fluid exchange, which aids in maintaining synovial fluid (SF) quality and thus cartilage nutrition and joint lubrication [24]. Further, proper strengthening of supporting joint structures reduces future injury risk [26].

However, as suggested previously, not all exercise regimens are necessarily beneficial. The specific loading environments of bones and joints must walk a fine line between benefit and detriment. Overloading these structures may result in irreparable injury and long-term impacts on soundness. Thus, how exercise is conducted is arguably just as important as whether or not exercise is provided at all.

Circular exercise, in particular, is common practice amongst most equestrian disciplines. For instance, this exercise may occur in the form of lunging or placing horses on a mechanical walker, often for the purpose of improving fitness, allowing release of pent-up energy, or as a perceived “safe” alternative to turnout [27-29]. Circular exercise also occurs frequently during ridden exercise, both during competition and training. Disciplines such as reining, barrel racing, or show jumping often include very small circles and tight turns performed at relatively quick speeds. Though exercising on a curve or circle is used frequently during training or competition, it may be at the detriment of the horse’s musculoskeletal system—making it a potentially counterintuitive practice.

As horses exercise on a curve, they increase their lean angle toward the center of the turn to aid in offsetting the new centripetal force and maintaining balance [30]. With this lean angle, which increases with greater speed and smaller turn radii, the forces acting on the distal limb are notably altered in comparison to exercise on a straight line [30,31]. As the horse abducts the limb from the body, the strain environment of the outside leg is increased—thus increasing the strain placed on the bone [30,32]. This strain is associated with increased peak vertical ground reaction forces and hoof loading area on the outside limb as speeds increase, indicating a “push off” motion [33]. Circular exercise also appears to have effects on the inside limb such as increased duty factor, greater limb inclination, and increased torsion [30,34]. Thus, circular exercise inherently results in asymmetric loading of the limbs. When combining the knowledge of this asymmetry with how bone and joint structures are influenced by their loading environment, concern is warranted for how this style of exercise influences the musculoskeletal system and risk for equine injury.

Amongst Thoroughbred racehorses, it has been documented that risk of fracture to the outside forelimb was greatest at tracks with the smallest turn radii [35]. Similarly, underbanked tracks for Standardbred racing have demonstrated increased strain on the outside limbs, with proper banking resulting in angle relief and decreased risk of injuries [36]. In contrast, a study of Thoroughbred racehorse injuries at Midwestern tracks in the United States found that injury risk was greatest to the inside forelimb [36]. In a recent study using calves as a model for horses, circular exercise resulted in changes of increased dorsopalmar diameter of the fused third and fourth metacarpal (MCIII and IV) on the inside leg and increased glycosaminoglycan (GAG) on the outside leg [37]. Thus, this group found that musculoskeletal development may indeed be impacted by circular exercise, potentially predisposing horses to injury risk or development of joint disease. Similar conclusions were found by another earlier study, in which sheep were used as a model species, that found greater macroscopic evidence of cartilage damage in circularly exercised animals but greater histological Osteoarthritis Research Society International (OARSI) scores for animals exercised on a straight line [38].

Measuring changes in bone and cartilage are necessary to truly determine the effects of exercise. Serum biomarkers such as osteocalcin (OCN) and crosslinked C-telopeptides of type I collagen (CTX-I) are useful in determining relative bone formation and resorption, respectively

[39]. Bone can further be assessed through width and mineral density measurements via computed tomography (CT) scan, which allows for greater insight regarding structural changes of bone [40]. Biomechanical testing of bone allows for measurement of the bone's ultimate strength, indicating how resistant it is to fracture [41]. Together, these factors create a comprehensive image of bone quality for researchers. Similarly, a number of factors can be analyzed to investigate effects of exercise on the joint environment. Synovial fluid prostaglandin E_2 (PGE_2) concentrations are often used as a marker of inflammation within the joint and have been found to be elevated in horses with OA or synovitis [22,42]. Cartilage DNA also provides insight into joint health as a relative marker of cellularity; damage to joints has been found to result in decreased cellularity, and subsequently, DNA content [43]. Similarly, cartilage GAG content is a useful marker of extracellular matrix (ECM) quality. These compounds contribute to proteoglycan content in the ECM, maintaining water balance and compressive strength [44]. In damaged cartilage, such as with OA or injury, GAG content has been noted to decrease [45].

Though many studies have investigated the effects of circular exercise on gait kinematics and kinetics, few have delved into the effects this exercise has on bone and joint quality. Thus, the objective of this study was to determine the influence of circle diameter and speed on markers of bone and joint quality in the distal forelimb. To do so, lambs were used as a model for young horses, which have previously been used as a successful model for musculoskeletal research. It was hypothesized that smaller circle diameter and faster speeds would result in more asymmetrical changes to bone and negative effects on joint quality.

MATERIALS AND METHODS

Animals and Housing

All use of animals was approved by the Michigan State University Institute of Animal

Care and Use Committee (IACUC PROTO202200234; approved September 16, 2022). Forty-two lambs (21 ewes and 21 wethers) aged 4±1 mo with an average weight of 40±1 kg were obtained from the Michigan State University Sheep Teaching and Research Farm. On d 0, lambs were measured for weight and height then striated into one of seven treatment groups. Each treatment group was composed of 6 animals: 3 ewes and 3 wethers. Exercise was conducted 4 d/wk on a mechanical walker (circular exercise) with a diameter of 12 m (small) or 18 m (large) or a treadmill (straight line) at a speed of 1.3 m/s (slow) or 2.0 m/s (fast) with one group serving as a non-exercised control (Figure 4.1). Circularly exercised animals tracked right (clockwise) to ensure inside and outside limbs remained consistent. Animals started exercise at 390 m/d and increased by 390 m weekly until they reached 2,340 m/d (Table 4.1). When not exercising, animals were housed in two straw-bedded pens with areas of 35 m^2 and 33 m^2 that allowed for *ad libitum* access to clean water and hay. Animals were fed a grain-mineral mix once daily at a rate of 0.65 kg/hd/d.

Figure 4.1: Treatment groups for animals based on exercise style (straight line, large circle, small circle, or no exercise) and speed (slow (1.5 m/s) or fast (2.0 m/s)). Image created with BioRender.com.

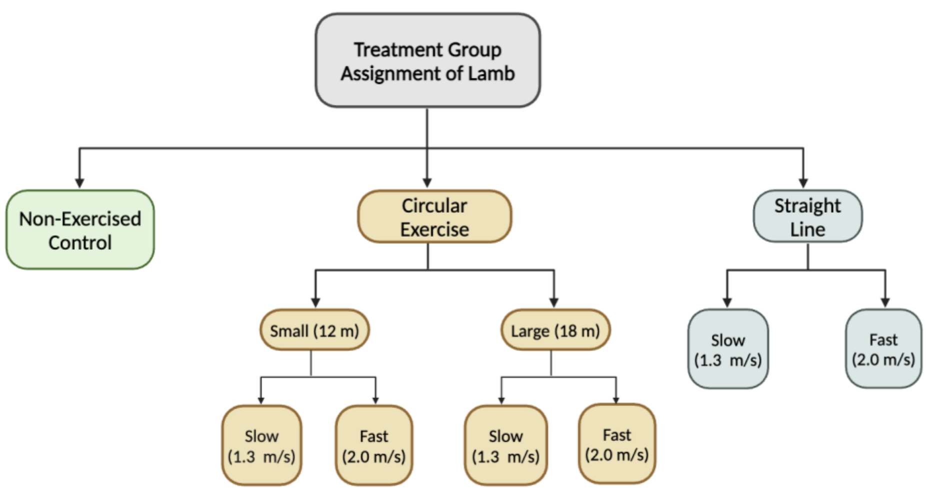

Table 4.1: Exercise protocol for treatment groups based on speed and total distance completed per exercise session weekly.

	Week of Treatment					
	1	**2**	**3**	**4**	**5**	**6-12**
			Slow Treatment Groups			
Time (min) at 1.3 m/s	5	10	15	20	25	30
Distance (m) at 1.3 m/s	390	780	1,170	1,560	1,950	2,340
Total Distance (m)	390	780	1,170	1,560	1,950	2,340
			Fast Treatment Groups			
Time (min) at 1.3 m/s	4	8	12	16	20	24
Distance (m) at 1.3 m/s	312	624	936	1,248	1,560	1,872
Time (min) at 2.0 m/s	0.7	1.3	1.95	2.6	3.25	3.9
Distance (m) at 2.0 m/s	78	156	234	312	390	468
Total Distance (m)	390	780	1,170	1,560	1,950	2,340

Sample Collection

A chute system was assembled near the housing pens, which was used to sort animals into their exercise groups, as well as perform blood draws every two wk beginning on d 0. Blood samples were collected via jugular venipuncture into a vacutainer tube. On d 83 to d 85, all animals were humanely euthanized at the Michigan State University Meat Laboratory. The distal forelimb was removed from the animal at the mid-radius. Synovial fluid (SF) was collected from the carpus of each leg primarily from the radiocarpal, but composite samples were accepted if SF was also collected from the intercarpal or carpometacarpal joints due to limited fluid volume. Once SF was collected, it was immediately placed onto dry ice and then stored at -80ºC until analysis. Each joint was then opened to expose the joint surface, and a cartilage sample was taken from the carpometacarpal joint with a scalpel. Cartilage was also placed on dry ice at time of collection, then stored at -20ºC until analysis. After samples were collected, legs were labeled and placed in a temporary chiller (4.8ºC) overnight until they were able to be computed tomography scanned. After computed tomography scans were completed, legs were stored in a freezer (-20ºC) until biomechanical testing of the fused third and fourth metacarpal (MCIII and IV) was completed.

Computed Tomography Scans

Computed tomography (CT) scans were completed at the Michigan State University College of Veterinary Medicine using a GE Revolution Evo Scanner (Boston, MA, USA). The right and left distal forelimbs of each animal were scanned within 36 h of collection. The position was set to a head-first supine position. Slice density was specified to 0.623 mm with settings of 120 kV and 320 mA. Each scan had a 100 mm field of view with a 512 $\times$ 512 matrix size, resulting in a 0.195 mm $\times$ 0.195 mm pixel and a subsequent voxel volume of 0.024 mm^3. A hydroxyapatite phantom was used in each of the scans to provide a reference for bone mineral density (BMD) with rows of 0, 75, and 100 $mineral/cm^3$. The average HU was recorded at 10 locations within each concentration of mineral density of the phantom. These HU values were then compared with the known concentration of mineral to create a regression line. The equation of this line was used to convert HU into mg $mineral/cm^3$ to determine BMD. This method for calculating BMD has been previously used and reported [37].

All CT scans were analyzed using Mimics 24.0 (Materialise, Leuven, Belgium) with measurements taken at the midpoint of the MCIII and IV. The midpoint was determined by measuring the total length of each bone. Bone mineral density, cross sectional area, cortical area, dorsopalmar diameter, mediolateral diameter, and cortical widths were all recorded.

Biomechanical Testing

All legs were moved from the freezer (-20˚C) to thaw for 12 h at 4.8˚C to excise the MCIII and IV and remove all soft tissue from the bone using a scalpel. Once cleaned, the MCIII and IV was measured for length as well as dorsopalmar and mediolateral diameters at 25%, 50%

and 75% of the total bone length. After measurements were taken, bones were each wrapped in a paper towel saturated with saline and replaced into freezer storage (-20˚C) until testing. Bones were removed from the freezer and allowed to thaw at 4.8˚C three d before testing. On the d of testing, bones were removed from refrigeration and allowed to warm to room temperature. Biomechanical testing of the MCIII and IV was performed at the Michigan State University Department of Plant Biology using a 3-point bending test performed with an electromechanical universal testing machine (Model 4202, Instron Corp., Canton, MA) equipped with a 10 kN load cell. Right and left MCIII and IV were placed individually with the palmar aspect of the bone facing upwards towards the force applicator and the dorsal aspect of the bone under tension. Bottom supports were placed 80 mm apart, with the upper force applicator applied centrally between the two bottom supports (40 mm from each). Samples were loaded until failure at a rate of 10 mm/min with a data acquisition rate of 100 Hz. Fracture force was determined to be the maximum amount of force applied before mechanical failure of the bone.

Bones were modeled as a hollow ellipse and all calculations were performed in accordance with guidelines from American Society of Agricultural and Biological Engineers (ASABE) and previous methodology of this laboratory [18,37,46,47]. Dimensions of bone necessary for calculations of moment of inertia, Young's modulus, and flexural rigidity were acquired from CT scan analysis using Mimics software. Moment of inertia (MOI, mm^4) was calculated as follows:

$$MOI = 0.0049[(B \cdot D^3) - (b \cdot d^3)$$

Where B is the outer lateromedial diameter of the bone, D is the outer dorsopalmar diameter, b is the inner lateromedial diameter, and d is the inner dorsopalmar diameter, and 0.0049 is a constant. Young's modulus (E, N/mm^2) was calculated as follows:

$$E = \left(\frac{F}{d}\right)\left(\frac{L^3}{48I}\right)$$

Where F is applied force, d is the displacement of the bone, L is length between lower posts, and 48 is a constant. Flexural rigidity (EI, N·mm^2) was calculated as the product of the moment of inertia and Young's modulus.

Osteocalcin

Serum samples from the sheep were analyzed for OCN concentration as a marker osteoblast activity and thus bone formation. Assays were performed using the commercially produced MicroVue Bone Osteocalcin Enzyme Immunoassay kit (Quidel, San Diego, CA, USA). All samples were diluted 1:8 with wash buffer, and analysis was performed following kit instructions. Results with a coefficient of variation of 10% or lower were accepted.

Crosslinked C-Telopeptides of Type-I Collagen

Sheep serum samples were evaluated for concentrations of CTX-1 as a marker of osteoclast activity and thus bone resorption. Assays were performed using the commercially produced Serum Crosslaps kit by Immunodiagnostics Systems (Gaithersburg, MD, USA). All samples were run neat, and analysis was performed following kit instructions. Results with a coefficient of variation of 10% or lower were accepted.

Ratio of Osteocalcin to Crosslinked C-Telopeptides of Type-I Collagen

A ratio of OCN to CTX-1 was calculated to gain an understanding of the relative relationship of bone formation and bone resorption. Values for the ratios were calculated as the OCN level divided by the CTX-1 level, both measured in ng/mL.

Prostaglandin E_2

Synovial fluid samples were analyzed for PGE_2 concentration as a marker of inflammation within the joint. Assays were performed using a commercially produced PGE_2

ELISA kit (Enzo, Farmingdale, NY, USA). Samples were first digested with a 50 μg/mL hyaluronidase solution with hyaluronidase source from bovine testes. After digestion, samples were diluted 1:2 with assay buffer. Due to some samples falling outside of the sensitivity range, select individual samples were rerun neat. Analysis was performed according to kit instructions Coefficients of variation at or below 15% were accepted.

Glycosaminoglycan

Cartilage samples were digested with papain for determination of GAG concentrations as a measure of ECM quality. To do so, a dimethylmethylene blue assay was used. This procedure has been previously validated and used in the methodology of previous investigations by this laboratory [37,48]. The cationic 1,9-dimethylene blue dye binds to anionic GAG, resulting in a colorimetric assay. A chondroitin sulfate standard was used for generation of a linear curve against which sample values were determined. Digested samples were diluted 1:25 with dilution buffer. Coefficients of variation at or below 10% were accepted.

DNA

Digested cartilage samples were also analyzed for DNA content as a measure of cellularity. Assays were performed using the commercially produced Qubit dsDNA High Sensitivity kit (ThermoFisher Scientific, Waltham, MA, USA). Fifteen µl of digested sample was added to 185 µl of working solution for a total tube volume of 200 µl according to kit instructions. Qubit 2.0 Fluorometer was calibrated using provided standards to ensure accurate reading of samples.

Statistical Analysis

All statistical analysis was performed in SAS 9.4 (SAS Institute Inc., Cary, NC). OCN and CTX-1 were run using Proc Glimmix with the main effects of day, treatment, and interaction of d by treatment. The ratio of OCN to CTX-1 was also analyzed using Proc Glimmix with the main effects of d, treatment, and interaction of d by treatment. Data from CT scans, biomechanical testing, and PGE_2 concentrations were run using Proc Mixed with the fixed effects of sheep, treatment, leg and interaction of treatment by leg. The GAG and DNA concentration data were grouped by leg and analyzed with the fixed effects of sheep and treatment. All data is reported as the mean $\pm$ the standard error of the mean (SEM). Significance was set at $p \leq 0.05$ and trends are discussed at $p \leq 0.1$.

RESULTS

Computed Tomography Scans

A significant treatment by leg interaction was found for the medial cortex of the MCIII and IV (p = 0.004), with the large slow treatment group having a lower medial cortex density for the left leg compared to the right (Table 4.2). Additionally, there was a trend for leg to influence lateral cortex density (p = 0.052), with left legs tending to have lower density than right legs. This appears to be driven by a significant effect of treatment by leg (p = 0.03), with the large slow treatment group having a lower lateral cortex density for the left leg compared to the right. There was no significant effect of treatment, leg, or the interaction of treatment by leg on dorsal or palmar cortex density (Table 4.2). There was no significant effect of treatment, leg, or the interaction of treatment by leg for midpoint whole slice density.

There was no effect of treatment or leg on bone length for the MCIII and IV. Similarly, there was no effect of treatment or leg for dorsal, lateral, medial, or palmar cortical widths nor whole slice, medullary mediolateral, or medullary dorsopalmar diameters. The interaction between treatment group and leg was insignificant for all measures of bone length and width. There was no significant effect of treatment, leg, or the interaction of treatment by leg for cortical area. There was also no significant effect of treatment, leg, or the interaction of treatment by leg for cross-sectional area.

Table 4.2: Bone mineral density (BMD, mg mineral/cm^3) of the dorsal, palmer, medial, and lateral aspects at the midpoint of the fused third and fourth metacarpal (MCIII and IV) of juvenile sheep by leg and treatment group.

Treatment	***Leg***	***Dorsal***	***Palmar***	***Medial***	***Lateral***
Control	Right	1320	990	1300	1320
	Left	1300	1010	1310	1270
Straight Fast	Right	1300	1010	1300	1340
	Left	1210	950	1280	1280
Straight Slow	Right	1320	1080	1330	1310
	Left	1300	1040	1330	1320
Large Slow	Right	1290	990	1320	1300
	Left	1100	880	1180*	1170*
Large Fast	Right	1320	1010	1320	1300
	Left	1300	990	1320	1340
Small Slow	Right	1300	1050	1320	1320
	Left	1330	1050	1330	1330
Small Fast	Right	1310	1020	1310	1320
	Left	1300	1040	1320	1330
SEM		48.5	45.4	27.5	29.2

No differences were found among treatments or legs. Leg tended to have an effect on lateral cortex density ($p < 0.1$). Treatment by leg interactions were found for the medial and lateral cortex densities of the large slow treatment group ($p < 0.05$). * indicates significant difference at $p \leq 0.05$.

Biomechanical Testing of the Fused Third and Fourth Metacarpal

Moment of inertia (MOI) was not significantly affected by treatment, leg, or the interaction of treatment by leg. Similarly, Young's modulus or the modulus of elasticity (E) showed no significant effect of treatment, leg, or the interaction of treatment by leg. There was no treatment effect or leg effect on flexural rigidity (EI), though there was a trend for a treatment by leg effect ($p = 0.08$) with the large fast exercise group tending to have greater EI for the left (outside) leg compared to the right. Similarly, there was no treatment or leg effect on fracture force (ultimate strength), though there was a trend for a treatment by leg effect ($p = 0.08$) with the large fast exercise group tending to have greater ultimate strength for the left leg compared to the right leg (Table 4.3).

Table 4.3: Fracture force (ultimate strength, N) of the fused third and fourth metacarpal (MCIII and IV) by treatment and leg.

Treatment	***Leg***	***Max Force (N)***
Control	Right	2140
	Left	2180
Straight Fast	Right	2280
	Left	2250
Straight Slow	Right	2270
	Left	2360
Large Slow	Right	2350
	Left	2280
Large Fast	Right	2220
	Left	2310 †
Small Slow	Right	2550
	Left	2550
Small Fast	Right	2430
	Left	2490
SEM		114

No differences were found for treatment or leg. There was a trend for a treatment by leg interaction for the large fast group ($p < 0.1$). † indicates a trend at $p \leq 0.1$.

Serum Osteocalcin

There was a significant effect of day ($p < 0.001$) and treatment ($p = 0.002$) on serum OCN concentrations. The small fast and straight fast treatment groups had greater OCN concentrations than the large fast, large slow, small slow, and straight slow treatment groups. Serum OCN was greatest on d-0 ($p < 0.001$). Concentrations of OCN decreased by d-14 and remained the same until d-28 ($p = 0.16$). Concentration of OCN then decreased again by d-42 ($p = 0.01$) and stayed the same for the remainder of the study. The interaction between day and treatment was insignificant (Table 4.4).

Table 4.4: Serum osteocalcin (OCN) levels in ng/mL measured biweekly across the 12-wk study period by treatment and day.

		Day						
		0	**14**	**28**	**42**	**56**	**70**	**82**
Treatment	***Overall Average***	**125[w]**	**102[x]**	**94[xy]**	**79[z]**	**84[yz]**	**91[xy]**	**84[yz]**
Control	**96[ab]**	131	98	91	81	79	98	92
Straight Fast	**104[a]**	145	108	104	90	93	95	93
Straight Slow	**85[b]**	117	97	77	74	72	84	76
Large Slow	**86[b]**	123	88	94	66	72	84	78
Large Fast	**92[b]**	111	108	84	79	88	95	79
Small Slow	**91[b]**	115	101	87	79	90	87	81
Small Fast	**104[a]**	135	111	115	84	95	96	92
SEM	4	4	4	4	4	4	4	4

There was an effect of both treatment and day on serum OCN concentrations ($p < 0.01$). No treatment by day interaction was found. [a,b] Treatments lacking a common superscript differ at $p \leq 0.05$. [w,x,y,z] Days lacking a common superscript differ at $p \leq 0.05$.

Serum Crosslinked C-Telopeptides of Type-I Collagen

There was a significant effect of day ($p < 0.001$) and treatment ($p < 0.001$) on serum CTX-1 concentrations. The large slow and small slow treatment groups had greater CTX-1 concentrations when compared to the control, large fast, small fast, and straight fast, and straight slow treatment groups. The straight slow treatment group also showed greater concentrations in comparison to the straight fast group. Serum CTX-1 concentrations remained the same from d-0 to d-28, with a slight increase on d-42. These levels then returned similar to baseline, followed by another increase on d-82 at which concentrations reached their peak. The interaction between day and treatment group was insignificant (Table 4.5).

Table 4.5: Serum crosslinked C-telopeptides of type-I collagen (CTX-1) levels in ng/mL measured biweekly across the 12-wk study period by treatment and day.

		Day						
		0	**14**	**28**	**42**	**56**	**70**	**82**
Treatment	***Overall Average***	**0.60^{yz}**	**0.57^{z}**	**0.55^{z}**	**0.65^{xy}**	**0.60^{yz}**	**0.59^{yz}**	**0.71^{x}**
Control	**0.59^{bc}**	0.66	0.53	0.53	0.66	0.58	0.53	0.66
Straight Fast	**0.53^{c}**	0.49	0.53	0.49	0.60	0.45	0.55	0.61
Straight Slow	**0.60^{b}**	0.60	0.61	0.60	0.65	0.52	0.54	0.71
Large Slow	**0.67^{a}**	0.72	0.58	0.61	0.67	0.77	0.60	0.75
Large Fast	**0.56^{bc}**	0.53	0.50	0.52	0.57	0.54	0.59	0.64
Small Slow	**0.71^{a}**	0.68	0.57	0.57	0.72	0.79	0.72	0.92
Small Fast	**0.60^{bc}**	0.54	0.65	0.52	0.66	0.55	0.58	0.69
SEM	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

There was an effect of both treatment and day on serum CTX-1 concentrations ($p < 0.01$). No treatment by day interaction was found. ^{a,b,c} Treatments lacking a common superscript differ at $p \leq 0.05$. ^{x,y,z} Days lacking a common superscript differ at $p \leq 0.05$.

Serum Ratio of OCN to CTX-1

The ratio of OCN to CTX-1 had a significant effect of day (p < 0.001) and treatment (p < 0.001), but the interaction of day by treatment was insignificant (Table 4.6). Day 0 had a greater ratio of OCN to CTX-1 than all other days. The ratio between concentrations remained the same from d-14 to d-28, then decreased again by d-42, and then increased by d-56. From d-56 to d-70, the ratio remained the same before a final decrease on d-82. Control, large fast, small fast, and straight fast exercise groups all showed greater ratios than the large slow and small slow exercise groups. Aside from the control treatment, values were also increased in comparison to the straight slow treatment group. The straight fast treatment group also showed greater values compared to the control treatment group.

Table 4.6: Ratio of osteocalcin (OCN) to crosslinked C-telopeptides of type-I collagen (CTX-1) measured biweekly across the 12-wk study by day and treatment.

		Day						
		0	**14**	**28**	**42**	**56**	**70**	**82**
Treatment	***Overall Average***	**216^{w}**	**189^{x}**	**179^{x}**	**126^{z}**	**152^{y}**	**166^{xy}**	**123^{x}**
Control	**167^{bc}**	200	196	177	127	138	190	144
Straight Fast	**199^{a}**	297	202	219	148	210	176	139
Straight Slow	**148^{cd}**	200	182	132	118	140	158	109
Large Slow	**132^{d}**	171	158	156	99	95	144	105
Large Fast	**177^{ab}**	220	229	173	138	169	185	126
Small Slow	**141^{d}**	175	181	160	117	124	133	93
Small Fast	**187^{ab}**	252	175	238	134	186	180	146
SEM	8	8	8	8	8	8	8	8

There was an effect of both treatment and day on the ratio of serum OCN to CTX-1 concentrations ($p < 0.01$). No treatment by day interaction was found. ^{a,b,c,d} Treatments lacking a common superscript differ at $p \leq 0.05$. ^{w,x,y,z} Days lacking a common superscript differ at $p \leq 0.05$.

Synovial Fluid Prostaglandin E_2

There was no effect of treatment, leg, or interaction of treatment by leg for SF PGE_2 concentrations (Table 4.7).

Table 4.7: Average PGE_2 concentrations from carpal synovial fluid by treatment and leg.

Treatment	*Leg*	*PGE_2 Concentration (pg/mL)*	*SEM*
Control	Right	458	166
	Left	381	166
Straight Fast	Right	484	152
	Left	501	152
Straight Slow	Right	520	152
	Left	779	166
Large Slow	Right	331	152
	Left	528	152
Large Fast	Right	476	152
	Left	410	152
Small Slow	Right	543	186
	Left	529	186
Small Fast	Right	454	152
	Left	338	152

No differences were found for treatments, legs, or the interaction of treatment by leg.

Cartilage DNA and Glycosaminoglycan

DNA and GAG were grouped by leg for analysis. There was no effect of treatment on cartilage DNA or GAG concentrations for right or left legs (Tables 4.8 and 4.9, respectively).

Table 4.8: Average DNA concentrations from cartilage slices collected from the proximal surface of the fused third and fourth metacarpal (MCIII and IV).

Treatment	*Leg*	*DNA Concentration (ng/mg)*	*SEM*
Control	Right	25.2	5.5
	Left	10.1	4.9
Straight Fast	Right	22.1	5.5
	Left	9.6	4.9
Straight Slow	Right	19.6	5.5
	Left	14.9	4.9
Large Slow	Right	23.8	5.5
	Left	19.2	4.9
Large Fast	Right	24.1	5.5
	Left	8.8	4.9
Small Slow	Right	28.6	5.5
	Left	13.8	4.9
Small Fast	Right	16.5	5.5
	Left	6.6	4.9

No differences were found among treatments.

Table 4.9: Average GAG concentrations from cartilage slices collected from the proximal surface of the fused third and fourth metacarpal (MCIII and IV).

Treatment	*Leg*	*GAG Concentration (µg/mg)*	*SEM*
Control	Right	30.5	6.4
	Left	53.9	8.4
Straight Fast	Right	39.6	6.4
	Left	50.7	8.4
Straight Slow	Right	32.2	6.4
	Left	54.2	8.4
Large Slow	Right	31.2	6.4
	Left	43.5	8.4
Large Fast	Right	36.4	6.4
	Left	57.7	8.4
Small Slow	Right	43.3	6.4
	Left	61.0	8.4
Small Fast	Right	30.4	6.4
	Left	56.2	8.4

No differences were found among treatments.

DISCUSSION

Using lambs as a model for young horses to observe changes that occur to the musculoskeletal system proved to be useful. The hypothesis that smaller circle diameter and greater exercise speeds would result in greater indication of bone alteration and joint damage was able to be partially accepted. Results from this study showed that the musculoskeletal system does adapt differently to various exercise styles, marked by differences found in bone density, fracture force, and markers of bone resorption and formation though no definitive changes to joint quality were measured in this study.

Computed tomography scans revealed no differences in bone length or cross-sectional area indicating that the sheep had relatively uniform conformation. However, sheep in the large slow treatment group were found to have decreased medial and lateral BMD of the left leg compared to the right leg. These results do appear to be a logical result regarding how these sheep exercised. When exercising on a circle, the outside leg is found to experience increased strain as lean angle increases, which would be expected to result in increased bone density. In this study, sheep exercised in a clockwise direction (tracking right), with their left leg on the outside. Thus, the decreased BMD of these cortices indicate that on a large diameter and at relatively slow speeds, the outside leg is not experiencing significant strain to promote increased bone density. Similar results have been found previously, with calves exercised on a 12-m diameter circle having smaller dorsopalmar diameter of the outside leg compared to the inside [37]. Therefore, while exercise on a large, slow circle does not appear to increase bone strength, it may be less damaging to joints than exercise performed at faster speeds on a smaller turn radius. While increased loading is generally thought to be beneficial for bone, it may be at the detriment of the joint as excessive loading of the cartilage has been shown to increase risk for chronic diseases such as OA. Thus, circular or curved exercise on a large turn radius at a relatively slow speed is unlikely to result in damage to the distal forelimb based on the results of this study, making it unlikely to result in joint damage and subsequent unsoundness. It should be noted that one CT scan from this treatment group had increased artifact and may have contributed to the decreased density measurements, but the observed difference agrees with the projected hypothesis of the study.

In the large fast exercise group, there was a trend for the left leg to have greater flexural rigidity (EI) and fracture force (ultimate strength) of the left leg in comparison to the right leg. In contrast to the large slow treatment group that had decreased bone density of the left leg, these results indicate that increasing speed on the same circle diameter can lead to the increased strain documented in other studies leading to increased strength of the bone. While the hypothesis of this study would suggest that this difference would be more likely to occur in the small fast treatment group, the large fast exercise group tended to be more willing to exercise. Though both treatment groups maintained average speeds of 2.0 m/s during the fast portion of their exercise sessions, the large fast treatment group was observed to maintain more consistent pacing which could contribute to this trend being found in this group and not the other. While only a trend, this further exemplifies asymmetric adaptations to exercise, and significant difference may be able to be detected with even faster speeds or a greater number of animals.

No treatment differences were found for BMD within each cortex or whole-slice analysis, nor were treatment differences detected for fracture force, MOI, E, or EI. This lack of difference among treatment groups may indicate that strain was relatively consistent among treatment groups as a whole and only able to be found when comparing right and left legs within a treatment. A limitation to this study is that strain was not measured, and thus differences in strain rate cannot be compared. However, this is not wholly unexpected as gait and subsequently speed is known to be a key factor in changing the bone environment. With each increase in gait, limb kinematics become more asymmetrical due to increased lean angle of the animal [30]. For instance, the greatest differences in strain and hoof loading area are detected at the canter compared to walk or trot [33]. This is notably a product of the speed at which each gait is performed, as these asymmetries have also been noted in Standardbreds that race at a trot or pace [36]—however, race speeds at these gaits are much greater than a standard working trot or jog. Further, when horses canter, they are performing a three-beat asymmetric gait in which the inside forelimb typically leads. Thus, it is possible that gait symmetry is another key factor when considering the effects of circular exercise. While speed was a key independent variable in this study, gait was not controlled, nor recorded, though sheep tended to perform a walk or trot at the slow (1.3 m/s) and fast (2.0 m/s) speeds, respectively. Thus, there may not have been great enough difference in speed, lean angle, or gait to elucidate measurable differences in bone among treatment groups.

Serum OCN concentrations, a marker of bone formation, revealed a significant effect of day, with concentrations generally decreasing over time. Concentrations decreased from d-0 to d-14, then remained consistent to d-28. Concentrations decreased again by d-42 then appeared to level out for the remainder of the study with small increase by d-70 that made levels comparable to those on d-14 and d-28. These values likely represent acclimation to exercise near the beginning of the study period and changes in rate of bone growth as animals matured. While there is no clear explanation for OCN levels increasing again on d-70 within the exercise protocol, it is possible that this could be attributable to the onset of cooler weather. This study was conducted from September to December in Michigan, USA. Thus, serum samples on d-70 were collected in late November, around the time more winter-like temperatures start to set in, with an average daily high temperature of 3.6 °C in the week leading up to d-70. For comparison, the average daily high temperature of the week prior to sample collection on d-56 was 17.6 °C. Cooler weather may have resulted in animals being more playful and exercising more willingly, as the sheep were likely experiencing decreased thermal load. Thus, all sheep may have experienced greater loading associated with more intensive free- and forced-exercised sessions, resulting in an increase in bone formation.

A significant effect of treatment was also found for serum OCN concentrations, with the straight fast and small fast treatment groups having greater concentrations in comparison to the straight slow, large slow, small slow, and large fast treatment groups. Though differences were not detectable with macroscopic measurements of bone quality such as density and biomechanical properties, these results indicate that faster exercise groups experienced greater loading resulting in increased bone formation. The fact that this was not observed in the large fast treatment group may be attributable to two factors. First, the large circle diameter—even at the fast speed—would not be expected to have induced as much strain on the bone as the small circle. This aligns with the hypothesis of this study, as smaller circle diameters are suspected to result in greater musculoskeletal adaptations compared to larger circle diameters. Second, though a large circle diameter is thought to be more comparable to straight-line exercise, differences in footing and pacing may account for increased formation in the straight fast treatment group but not in the large fast treatment group. Sheep in the straight-line exercise groups were exercised on a treadmill, resulting in harder footing compared to the sand footing in the mechanical walker for the circularly exercised sheep. This could result in greater concussion with each step on the treadmill, ultimately increasing loads placed on the bone. Additionally, sheep exercised on the treadmill had to keep up with the belt speed and had no opportunity to stop or turn around. In contrast, sheep exercising in the mechanical walker did have the space and opportunity to either stop or turn around briefly, though this was readily corrected by human handlers. Thus, it is possible that the pace of sheep in the large fast treatment group was less consistent than the straight fast treatment group, though average speed was the same. As for the control sheep having similar concentrations of serum OCN to all other treatment groups, it is possible that free exercise in the form of play was enough to induce similar loads to the bone when compared to the small fast and straight fast exercise groups. Similar results have been found in horses previously, where non-exercised control animals engaged in enough free exercise to have similar bone density to their exercised counterparts [49].

Serum CTX-1 concentrations, a marker of bone resorption, also showed a significant effect of day. Values remained the same from d-0 to d-28, with a slight increase on d-42 compared to d-14 and d-28 but not surpassing baseline concentrations on d-0. Levels then decreased slightly again to levels during the first four wk of exercise, followed by another increase on d-82 in which they surpassed baseline concentrations on d-0 but did not exceed levels on d-42. Interestingly, this pattern appears to be the opposite of those observed with OCN concentrations, with noticeable increases on d-42 and d-82 for CTX-1 and noticeable decreases on d-42 and d-82 for OCN. Thus, it appears that these increases in bone resorption may be attributable to decreases in bone formation following acclimation to exercise, increased activity with the onset of cooler weather, and natural changes in bone growth rate. Similar findings have been found regarding bone resorption markers increasing after acclimation of exercise [37].

A significant effect of treatment was also found for serum CTX-1 concentrations with large slow and small slow exercise groups having increased values compared to the control, large fast, small fast, straight fast, and straight slow treatment groups. The straight slow treatment group also had greater concentrations when compared to the straight fast treatment group. Though differences in bone among treatment groups was not detected with macroscopic analysis of bone quality, these data indicate that slow treatment groups had greater resorptive activity than fast treatment groups. This supports the idea that slower speeds and larger turn radii (in the case of the large slow exercise group) result in reduced strain placed on the bone when compared to faster speeds and smaller turn radii. Though the large slow treatment group also had greater resorptive activity than the straight slow treatment group, this could likely be due to differences in footing and pacing between sheep exercised in the mechanical walker versus the treadmill as discussed previously. Though it was surprising to see lower resorptive activity in non-exercised lambs, this may be due to these sheep engaging in free exercise (play) with their pen-mates as similarly hypothesized regarding their OCN concentrations being similar to the large fast and straight fast treatment groups.

A comparison of ratios of OCN to CTX-1 further exemplified the patterns seen with OCN and CTX-1 concentrations individually, with fast exercise groups having greater ratios of OCN to CTX-1 in their serum than slow treatments, indicating more bone formation activity. Further, over time, this ratio decreased indicating less bone formation and greater resorption as the study progressed. This reflects the idea that the beginning of the study resulted in greater formation activity as animals acclimated to exercise. The exception to this was d-56 and d-70, when the ratio increased again following a decrease on d-42. However, as discussed, this increase may be associated with greater osteoblast activity with the onset of cooler weather which could have influenced sheep behavior, making them more willing to participate in free and forced exercise.

Though no significant differences were found regarding treatment, leg, or treatment by leg for PGE_2, nor effects of treatment on DNA or GAG as markers of joint health, it cannot be ruled out that circular exercise may have detrimental effects on joints. In this study, sheep were exercised at relatively slow speeds. Thus, at these circle diameters, it is possible that the lean angle of the animals did not reach a degree that would elucidate clear treatment or leg differences, such as that of cantering or galloping in a horse. Similarly, for the sheep exercised in the treadmill treatment groups, it was not expected that a speed of 2.0 m/s would result in overloading of the joints and subsequent damage. However, evidence from bone density analysis, fracture force, and serum markers of bone formation and resorption indicates that exercise regimens did elicit differences in loading patterns between legs or treatment groups. This is important to keep in mind, as what is beneficial for bone may not necessarily be as beneficial for joints. It is known that injuries to supporting joint structures may result from overuse or overloading and can subsequently result in osteochondral damage [20-22]. Similarly, abnormal loading on otherwise healthy cartilage can result in damage noted by synovitis and eventual development of conditions such as OA [7]. Therefore, changes in loading patterns that are significant enough to elicit changes to bone could also be significant enough to elicit changes to the cartilage or risk injury to supportive structures. Even during normal, symmetric loading of the limbs, loads placed on cartilage are not uniform across the joint surface [50,51]. Thus, as loading of the limb increases, focal points on the joint surface may experience excessive force even if below the strain threshold of the bone. In the case of circular exercise, which results in asymmetric kinematics as well as asymmetric musculoskeletal adaptations as evidenced by the results of this study and others, the risk for joint damage may be increased further. Asymmetric joint changes have been documented previously in response to circular exercise by Logan et al.

in 2022, with GAG concentrations of cartilage from the proximal surface of the MCIII and IV being greater for the outside leg than the inside leg of calves exercised on a small diameter circle [37]. However, conclusive evidence regarding the effects of circular exercise on joint health and characteristics has yet to be found. A limitation to this study is that data for DNA and GAG analysis were required to be grouped by leg, as preliminary statistical analysis found leg differences across all treatment groups. This is suspected to be due to samples from the right and left legs each being collected by different individuals resulting in an erroneous statistical finding due to the sampling procedure. Thus, treatment differences were analyzed for right and left legs independently. Further, it is possible that differences were not found due to sampling a single joint location, and changes could have occurred at different anatomical locations. However, these data still provided valuable insight to effects on joint health under these varying exercise protocols and provide direction for future investigations.

Effects of circular exercise on bone and joint health may be able to be further determined by future studies in which greater lean angle is induced either by faster speeds, smaller circle diameters, or both. Further, data collection on stride rate and gait kinematics that allow for calculation of strain rate would be beneficial in determining the specific loads placed on the distal forelimb. Future considerations should also include how these effects are influenced by presence of a rider and frequency or intensity of circular exercise as it relates to different riding disciplines. However, even in the current study at relatively slow speeds and moderate circle diameters, leg differences were found regarding BMD and fracture force while treatment differences were seen regarding markers of bone formation and resorption, indicating that circular exercise is likely to result in asymmetric musculoskeletal adaptations.

CONCLUSIONS

Circular exercise is frequently used across many equestrian disciplines, but it may be at the detriment of joint health for the horse and result in asymmetric bone adaptations. Based on the results of this study, circular exercise influences musculoskeletal characteristics indicative of bone quality, likely through asymmetric loading. Fracture force of the MCIII and IV tended to be higher in the outside leg of the large fast exercise group, and BMD was decreased on the outside leg of the large slow exercise groups. Analysis of serum biomarkers also indicated that greater exercise speeds result in greater bone formation activity and slower speeds resulting in greater bone resorption activity. These changes in loading may also influence joint health when exercise is performed at faster speeds or smaller circle diameters. Thus, the combination of speed and circle diameter is an important factor to consider when designing a training program as well as in the evaluation of common practices within a discipline. Further studies are needed to determine the full effects of circular exercise on bone and joint health, though the results from this study and similar ones in the available literature suggest that circular exercise does indeed result in physiological changes to the musculoskeletal system.

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CHAPTER 5: OVERALL DISCUSSION AND CONCLUSIONS

The objective of this study was to determine the effects of varying circle diameters and exercise speeds on markers of bone and joint health. The results indicate that circular exercise does indeed cause varying adaptations when compared to straight line exercise, such as changes in bone mineral density and fracture force (Chapter 4). These results were further supported by treatment differences of osteocalcin and crosslinked C-telopeptides of type-I collage (CTX-1) as markers of bone formation and resorption, respectively (Chapter 4). Therefore, this study was successful in adding to known information surrounding the effects of circular exercise on the musculoskeletal system.

It was originally hypothesized that smaller circle diameters and faster speeds would be at greater detriment to joint health and alterations to bone from asymmetric loading. In functional assessments of bone, differences in bone mineral density were only found between right and left legs of the large slow treatment group (Chapter 4). Similarly, fracture force only had a trend of being different between the right and left legs of sheep in the large fast treatment group (Chapter 4). While these data still contribute to furthering this area of research and allows for further inferences to be made regarding effects of circular exercise, the hypothesis can only be partially accepted as asymmetric changes to bone were found.

Further, there were no differences found for leg or treatment for markers of joint health (Chapter 4). The DNA and glycosaminoglycan content did have to be grouped by leg for statistical analysis, as a preliminary analysis found that right and left legs differed across all treatment groups. This finding appeared to be erroneous, and the difference is suspected to be due to two people collecting cartilage from the right and left legs, respectively. This is a limitation of this study, as it made leg effects impossible to detect for DNA and GAG. Further, a high number of lesions were recorded across all animals, which was an unexpected finding (Chapter 4). It is possible that there may have been macroscopic evidence of damage to cartilage, but that the high level of developmental lesions masked any leg or treatment effect. However, this finding in and of itself was interesting, as it suggests sheep may be a potentially useful model for studying other musculoskeletal conditions in the horse such as osteochondrosis.

To build from this work, studies with larger treatment groups may prove beneficial as it is possible that six animals per treatment was not sufficient to detect small changes at this combination of circle diameter and speed. Similarly, another option going forward would be to reduce the circle size further, increase speed, or both. In this study, animals exercised on either a 12 m or 18 m diameter circle at a speed of 1.3 m/s or 2.0 m/s. This resulted in the sheep either walking or trotting. Thus, the lean angle of the animals may not have increased enough to elicit strong differences across all parameters. In horses, circles may often be performed on a smaller diameter—for instance, it is common in dressage to perform a 10 m circle and in timed speed events such as barrel racing, turns are performed in the smallest radius possible. Similarly, though a speed of 2.0 m/s did result in a trot for the sheep, a horse on the same circle diameter at a trot would be moving at faster velocity, likely resulting in a greater lean angle.

Another factor that may have contributed to not finding more definitive evidence to changes of bones or joints was the walker itself. Due to being designed for horses, the track width was wide enough that sheep were easily able to turn around, which affected their pace. Further, at the beginning of the study, the sheep were small enough that the push panels could pass over them. They quickly became desensitized to the panels and learned that they could stop. Even as sheep grew larger, they would allow the panels to drag over their backs, even with the electricity turned on. This resulted in sheep needing to have handlers walking or running behind them to maintain speed. Though average speeds of 1.3 m/s or 2.0 m/s were maintained as prescribed for each exercise session, sheep were still able to occasionally stop or turn around, which may have reduced the overall intensity of their exercise session than if they maintained a consistent 1.3 m/s or 2.0 m/s pace for the entire duration of exercise.

Despite these limitations, the results of this study provide valuable information to the equine community regarding the impacts of circular exercise, as it does appear that bone and joints may be affected differently than when animals are exercised on a straight line. Building from this study, future research should include investigations of specific lean angles as this is suspected to be the critical factor in potentially damaging effects on bone and joints. It is clear, though, that circular exercise is not likely to be eliminated as a common practice within equestrian disciplines anytime soon. However, with the information provided from previous studies and the results of the present one, it is clear the frequency and style of circular exercise is something to be carefully considered in the training and exercise regimens of equine athletes.