Exercise at Young Age May Influence the Final Quality of the Equine Musculoskeletal System

P. René van Weeren, DVM, PhD, Dipl.ECVS; Pieter A. J. Brama, DVM, PhD; and Albert Barneveld, DVM, PhD

During the first months of life the equine musculoskeletal system passes through a dynamic period of growth and remodelling. The musculoskeletal tissues, including those notorious for their lack of regenerative capacity in the mature individual, such as articular cartilage and tendons, seem to be molded in this period by the amount and character of exercise. In a study comparing young foals kept in boxes, with and without additional exercise, and raised at pasture with free exercise, the pasture-raised foals appeared to develop stronger musculoskeletal tissues. Authors' address: Department of Equine Sciences, Utrecht University, Yalelaan 12, NL-3584 CM Utrecht, The Netherlands. © 2000 AAEP.

1. Introduction

At birth the foal leaves its protected uterine environment and becomes exposed to the influences of the outside world. Then, a period of rapid growth and development follows during which genetic and environmental factors gradually shape the mental and physical abilities which will finally determine the athletic potential of the animal.

One of the most important factors in terms of athletic potential in the horse is the quality of the musculoskeletal system. The basis of the equine musculoskeletal system is initiated during embryonic and fetal development; this process continues after birth during growth which is fastest in the early postnatal period.

Of the four major constituent tissues of the musculoskeletal system (cartilage, tendon and ligaments, muscle and bone) the last two are known to easily respond to external influences and to have a high inherent capacity for rapid regeneration or repair which in most cases will lead to complete functional recovery. However, with respect to cartilage Hunter stated in 1743 that “from Hippocrates to the present age there is universal agreement that ulcerated cartilage is a troublesome thing that once destroyed is not repaired,” an observation that still holds today. For tendons, another tissue with a high extracellular matrix to cell ratio and limited vascularization, the same principle applies.

There is mounting evidence that the biochemical composition and ultrastructure of some musculoskeletal tissues in the neonate is considerably different from the mature animal and may be modulated by exercise through biomechanical loading. As the biochemical composition of tissues is directly related to biomechanical functionality, it may be hypothesized that, for some tissues, biomechanical loading in early life will influence the final quality of the tissues and hence the ability to respond to athletic demand and/or resistance to injury. This will be particularly important for
those tissues known to have a very limited, if any, capacity for repair or remodeling in the adult, such as tendons and articular cartilage.

This report is the synopsis of the outcome of a large project (setup and results of which have been extensively discussed in a recent Supplement of the Equine Veterinary Journal\textsuperscript{7,8}) that aimed at the comprehensive evaluation of the effect of exercise during early life on the development of all four major constituent tissues of the equine musculoskeletal system. To this end, a large group of newborn foals was divided into 3 groups. One of these groups was withheld exercise from birth to 5 months of age and kept in a box stall, the second group was kept under the same circumstances but given an additional exercise regimen, and the third group was given free (pasture) exercise serving as a control. Musculoskeletal development was assessed during this period using non- or minimally invasive techniques and, after sacrificing a number of foals at 5 months, with help of a large number of histologic and biochemical methods. To determine whether any induced effects were reversible, a number of foals from all 3 groups was kept under a similar exercise regimen for an additional 6 months after which the tissues were analyzed in the same way.

2. Material and Methods

Experimental Design

The study utilized 43 Dutch Warmblood foals born between March 29 and July 23 1996 (23 males, 20 females). Their dams belonged to the herd of the Institute for Horse Husbandry (Lelystad, The Netherlands).

After birth, all foals remained with their dams in a paddock for one week, after which they were divided into 3 groups that were subjected to different exercise regimens until weaning at 5 months. Allotment to the exercise groups was random, but the foals were blocked for sex and sire. Group\textsubscript{box} (n = 14) was confined to a box stall of 3 × 3.5 m throughout this period. Group\textsubscript{training} (n = 14) was kept in box stalls of the same size, but was given an increasing number of gallop sprints in a 48 × 15 m enclosure with a concrete floor covered by a thick sandy top layer that provided an adequate cushion during running. Two persons at the opposite ends of the enclosure chased the mares between them. The foals would follow the mares. Exercise started the day after their allotment to Group\textsubscript{training} (D7) and consisted of 12 sprints of about 40 m each. From D8–D24 the number of sprints was 16. From D25–D38 they made 24 sprints and from D39 until weaning at 5 months 32 and 16 sprints on alternate days. After the sprint training, the foals were allowed an additional 0.5 h of free exercise in the enclosure. Training was given 6 days a week from Monday to Saturday. Group\textsubscript{pasture} (n = 15) was kept at pasture 24 h a day, all 15 mares and their foals grazing together.

All foals were weaned at 5 months. From each group 8 foals were selected randomly (again blocked for sex and sire) and subjected to euthanasia directly after weaning. The remaining 19 foals were kept under exactly the same conditions in 2 groups (according to age), in an open loose box with access to a small paddock. None of them were trained, and all received the same amount of exercise. When they had reached age 11 months these foals too were euthanized.

Apart from the exercise regimen, all other conditions were kept as similar as possible for the 3 groups. The boxed and trained groups were housed in similar box stalls in the same building. They were fed fresh grass harvested from the same pasture in which the pasture group was confined. No concentrates were given. All studies were approved by the Utrecht University Ethical Committee.

Experimental Procedures During the In Vivo Period

Tendon Biopsies

At age 2 months, a single biopsy (1 × 0.1 cm) was taken from the peripheral fibrils of the superficial digital flexor tendon, under general anaesthesia.

Muscle Biopsies

Muscle biopsies were taken bilaterally from the gluteus medius and semitendinosus muscles in the standing animal after sedation and the application of local infiltration anaesthesia at 0, 2, 4, 8, 22, and 48 wk after birth.

Gait Analysis

Kinematic data of the foals were captured at age 5 months and in the remaining 19 foals at 11 months using a MacReflex\textsuperscript{9} gait analysis system.

Euthanasia and Further Processing of the Experimental Material

Euthanasia

Euthanasia was performed using an overdose of barbiturates (Euthesate\textsuperscript{8}), 100 ml/animal IV, after sedation with 0.1 ml/100 kg detomidine (Domosedan\textsuperscript{9}).

Processing of Cartilage and Subchondral Bone

Cartilage samples were taken from the right metacarpophalangeal joint for the determination of biochemical parameters (DNA, water, glycosaminoglycans [GAGs], total collagen and, for the characterization of post-translational modifications of collagen, hydroxylsine and hydroxylysylpyridinoline ([HP] crosslinks).

From the right femoropatellar/femorotibial and tibiotarsal joints, cartilage samples were harvested from predefined sites for explant studies in order to assess the metabolic status of the chondrocytes (incorporation of 35S directly ex vivo and after stimulation; proteoglycan release).
Processing of Bone

A 10 mm thick transverse slice from the left mid-metacarpal region was sawn and analyzed for bone mineral density (BMD) using quantitative computer tomography. The same technique was used to analyze BMD at three levels of the proximal sesamoid bone. Surface BMD was measured in the third carpal bone and distal radius using dual X-ray absorptiometry. Volumetric BMD was determined in the third carpal bone only.

Processing of Tendon

The tendon biopsies taken at 2 months and samples harvested at euthanasia at 5 and 11 months were investigated with respect to fibril diameter distribution using electron microscopy. The biochemical composition of the tendons from the 5 and 11 month groups was determined for glycosaminoglycans, DNA, hyaluronic acid, total collagen, and post-translational modifications (hydroxylysine, HP crosslinks) and cartilage oligomeric matrix protein (COMP).

Processing of Muscle

Muscle biopsies were analyzed with regard to fiber composition as reflected in myosin heavy chain (MHC) isoforms with the aid of monoclonal antibodies. Furthermore, succinate dehydrogenase and myofibrillar ATPase activities were measured in different fiber types. The effect of training was assessed by measuring the Na\(^+\),K\(^+\) ATPase concentrations using the \(^{[3H]}\) ouabain binding technique.

3. Results

Articular Cartilage

Many differences in biochemical characteristics were found between cartilage from the 5-month-olds and the 11-month-olds, reflecting the profound changes that take place in the extracellular matrix of the articular cartilage during this period of rapid evolution.

DNA content, GAG content and water content significantly decreased from 5 to 11 months, whereas collagen and the number of HP crosslinks significantly increased.\(^9\) The rise in HP crosslinks indicates that the tensile strength of the cartilage increases, but also that the extracellular matrix becomes more tightly organized and less penetrable to catabolic enzymes. Basal metabolism (\(^{35}\)S uptake) was not significantly different between 5-month-old and 11-month-old foals. However, when the cells were serum stimulated maximal absolute values in the 11-month group were significantly lower than in the 5-month group, indicating that maximal synthetic capacity had substantially decreased in the older group.\(^10\)

Exercise appeared to influence only GAG content, which increased with exercise (Fig. 1).\(^9\) There was a significantly higher basal metabolic rate of the chondrocytes in the trained group than in the other two groups.\(^10\) At 11 months, GAG content of the
former box and pasture groups were at a similar level, which was not unexpected 6 months after the cessation of the different exercise regimens. In the previously trained group GAG levels were reduced which could be explained only by the results of the explant study. From this study it resulted that the basal metabolism of all three exercise groups was similar, but that the chondrocytes of the trained group could virtually not be further stimulated, implying that the maximal production capacity of this specific group was severely impaired (Fig. 2).

Bone

BMD in the third carpal bone increased with age (Fig. 3). In the compact bone of the proximal sesamoid bone a similar effect was demonstrated, but in the cortex of the metacarpus BMD in fact decreased with age, agreeing with the results of Stover et al. who found that BMD at this site was more compact in fetuses than adults. However, as cross-sectional area (CSA) of the metacarpus increased considerably from 5 to 11 months and with it the product CSA*BMD, there is no doubt that actual strength increased, as expected. In general, the sprint training had a larger effect on BMD than did pasture exercise, leading to the conclusion that factors such as speed, impact, and strain rate are more important than duration of exercise.

Six months after the cessation of the different exercise regimens, most differences in BMD had disappeared due to a sharp increase in BMD in the box-rested foals, a slight increase in the pasture group, and a stationary situation or even decrease in the trained group. The sharp increase in the box rest group is thought to be caused by the increase in exercise in this group; in the pasture group the lesser increase is probably the normal age-related increase. However, there is no obvious reason why at some sites (especially the trabecular component of the proximal sesamoid bone at various levels), BMD in the trained group at 11 months dropped to levels well below those found in the pasture group (Table 1).

Tendons

Hyaluronic acid (HA), hydroxylsine (hyl), HP crosslink and COMP levels in tendon tissue were found to rise with age. In the 5-month-olds GAG content increased in the order box rest–training exercise–pasture, identical to the pattern seen in the extracellular matrix of articular cartilage in the fetlock joint. At 11 months of age, tendon GAG content in the former trained group was significantly lower than in both other groups, again similar to the extracellular matrix of articular cartilage. For HA an identical pattern was found between the exercise groups in tendon tissue. Interestingly, COMP levels in the 5 month group were lowest in the trained foals and highest in pastured animals.

From these data it is concluded that lack of exercise may inhibit the development of the superficial digital flexor tendon, but inappropriate or excessive exercise may also damage developing tendon, resulting in a reduced synthesis or increased loss of COMP. The lower concentration of some non-collagenous extracellular matrix components in the trained group 6 months after cessation of exercise indicates that this exercise regimen had a profound impact on tendon metabolism.

For electron microscopic fibril diameter assessment a biopsy was taken using a new technique at

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**Table 1. Bone Mineral Density (MBD, Mean ± SD, mg/cm²) of Trabecular Bone at the Apical Level of the Medial Proximal Sesamoid Bone in the 3 Exercise Groups at Ages 5 and 11 Months**

<table>
<thead>
<tr>
<th>Age</th>
<th>5 Months</th>
<th>11 Months</th>
<th>5 Months</th>
<th>11 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Box-Rest</td>
<td>Training</td>
<td>Pasture</td>
<td>Box-Rest</td>
</tr>
<tr>
<td>BMD</td>
<td>457 ± 50</td>
<td>521 ± 33</td>
<td>506 ± 30</td>
<td>582 ± 30</td>
</tr>
</tbody>
</table>

¶Significantly different between box-rested and trained foals (¶ = p < 0.05; ¶¶ = p < 0.01); § = significantly different between box-rested and pastured foals (p < 0.05); ¶¶ = significantly different between trained and pastured foals (p < 0.05). *** = significantly different between 5 and 11 months group (p < 0.001).

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an age of 2 months and entire tendons became available for study in 24 animals at 5 months. At 2 months a unimodal distribution pattern was found with a mean diameter of approximately 120 nm. At 5 months the number of smaller fibrils had increased in both exercised groups while the box rested foals still had a unimodal distribution (Fig. 4). It was interesting to note that there was no influence of exercise on the collagen fibril diameter in the post-biopsy scar tissue. All samples showed the same small diameter fibrils, which may have been due to the young age of these fibrils or to the location within the tendon (peripheral versus central core). The latter aspect merits further investigation as outspoken regional differences exist in the occurrence of tendon lesions.

Muscle

Using human monoclonal antibodies the presence of two isoforms of myosin heavy chain (MHC) in equine skeletal muscle could be demonstrated: Cardiac-α and Developmental MHC (Fig. 5). Also, a high degree of MHC co-expression was found, especially during the first months after birth, again indicating that for muscle this period is a time of intense activity and rapid transformation. In this study only foals from the box rest and the training group were compared. There was a trend towards a sharper increase in IIa fibers in the trained foals, but the differences were not statistically significant (p = 0.06), probably because of low numbers. Exercise in the form of sprint training (training group) appeared to significantly increase the number of [H]ouabain binding sites and hence the capacity of the Na⁺/K⁺-pump. However, the more gradual exercise the pastured foals were exposed to had no such effect (Fig. 6). These results clearly indicate that different training techniques will result in different responses by the equine musculoskeletal system.

Gait

An effect of training, or rather an effect of lack of training, was demonstrated in the 5-month-old foals. Gait characteristics of the box-confined animals showed an exaggerated, hypermetric movement pattern. The pastured group showed a flat, efficient locomotion pattern not unlike the pattern found in the control group in a study on the effect of a 70-day training program in two-year-olds. The trained group in the present study, however, showed a remarkable difference between forelimb and hind limb kinematics. Locomotion in the hind limb was as in the pastured foals, in the fore limb the pattern more resembled locomotion in the box-confined group. This indicates that the specific training of gallop sprints was more effective in developing the hind quarters. The fact that the exercise-induced differences in locomotion pattern had disappeared at 11 months, showed that no lasting effect of different exercise programmes on gait can be expected. At 5–6 months the foal apparently is still capable of changing its coordination pattern.

4. Discussion and Conclusions

The present study has clearly shown that the period from birth to 5 months is a turbulent period of rapid change for all major constituents of the musculoskel-
In this period of fast growth and rapid development a large number of dynamic remodelling processes take place, which profoundly influence the histological, biochemical, and biomechanical characteristics of the tissues. This not only applies to those tissues known to have a high capacity for remodelling and repair in the mature animal, such as bone and muscle, but also to articular cartilage and tendons which are notorious for their low turnover rate and insufficient repair. In the period from 5 to 11 months these processes continue, though at a slower rate, and there are indications that in some tissue components (such as articular cartilage collagen) most of the final constitution has already been formed at 5 months.

Differences in exercise level during the first months of life induced significant effects in all components of the musculoskeletal system examined (articular cartilage, bone, tendon, and muscles), including a “product” of this system: gait.

The sprint exercise given could be classified as not excessively demanding with respect to the total amount of exercise given. The maximal number of sprints (32 per day) equalled a little more than 3 min of galloping. Foals allowed free pasture exercise have been shown to gallop on average for about 3.5 min per 24 h, divided over approximately 40 sprints. However, it should be kept in mind that in the case of the trained foals the sprint exercise was superimposed on a basis of box confinement, and not on low-intensity exercise in the form of much walking and a little trotting as in the pastured foals.

In general, the enforced withholding of exercise (box rest group) led to a retardation in development. The effects of the other two regimens were diverse, varying with the type of tissue and the parameters investigated. While pasture exercise led to the highest glycosaminoglycan content in articular cartilage and tendon, sprint training enhanced bone mineral density in trabecular bone more than did pasture exercise. The same applied to the induction of the \( \text{Na}^+/\text{K}^+ \)-pump in muscular tissue, while the influence on gait of the latter type of exercise was limited mainly to the hind quarters.

After a period of six months of similar exercise, the tissues of the foals from the three exercise groups responded in different ways.

The foals from the box rest group appeared to have made up the lost ground with respect to those parameters known to be still responsive to external influences at older age, such as GAG content in articular cartilage and tendon and bone mineral density. Interestingly, this was not the case for a number of collagen characteristics in articular cartilage, indicating that in this case the withholding of exercise had a lasting effect on the eventual biochemical make-up of the tissue. This observation is of paramount importance as the biochemical composition of the extracellular matrix is known to be strongly related to the biomechanical behavior which in itself is widely supposed to be an important determinant for injury resistance.

In the trained group a singular observation was made with respect to articular cartilage, bone, and tendon. In all these tissues there were indications that tissue vitality and/or quality was less at 11 months than in both other groups. Though no conclusions can be drawn with respect to eventual permanent damage and consequences for later performance as the foals were not followed...
for more than 11 months, these consistent findings in various tissues were interpreted as a warning that the rather heavy exercise on top of a basic box rest regimen these foals had been subjected to was detrimental.

Apparently, a certain amount of exercise is essential to ensure an optimal development of musculoskeletal tissues. However, this exercise should be well-distributed as imbalanced training regimens may have a negative effect. There is no doubt that of the options in this study, pasture exercise was superior.

It is concluded that during the first year of life, and especially during the first months of this period, the equine musculoskeletal system passes through a very dynamic period of growth-related development and intense alterations. In this period the system is sensitive to biomechanical loading resulting from exercise. In fact, the tissues seem to be molded by the amount and character of exercise. This is even true, at least in the very first months of life, for those tissues notorious for their lack of remodelling and repair capacity in the mature animal. Though it has become clear from the present study that any exercise protocol at a very young age should be extremely well-balanced to avoid adverse effects, this offers the exciting possibility to substantially modify the make-up, and hence manipulate the resistance to, injury of especially those tissues that are, once injured, the major causes of wastage. Influencing the biochemical make-up of lesion-prone tissues in the musculoskeletal system of the horse may contribute significantly to the prevention of injuries and hence may have huge benefits, both in economic terms and in terms of animal welfare.

References and Notes

3. Little CB, Ghosh P. Variation in proteoglycan metabolism by articular chondrocytes in different joint regions is determined by post-natal mechanical loading. Osteoarthritis and Cartilage 1997;5:49–62.

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