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Original Research

Evaluation of the Effects of Chiropractic on Static and Dynamic Muscle Variables in Sport Horses

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ABSTRACT

The objective of this prospective controlled experimental study was to assess the effect of chiropractic on static bioimpedance (BI) and dynamic acoustic myography (AMG) of paired muscle groups in healthy sport horses. BI measures the passage of current through muscle and gives information relating to the degree of contraction, whereas AMG records and analyzes low-frequency sounds created during muscular activity. Bioimpedance and AMG recordings, in addition to subjective and objective gait analysis, were taken from 6 horses before and at 24, 48 and 72 hours after chiropractic treatment. Signs of gait asymmetry were detected objectively in all horses throughout the course of the study. BI: Bioimpedance scores for the left trapezius muscle were significantly altered at 24 and 72 hours. AMG: The balance score of the GM muscle at walk was significantly affected by time, as was its efficiency at the trot. This small pilot study reveals that some objective measurements of muscle function are altered following chiropractic, lasting for at least 72 hours after treatment. This warrants further investigations in a larger number of subjects and in additional muscle groups.

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1. Introduction

Chiropractic, a form of manual therapy utilizing the application of controlled force to specific anatomic areas to modulate pain and improve function, has become widely used in equine medicine [1,2]. Only a handful of studies have reported on the effects of manual therapies on equine muscle tissue. Wakeling et al. (2006) used electromyography (EMG) to assess the effects of two manipulative therapies (spinal manipulations and reflex inhibition therapy) on the longissimus dorsi (LM) in 17 horses and found that total EMG intensity within this muscle showed a significant decrease immediately after manipulative therapy, suggestive of decreased muscle tone [3]. Langstone et al. (2015) also found a significant decrease in surface EMG (sEMG) readings of the splenius muscle following manual chiropractic treatment [4]. Longer-term responses to treatment were not assessed in either of these studies.

Electromyography, the study of motor unit action potentials associated with muscle activity during movement, has been used widely as a clinical and research tool in several species, including horses [5–7]. Extensive guidelines exist for the use of sEMG in humans [8]. In one study investigating the repeatability of phasic muscle activity using both surface and intramuscular EMG electrodes in human subjects, it was determined that the reproducibility and reliability of measurements were superior for surface electrodes when compared with more invasive needle electrodes [6]. In horses, sEMG has been successfully used to assess the response of a variety of muscle groups, including the gluteus medius (GM) to exercise on the high-speed treadmill across multiple readings [9–13].

Acoustic myography (AMG) measures the low-frequency sounds created during muscular activity, which reach the skin surface as mechanowaves that can be detected by a piezoelectric transducer placed on the skin overlying the muscle [14]. This is then used to generate information regarding the duration of time that a muscle is active throughout a movement (“efficiency” [E]), the number of

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Animal welfare/ethical statement: This study adheres to the Principles of Veterinary Medical Ethics of the AVMA. The work in this study was carried out in accordance with *EU Directive 2010/63/EU for animal experiments* and uniform requirements for manuscripts submitted to *Biomedical journals*.

Conflict of interest statement: None.

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active muscle fibers, derived from the amplitude of the sound signal ("spatial summation" [S]), and the frequency with which they contract, derived from the signal frequency ("temporal summation" [T]). These data can then be added together to give a total "ESTi" score for each muscle (Table 1) [14,15]. This technique was first validated in humans in the 1980s, when it was found that an AMG signal from a motor unit was reproducible and reliable when compared with needle EMG readings [16]. However, this study also revealed that the technique was more susceptible to interference caused by overlapping motor unit signals, and the technique fell out of favor within the medical community [16]. Recent technological advances have enabled many of the limitations associated with the use of AMG to be addressed. Harrison et al. (2013) assessed the biceps brachii muscle of human subjects using an improved AMG device with filtering capabilities and found that the unit was able to isolate the signal from the contracting biceps muscle and that signal was incrementally increased with increased workload in this muscle [1]. This study also validated AMG alongside sEMG in the gastrocnemius muscle, showing a highly comparable initiation and completion time for both signals. In addition, gastrocnemius readings were not significantly altered by short-duration exercise (amplitude 0.17 $\pm$ 0.01 mA before exercise and 0.17 + 0.04 mA after 20-minute run). It is the author's understanding that similar work to validate AMG alongside sEMG has been performed but not published in equines (Harrison, pers. comm.). AMG has also been used in horses to locate areas of muscle injury, assess the response to acupuncture treatment, and in the diagnosis of proximal suspensory ligament desmopathy [17–19].

There are several advantages of AMG over sEMG for the assessment of muscle activity. One practical difference between the techniques is that, unlike for sEMG, the reliable placement of AMG electrodes does not require the skin to be shaved before application [20]. Second, the biphasic AMG signal enables independent analysis of the initial concentric contraction (low-amplitude) phase, followed by the eccentric contraction (high-amplitude) phase [20]. In addition, the AMG signal is not as sensitive to either noise or sensor placement as sEMG [20]. Electrodes used for sEMG measurements may also be affected by local electronic equipment and electromagnetic radiation [6]. Moreover, sEMG signals may be distorted by crosstalk of motor unit action potentials from neighboring muscles due to skin displacement associated with poor electrode contact [6]. In contrast, movement of the AMG sensor to various different regions of an active muscle result in the measurement of comparable signal amplitudes, reflecting general muscle activity of a certain muscle group [20].

In the standing horse, BI uses various measurements of the passage of current through cell membranes and tissue interfaces to infer information regarding the degree of contraction and health of the selected muscle (center frequency [fc]) [21]. This technique has been validated for the assessment of a number of muscle groups, including the GM and LM in healthy equine subjects [21]. In this validation study, identical readings were obtained for all variables measured over multiple recordings, and minimal intersubject variation was seen (mean fc of 37.1 $\pm$ 0.9 SEM for GM muscle [n = 10]) [21]. Bioimpedance analysis has also been used successfully to assess the response to myofascial release therapy in equines [22].

The objective of this study was to assess the effect of chiropractic treatment on static BI and dynamic AMG of paired muscle groups in healthy sport horses. It was hypothesized that chiropractic would significantly alter muscle activity, as measured by BI and AMG.

2. Materials and Methods

Six client-owned, professionally trained show jumping sport horses (1 mare, 5 geldings; 1 Dutch Warmblood, 1 Belgian Warmblood, 1 Irish Sport Horse, 1 Thoroughbred, 1 Hanoverian, and 1 Warmblood $\times$ Thoroughbred cross) were included in the study after approval by the Animal Care and Use Committee of the University of California, Davis. All horses were considered healthy based on physical examination, with vital parameters (heart and respiratory rates and temperature) within normal limits. All horses were without recent history of lameness issues or known neck or back pain or pathology, although diagnostic imaging to exclude the presence of such pathology was not performed before inclusion. They were in full jumping training and were competing at moderate levels ranging from 1.0 to 1.2 m. The horses were evaluated at their respective training facilities (two locations).

Bioimpedance data were recorded for four muscle groups bilaterally for each horse at rest: trapezius (both cervical and thoracic components), latissimus dorsi, LM, and GM. A conductive paste (Ten20; Weaver and Company, 565 Nucla Way, Unit B, Aurora, Colorado, 80011, USA) was applied to the haired skin overlying the muscle of interest, onto which platinum electrodes (MyoDynamik ApS, Thorvaldsensvej, 19, 2. th 1871 Frederiksberg, Denmark) were placed (Fig. 1). A multifrequency BI unit (ImpediVET Unit 1, 50 Parker Court, Pinkenba, QLD 4008, Australia) providing 800 μA of current was subsequently attached to the electrodes, and recordings were carried out. Measurements were taken over a range of 256 frequencies (4 kHz–1000 kHz) and repeated six times with a 1-second interval.

A diagnostic acupuncture palpation examination (DAPE) was then performed by a consistent observer (S.L.J.) on each horse. This consisted of applying manual pressure along the areas outlined in Fig. 2 in a consistent manner as previously described [23]. Responses to manual pressure in the form of muscle fasciculation associated with any of the acupuncture points palpated warranted repeat, localized palpation, which was then recorded using a scale of 1–4 (Table 2). As seen in Fig. 2, the DAPE scan included palpation of portions of all of the muscle groups investigated in this study.

Following DAPE scan, each horse was instrumented with two commercially available systems—the CURO system (MyoDynamik

Table 1
Description of "E," "S," "T," and "ESTi" values.

Variable	Represents	High Value	Low Value
E—Efficiency (s)	Synchronization of muscle fiber activation during movement	Coordinated and synchronized fiber contractions	Poor coordination of fiber activation
S—Spatial Summation (mV)	Number of muscle fibers recruited to generate movement	Few fibers recruited (low amplitude)	Many fibers recruited (high amplitude)
T—Temporal Summation (Hz)	Frequency of muscle fiber activation required to generate movement	Low firing rate (low frequency)	High firing rate (high frequency)
ESTi Score	Overall efficiency of muscle activity during movement	Efficient muscle activity	Inefficient muscle activity

Adapted from Fenger (2017) [15].

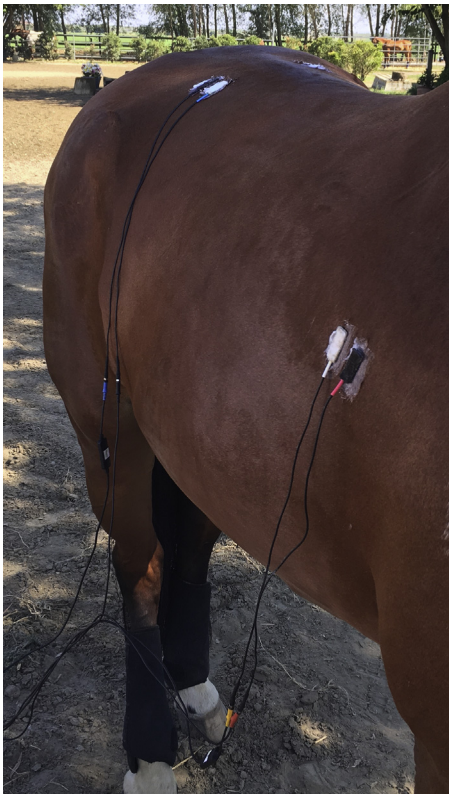

Fig. 1. Image of horse undergoing bioimpedance analysis of the latissimus dorsi muscle.

ApS, Denmark) to record AMG data and the Lameness Locator system (Equinosis LLC, MO, USA) for objective lameness data recording. The CURO system consisted of four single-use calibrated sensors, two placed 3 inches abaxially to the pelvic midline over the GM and two placed over the LM at the level of the 12th thoracic vertebra bilaterally (Figs. 3 and 4). Each of these sensors was placed directly onto unclipped skin, which had been prepared with ultrasound gel to increase contact with the sensor. The sensors were held in place via adhesive foam tape. The sensors were attached to a custom built AMG unit capable of recording at a distance of 100 m and with a sampling rate of 96000 Hz (Fig. 4). This unit was placed in a pouch and attached to a surcingle for real-time recording. Data were digitally sampled at 2–250 Hz in real time and analyzed by the CURO software. Acoustic myography recordings were transformed into E, S, and T values by the CURO AMG system. The ESTi score was calculated by taking the mean value of the E, S, and T values for each muscle on each side of the horse.

Table 2
Criteria for acupoint sensitivity during DAPE [22].

Grade	Criteria
1	An inconsistent localized muscle fasciculation
2	A mild consistent localized muscle fasciculation
3	A moderate consistent localized muscle fasiculation
4	A severe consistent localized muscle fasiculation

Muscle groups were chosen based on their superficial location, which was necessary for the use of BI and AMG, for which electrodes must be placed directly overlying the muscle of interest. The LM muscles were chosen because of their important role in bending and stabilizing the spine during movement [24]. The latissimus dorsi, trapezius, and GM muscles were selected because of their importance for locomotion [25,26].

The Lameness Locator system consisted of three inertial sensors. A uniaxial (vertical) accelerometer sensor was placed over the poll and between the tuber sacrale on dorsal midline, and a uniaxial gyroscopic sensor on the dorsal surface of the right forelimb pastern. An identical “sham” sensor was placed on the dorsal surface of the left pastern but was not switched on, to minimize any possible effects of the presence of the sensor and its wrap holder on movement of the horse.

Data were recorded at the walk and trot in a straight line before and after chiropractic adjustments on a surface that was consistent between the various trials for each horse, but not consistent for all horses. The two surfaces were sandy arena footing. AMG data were recorded at the walk, and AMG and inertial sensor data were recorded at trot in a straight line immediately before and at approximately 24 (range 20–25.25) hours, 48 (range 42.75–52.25) hours, and 72 (range 66.75–72.75) hours after chiropractic treatment for five horses. For one horse, due to time constraints, data were not collected at 72 hours. DAPE scan was performed immediately and at 24, 48, and 72 hours after chiropractic treatment.

Chiropractic adjustments were performed on all horses by the same veterinarian certified in chiropractic (S.L.J.). Vertebral

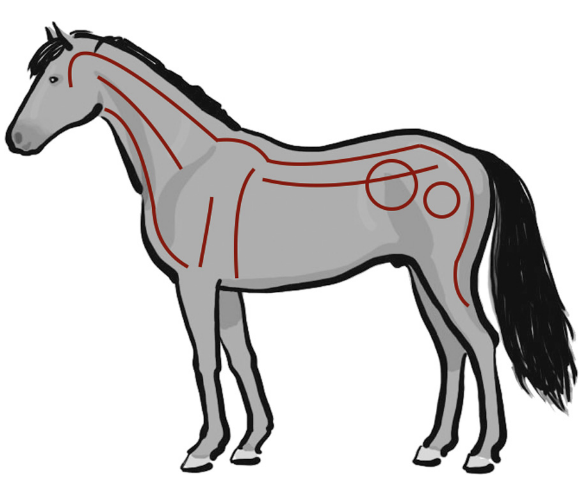

Fig. 2. Diagram depicting the sites of pressure application for the diagnostic acupuncture palpation examination (DAPE).

Fig. 3. Image of horse instrumented with the CURO system—white foam patches cover electrodes in place over gluteus medius muscles.

Fig. 4. Image of the CURO system, including electrodes courtesy of MyoDynamik.

segments requiring treatment were identified based on motion palpation of each segment, beginning at the pelvis. Any areas of motion restriction were adjusted using short lever, high velocity, low amplitude, and controlled thrusts.

Data were screened for significance ($P = .05$) using a repeated-measures ANOVA, and then Dunnett's test was used for pairwise comparisons.

3. Results

All horses tolerated the procedures well. Sensitivity at acupuncture points was present initially in 5 of 6 horses and was abolished immediately after and at 72 hours after chiropractic treatment in all of them (Table 3). Mild positive responses to the DAPE scan were seen again in one horse at 24 hours and two horses at 48 hours after treatment. Adjustments were performed based on motion palpation examination (Table 4). Signs of mild lameness were detected objectively (Lameness Locator) in all horses before chiropractic treatment and throughout the course of the study.

Center frequency (fc) scores for the left trapezius muscle were significantly ($P < .05$) increased relative to pretreatment scores at 24 and 72 hours after chiropractic treatment as detected using Dunnett's test (Fig. 5). There was a significant ($P < .05$) effect of time on the AMG balance score of the GM muscle at walk detected via RM ANOVA; however, no statistically significant pairwise comparisons were detected using Dunnett's test (Fig 6). There was also a significant ($P < .05$) effect of time on temporal summation scores for the left GM at trot detected via RM ANOVA (Fig. 7). Statistical significance was not detected in any of the remaining data.

Table 4
Chiropractic adjustments performed in each horse.

Horse	Chiropractic Adjustments
1	Anterior-superior ilium left and right T18–L3 posterior right
2	Anterior-superior ilium left and right
3	Anterior-superior ilium left and right L3–L4 posterior left L2–L6 posterior right
4	Anterior-superior ilium left and right Sacral apex left L4 posterior right
5	Anterior-superior ilium left and right L6 posterior left Intertransverse L6–S1 left C5 body left L5 posterior left
6	Anterior-superior ilium left and right L5 posterior left Atlas posterior right L2 posterior right L4 posterior right

Adjustment performed in cervical (C), thoracic (T), and lumbar (L) spine, as well as pelvis.
Description of adjustments is as described by the International Veterinary Chiropractic Association [27].

4. Discussion

This study demonstrates that BI and AMG modalities may be used to detect changes in the muscle function of equine athletes for several days after chiropractic treatment. The changes seen in this population included a significant increase in the center frequency (fc) of the left trapezius muscle and a significant effect of time on gluteal muscle function after chiropractic treatment, as measured by AMG recording.

The use of BI and AMG technology to measure muscle function has been validated in humans and horses [14,21]. These modalities have also been used in horses to assess the response to various complementary therapies and have demonstrated that these treatments are capable of causing measurable alterations in muscle activity [22,23].

A 2015 study used BI to assess the effects of myofascial release therapy in six horses. Treatment was found to induce a 58% decrease in fc values across multiple muscles more than 48 hours after treatment, representing muscular relaxation [22]. A recent study was performed to investigate the effects of acupuncture on AMG recordings of GM muscle function in sport horses. This study

Table 3
Results of DAPE in each horse.

Horse	Before Chiropractic		Immediately After Treatment		24 h		48 h		72 h	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	No response	BL39-41 (1)	No response	No response	No response	No response	BL20-21 (1)	BL39-40 (2)	No response	No response
2	No response	BL20-21 (2)	No response	No response	BL 20-21 (1)	No response	No response	No response	No response	No response
3	BL20-21 (2)	BL20-21 (2) BL39-40 (2)	No response	No response	No response	No response	No response	No response	No response	No response
4	BL20-21 (2)	BL20-21 (3) BL54 (3)	No response	No response	No response	No response	BL18-19 (1)	BL21-23 (1)	No response	No response
5	St7 (2) BL20-21 (1)	St7 (2) BL20-21 (2)	No response	No response	No response	No response	No response	No response	No response	No response
6	No response	No response	No response	No response	No response	No response	No response	No response	No response	No response

Sensitivity was observed at bladder (BL) and stomach (St) points.
Numbers denote severity of response to palpation according to the criteria outlined in Table 2.

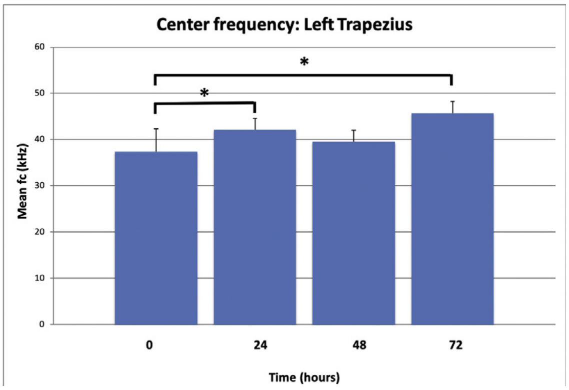

Fig. 5. Graph displaying mean center frequency (fc) scores for the left trapezius muscle. *$P < .05$.

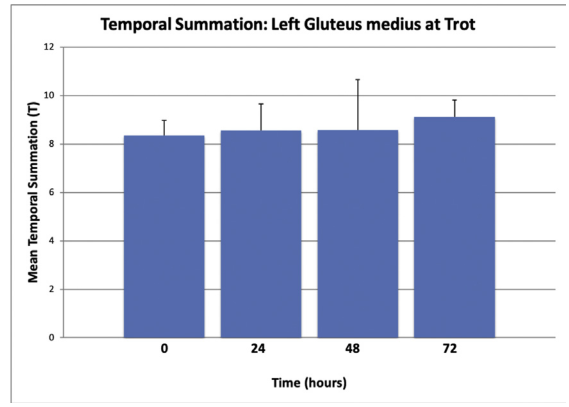

Fig. 7. Graph displaying mean temporal summation for the left gluteus medius muscle at trot. Significant effect of time detected using repeated-measures ANOVA; $P = .05$.

found that ESTi scores were increased in all horses at 48 hours after acupuncture treatment and that these increases persisted for a week in three of the five horses studied, although statistical analyses of these data were not performed because of the low number of horses included in the study [23].

The present study revealed that chiropractic adjustment induced statistically significant changes in the trapezius muscle that were measurable via BI. Center frequency (fc) is indicative of the amount of energy required to send 800 μA of current through the tissue [21]. When a muscle is more contracted, a higher energy is required for this current to pass through the muscle [28,29]. In this study, fc increased significantly at 24 and 72 hours after chiropractic treatment in the left trapezius muscle, indicating a more contracted state at rest compared with baseline at these time points. The reason for, and consequence of, this is not known. Previous studies have shown evidence of relaxation of various muscle groups in response to equine chiropractic treatment, although not the trapezius muscle [4,16]. Perhaps, rather than inducing relaxation alone in muscle groups adjacent to treatment sites, a more complex interaction of muscle groups occurs resulting in a more contracted state in some muscles when analyzed in isolation. In addition, we found significant changes in muscles that were not directly overlying the areas of adjustment. This might indicate that although relaxation may be induced in muscles in close proximity to the adjustment, this is not the case in more distant muscle groups.

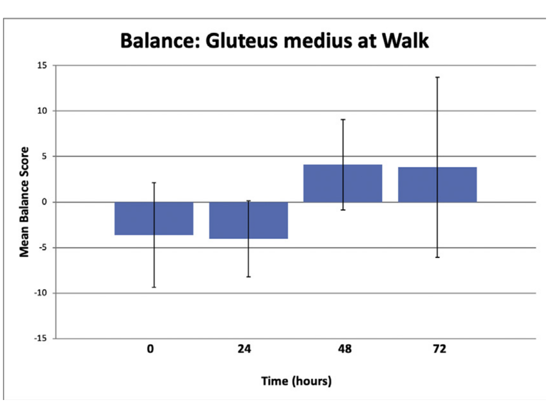

Fig. 6. Graph displaying mean balance scores of the left gluteus medius muscle at walk. Significant effect of time detected using repeated-measures ANOVA; $P = .05$. Negative values indicate greater activity in this muscle on the right side of the horse; positive values indicate greater activity on the left.

The AMG data collected in this study revealed that significant changes are seen in the gluteal muscle in association with time after chiropractic treatment. The ESTi score generated by the AMG CURO system is the mean of the E, S, and T scores. It represents the overall activity level of the muscle, with a higher ESTi score representing more efficient muscle function [20]. The AMG balance score is calculated by subtracting the ESTi score for a particular muscle on the right side of the horse from the score of the same muscle on the left side. This score therefore indicates how balanced each muscle group is in terms of its relative activity on either side of the horse. There was a significant effect of time on the balance score of the GM muscles at walk across the six horses. In this muscle group, the right side had a higher ESTi score before chiropractic treatment (5.6 ± 1.7 right vs. 4.9 ± 1.3 left) and this remained the case at 24 hours after treatment (7.7 ± 0.9 right vs. 6.4 ± 1.2); however, at 48 and 72 hours after chiropractic treatment, the left gluteus muscle had a higher ESTi score (7.3 ± 1.6 left vs. 5.9 ± 1.0 and 7.0 ± 2.8 left vs. 5.7 ± 2.1 right, respectively). The reason for—and significance of—this switch is unclear. Theories of “handedness” in relation to horses have been presented, and it is well recognized that horses typically favor one side of the body over the other. Similarly, each horse in the present study was noted to have an underlying asymmetry according to the objective gait analysis (Lameness Locator) system. Such asymmetries confound interpretation of the balance data.

There was a significant effect of time on temporal summation scores for the left GM muscle at trot. Temporal summation scores reflect the speed with which muscle fibers are activated repeatedly during a movement (high score = low activation frequency; see Table 1). These values increased over time in this muscle, representing a decreased frequency of fiber activation for the required workload subsequent to chiropractic in these horses at trot. The GM muscle is one of the major propulsion muscles of the hind limb [26]. This suggests that chiropractic may have induced more efficient activity within this muscle. Although a significant change was seen only in the left side in the present study, it is possible that this increased activity is bilateral but that our sample size was too small to detect significance on the right side. It is also possible that these data were also affected by the horses' preexisting asymmetries.

Bearing in mind the small sample size, it is the authors' opinion that the fact that significant differences were found in these horses' muscles following treatment is worthy of note and relevant to a discussion regarding the possible effects of chiropractic on muscle activity. There has been limited scientific evidence that chiropractic influences muscle activity in particular and this study contributes toward this literature. The fact that the BI results were somewhat counterintuitive, with increased muscular contraction being noted in some muscles, has potential implications for how horses should be managed and worked following chiropractic treatment—would it be beneficial to incorporate passive or dynamic stretching at the time of treatment to mitigate this increased muscle contraction; or is this part of the therapeutic effects of treatment? More studies investigating the effects of chiropractic on the BI of various muscle groups to elicit more information regarding the relative changes in these would therefore be beneficial. Muscle activity was seen to increase in the gluteus and trapezius muscles following chiropractic—this might point toward the regular use of chiropractic in horses looking to build topline and muscle mass in these regions in particular. Using AMG before and after subsequent chiropractic treatments might be helpful in objectively measuring the effects over time of regular chiropractic treatment on such muscle activity.

As discussed previously, a limitation of the present study is that despite having no history, or visible evidence, of lameness, all of the horses used were found to have objective gait asymmetry. This complicates interpretation of the data. However, finding a group of subjects with total symmetry would likely prove challenging because most horses in demanding exercise programs are likely to have small, underlying irregularities of movement. Instead, this study is more representative of the genuine effects of chiropractic in competing equine athletes.

Another limitation of the study is that the same surface was not used for all horses. It is well documented that different ground surfaces can affect a horse's gait; however, because each horse was repeatedly assessed on the same surface, this is unlikely to have influenced the results of our chiropractic treatment on each individual [30–32]. In addition, each horse was examined on its usual training surface, and it was thought that the horse was likely to move more normally in a familiar environment.

Radiographs of the spine of each horse assessed were not obtained before inclusion in the study, and therefore, the presence of pathology in these areas could not be excluded. However, poor correlation between radiographic and clinical signs of neck and back pathology in horses has been documented; therefore, the lack of history of neck or back problems and the absence of pain on palpation were considered to be stringent enough inclusion criteria [33,34].

The use of a control group that did not receive chiropractic treatment may have provided more information regarding the effects of this therapy. However, the techniques used to measure muscle function in the present study have previously undergone temporal validation in which intrasubject values were seen to remain static over multiple readings [14,21]. In the present study, both BI and AMG electrodes were not left in place for the duration of the study but were reapplied at each time point. This technique has been used in previous studies, which have demonstrated that there is insignificant day-to-day variability in these measurements despite this reapplication [14,17,21]. The electrodes were placed by the same operator for all readings to ensure consistency. For each BI recording, a mean of six consecutive measurements was taken to minimize any movement artifact. Variability of BI measurements has been shown to be minimal in healthy horses. One study investigated the GM and LM muscles in 10 healthy horses and found mean fc to be 34.5 ± 1.3 SEM and 37.1 ± 0.9 kHz, respectively. They also found that repeat measurements taken over several hours were identical for all of the parameters measured [21]. Significant intraindividual variability in sEMG reading in the horse has been reported (up to 20.5%; Robert et al. 2001 [35]). In the published data regarding AMG, sequential readings taken from one horse revealed a variability of 7.9%–12.7%, suggesting that this modality is equally if not more reliable that sEMG for repeated evaluation of muscle activity in the same horse, as performed in the present study [14]. With regard to the possible effects of the DAPE scan on AMG readings, this method of evaluating horses has been previously used in another study, in which it was found that performing the DAPE scan did not influence subsequent AMG recordings (le Jeune, pers. comm.).

Despite some of the data generated in the present study reaching statistical significance, the sample size of the present study is small. A similar study with a larger numbers of subjects might reveal further significant differences that are only reflected as trends in the data contained within the present study. Power analysis based on the data obtained in this study suggests that the sample used (6 horses) provided a power of 0.8 with the alpha level set at 0.05 to detect a 20% treatment effect on the BI central frequencies. However, for the AMG variable ESTi, the power achieved with 6 horses to detect a 20% treatment effect was only 0.3, and it is likely that the lack of statistical significance is related to a type II statistical error for at least some of the comparisons. Eighteen horses would be necessary to achieve a power of 0.8 using the same criteria.

5. Conclusions

The objective of this study was to assess the effect of chiropractic treatment on static BI and dynamic AMG of paired muscle groups in healthy sport horses. Based on the data presented here, chiropractic treatment induces significant changes in objectively measureable parameters of muscle activity within some muscle groups. Some of these changes are sustained for at least 72 hours. The clinical significance of these changes is unclear, and this warrants further investigations in a larger number of subjects and in additional muscle groups.

Acknowledgments

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