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SKELETAL ADAPTATION DURING GROWTH AND DEVELOPMENT: A GLOBAL RESEARCH ALLIANCE

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Introduction

Musculoskeletal diseases in racehorses are common and can lead to catastrophic injuries requiring euthanasia of the horse. Consequently, results of intensive studies concerning the pathogenesis of these injuries have revealed that many of these problems are due to chronic fatigue damage in the tissues from repetitive stress of racing and training (Kawcak et al., 2001). A horse's predisposition to tissue damage may be due to high mechanical loads imposed on a particular tissue, relatively poor material properties of a specific tissue, or both. Abnormal mechanical loading on a particular tissue can be due to a number of factors, including neurologic dysfunction and remote pain leading to overload of an opposing limb. In addition, we know clinically that conformation can greatly influence the forces on a specific joint, tendon, or ligament, often leading to clinically detectable diseases. Recent evidence is also starting to show that certain joints may be predisposed to high mechanical loads due to subtle geometric differences within the joint tissues (Muller-Gerbl, 1998). The material properties of a tissue, whether it is bone, articular cartilage, ligament, tendon, or muscle, are dictated by its collagenous and noncollagenous protein characteristics. For bone, the material properties are also dictated by the quantity and quality of mineral (Kawcak et al., 2001). Therefore, aberrant characteristics in any of these matrix components can lead to reduction in strength of the tissues.

Matrix components within tissues can be influenced by things such as genetics, nutrition, and physical loading history. As an example, in humans, there is considerable evidence that genetics is a strong factor in dictating the presence of osteoporosis (Runyan et al., 2003; Seeman et al., 1996). Nutrition is also a factor, which is suggested in horses as well (Runyan et al., 2003). Recently, exercise has been shown to strongly influence tissue material properties. In humans, it has been shown that, regardless of genetic and nutritional influences, people with a long history of moderate levels of exercise have a protective effect for osteoporosis (Runyan et al., 2003; Daly and Bass, 2006; Micklesfield et al., 2005). To further those investigations, it appears that when exercise was imposed during the greatest growth period, bone strength was maximized later in life, providing a protective factor from osteoporosis (Cooper et al., 1995; Janz, 2002; McKay et al., 2005; Wang et al., 2005; MacDonald, et al., 2006). In addition to these clinical results, experimental studies in several different species show, in general, that there is a threshold of exercise beyond which tissues are strengthened, but as importantly, a threshold above that which can be damaging (Kawcak et al., 2000; Kawcak et al., 2001).

The problem with these findings is that usually only one tissue such as bone is studied, and there are no conclusions as to the effects of a particular exercise level on all tissues.

Exercise studies in horses have shown that beyond a certain level, tissue damage can occur to certain tissues (Kawcak et al., 2000; Kawcak et al., 2001). In addition, limited exercise or nonweight-bearing events, such as casting, can also cause tissue damage (Richardson and Clark, 1993; van Harreveld et al., 2002a; van Harreveld et al., 2002b). Therefore, it appears that the results for horses are similar to other species. However, in order to use physical loading to positively affect tissue material properties, guidelines are needed to show whether loading during growth will be protective of all tissues.

The goal of the current study was to determine the effects of exercise at an early age on musculoskeletal tissues in the horse. Our hypothesis was that early imposed exercise would strengthen all tissues, thus preventing tissue damage later in life.

Materials and Methods

Thirty-three Thoroughbred foals were divided into two groups and subjected to different exercise regimens. In phase 1 (Figure 1), from birth until 18 months of age, the conditioned group was raised on pasture as well as subjected to a conditioning program (1020 meters) of increasing exercise level from approximately 10 days of age. The control group exercised spontaneously at pasture. At 18 months of age, 6 random foals from each group were euthanized for postmortem analysis. The remaining 21 foals entered phase 2, during which they were trained for two-year-old racing.

Horses were observed daily for general health, and clinical examination was carried out by a veterinarian at approximately four days of age and monthly thereafter in phase 1. This examination consisted of a general physical and lameness examination. At the end of phase 1, horses underwent clinical examinations, together with full radiographic, scintigraphic, and ultrasononographic examinations at the Massey University Equine Hospital. The behavior and plasma cortisol levels of the foals between average ages of three and five months were quantified (Crowell-Davis, 1986; Crowell-Davis and

Houpt, 1986). The following detailed evaluations have been completed on the tissues acquired at 18 months.

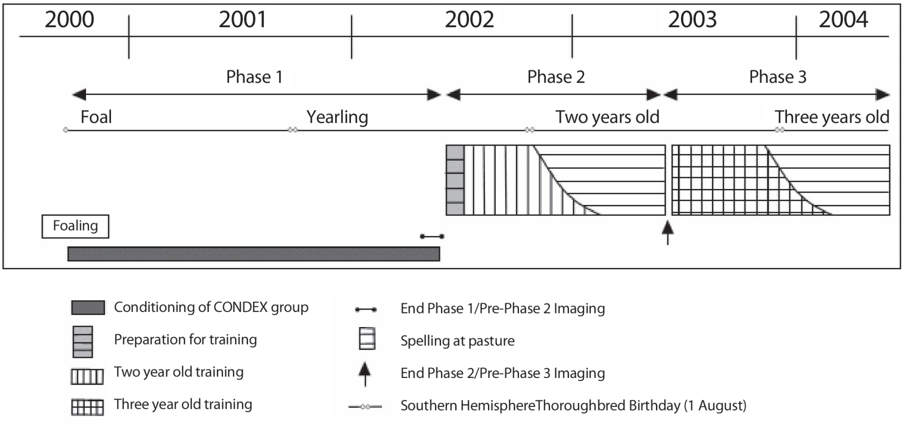

Figure 1. Timeline showing the three phases of the project (Rogers et al., 2006).

EFFECTS OF EARLY EXERCISE ON ARTICULAR CARTILAGE VIABILITY

In order to determine the effects of early exercise on articular cartilage and subchondral bone in specific sites of the metacarpophalangeal and metatarsophalangeal joints of young Thoroughbred horses, articular cartilage samples from four sites of the distal third metacarpal/metatarsal bones were stained with calcein-AM and propidium iodide. Confocal laser scanning microscopy was used to count live and dead cells. Proteoglycan scoring and modified Mankin scoring were also determined. The subchondral epiphyseal bone mineral density at the sites was measured using computed tomography (Dykgraaf et al., 2006).

EFFECTS OF EARLY EXERCISE ON MECHANICAL PROPERTIES OF ARTICULAR CARTILAGE

The objectives of the study were to determine (1) the site-associated response of articular cartilage of the equine distal metacarpal condyle to training at a young age as assessed by changes in indentation stiffness and alterations in cartilage structure and composition, and (2) relationships between indentation stiffness and indices of cartilage structure and composition. Four osteochondral samples were harvested per metacarpal condyle from dorsal-medial, dorsal-lateral, palmar-medial, and palmar-lateral aspects. Cartilage was analyzed for India ink staining (quantified as reflectance score), short-term indentation stiffness (sphere-ended, 0.4 mm diameter), thickness, and biochemical composition (Nugent et al., 2004).

EFFECTS OF EARLY EXERCISE ON SUBCHONDRAL MINERALIZATION PATTERN IN THE THIRD METACARPAL CONDYLES

Metacarpophalangeal joints were scanned using a conventional computed tomographic scanner and the files were exported to a custom-designed program for three-dimensional analysis of the joint. In the computer program, the third metacarpal condyles were disarticulated and analyzed. The bones were further cut into slices at 20, 30, and 40 degrees palmar from the mid-frontal plane. This allowed analysis of bone density and density pattern in the area most susceptible to injury.

EFFECTS OF EARLY EXERCISE ON OSTEOCHONDRAL TISSUES

Articular cartilage was harvested for analysis of glycosaminoglycan content and synthesis. In addition, synovial membrane, articular cartilage, and osteochondral samples were collected for histologic analyses using published techniques. All data were analyzed to determine the effects of exercise and site within the joint on dependent variables.

Results

Ten colts and 23 fillies were born with an average birth weight of 56.4 ± 5.54 kg. Foals were weaned at a mean age of 133 ± 9.51 days. No significant differences in behavior, plasma cortisol levels, or body weights existed between groups. Control foals had significantly higher condition scores compared to conditioned foals at 3 and 4 months.

The analysis of these clinical data indicated that conditioning increased the risk of joint effusion in the metacarpophalangeal, intercarpal, and radial carpal joints, and was protective for tarsocrural effusion. Significant differences in physitis scores for the distal radius between the conditioned and control groups were observed in months 2, 3, 4, 6, and 10. Analysis of the third metacarpal scores identified significant differences between the control and exercise groups at 2, 4, 6, 7, and 8 months of age ($p<0.05$).

EFFECTS OF EARLY EXERCISE ON ARTICULAR CARTILAGE VIABILITY

The mean number of viable chondrocytes was 14% more in the exercised horses than non-exercised horses (88% ± 1.3 vs. 74% ± 1.3, p=0.001), and 34% greater at the dorsal sites of the exercised horses than dorsal sites of non-exercised horses (87% ± 1.2 vs. 53% ± 2.1, p=0.001). The exercised group had a greater overall proteoglycan staining score than the non-exercised group (2.0 ± 0.1vs 1.2 ± 0.1, p=0.001).

EFFECTS OF EARLY EXERCISE ON MECHANICAL PROPERTIES OF ARTICULAR CARTILAGE

Cartilage structural, biochemical, and biomechanical properties varied markedly with site in the joint. Sites just medial and just lateral to the saggital ridge showed signs of early degeneration, with relatively low reflectance score, indentation stiffness, and collagen content, and relatively high water content. Effects of exercise and side (left vs. right) were not detected for any measure. Overall, indentation stiffness correlated positively with reflectance score and collagen content, and inversely with thickness and water content.

EFFECTS OF EARLY EXERCISE ON SUBCHONDRAL MINERALIZATION PATTERN IN THE THIRD METACARPAL CONDYLES

Results showed that there was a modest increase in bone density in the fetlock joints of horses that were exercised from an early age. In addition, there appeared to be a density pattern that might predispose some horses to condylar fracture. There was no effect of exercise on this pattern, but it was concerning that some horses showed a significant density gradient in the parasaggital groove of the third metacarpal condyle–the area where condylar fractures occur.

There were no significant effects of exercise on synovial membrane or articular cartilage histologic parameters. There was a trend for articular cartilage glycosaminoglycan content to be higher in exercised horses, although a trend existed for articular cartilage glycosaminoglycan synthesis to be higher in control horses. Analysis of subchondral bone parameters showed minimal effect of exercise on differences in bone fraction in each area, and significant increase in bone formation in the subchondral bone areas of the lateral and medial aspects of the third metacarpal condyles.

Discussion

Exercise beginning at 10 days of age did not have a detrimental effect on clinical, histologic, and biochemical parameters of musculoskeletal tissues. However, only osteochondral tissues were evaluated in this report, and results of tendon and ligament analyses are pending. One concern during development of this protocol was that early exercise might be detrimental to tissues. However, the level of exercise used in this study did not adversely affect tissues, but also had only a mild effect on positive tissue characteristics. Therefore, exercise levels beyond that used in this study would be recommended for future studies.

Early exercise had a beneficial effect on cellular and matrix features of the articular cartilage. The sequence of events leading to articular cartilage change appeared to be unrelated to SCB sclerosis. These findings may have implications for the use of exercise to condition developing mammalian articular cartilage. Increased chondrocyte viability and matrix quality could improve resilience of the articular cartilage to injury (Dykgraaf et al., 2006). However, exercise-imposed mechanical stimulation did not markedly affect articular cartilage function or structure. The marked site-associated variation suggests that biomechanical environment can initiate degenerative changes in immature cartilage during joint growth and maturation (Nugent et al., 2004).

Early exercise did induce significant increase in bone formation rate when the bone labels were given at 8 months of age. However, the overall effect on bone fraction at the end of the study (18 months) was minimal. Therefore, it appears that control horses may have regulated bone content with normal pasture exercise and growth. There were modest increases in bone density in certain areas of the metacarpal condyles; therefore, some protective effect may have occurred due to early exercise.

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