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# DEVELOPMENT AND RESPONSES OF EQUINE MUSCULO-SKELETAL CONNECTIVE TISSUES

**A Collection of Published Papers Presented in Application**

**for the**

**Degree of Doctor of Science at Massey University**

**Elwyn Firth, BVSc, MSc, PhD**

**November 2006**

# Declaration

**I declare that the material to be examined in this thesis has not been submitted by me to any other university for the award of any degree.**

**Elwyn C Firth**
**November 2006**

# Acknowledgements

The scientific papers presented here show my interest in a particular area of equine research, and also in my active collaboration with many different colleagues over a period of some 25 years. The work was conducted when I was a staff member at Utrecht University, The Netherlands, and it continued at Massey University after I returned to New Zealand in 1988.

The part played by others in the work recorded here is indicated by their authorship or in the acknowledgement section of the papers. Although in almost all cases the papers are the result of studies which I conceived, planned and was actively engaged in, more than half do not bear my name as first author. This is because almost all the work involved collaboration with colleagues outside my own academic organisation and country of residence, and much of it involved postgraduate students who were engaged in research projects which I supervised.

A most enjoyable feature of research is engaging with other minds, and I thank all with whom I have had the great pleasure of working in this way. The joy of discovering new things is exceeded only by the pleasure of discovering them with other scientists. I state this here, because such fulfilment is rarely communicated to young people, who apparently find it difficult to contemplate enjoyment in science when they are choosing a career.

For their assistance and collaboration in my research work, I express my deep appreciation to all past and present staff in the above institutions and in other groups in which I have worked. In particular, I thank Professors J T Vaughan, A W Kersjes, Neil Waters, Des Feilden and Grant Guilford, the first three of whom appointed me, and all of whom have been generous in assisting with access to university facilities and have encouraged and supported me in research in many different ways.

Finally, for their love and support I thank my dear family, Sharmian, Jos and Tom, to whom I dedicate this work.

# Introduction

This application for assessment for examination for the Doctor of Science degree (Massey University) consists of a collection of papers published in international peer-reviewed scientific journals. The work was conducted over the last 25 years at Utrecht University, The Netherlands and Massey University, New Zealand. Also in the list of publications are papers (#) originally published in my PhD thesis (1983), and peer-reviewed abstracts of selected presentations at international conferences (*). They are included not for assessment, but to provide the reader with an insight into the origins of the work and the direction in which it is continuing.

My research focus began to take shape in the 1980s, when I first became interested in the reasons for the site predisposition of infective disease within bone, and in the differences in the responses of bone to mechanical forces. This collection of papers thus focuses on the responses of the musculo-skeletal connective tissues - bone, tendon and cartilage - to various influences. This research area remains important to both equine and human health, since the ways in which connective tissues respond to growth, disease, exercise and lack of exercise largely determine their subsequent structural integrity, and if and when they will be injured during the exertions of athletic training and competition. The work presented here does not include my research in fields less related to the subject of the development and responses of connective tissues.

# Summary

The focus of these studies has been the response of bone, cartilage and tendon to growth, exercise, and infective and aseptic disease. Study of the behaviour of these connective tissues has centred on their responses to various stimuli, which can be classified as either natural (for instance, infective disease, aseptic disease, growth, natural exercise), or induced under laboratory conditions (forced exercise, alterations in nutrition).

This collection of papers, entitled "Development and responses of equine musculo-skeletal connective tissues", thus has three major sections: infection, cartilage development, and exercise. However, division of the papers into separate chapters would be inappropriate because these influences overlap to some extent. For instance, normal growth and development requires exercise, and the propensity to cartilage and bone infection in foals is related to the stage of maturation of the tissues, which in turn may be related to exercise.

The publications are presented in the order in which the candidate successively conducted studies of how the above stimuli act on connective tissues. The studies appear in the reference list in the order in which they appear in this summary.

## Infection

The initial work was through the clinical, radiological and pathological study of many foals which apparently had infective polyarthritis, but which were shown to also be afflicted with osteomyelitis. Until then, it had been assumed that bone infection was a sequel of chronic infection of articular cartilage extending into the underlying bone. Epiphyseal osteomyelitis was found to be common in foals, often present in subchondral bone without evidence of infection at the surface of joint cartilage. The epiphyseal form of haematogenous osteomyelitis is rare in children, and had not been described previously in foals. The then new classification system of the various combinations of infective arthritis, epiphyseal osteomyelitis, metaphyseal osteomyelitis (also referred to as metaphysitis), and tarsal osteomyelitis (1,2,3, 4) is still used. These disease entities were recognized in calves and the same classification system was applicable (5).

The reason for predilection of the osteomyelitis for particular joints, and for particular sites within those joints (6) was studied with specific reference to the vascular anatomy (7,8), cartilage thickness (9), cartilage maturation (10), cartilage morphology (11), and the varying shape of the physis in the growing animal (12). Besides the classically described bone infection originating at the junction of physeal cartilage and metaphyseal bone (metaphysitis), infection was also demonstrated to originate on the epiphyseal side of the physeal cartilage.

Transphyseal vessels were a significant route of extension of infection, allowing extension of bone infection from one side of the physis to the other. Epiphyseal bone infection was shown to originate at the chondro-osseous junction of the deepest layer of cartilage and the underlying immature bone. The infection almost always began at sites with thick epiphyseal cartilage, and the latter was found to be where intra-cartilaginous vessel arcades persist for longest and the process of expansion of the secondary ossification centre of the epiphysis lasts longer than in areas of thin cartilage. This relationship of the predilection site of haematogenous epiphyseal bone infection to cartilage thickness was a novel finding, largely explaining why the disease occurs in specific sites, and only in foals up to a few weeks old.

The special nature of the terminal vascular arrangements in the thickest unossified epiphyseal cartilage led to the first studies of the tissue pressures in bone of both foals (13,14) and older ponies (15), since the conditions for bacterial colonization most likely include low pressure and low blood flow velocity in the areas shown to be susceptible to establishment of infection.

Consideration of the clinical implications of studies up to that time prompted investigation of the early diagnosis of infective metaphysitis in foals (16), and the features of metaphysitis caused by Rhodococcus sp (17). This resulted in a hypothesis that transphyseal vessels probably have a reserve function in the repair of metaphyseal retained cartilage and possibly other lesions on either side of the physis (18). A separate form of bone infection, diaphyseal osteitis, was also documented in terms of its frequent prevalence, relationship to penetrative trauma or other sources of infection, predilection site, and management (19).

At the time, the rationale for clinical treatment of infective arthritis relied on investigation of synovial fluid concentrations of systemically administered antibiotics, using normal horses, and in some laboratories in horses with induced bacterial infection. The latter was undesirable, and was replaced by a model using endotoxin-induced inflammation (20,21) which could be accurately quantified and minimized in terms of severity and duration. This allowed induction of extremely mild bone inflammation (16) which is still used in orthopaedic research, thus improving the welfare of animals. The interest in pursuing treatment led to collaborative work investigating aspects of optimizing factors to increase concentrations of antibiotic at the focus of bone infection (22,23,24,25,26,27). This was a prime focus before other methods such as regional intravenous, intra-articular, and regional intra-osseous administration were examined in detail.

## Cartilage Development and Osteochondrosis in foals

Careful pathological examination of bone sections of very young foals without evidence of infection revealed the incidental finding of small retained cartilage lesions (28) at particular sites of the epiphyseal and metaphyseal aspect of the physeal cartilage (12). Therefore, induction of cartilage retention was attempted so that the relationship of retained cartilage to angular limb deviation could be studied. After altering the foot conformation by applying a plastic wedge on the

outside (lateral) wall, the bone strain increased on the lateral aspect of the third metacarpal bone by 100%, and decreased by 40% on the medial aspect (compared to values before the wedge was applied). Within 10 days, the strains had equalized (29) and the hoped for non-invasive model of angular limb deviation did not eventuate. However, the work was the stimulus for the author, many years later, to study the effects of altered forces on connective tissues.

From the studies conducted to this stage, involving dissection and examination of hundreds of foal joints, it was clear that the sites with thickest cartilage were those of predilection to not only bacterial infection but also to articular osteochondrosis. The latter disease is part of the Developmental Orthopaedic Disease complex, is said to be the biggest problem facing horse breeders world-wide, and is still increasing in prevalence. This stimulated particular interest in the stage of skeletal maturity at birth and in subsequent development as immature structures develop into those capable of sustaining the forces applied during athletic locomotion. The degree of maturation at birth and subsequent growth and development also determine the likelihood of infection (4) in the first weeks of life. Also, abnormal endochondral ossification remains the acknowledged reason for the development of lesions of osteochondrosis.

At the time, it was vigorously contended that dietary concentration of copper was of central importance in the pathogenesis of equine osteochondrosis. This was of considerable importance to the New Zealand horse-breeding industry, since most of our pastures have much lower copper concentration than is recommended by the National Research Council and by feed companies in the Northern Hemisphere. Feeding copper to late pregnant mares resulted in higher liver copper stores in their newborn foals, compared to those of non-supplemented mares (30) and some foals from supplemented mares had much greater storage of copper, which they retained for longer (31). Osteochondrosis lesion scores were lower in foals whose dams had been supplemented with copper, but the lesions were very small and of unlikely clinical significance (32). The need for supplemental copper appears to have been overstated, at least in terms of pathogenesis of osteochondrosis, although higher copper status of the foal was associated with superior healing of early osteochondrosis lesions in Dutch Warmblood horses (33). The growth rate at 3-4 months was significantly higher in foals with more severe osteochondrosis, and in severely affected young horses the bone mineral density was less (perhaps indicating greater bone fragility) than in the same sites in horses with less severe osteochondrosis (34). The mechanisms through which growth affects cartilage to cause osteochondrosis lesions, and why some lesions heal and others do not, remain open questions.

Because of the known effect of concentrate feeding regimens on the risk of osteochondrosis, study was undertaken to determine if the dietary requirements of New Zealand thoroughbred foals could be realized when raised on well-managed pasture alone. Although accurate measurement of the dietary intake of individual animals at pasture remains impossible, very good estimates of intake and requirements of weanlings (35), yearlings (36) and mares (37), and of the total body mineral composition of foals (38) and of mare's milk (39) provided a scientific basis for Thoroughbred pasture nutrition. Because of the possible relationship between excess body weight and pathogenesis of juvenile orthopedic

diseases, whole body fat content was estimated in Thoroughbred weanlings (40). Enlargements above the fetlock area, commonly referred to as epiphysitis and said to be a form of osteochondrosis, were common and devoid of indications of being inflammatory, instead appearing to be a normal response of bone tissue (41). In these same 5 month old foals, only subtle articular osteochondrosis lesions could be detected (42). In other foals, higher growth rates appeared to be associated more with season-related nutritional effects (43) than with puberty, the onset of which occurred in both fillies and colts within a very narrow interval in spring (43,44,45).

## Exercise

Various factors induce change in the structure, composition and resistance to deformation of connective tissues. In an age in which most horses are used for intermittent athletic or recreational purposes, the most obvious and significant factor affecting equine tissues appears to be exercise, in the form of athletic preparation and training and competition, or **lack** of exercise through confinement in either pasture or stall. If the training stimulus is too severe, or if adaptive change to a suitable stimulus is inappropriate, then accumulation of micro-damage can result in injury, of either subtle or catastrophic nature. The equine scientific literature indicates that muscle appears to be less often injured than tendon, bone and joint cartilage, so attention was thus given to the latter three tissues.

The response of tissues to growth, ageing, or exercise were documented in cross-sectional study of tendons of wild (46) and domesticated (47,48) horses, and articular cartilage and subchondral bone of wild horses (49). Controlled studies involving treadmill training showed obvious change in the collagen properties in the central but not the peripheral region of the superficial digital flexor tendon of exercised horses that were exercised for 19 months, but not 19 weeks (50), compared to control horses which were walked and trotted only (51,52).

In carpal bones, the bone mineral density was greater in the dorsal compressive load path of the carpal bones, compared to the more palmar regions of the joint. In some regions of interest, mean density was more than 30% greater in the trained compared to the control group (53), and such changes were evident in horses trained for only 19 weeks (54). Foals bred from osteochondrosis-afflicted parents were sprint-exercised and compared to a group of foals kept in a large stall, and to another group which was at pasture. Again, the exercise, and lack of it in the boxed group, resulted in clear differences in bone mineral content in the distal radius and in the third carpal bone (34).

Neither of the exercise regimens in the above studies were typical of management used in New Zealand. The Massey University Grass Exercise Study (MUGES) was the first to determine the effect of conventional training exercise conducted at a racetrack on bone, tendon, and joint cartilage (55). The exact distance that each horse worked at various velocities was determined (56). The cross sectional area (57) and volume (58) of the superficial digital flexor and common digital extensor tendons were slightly greater in the trained than untrained group; the lower density

of the former tendon is a novel finding. Wear lines and osteochondral fragmentation in articular cartilage were few and mild in the carpus, but obvious in the metacarpo-phalangeal joints (55), associated with altered collagen biochemical characteristics (59). Carpal hyaline cartilage was thicker in trained horses, which indicated that cartilage responded to increased load (60). Bone accretion rate in the metacarpal diaphysis was determined by confocal microscopy after serial injection of bone markers (61). Diaphyseal size increased after bone density increased, leading to the first recognition that different gaits or gait velocity may stimulate different bone properties contributing to bone strength (62). After the training period of only 12-14 weeks, increased bone density in the third metacarpal condyle was established to be via deposition of new bone on pre-existing internal surfaces without prior resorption (63), in the form of progressively thicker columnar structures in the third carpal bone (60). Other novel findings included the physical communication between cartilage and bone through the calcified articular cartilage (64).

Because of the deficiencies demonstrated in one conventional imaging modality (65) and reviewed in others (66), more sophisticated imaging modalities are required to study changes in horses that are training and/or growing. Also, spontaneous lesions that would have prevented successful racing careers had been observed in untrained young horses, and worse lesions were found in horses that had been trained, although their training was at lower intensity than usually utilized in commercial training. These articular cartilage changes are probably the most important factor causing the high wastage in Thoroughbred training and racing, and require more sensitive detection techniques (55, 62).

Collaborative work (67) gave support to the hypothesis that injury and wastage might be as dependant on the factors, summarized in an invited review (68), which affect tissue development and health **before** training, and not just on what occurred **during** training. This required monitoring of cartilage, bone and tendon development in foals exposed from a very early age to exercise of a lower intensity than the regimen known to have caused long-term damage to articular cartilage in the confined foals described above. In the largest study of its kind, in collaboration with Dutch, English and American investigators, 16 of 32 foals raised on pasture were exercised on a grass track 5 days per week until 18 months of age, and the other 16 exercised only spontaneously in the paddock. An imaging method relatively new to equine research, peripheral quantitative computed tomography, was used to measure bone mineral content of the proximal phalanx, third metacarpal bone and distal radius at 0, 2, 4, 12 and 18 months of age. The early conditioning exercise resulted in a significant increase in bone size and strength and mineral content in the trained group compared to the control group, and the effect of spelling after race training of the horses as two and three year olds was determined (69). Computed tomography in the standing horse (as opposed to the anaesthetized laterally recumbent animal) is thus a rational option for quantifying the effects of growth, early exercise, athletic competition and withdrawal from competition, but magnetic resonance imaging is required for assessing articular cartilage.

The foals exposed to early exercise were also studied *ex vivo* to ensure that the conditioning regimen had caused no ill-effect on the most susceptible connective

tissue, articular cartilage. The features of articular calcified cartilage were shown, using combined forms of microscopy (70), to be different in conditioned and control foals (71). In the hyaline cartilage of the third metacarpal bone condylar surface, conditioning exercise was associated with a lower proportion of dead chondrocytes than in the cartilage of the control foals, which had exercised freely at pasture (72). This is an important observation and indicates that cartilage responds like other musculo-skeletal system tissues, that moderate exercise in the young is beneficial, and that managing horses in paddocks may not be as natural as it appears, in that some degree of sedentarism occurs.

The studies in this thesis began with the neonatal foal, later concentrated on the training of the young equine athlete, and most recently returned to the study of exercise in the very young as a way of altering the capability of tissues to later athletic training. However, the function of tissues largely depends on their structure, and structure is determined by many interacting influences, including genetic factors which can be modified in gestational or early post-natal life by various environmental stimuli, including maternal size and constraint, maternal and fetal nutritional status, and the pre- and post-natal exercise environments. Study in this exciting arena of very early growth and development, and its effect on later musculo-skeletal health, has begun (73, 74).

# List of publications

Five papers (#) originally published in the candidate's PhD thesis (1983) and peer-reviewed abstracts of selected presentations at international conferences (*) are included not for assessment, but to provide the reader with an insight into the origins of the work and the direction in which it is continuing.


1. **Firth EC, Dik KJ, Goedegebuure SA, Hagens FM, Verberne LRM, Merkens HW, Kersjes AW. (1980) Polyarthritis and bone infection in foals.** ***Zbl Vet Med. B 27, 102-124.***

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4. **Firth EC, Goedegebuure SA, Dik KJ, Poulos PW. (1985) Tarsal osteomyelitis in foals.** ***Veterinary Record 116, 261-266.***

5. **Firth EC, Kersjes AW, Dik KJ, Hagens FM. (1987) Haematogenous osteomyelitis in cattle.** ***Veterinary Record 120, 148-152.***

6. **Firth EC, Goedegebuure SA. (1988) The site of focal osteomyelitis lesions in foals.** ***Veterinary Quarterly 10, 99-108.***

7. **#Firth EC, Poulos PW. (1982) Blood vessels in the developing growth plate of the equine distal radius and metacarpus.** ***Research in Veterinary Science 33, 159-166.***

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