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ORIGINAL ARTICLE

The use of electromyography interference pattern analysis to determine muscle force of the deep digital flexor muscle in healthy and laminitic horses

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ABSTRACT

Background: In equine laminitis, the deep digital flexor muscle (DDFM) appears to have increased muscle force, but evidence-based confirmation is lacking.
Objectives: The purpose of this study was to test if the DDFM of laminitic equines has an increased muscle force detectable by needle electromyography interference pattern analysis (IPA).
Animals and Methods: The control group included six Royal Dutch Sport horses, three Shetland ponies and one Welsh pony [10 healthy, sound adults weighing 411 ± 217 kg (mean ± SD) and aged 10 ± 5 years]. The laminitic group included three Royal Dutch Sport horses, one Friesian, one Haflinger, one Icelandic horse, one Welsh pony, one miniature Appaloosa and six Shetland ponies (14 adults, weight 310 ± 178 kg, aged 13 ± 6 years) with acute/chronic laminitis. The electromyography IPA measurements included firing rate, turns/second (T), amplitude/turn (M) and M/T ratio. Statistical analysis used a general linear model with outcomes transformed to geometric means.
Results: The firing rate of the total laminitic group was higher than the total control group. This difference was smaller for the ponies compared to the horses; in the horses, the geometric mean difference of the laminitic group was 1.73 [geometric 95% confidence interval (CI) 1.29–2.32], and in the ponies this value was 1.09 (geometric 95% CI 0.82–1.45).
Conclusion and clinical relevance: In human medicine, an increased firing rate is characteristic of increased muscle force. Thus, the increased firing rate of the DDFM in the context of laminitis suggests an elevated muscle force. However, this seems to be only a partial effect as in this study, the unchanged turns/second and amplitude/turn failed to prove the recruitment of larger motor units with larger amplitude motor unit potentials in laminitic equids.

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Horse; equine; deep digital flexor muscle; electromyography; laminitis

1. Introduction

Laminitis, which causes the lamellae of the hoof to become inflamed and weakened, has a severe impact on horse welfare. The traction of the deep digital flexor (DDF) muscle–tendon unit on the distal phalanx (Ph3) might cause it to rotate because there is no opposition because of lamellar failure (Morrison 2004). The traction of the DDF on Ph3 in a founder is thought to be very painful as the descended phalanx places pressure on the sole (Politt 1995a). To reduce this traction on Ph3, a tenotomy of the DDF can be performed (Cripps and Eustace 1999; Carter and Ben Renfroe 2009; Eastman 2010; Morrison 2011). However, Morisson (2011) described the outcome of 245 cases of DDF tenotomy and found a 51% success rate. Among the successful cases, only 13% returned to some form of athletic soundness (Morrison 2011). Some clinicians, researchers and horse owners have suggested that increased muscle tone or even contracture of the DDF muscle arises from laminitis. This symptom would be most obvious at a chronic stage of the disease when the pain induces contracture of the DDF muscle (Pollitt 1995b; Parks 2003). However, to our knowledge, this increased muscle tone has never been proved by evidence-based research.

Clostridium botulinum toxin type A has been used as a therapeutic agent in human medicine for several years to treat muscle over-activity, among other disorders. In addition, *C. botulinum* toxin has been successfully used to reduce the muscle tone of several equine skeletal muscles (Adam-Castrillo et al. 2004; Wijnberg et al. 2009). *Clostridium botulinum* toxin type A injections into the DDF muscle of healthy, sound horses significantly reduced muscle activity without inducing lameness (Hardeman et al. 2013; Wijnberg et al. 2013). The hypothesis is that laminitis weakens the lamellae due to inflammation and the tension of the DDF on Ph3 displaces it. Relieving the pull of the DDF might

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give results similar to a tenotomy, but the *C. botulinum* toxin type A action would only last for 3 or 4 months and would not damage the DDF tendon (Ney & Joseph 2007; Wijnberg et al. 2009). If this study supports the hypothesis that laminitis causes the DDF muscle to have increased muscle force, then treating equine laminitis with *C. botulinum* toxin type A injections into the DDF muscle could be substantiated.

Electromyography (EMG) has been successfully used in horses for over a decade (Wijnberg et al. 2003; Wijnberg et al. 2004; Westermann et al. 2007). However, the use of electromyography interference pattern analysis (IPA) in the horse is nascent (Wijnberg et al. 2011; Jose-Cunilleras & Wijnberg 2015). In human medicine, IPA has been an important diagnostic tool for several years (Fuglsang-Frederiksen 2000; Finsterer 2001). The interference pattern is composed of motor unit potentials representing the electric activity of the motor units, and it is based on the recruitment pattern of the potentials (Kimura 2001). The interference pattern can be analysed subjectively or objectively by using quantitative computer-based methods like turn/amplitude analyses (TAA) which measures turns/second (T) and amplitude/turn (M) and permits calculating the M/T ratio. Amplitude can be described as the mean voltage difference between turns, while a turn is a change in polarity occurring at each positive or negative peak (Sanders et al. 1996; Farrugia & Kennett 2005). Several IPA parameters are influenced by muscle force (Sanders et al. 1996; Fuglsang-Frederiksen 2000; Finsterer 2001). If force increases, the first motor unit to be activated is a small one with a low frequency. If force increases, the frequency of the small motor unit increases and once it hits a certain frequency, a larger motor unit will be activated (Sanders et al. 1996). With additional force enlargement, both motor units increase their firing rate and another motor unit becomes activated (Sanders et al. 1996). The activation of motor units is based upon a 'size principle' described by Henneman in 1965: the motor units that are recruited first are small and fatigue resistant (type I muscle fibre), while the motor units activated later are larger and can fatigue earlier (type II muscle fibre) (Henneman & Olson 1965; Sanders et al. 1996; Kimura 2001). Thus, the firing rate will increase as the force becomes greater. Moreover, if the force becomes greater, several mechanisms cause the interference pattern amplitude to increase. Specifically, there is a larger chance of recruiting a motor unit close to the electrode, and larger motor units with larger territories and muscle fibre diameter (type II muscle fibres) are recruited later when force increases. In addition, there is more summation of motor unit potentials (Sanders et al. 1996; Finsterer 2001; Farrugia & Kennett 2005). The number of turns increases as force grows because the number of motor unit discharges becomes higher. The number of turns increases until approximately 50% of maximum force and levels off thereafter due to summation of motor unit potentials (Christensen et al. 1984; Sanders et al. 1996).

The purpose of this study was to test if the DDF muscle shows an increased muscle force in horses with laminitis that is detectable by needle electromyography IPA.

2. Materials and methods

2.1. Horses

The control group included six Royal Dutch Sport horses, three Shetland ponies and one Welsh pony [adults weighing 411 ± 217 kg (mean ± SD) and aged 10 ± 5 years]. All horses were found to be clinically healthy and sound upon orthopaedic examination. The study was approved by the Animal Welfare Committee, Utrecht University (approval number 2008.III.07.061 and 2013.III.01.012).

The laminitic group included three Royal Dutch Sport horses, one Friesian, one Haflinger, one Icelandic horse, one Welsh pony, one miniature Appaloosa and six Shetland ponies (adults weighing 310 ± 178 kg and aged 13 ± 6 years) with acute or chronic laminitis. All horses were privately owned, and owners provided written consent. To enable distinguishing the different severities of laminitis, all horses and ponies underwent a clinical examination that included orthopaedic examination before electromyography. Patients in this study had laminitis with some degree of characteristic lameness, and symptoms included strong digital pulses, toe relieving stance and weight-shifting; some cases were complicated by acute/chronic founder or sinker (Eustace 2010). Patients were classified on a four-point scale as described by Obel (1948) and Dutton et al. (2009). Some horses or ponies of the laminitic group were receiving medication (different dosages, mostly based on severity of the laminitis as judged by the owner or owner's veterinarian): six were receiving non-steroidal anti-inflammatory drugs (NSAIDs; meloxicam or fenylbutazone), three were receiving anticoagulants (salicylic acid-acetate), one of the three was also receiving acepromazin and tramadol.

2.2. Preparations for EMG recordings

In preparation for EMG recording, the caudal part of the left forelimb between the olecranon and the carpal joint was shaved and cleaned on each horse or pony. Measurements were performed at three different sites in the widest part of the DDF muscle belly. To determine the sites, the distance between the top of the olecranon and the os carpi accessorium was measured using a tape measure. Then, data from an earlier study

Table 1. M. flexor digitorum profundus insertion sites based on data from an earlier study on cadaveric limbs.

Distance top of the olecranon – os carpi accessorium (cm)	Insertion sites: distances from os carpi accessorium (cm)		
	1	2	3
20	7.3	8.5	9.8
21	7.6	8.9	10.2
22	8.0	9.4	10.7
23	8.3	9.8	11.2
24	8.7	10.2	11.7
25	9.1	10.6	12.2
26	9.4	11.1	12.7
27	9.8	11.5	13.2
28	10.2	11.9	13.7
29	10.5	12.3	14.1
30	10.9	12.8	14.6
31	11.2	13.2	15.1
32	11.6	13.6	15.6
33	12.0	14.0	16.1
34	12.3	14.5	16.6
35	12.7	14.9	17.1
36	13.1	15.3	17.6
37	13.4	15.7	18.0
38	13.8	16.2	18.5
39	14.1	16.6	19.0
40	14.5	17.0	19.5
41	14.9	17.4	20.0
42	15.2	17.9	20.5
43	15.6	18.3	21.0
44	16.0	18.7	21.5
45	16.3	19.1	22.0
46	16.7	19.6	22.4
47	17.0	20.0	22.9
48	17.4	20.4	23.4
49	17.8	20.8	23.9
50	18.1	21.3	24.4

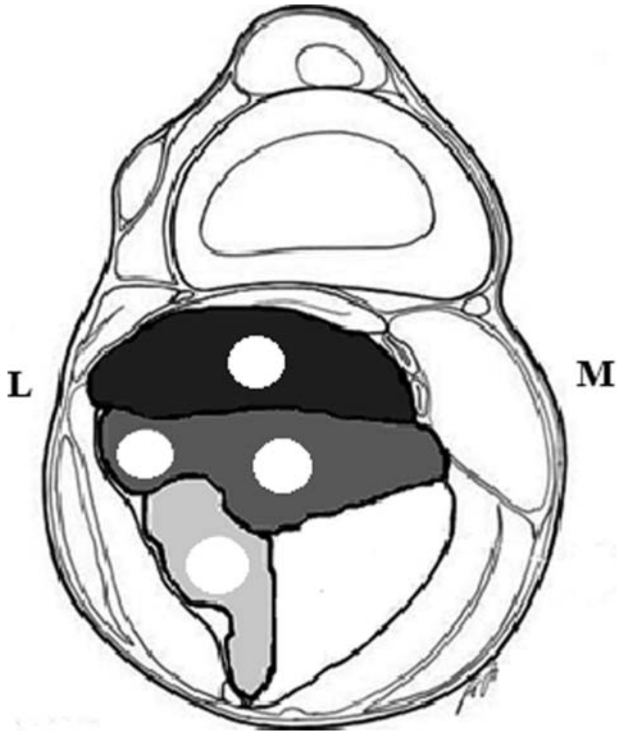

Figure 1. Different depths of the deep digital flexor muscle used for electromyography recordings (white points). Black shaded = deep part of deep digital flexor muscle. Dark-grey shaded = middle part of the deep digital flexor muscle. Light-grey shaded = superficial part of the deep digital flexor muscle. M, medial aspect of forelimb; L, lateral aspect of forelimb. Adapted from Zarucco et al. 2004 with permission of Am J Vet Res.

on cadaveric limbs were used to determine the specific sites in the widest part of the muscle belly (Table 1). The skin and subcutis were anaesthetised at each with an injection of 10 mg of lidocaine hydrochloride with 5 µg of adrenaline (Alfacaine 2% + adrenaline, Alfasan, Woerden, the Netherlands). Afterwards, the limb was disinfected using 70% alcohol. As previously mentioned, some laminitic patients were receiving NSAIDS or anticoagulants to treat laminitis. All control horses and all laminitic patients who were not already receiving NSAIDs, were injected with 1 mg/kg BW flunixin meglumine (Bedozane, Eurovet Animal Health, Bladel, the Netherlands) intravenously. The NSAIDS and lidocaine were administered in order to allow for the deep intramuscular insertions. After EMG measurements, the forelimb was cooled using cold water for 10 min.

2.3. EMG recordings

A portable electromyography apparatus (Viking Quest EMG system, Nicolet Biomedical, Fitchburg, Wisconsin USA) and a 23 G concentric needle (length 75 mm; diameter 0.60 mm; sampling area 0.068 mm^2) (MedCat, Erica, the Netherlands) was used to record electromyographic signals. The ground electrode consisted of a surgical pad attached to the horse with a girdle and connected to the preamplifier.

The electromyography needle was inserted and measurements were performed at three different depths of the DDF muscle as shown in Figure 1. At the middle insertion site, a fourth measurement in the middle part of the DDF muscle was performed. The needle was inserted in the groove between the Musculus flexor carpi ulnaris and the Musculus flexor digitorum superficialis.

For the analysis of the firing rate, the sensitivity was set at 200 μV. Measurements were performed at three sites in the DDF muscle at three or four different depths as already described. At all measurement points, three recordings with an epoch length of 100 ms were performed, and firing rates per epoch (Hz) were manually recorded. The threshold level was set beneath 100 μV (Fuglsang-Frederiksen 2000) based on macroscopic evaluation of the contraction pattern to exclude the influence of background noise.

For IPA, sensitivity was again set at 200 μV. The epoch length was maintained at 100 ms. At each insertion site, 3 contractions of the DDF muscle were measured, resulting in the measurement of 30 contractions in total. The electromyography software calculated turns/second (T) (Hz), amplitude/turn (M) (μV) and M/T ratio (%) automatically (Finsterer 2001).

3. Statistical analysis

Data were analysed using R version 3.1.1. All outcome variables were transformed to natural logarithm, and normality was checked using Q–Q plots. A general linear model was used with the outcome variables firing rate, turns/second, amplitude/turn and M/T ratio, respectively. The independent factors were group (control or laminitis), breed (horse or pony) and the interaction between group and breed. Likelihood ratio tests were used to select the best model. A 95% confidence interval instead of *p*-values was used to interpret the data (Cumming 2008). Outcomes were back-transformed to geometric means and geometric confidence intervals in order to describe the data more comprehensible.

4. Results

All patients showed clinical signs of laminitis, and Obel scores varied from grade 1 to grade 3. Duration of the laminitic episode varied from 0.5 to 312 weeks. Among the horses, the mean duration was 6 weeks, and among the ponies it was 75 weeks. The mean ($\pm$ SD) firing rates of the control and laminitic group were 53 $\pm$ 11 and 72 $\pm$ 22 Hz, mean turns/second values were 112 $\pm$ 57 and 102 $\pm$ 40 Hz, mean amplitude/turn values were 284 $\pm$ 51 and 249 $\pm$ 33 μV and mean M/T ratios were 0.39 $\pm$ 0.17 and 0.41 $\pm$ 0.16%, respectively.

According to the likelihood ratio tests, significant independent factors for firing rate were group, breed and the interaction between group and breed. For turns/second, breed was the significant independent factor. For amplitude/turn and M/T ratio, there were no significant differences between the means of the groups, resulting in a model with no significant independent factors.

Figure 2 shows the boxplots of the EMG parameters subdivided into control or laminitic and horse or pony. The firing rate of the total laminitic group was higher than the total control group. This difference was smaller for the ponies compared to the horses; in the horses, the geometric mean difference of the laminitic group was 1.73 [geometric 95% confidence interval (CI) 1.29–2.32], and in the ponies this value was 1.09 (geometric 95% CI 0.82–1.45). The number of turns/second of the ponies was slightly lower compared to the horses (geometric mean difference 0.70, geometric 95% CI 0.50–0.99). Other differences were not significant.

5. Discussion

The objective of this study was to test if increased muscle force detectable by needle electromyography IPA occurs in the DDF muscle in association with equine laminitis. Our study data show an increased firing rate in the laminitic group compared to the control group. This increased firing rate is a characteristic of increased muscle force that has been demonstrated in human medicine (Sanders et al. 1996; Finsterer 2001). Thus, the increased firing rate of the DDF muscle in the context of laminitis suggests an elevated muscle force. However, this seems to be the only evidence for altered muscle force as the turns/second and amplitude/turn did not change and thus provided no evidence for the recruitment of larger motor units with larger amplitude motor unit potentials. The discrepancy in firing rate between the control and laminitic groups of the horses and ponies could be caused by a difference in bodyweight or physique. In addition, the dissimilar duration of laminitis (6 and 75 weeks mean duration in horses and ponies, respectively) might have influenced the muscle force of the DDF.

As described by Finsterer (2001), muscle temperature influences the outcomes of electromyography IPA. However, in clinically healthy humans, the need for temperature measuring was unnecessary (Finsterer & Mamoli 1996). Because none of our patients showed a body temperature outside the reference values upon clinical examination, the outcomes of our study can be compared without muscle temperature monitoring data. Next to muscle temperature, the age of the subject might influence electromyography IPA outcomes (Fuglsang-Frederiksen 2000; Finsterer 2001). In human medicine, test results within an age range of 20–65 years were not age dependent (Haridasan et al. 1979; Sanders et al. 1996). Extrapolated from these results, only adult horses, ages ranging from 4 to 20 years, were included in our study. In our study, results of horses and ponies of all types of breeds were combined. Back et al. (2007) found a difference in load distribution of the front and hind limbs between Quarter horses and Warmbloods. If front limbs are loaded with a larger part of the bodyweight in a particular breed, it might result in more muscle force of the DDF, resulting in higher IPA values. However, the measurements by Back et al. (2007) were performed in trotting horses and extrapolation of these data to standing horses is questionable.

The IPA in horses has only been investigated twice for the purpose of comparing normative data of several skeletal muscles using among others IPA cloud analysis (Wijnberg et al. 2011; Jose-Cunilleras and Wijnberg 2015). However, study designs differed from this study, impeding the possibility of comparing data. Although the use of interference pattern TAA has been shown to be useful in determining the effect of *C. botulinum* toxin type A injections into the DDF muscle (Wijnberg et al. 2013), the variance of these parameters in this study led to non-significant results. This high variance might have been influenced by the personality and the corresponding behaviour of the horses and ponies during the test. In human medicine, increased

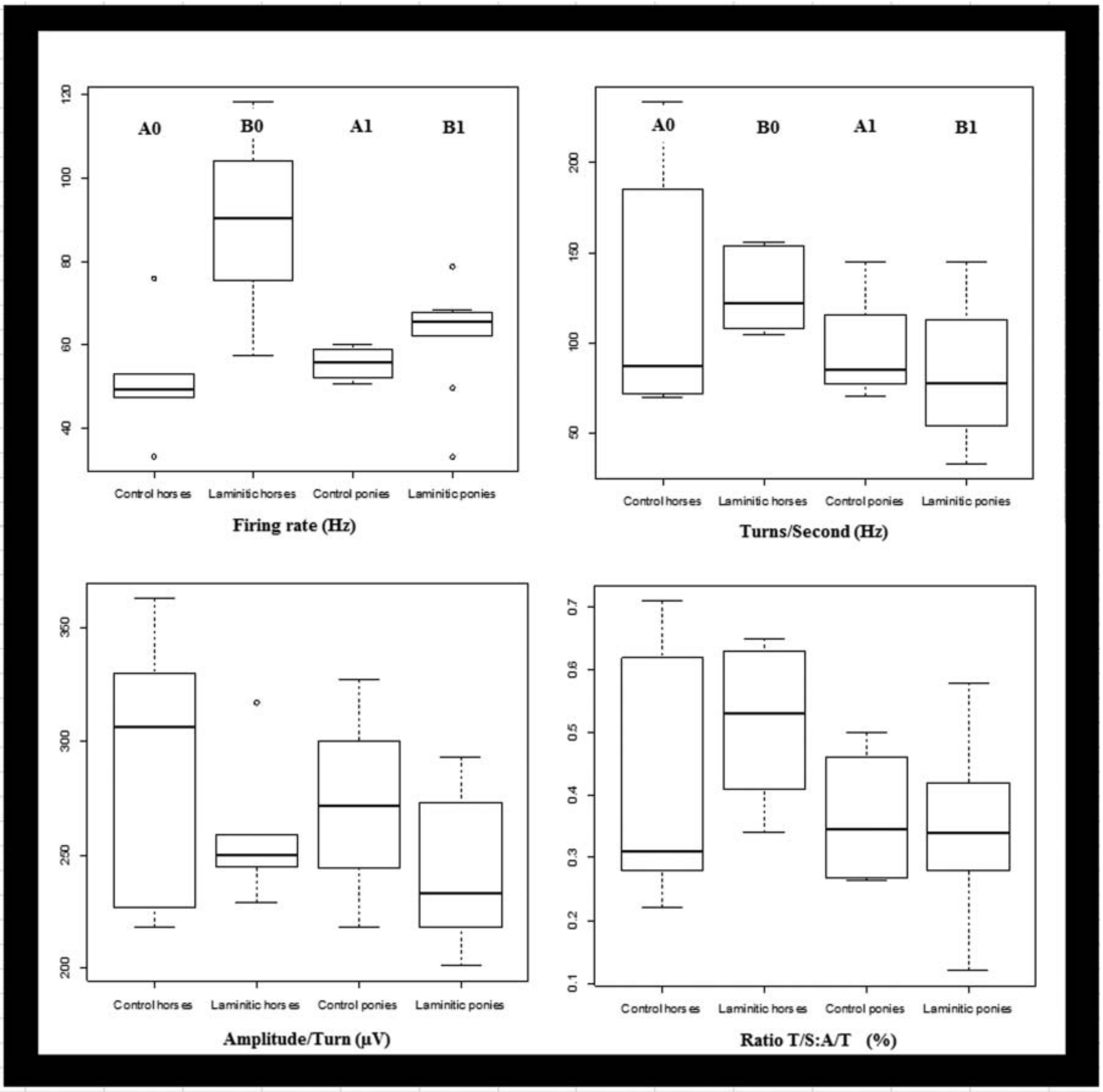

Figure 2. Boxplots of the electromyography interference pattern firing rate (Hz), turns/second (T) (Hz), amplitude/turn (M) (μV) and M/T ratio (%) of the deep digital flexor muscle of the horses and ponies of the control group (n = 10) and the laminitic group (n = 14). The firing rate of the laminitic group (B0 + B1) was higher compared to the control (A0 + A1). This difference was smaller for ponies (A1 + B1) compared to horses (A0 + B0). The number of turns/second of the ponies (A1 + B1) was slightly lower compared to the horses (A0 + B0). Other differences were not significant.

muscle tension appeared to be a uniform finding related to anxiety (Hoehn-Saric et al. 1997; Pluess et al. 2009). This notion might partly explain the high variance in our data, given that we were measuring horses and ponies of different ages and personalities.

In general, this study's results provide some evidence of an increased muscle force in the DDF associated with laminitis based on the increased firing rate. This finding might provide some support to the hypothesis that reducing the muscle tone of the DDF by using *C. botulinum* toxin type A might be a successful supportive therapy in treating equine laminitis. As the number of turns/second and amplitude/turn of the control and laminitic groups were not dissimilar, larger motor units with larger amplitude motor unit potentials were apparently not recruited. Therefore, it can be concluded that there is only a partial increase in muscle force. A larger test group in a future study would narrow the 95% CI, thereby estimating the mean of the population more precisely. Furthermore, a larger group would enable subdividing the laminitic group based on, for example, duration and severity of the laminitis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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