This review documents some new advances in the field of Veterinary medicine with specific focus on the exciting research area of myo-fascia. It presents some of the latest findings in structure as well as function of these interconnected tissues, as well as highlighting the effects and benefits of treatment for such underlying issues as regional stiffness affecting performance, and the role of myofascial kinetic lines. It likewise addresses some of the new and non-invasive techniques such as accelerometry, multi-frequency bioimpedance and acoustic myography that have emerged over recent years, and illustrates how they can be readily adopted in the Veterinary clinic to assess muscle imbalance and injury as well as to direct such treatment as myofascial release therapy, acupuncture and proprioception and to follow rehabilitation.
Introduction:

With new technology come new possibilities of detecting, diagnosing and even treating injuries, illnesses and diseases, not only in the field of human medicine, but also that of veterinary medicine. However, new advances can also come with new insights, improvements and understanding of existing techniques or even more careful and detailed dissection procedures (Elbrønd et al 2015; Harrison et al 2018; Elbrønd & Schultz, 2015; Elbrønd & Schultz, 2018; Jensen et al 2018; Chavers et al 2018).

Fascia:

Recently, dissection work in the horse has led to the discovery of functional interconnected lines and structures that are comparable to the so called “anatomical trains” discovered in humans and reported by Meyers (2009) (Elbrønd & Schultz, 2015). These so called “myofascial kinetic lines” are now being accepted as being at the core of equine biomechanics and functional anatomy and they are revealing new insights into not only static but also dynamic posture (Skalec & Egerbacher, 2017; Elbrønd & Schultz, 2018). What makes them even more important perhaps is that they constitute a readily useable tool that can be applied by Veterinary practitioners to not only track the underlying cause of a locomotory problem, but also to understand compensatory patterns in a horse with impaired performance (Elbrønd & Schultz, 2015).

With this new understanding of the plasticity as well as the mobility of body structures, many of which have importance for the locomotion system, comes a new concept, namely that of “slide and glide” (see Fig 1). This quickly understood and accepted phenomenon defines the ability of muscles and other structures to move individually or in groups with minimal resistance to one another. The latter is of great importance as it is crucial for protection, for energy conservation and the facilitation of coordinated body movements. Moreover, when taken together with what is now known about the fascia, as well as muscle interconnections in the body (Huijing & Baan, 2008; Huijing, 2009), one cannot help but think away from the common and generally accepted idea of single/individual muscle function and its interaction with motion within one region of the body, towards one that rather embraces a flow of activity through the body (Carvalhais et al 2013). This flow not only involves different regions of the body, but also different muscles, both synergistic and antagonistic, with force being transferred smoothly and in a coordinated way from one muscle group to another, whilst other regions of the body provide stability and support.

Indeed, it has recently been shown that hyaluronan is a major factor facilitating the concept of “slide and glide” (Stecco et al 2018). Whilst both equine dissections and histology studies support this new concept (Elbrønd & Schultz, 2018; Krasnodebska et al 2015; Ahmed et al 2018) they have also drawn attention to a point of clinical relevance, that of inter-structure mobility and its impairment as the result of scar tissue, a topic that now warrants further investigation.
Figure 1: Panel A: Shows an epimysial lining and surrounding fascia with a clear blue stained line of hyaluronic acid – it indicates the potential for “sliding and gliding” between muscle and surrounding tissues. Panel B: Shows the epi- and perimysial lining between two fascicles of a muscle – facilitating free movement between them. Scale bar for both panels denotes 100 μm. The lower schematic illustrates the concept of “slide and glide”, showing regions of muscle fibres separated by a wavy line of intermuscular fascia – the perimysium. It highlights the ability of adjacent muscles to move independently of each other whilst maintaining tension/force (muscle image scale bar = 50 μm). Staining: H&E and Alcian Blue. Images: APH & VSE.

Muscle:

Of course being able to identify the links between muscles and present them as a kinetic line enables Veterinary practitioners the opportunity to begin to treat locomotory problems in horses. In a recent study, advantage was taken of a new and non-invasive means of myofascial
release therapy (Elbrønd et al 2015). Myofascial release is a soft tissue therapy for the treatment of skeletal muscle immobility and pain (Barnes, 1990). The therapy relaxes contracted muscles, improves blood and lymphatic circulation and stimulates the stretch reflex in muscles. Myofascial release was undertaken using a multifrequency “AtlasOrange1” device which delivers three vibration cycles of alternating frequency and intensity to specific muscle sites, through a carefully designed semi-hard tip – in many ways providing an alternative to massage treatment. In this study, an “AtlasOrange1” device was used as a novel means of mechanical physiotherapy massage, being applied to just two anatomically identifiable myofascial regions on a total of 6 horses, for a period of treatment lasting only 2 minutes. The transmitted oscillations were found to be sufficient to induce an immediate as well as a more long-term change in the resting tension of the treated muscles (Elbrønd et al 2015).

What is interesting with this particular study of myofascial release therapy (Elbrønd et al 2015), is that firstly, regions of increased myofascial tonus/stiffness were detected manually as well as confirmed scientifically by a blinded and independent measurement obtained through multi-frequency bioimpedance (for details see next section). Measurements were noted for all 6 horses by an experienced equine Veterinarian. Treatment was found to induce a significant 58% decrease (P=0.003) in resting tension over a 48 hour period, post treatment, as measured using a commercial mf-BIA unit (Elbrønd et al 2015). mf-BIA recordings can be analyzed using a Cole–Cole plot (showing resistance against reactance), where the centre frequency (fc) is the frequency at which the maxima reactance (Xc) is recorded (Ivorra 2003). Thus, fc is indicative of the amount of energy needed to send a set alternating current through a tissue of interest. With a higher level of contraction or resting tension in a tissue, the greater the density of that tissue and the higher the energy needed to penetrate it, i.e. a higher frequency is required for the current to pass through that tissue. If the fc of a given muscle decreases over time, then it indicates that the muscle has become less tense i.e. it has become more relaxed.

The study by Elbrønd and colleagues (2015) is also unique in being able to show how myofascial release therapy can cause an almost immediate release of tonus/stiffness in one region with a form of compensatory stiffness being temporarily noted in other parts of the horses body in response to treatment. This observation, which was found to be statistically significant, once again serves to highlight the functional role and connection between muscles in a myofascial kinetic line.

The study is further unique in that myofascial tonus/stiffness was measured in a quantifiable way using the new, novel and validated method of multi-frequency bioimpedance (Ivorra, 2003; Nescolarde et al 2013; Riis et al 2013; Elbrønd et al 2015; Harrison et al 2015; Harrison et al 2018).
**Bioimpedance:**

Bioimpedance (BIA) has been applied in the human clinic and in the field of sports training for assessment of human muscles (Salinari et al 2002; Stahn et al 2008; Carter et al. 2009; Rebeyrol, 2010, Kim & Kim 2013; Nescolarde et al 2013). The method has the advantage of being non-invasive and easily transportable.

Multi-frequency BIA (mf-BIA), that is to say 256 measurements obtained over a frequency range of 4 to 1000 kHz, has now extended the application of BIA to include a much more in depth analysis of the muscle state in terms of training state, exhaustion, injury and metabolic state (Stahn et al 2008; Nescolarde et al 2013; Ward et al 2016) and mf-BIA has recently been shown to be useful in diagnosing muscle damage and atrophy in the equine Veterinary clinic (Riis et al 2013; Ward et al 2016).

One of the useful parameters in terms of myofascial tonus/stiffness, is the centre frequency ($f_c$), that as mentioned in the previous section, is the frequency needed to enable an alternating current to travel through a tissue of interest and to obtain the highest possible reactance ($X_c$) value. BIA parameters measured over the β-dispersion range, that is to say the range of frequency covering 10kHz–10MHz has revealed that the centre frequency ($f_c$) parameter can be used to reveal the effects of myofascial release treatment. Conversely, it has been found that compression of soft tissue has an adverse effect on the Cole-Cole plot in general and both the reactance and centre frequency parameters (Dodde et al 2012). In a study of the application of mf-BIA in equine muscle assessment (Harrison et al 2015) it was found that the $f_c$ at rest prior to myofascial release treatment was 60.9 kHz and that this value fell to 51.6 kHz an hour after treatment, falling again to 46.8 kHz at 24 h post treatment. This form of measurement in connection with myofascial release has been repeated on other horses, with similar highly significant results; 58% decrease in $f_c$ ($P=0.003$) over a 48 hour period post-treatment (Elbrønd et al 2015; Harrison et al 2015).

Thus mf-BIA offers a quick, non-invasive and pain free means of not only assessing muscle mass and health, but also a way of quantifying the beneficial effects of various forms of treatment, among them myofascial release therapy (Riis et al 2013; Elbrønd et al 2015; Harrison et al 2015).

**Biomechanics:**

Static assessment of animals in the Veterinary clinic is of course very important, but it is mostly when animals are physically active that problems become visible and empirically detectable. It is therefore essential that a dynamic means of assessing performance and the use of the locomotor system is available to Veterinarians.
For example, it is known that certain types of muscle contraction associated with specific gaits have the ability to store and convert energy (Alexander & Bennet-Clark, 1977). An extension of which, the “Spring-Mass” model, as proposed by Blickhan (1989) has led to a deeper understanding of the energetic aspects of walking, trotting and cantering.

When walking, the center of body mass of a dog, for example, is actively raised during the first half of the stance phase to a point above the limbs, and as it subsequently moves back downwards, potential energy is transformed into kinetic energy (Alexander, 1976). In a more recent study, Usherwood and colleagues (2007), showed that dogs, during a slow walk, have relatively stiff limbs, but that as they begin to walk at a faster pace, their limbs begin to increasingly impact with the ground and in doing so behave elastically.

In the trot, the body’s centre of mass behaves differently. Rather than being raised in the first half of the stance phase, it is lowered and the body moves as a wave, often with minimal oscillation in those breeds of dogs that are used to trotting for long periods of time (Jayes & Alexander, 1978). Here potential energy is converted into elastic energy and stored upon impact with the ground by passive stretching of tendons and muscles, to be released later on in the stance phase. The study undertaken by Gregersen and colleagues (1998), not only confirms this “Spring-Mass” model, it also reveals that the work performed by every joint of the fore- and hind-limbs is more efficient due to a reduced “cost” of physical activity resulting from the release of elastic energy – in the trot three quarters of the work performed by dogs is recoverable in this way (Gregersen et al 1998).

In recent years monitoring of biomechanics with the aid of various slow-motion cameras, sensory pads, force plates, accelerometers etc. has brought a great deal of understanding to the field of biomechanics, yet despite this, up until very recently it has still been impossible to accurately measure muscle function in real-time.

In recent years new technological developments have seen acoustic myography become a realistic and accurate means of assessing not only such parameters as spatial and temporal summation of individual muscles, but also the efficiency of their use (coordination) (Fenger & Harrison, 2017). It is known that contracting muscle fibres generate vibrations – this is seen most clearly when people shiver in the cold (Harrison et al, 1994). These vibrations produce pressure waves, which can be recorded at the level of the skin above a muscle of interest. For example, early AMG recordings were often obtained using piezoelectric microphones with an air cavity between the skin and the sensor (Barry et al, 1985). This new form of technology which is not only non-invasive but also easy and quick to use, has begun to reveal aspects about muscle function that have not been hitherto possible. In dogs, it has led to the confirmation and quantification of muscle function changes relating to elastic strain energy adaptation in muscles during a change of pace from walk to trot (Fenger & Harrison, 2017), supporting and documenting the “Spring Mass” model. Whilst in horses, it has revealed very slight imbalances in
muscle groups left-hand versus right-hand side, and quantified this imbalance during such gaits as walk, trot and canter (Jensen et al 2018). This recent research has shown how a slight imbalance during walking can become greater at the trot and even more prominent at the canter. It likewise helps to identify handedness, indicating whether an imbalance in muscle function becomes worse on a right-hand circle rather than a left-hand circle (Jensen et al 2018).

**Injury:**

It is known that sound horses measured using a pressure plate show a high degree of symmetry in terms of ground reaction force at the walk and trot (Oosterlinck et al 2013), presumably because of balanced and coordinated muscle contractions in their left and right limbs. Likewise, lameness in a horse can be measured through analysis of the kinetic gait with the aid of a static force plate (Ishihara et al 2009), although such diagnostic tests can prove challenging, time consuming, and expensive. It is not every Veterinary clinic that has access to a pressure plate system, despite frequent cases of recurring and shifting lameness. Indeed, just such a problem often faces sports teams, where monitoring of individuals health and fitness is vital, as is following their recovery once an injury has occurred (Nescolarde et al 2013). In a recent case study, Riis and colleagues published their findings from the application of new techniques in combination with regard to a 12-year-old Danish Warmblood that only 3 months after being purchased, presented with prolonged periods of lameness (Riis et al 2013). The authors report how acoustic myography, assessing both the amplitude and frequency of active muscles, was employed to locate a specific area of muscle injury (gluteal muscles - right hind leg), and that how the use of a small yet precise accelerometer attached to the horse just above the vertebral column at the level of the hips was able to reveal a normal step interval for the injured leg when compared with the contralateral, but a weaker acceleration and strike force. Finally, these authors performed a detailed assessment of muscle resistance (R) and reactance (Xc) using bioimpedance, confirming a regional loss of gluteal muscle mass and a loss of cellular integrity compared with the contralateral limb – findings that were comparable with a grade II muscle injury (Järvinen et al 2005; Nescolarde et al 2013). In another equine case, namely that of a 6-year-old Danish Warmblood, these techniques were used once again in combination to assess a horse with recurring and shifting lameness (Harrison et al 2018). An x-ray examination of the back region revealed “Kissing spine” lesions, also referred to as dorsal spinous process impingement (DSPI) or Basstrup’s disease. Acoustic myography measured a normal muscle signal for both regions T10/11 and L3-4, whilst for region T14/15, where the worst “Kissing spine” lesion was identified using x-ray, there were signs of highly elevated muscle contractions, serving to stabilize this painful region. The bioimpedance data showed that the impedance, resistance and reactance were lower on the left- cf the right-hand sides of the back, indicative of a reduced muscle mass, inflammation and swelling as well as
impaired cellular energy storage, respectively. The data collected using a small accelerometer detected a change in gait in this horse that was found to disappear after a local anaesthetic was applied to the region identified by x-ray as being the worst “Kissing spine” lesion (Harrison et al 2018). These authors concluded that acoustic myography, bioimpedance and accelerometry in combination may provide a useful set of diagnostic data that could potentially assist in not only rapidly determining the site and extent of muscular injury but quite possibly in directing treatment and rehabilitation in afflicted horses.

**Treatment:**

One thing is the ability to detect and quantify a muscle imbalance in an animal, another is the ability to correct it. Of interest in recent years has been the role of proprioception, that is to say the sense of the relative position of one’s own body parts and the physical effort used at rest and during movement (Lephart et al 1997). A study performed a few years ago by Clayton and colleagues revealed a new form of therapeutic treatment (proprioception) intended to activate the hind limbs of horses to a greater extent, thereby removing any imbalance during the engagement of these limbs (Clayton et al 2010). Using both proprioception and the new analytical tool of acoustic myography, it was very recently shown that not only could proprioception be used to correct slight muscular imbalances in equine hind limbs, confirming the earlier work of Clayton and colleagues (2010), but that this form of treatment could be illustrated and quantified (Jensen et al 2018). The authors concluded, that not only could functional musculoskeletal asymmetry be measured during periods of activity, and done so in an accurate manner using acoustic myography, but that there is a clear association between a program of proprioceptive training and an improvement in muscular imbalance (Jensen et al 2018). To this end, an earlier study has shown effects of light tactile stimulation on sensitive regions of the equine hindlimb, specifically the pastern and the coronet, which are thought to act via cutaneous mechanoreceptors (Sherrington, 1910; Clayton et al 2010). It is documented that adaptations occur in muscles and joints as the result of disturbances induced by training itself (Gruber et al 2006). Moreover, perturbation training in human subjects has revealed alterations in muscle co-contraction and increased peak flexion of joints (Chmielewski et al 2005). These changes are thus most likely grounded in kinematic alterations in response to the training protocol adopted in this study and the improved hind limb balance, observations reported first by Dr Vladimir Janda exploring sensorimotor training in connection with patterns of muscle imbalance (Page, 2006). Indeed, it was Dr Janda who was the first to propose proprioception as part of the rehabilitation process, with implications for equine locomotor pathology, using sensorimotor training to emphasize correct postural control and gradually restore normal motor programs in subjects (Schwanda & Peham, 2013).
Another form of treatment increasingly being adopted by Veterinarians includes acupuncture. Acupuncture (ACU) is part of Traditional Chinese Medicine and involves stimulating points on the body surface with acupuncture needles. The effects of ACU cannot be explained in terms of a single mechanism, but involves a series of interactions between the nervous, endocrine, and the immune systems. Mechanisms of action are still not fully understood but studies have shown that needle stimulation of acupuncture points causes microtrauma that produces a local inflammatory reaction, which results in an increased local tissue immune response, improved local tissue blood flow, and muscle relaxation (Dunkel et al 2017). Moreover, studies in humans, have suggested that ACU might stimulate motor nerve fibers, and as such, increase muscle activity (Chou et al. 2009; Dar & Hicks, 2016). Recently, a trial at UC Davis, coordinated by Le Jeune and colleagues (Millares et al 2017), applied acupuncture to five healthy sport horses. Acoustic myography recordings were taken while the horses were walked and trotted by hand, and it was found that not only did all the horses tolerate the procedures well, but that a) sensitivity at ACU points was present initially in 3 horses and was abolished immediately after ACU in all of them, and b) all horses had an increased Efficiency, Spatial, and Temporal score (ESTi-score) after 48 hours, of which 3 had a persistent increase for 1 week afterwards, suggesting a greater speed of activation of muscle fibers after ACU. Whilst these data are from a trial and there is now need for a more detailed study with a larger sample size, the findings of Millares and colleagues (2017) suggest that not only was acoustic myography a non-invasive technique, which was well tolerated and easily performed in a routine clinical setting, but more importantly that a change in acoustic myographic data appears to occur after ACU treatment (Millares et al 2017).

Concluding remarks:

This review has revealed how new advances in the fields of dissection and functional anatomy, and the positive potential of new technological developments such as non-invasive measurements of myo-fascial function using a combination of acoustic myography, accelerometry and bioimpedance can provide a useful set of diagnostic tools for rapidly determining myo-fascial issues and injuries in the modern Equine Veterinary clinic. It also serves to make the reader aware of new and emerging forms of treatment, such as myofascial release therapy, acupuncture and proprioception.

Acknowledgements:

The authors wish to thank their collaborators in these many diverse projects, as well as the horse and dog owners for their kind assistance.


References:


