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Surface Electromyography During Both Standing and Walking in *m. Ulnaris lateralis* of Diversely Trained Horses

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1. Introduction

The primary aim of any conditioning program in athletic horses is to improve performance by inducing physiological changes within the animal's body, including limb muscles and tendons (Rivero, 2007). To this end, electromyography (EMG) has been used in human studies to determine muscle fitness and general muscle strength, but it also serves as an indicator of muscle injury and/or disease (Licka et al., 2009; Heaf et al., 2010).

A good many studies have relied on needle electromyography (Tokuriki et al., 1989; Wijnberg et al., 2003a; Winjberg et al., 2003b), which in many ways is a precise technique, giving access to information about individual motor units within muscles, but this technique does not lend itself readily to muscles during periods of rapid and forceful contraction. Under such situations surface electromyography (sEMG) is preferred and in the equine, studies of primarily the back muscles have been undertaken (Licka et al., 2001; Licka et al., 2004; Peham et al. 2001), although sEMG has been used in ponies to identify forelimb muscle function (Jansen et al., 1992).

Being a rapid, painless and non-invasive method of measuring muscle activity, surface electromyography (sEMG) is potentially useful in equine athletes. For horses competing in sports events, it is essential that their limbs remain healthy and sound. Indeed, lameness and training induced injuries of the forelimb have become increasingly more common in competition horses (Kohnke, 2004; Firth, 2006), mainly as a result of a raised athletic level and increasing numbers of horses participating in competitions. It is not surprising therefore that sEMG has already begun to be used to supplement clinical findings in injured horses, and is very often explored as an informative technique when other means of clinical examination fail (Wijnberg et al., 2004).

Perhaps of greater importance, however, is the very real potential to use this technique as a measure of muscle efficiency during locomotion/exercise and in so doing, provide an insight into a horses muscle fitness and health at any given point in time, as well as enable a rider or owner to follow an individual animals response to training. sEMG reflects the force and degree of activity/coordination of a muscle during a gait cycle, revealing bursts of activity when a muscle is active (Robert et al., 1999; Butcher et al., 2009). Yet, despite this

very real potential to assist with improving and monitoring training programs, very few data exist with regard to horses, whether exercised or not. The purpose of this study was, therefore, to validate sEMG recordings on worked and non-worked animals, and to generate reference data for further use in equine training studies. The underlying hypotheses in this study were; 1) muscle efficiency, as measured using sEMG signal analysis, is greater in worked than non-worked horses, 2) increasing age and body weight reduces muscle force and efficiency, and 3) injured horses present with a sEMG signal that is very different from that of un-injured horses.

2. Materials and methods

2.1 Animals

This study recruited 18 horses of different breed, age and training level, the later being established from FEI competition tests, as VO_{2max} could not be measured. Horses were weighed on a large animal scale where possible, whilst others were assessed using a measuring band placed around their girth (see Table 1). Of the 18 horses, numbers 1-6 were considered to be “non-worked” (NW), and these were stabled at the Animal Hospital facility as teaching horses for veterinarians (Department of Large Animal Sciences, Medicine & Surgery, Taastrup, Faculty of Life Sciences, Copenhagen University).

Horse No.	Exercise/training level	Name	Age (years)	Body weight (kg)	Height (cm)	Body Mass (kg/m ²)
1	NW	Bella	9	558	155	248.0
2	NW	Heksehyl	6	635	160	282.2
3	NW	Nathalie	7	625	160	277.8
4	NW	Mary	7	655	155	291.1
5	NW	Ingrid	9	473	150	210.2
6	NW	Benedikte	7	550	150	244.4
7	W	Akina	6	470	164	174.7
8	W	Telle	8	592	180	182.7
9	W	Zozet	13	552	166	200.3
10	W	Madonna	20	550	167	197.2
11	W	Kento	8	498	166	180.7
12	W	Kojak	26	470	141	236.4
13	HW	Camixa	6	511	177	163.1
14	HW	Laval	13	504	170	174.4
15	HW	Akeem	14	596	171	203.8
16	HW	Gallion	16	524	165	192.5
17	HW	Lambourghini	14	478	168	169.4
18	HW	Maxi	7	448	148	204.5

Table 1. Horse data including an estimate of training/exercise level and body mass (BM). NW = non worked, W = worked to a low level, and HW = hard worked e.g. Grand-Prix standard.

Horses numbers 7-12 were measured at a private stable (Tune, Roskilde) and were fed according to their level of exercise and training, which was deemed to be “worked” (W). These horses were sent out to pasture every day and trained/exercised on a daily basis. Horses numbers 13-18 were measured at another private stable (Kokkedal), and were provided with feed appropriate to their level of work, which was assessed as being “hard worked” (HW). These were horses that were typically used for competition (primarily dressage competitions at a National level) and therefore used to travelling.

2.2 Procedures

Horses were shaved in the region of the muscle *Ulnaris lateralis* (UL) and sEMG recording electrodes were applied. A ground or reference electrode was applied to the *medial styloid process* on the tip of the radius bone. Self-sticking (Co-plus) bandaged was wrapped around the leg in the region of the electrodes in order to prevent electrodes from being dislodged.

2.2.1 sEMG measurements

This study followed the guidelines laid out in the *European Recommendations for Surface ElectroMyoGraphy* as detailed by the SENIAM project (Hermens et al., 1999). The recorded surface EMG signal was assessed as described previously in terms of signal frequency (Hz), peak-to-peak amplitude (mV), mean amplitude frequency (mVs⁻¹) and the maximum power (mV²) using Chart analysis software (AD Instruments, Chalgrove, Oxfordshire, UK).

A double differential electrode configuration, with electrodes (N-00-S & R-00-S; Blue Sensor, Medicotest A/S, Ølstykke, Denmark) was adopted as described previously (Heaf et al., 2010; Andersen et al., 2008). sEMG recordings were taken *via* an ML 132 amplifier connected to a ML780 PowerLab/8s A/D converter (ADInstruments, Chalgrove, Oxfordshire, UK) with a further connection to a MacBook Air with Chart v. 5.5.6/s Software, Peak Parameters and Spike Histogram extensions. Input impedance was 200 MΩ differential, and a high and a low pass filter of 3 Hz and 500 Hz, respectively, were used. Sampling speed was set to 100,000 per second.

Background noise from the recording environment in which the horses were measured was checked to ensure an adequate signal was obtainable. Prior to data recording for standing and walking, the background noise was evaluated for each horse, however, none of the stable environments exhibited unduly high levels of background noise or electrical interference.

Measurements, taken during standing, were recorded for about a minute, after which the horse was walked (5-8 strides) to obtain a stable recording of ambulatory activity in the UL muscle. Horses were usually walked twice so as to obtain a more representative measurement. Leg posture was examined and noted as well as photographed. All horses except one were found to be sound and had no history of leg illnesses that we were informed of. One lame horse (no. 16) was measured on both forelimbs, since it had suffered an injury to the right forelimb at the region of the proximal phalanx. In this particular animal, the UL appeared to the naked eye to be enlarged (swollen/inflamed) in the right- *cf* the left- forelimb. In the case of horse No. 16, a recording of the superficial digital flexor tendon was made on both fore limbs in conjunction with a simultaneous recording from UL. The superficial digital flexor tendon recording was in the form of a movement artefact, representing a change in the position and thickness of the tendon relative to the fixed recording electrodes on the skin, during a period of muscle contraction.

2.3 Statistical analysis

Data are presented as mean ± SEM. Differences between means were tested for statistical significance with the use of GraphPad Instat 3 for Mac (Version 3.0b, 2003; GraphPad Inc., La Jolla, CA), with an additional test for Gaussian normal distribution. A one-way ANOVA was used to measure statistical differences between the means for all of the horse groups. Differences between means showing a *P* value >0.05 were considered non-significant.

3. Results

Ulnaris lateralis is a lateral palmar forelimb muscle connected to both the accessory carpal bone and the fourth metatarsal bone, and to the lateral humeral epicondyle. It is a muscle that is mainly comprised short fibers and is strong and stiff, with a peak isometric force of $F_{max} = 5731\text{ N}$, serving to stabilize the carpus during the stance phase of gait (Brown et al., 2003). In Figure 1 (see below) individual sEMG amplitudes and frequencies are presented for UL of the mean of the group.

Fig. 1. (Continued)

Fig. 1. A: Individual sEMG signal amplitude values for horses in this study during periods of both standing and walking compared to the mean value of the whole group. B: Individual sEMG signal frequency values for horses in this study during periods of both standing and walking compared to the mean value of the whole group. Worked (W), hard worked (HW) and non-worked (NW) horses.

3.1 sEMG amplitude data

The standing mean amplitude for the sEMG of UL was measured to be 0.17 ± 0.03 mV for HW horses, 0.09 ± 0.01 mV for W horses and just 0.07 ± 0.01 mV for the NW horses. The sEMG signal amplitude during walking was found to be more than three-fold that of the standing data with 0.50 ± 0.09 mV for HW horses, 0.33 ± 0.05 mV for W horses, and 0.27 ± 0.06 mV for NW horses (see Figure 2 below).

Fig. 2. Mean sEMG signal frequency (Hz) and amplitude (mV) values for *Ulnaris lateralis* during periods of standing and walking for Worked (W), hard worked (HW) and non-worked (NW) horses. Note that an increase in the level of training/exercise results in an improved degree of coordination such that the CMAP frequency falls and the amplitude rises – indicative of fast, coordinated recruitment of large MU’s.

sEMG signal amplitude varied between individual horses, and in particular among highly trained horses, which were above the mean, and where a greater individual difference between horses in terms of the degree of training/exercise was noted.

3.2 sEMG frequency data

A mean sEMG signal frequency for all the horses during standing and walking revealed values of 57.89 ± 11.83 Hz and 114.74 ± 24.78 Hz, respectively.

The mean frequency difference between standing and walking for HW horses was 39.79 Hz, whilst for W horses this was 45.69 Hz. NW horses were found to have twice the mean difference e.g. 89.43 Hz (see Figure 2). Indeed, there was a significant difference between NW and HW horses ($P < 0.01$), and between W and HW horses ($P < 0.05$) in terms of the sEMG signal frequency. Moreover, a positive correlation ($R^2 = 0.722$) was found between sEMG signal frequency and body weight of the W horses.

3.3 Leg posture/ lameness

A couple of horses (No.'s 2 & 16) were found to have an unusual leg posture, whilst one horse had been lame for some time. Indeed, these horses were recorded as having a very different sEMG signal from that of the healthy horses, and as a result they were excluded from most of the calculations. Horse no. 2 presented with a leg posture for the forelimbs in which it distributed its body weight to the leading edge of the hoof giving it a constant forward angle of both carpus. This resulted in a sEMG signal amplitude of 0.66 mV whereas the mean for the non-worked group was 0.07 mV during standing.

Horse no. 16 had been diagnosed lame due to right front fetlock osteoarthritis but it was still being ridden with care. Likewise, this horse was found to differ in its sEMG signal from that of the other horses in the HW group in terms of both amplitude and frequency. Furthermore, this particular horse presented with a left forelimb that performed during walking as per a normal HW horse in terms of its sEMG signal, whereas the right forelimb (injured leg) yielded a sEMG signal with a higher CMAP frequency, more typical of that for a W or NW horse; left forelimb 90 Hz & 0.39 mV *cf* right forelimb 122 Hz & 0.30 mV.

4. Discussion

This study, which endorses the sensitivity of sEMG as a non-invasive measure of muscular function in horses, confirms that muscle efficiency (short coordinated bursts), as measured using sEMG signal analysis, is greater in worked than non-worked horses. Likewise, this study has revealed that injured horses present with a sEMG signal that is very different from that of un-injured horses.

4.1 Exercise and the sEMG signal

Adaptation to physical training in both human and equine studies, has documented improvements in both neural activity patterns as well as fibre changes (Felici, 2006). In a recent study it was shown that exercise positively influences hypertrophy and neuromuscular excitability in humans and horses (Mileva et al., 2010; Wijnberg et al., 2008). Indeed, both of these authors found an increase in amplitude with exercise, a finding that is in agreement with the present study (see Figure 2). Wijnberg and colleagues (Wijnberg et al., 2008) found, for the horses that they tested, an amplitude of 0.23 mV in *m. vastus lateralis* at rest (standing), a value that is very comparable with that of the HW horses in this study taken at rest/standing (mean amplitude of 0.17 mV in UL).

The short yet coordinated bursts of sEMG signal activity in the HW horses in the present study reflects a training induced synchronization of the individual compound muscle action potentials (CMAPs) within the UL muscle measured (Mileva et al., 2010; Wijnberg et al., 2008).

Indeed, CMAP synchronization has been cited as a means of increasing muscle strength or power (Leisson et al., 2008). To which end, the trained horses in the present study were found to have a much higher power (0.75 mVs^{-1}) than non-trained horses (0.3 mVs^{-1}). sEMG signal frequency was also affected by training, such that HW horses had a lower frequency than NW horses ($P < 0.01$). This is not surprising in many ways, as muscle efficiency is very dependent on motor unit (MU) firing rate, with efficient muscles using a lower frequency (Hz) range when recruiting MU's in a highly synchronized fashion, thereby producing a greater force (Winjberg et al., 2003b). Indeed the findings of the present study suggest that training/exercise induces changes in the sEMG that result in the very fast and coordinated recruitment and firing of large motor units. This study has also found differences in the pattern of the sEMG signal over a given period of time, with respect to the level of training/exercise (see Figure 3). The horses assigned to the NW group were typically found to present with a sEMG signal in which short

Fig. 3. (Continued)

Fig. 3. Raw sEMG data for one horse in each of the three training/exercise groups; Worked (W), hard worked (HW) and non-worked (NW) horses. Data have been taken for the largest positive spike when walking. Note the difference in sEMG signal with training/exercise level of the horses - HW horses show a more controlled and immediate burst of activity of the other groups where the sEMG signal is slower to reach a peak and of a longer duration. Scaling differs for mV on the Y-axis and for seconds on the X-axis between panels.

intermittent bursts of contraction were found to give rise to a constant (low-level since weak amplitude) activation of the UL muscle - something that can be considered an inefficient use of this particular muscles energy. The W horses on the other hand showed long bursts of activity of the sEMG signal followed by periods of inactivity giving rise to periods of contraction and relaxation. Finally, the HW horses typically showed a sEMG pattern comprising very short and coordinated bursts of activity with long periods of inactivity (relaxation) and a considerable signal amplitude; 1.4 mV of 0.9 and 0.5 mV of the W and NW horses, respectively.

4.2 Injury and the sEMG signal

The analysis of the sEMG data collected in the present study, reveals a close similarity of values between individual horses when standing. It is really only when horses walk that differences in sEMG amplitude and frequency become more obvious.

Ulnaris lateralis has a pennation angle of 10-56°, and it is this relatively small pennation angle that gives this muscle its stiffness, contributing considerably to the stability of the carpus joint rather than producing active force as for example *Gluteus medius*. These findings along with previously published electromyography recordings indicate that *ulnaris lateralis* actively prepares for ground contact in the stance phase (Jansen et al., 1992; Brown et al., 2003). Moreover, this muscle has been found to differ between breeds, since thoroughbred horses have a greater muscle thickness than elite standard-bred horses; 70 mm versus 61 mm, respectively (Kearns et al., 2002).

It is of interest therefore that the sEMG signal analyses in this study have been able to determine significant changes in UL of horses presenting with signs of misuse or injury to their forelimbs. Horse no. 16 presented with a clear difference in forelimb muscle fitness,

with the sEMG signal analysis of its own counterpart forelimb serving as a healthy control (see Figure 4). These findings are important for the future use of sEMG as a research tool in the assessment of muscle health. Injuries, like those of horse no. 16, in which the UL of the right forelimb had a higher firing frequency than that of the left forelimb are commensurate with its role in providing stability to the carpus joint, especially when such a joint is deficient in cartilage, and such changes can be revealed without any invasive and perhaps more damaging procedures or techniques. Indeed, if caught early enough a full and speedy recovery of this forelimb joint may very well be achievable, perhaps through the aid of orthopaedic shoes or either use of IRAP (Interleukin-1 Receptor Antagonist Protein), or PRP (Platelet Rich Plasma).

Fig. 4. Photograph of horse no. 16 showing the placement of recording electrodes on the forelimbs – both the healthy and the injured leg. Note too that electrodes were placed over the superficial digital flexor tendon on both legs, and recordings of muscle:tendon (*m. Ulnaris lateralis* vs. Superficial digital flexor) activity were taken simultaneously.

4.3 sEMG reference data

One could imagine the use of sEMG on horses suspected of having various muscle diseases in order that injuries might be caught before they become lethal, and here it is imperative

that we have a clear working knowledge of normal and healthy sEMG signals for an array of diverse skeletal muscles so as to rule out any misinterpretation or misdiagnosis. To date there exists a very limited array of data concerning normal equine muscle sEMG paramaters (see Table 2). For instance, Wijnberg and colleagues (Wijnberg et al., 2004) found that myopathogenic muscles have a frequency of <150 Hz, which is far from informative, especially since all the healthy horses with normal muscle function, as defined by the present study, exhibit a frequency below 150 Hz. There is therefore clearly a great need of a reference set of data combining sEMG signal amplitude and frequency along with the horses full anatomical and medical history and work program if the true potential of this technique is to be realized.

EMG data	Muscle	Standing Frequency (Hz)	Walking Frequency (Hz)	Standing Amplitude (mV)	Walking Amplitude (mV)	Reference
Normal NW	Ulnaris lateralis	50	140	0.07	0.27	This study
Normal NW	Hind leg			<0.02		Schuurman <i>et al</i> 2003
Normal W	Ulnaris lateralis	75	120	0.09	0.33	This study
Normal HW	Ulnaris lateralis	45	85	0.17	0.5	This study
Normal HW	Hind leg				0.19-0.23	Wijnberg <i>et al</i> 2008
Joint Arthritis	Ulnaris lateralis	88	122	0.14	0.3	This study
Myopathogenic muscle	Subclavian	<150		0.28		Wijnberg <i>et al</i> 2004
Neuropathic muscle	Subclavian	>150		0.36		Wijnberg <i>et al</i> 2004
Uneven standing	Ulnaris lateralis	38		0.66		This study
Fore Head	Temporalis	19-20		0.06		Kruljc & Nemec 2006
Neck NW	Splenius			0.04-0.34		Giovangnoli <i>et al</i> 2002
Back NW	Longissimus dorsi				0.07-0.11	Licka <i>et al</i> 2009

Table 2. Comparative sEMG data for equine muscles during periods of standing and walking.

4.4 sEMG to tendon artefact

An sEMG signal can be altered/affected in many ways by what have been defined as causative, intermediate and deterministic factors (De Luca, 1997). For a more detailed explanation see the Chapter by Harrison and colleagues in this edition. The causative factors include such parameters as skin thickness and subcutaneous tissue composition

and depth, blood flow etc. Thus, by placing a pair of recording electrodes over a tissue that not only moves during a period of contraction, but also changes its thickness and potentially the flow of blood supplying it, one is able to record an artefact. Just such an artefact has been recorded in this study, through the placement of two recording electrodes over the Superficial digital flexor tendon of the forelimb of horse no. 16. In the resting state (standing) the measurement obtained from the two recording electrodes remained very stable, but as the Superficial digital flexor tendon moved relative to the electrodes on the skin's surface above the tendon (walking), an artefact was recorded – seen as a slight and transient deflection in the recorded signal (see Figure 5). In the case of horse no. 16, it was noted that the peak of this tendon artefact was significantly different in the healthy *cf.* the injured forelimb, such that the limb that had been considerably injured showed a very small time difference (δ - mSec) between the start of the sEMG signal in the *m. Ulnaris lateralis* and the peak of the tendon artefact recorded above the Superficial digital flexor tendon.

In the case of horse no. 16, the healthy left forelimb showed a greater δ compared with the injured right forelimb; 106 mSec *vs.* 49 mSec, respectively (see Figure 5). The greater the time lag between the start of the sEMG signal and the peak of the tendon artefact must be considered as indicative of a greater degree of elasticity in the muscle:tendon complex. Thus, with injury and inflammation one can expect the δ value for the muscle:tendon measurement to be reduced. Indeed, palpation of the two forelimbs gave just such an impression, and the fact that the δ for the injured forelimb was 50% that of the healthy forelimb indicates that in this forelimb very little elasticity remains in the muscle and tendon complex.

Should this condition persist, one might anticipate inflammation and damage of the tendon to bone interface and a permanent change in the tendon structure, towards one that is relatively less- to non-elastic and painful. In support of which the theory of microtrauma proposes that overuse and inflammation gives rise to joint damage e.g. tennis elbow (Roetert et al., 1995). The theory proposes that if a muscle is already at near maximum contraction, vibrations and twisting movements may be transferred directly through the muscle (muscle stiffness at this point being considerable) to the tendinous insertion, causing repeated microtrauma (Roetert et al., 1995). Clearly, there is a need for more detailed studies into muscle:tendon interactions and the incidence of tendon damage. However, studies involving rats have shown that early immobilization diminishes macrophage accumulation and leads to improved tendon-bone healing (Dagher et al., 2009), whilst untreated swollen tendons give rise to localized calcification (Ring et al., 1994).

5. Conclusion

sEMG lends itself to the detection of muscular differences in the forelimb muscle UL of diversely trained horses. Moreover, such information can be used by the owner/rider to help assist in improving and monitoring training programs. There is also a clear benefit of using this technique in the early detection of muscular/leg damage and injury. Indeed, one could envisage the use of sEMG in the assessment of diverse riding styles e.g. “low, deep and round” or “rollkur” particularly since the latter has been suggested as increasing the strain on the neck and back muscles of competing horses. Clearly, more research is now needed in this area to further validate the sEMG method in terms of

individual muscles, and to provide a bio-bank of data to assist in training and post injury programs.

Fig. 5. The raw sEMG signal of *m. Ulnaris lateralis* in relation to the artefact signal from the Superficial digital flexor tendon. Note the significantly shorter time (δ - mSec) for the right injured leg *cf.* that for the left healthy leg. The tendon artefact represents the movement of tissue (tendon) relative to the recording electrodes during a period of muscle contraction and leg movement.

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