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Original Research

An Investigation Into the Short-Term Effects of Photobiomodulation on the Mechanical Nociceptive Thresholds of *M. Longissimus* and *M. Gluteus Medius*, in Relation to Muscle Firing Rate in Horses at Three Different Gaits

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ABSTRACT

Back pain is a common condition in horses, yet despite this, quantitative assessments of the efficacy of treatment are scarce. Mechanical nociceptive thresholds (MNTs) and acoustic myography (AMG) recordings were obtained, both preinterventionand postintervention, from the left and right epaxial muscles in eight healthy general riding horses (mean age 17 ± 6 yrs). Using an algometer, MNT readings were taken at each of the 6 preselected points along the thoracolumbar *M. longissimus* and *M. gluteus medius* region. AMG recordings of the *M. longissimus* and *M. gluteus medius* were taken while walking, trotting, and cantering on a left or right hand 20m circle on a longe, on a waxed sand surface in an indoor arena. Horses were then treated using a class 1 laser. Therapy was applied for 1 minute at 1000 Hz to the same preselected points from which MNT measurements had previously been taken. Measurements were subsequently taken 1 hour and 24 hours post-treatment for MNT reading, and only 24 hours after for AMG measurements. No significant effect of treatment was noted for the MNTs. The AMG results were analyzed in terms of their temporal summation (T-score), where statistically significant improvements in the T-scores for *M. longissimus* and *M. gluteus medius* were noted for the different gaits. It is concluded that cold laser therapy has a positive effect on horse muscles that reveals a change in their firing frequency that is commensurate with changes seen with analgesia in subjects experiencing pain.

1. Introduction

Thoracolumbar sensitivity in ridden horses is commonly noted by veterinary physiotherapists and veterinary practitioners [1]. However, despite its prevalence, this condition, which often lacks objective parameters, is typically complex in nature (Dyson, 2015). Manual palpation remains the most reliable method for the diagnosis of back pain in a clinical setting [2], however, recent studies have documented the use of a number of diagnostic tools to objectively assess musculoskeletal sensitivity in horses, among them being pressure algometry (PA) [3,4]. This technique relies on the application of pressure to a specific region, with recognition of the subsequent reaction from the animal as being individual, in terms of a mechanical nociceptive threshold (MNT) response [5–7]. However, it is recognized that potentially this approach is not

Animal welfare/ethical statement: Writtle University College approved the study design. The horse owners were informed about the study and were able to ask questions in a private setting before consenting to participate. The measuring equipment, which was noninvasive, complied with both CE and FCC regulations.

Conflict of interest statement: APH is currently trying to commercialize the CURO system (CURO-Diagnostics) and is establishing a company to cover the costs of future development. The CURO system was provided to Writtle College University at no cost. Writtle College University and its staff was not compensated for this study.

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without examiner and subjective factor influence, which can affect the pressure algometry results obtained [6,8].

Photobiomodulation therapy (PBMT), commonly known as cold laser or low-level laser therapy (LLLT), is becoming increasingly popular for treating horses [8,9]. Practitioners are using such lasers for multiple purposes including wound healing, pain management, prophylactic care as well as improvements in sports performance in horses and dogs alike [10]. Photobiomodulation using super pulse lasers is nonthermal due to the nanosecond pulse durations used, while the continuous beam administered by conventional class IV lasers has shown evidence of heating due to their high energy power output [11]. Indeed, Brondon and co-workers [12] reported that super-pulsed lasers penetrate the skin more efficiently than class IV lasers, and Joensen et al [13] working with rats found that super-pulsed 904 nm waves penetrate the skin barrier more easily than a continuous wave of 810 nm. Recently, Luna et al [14] showed that photobiomodulation devices used with horses have a greater capacity for energy penetration than a class IV laser used on unclipped light or dark skin, suggesting that photobiomodulation may be more therapeutic. It is furthermore documented that low-level laser therapy reduces pain and inflammation [15–17] and has been shown to relieve plantar fasciitis in human subjects [18]. Moreover, it penetrates deep into tissue where it improves tissue healing and regeneration [19,20]. The effect of low-level laser therapy at a cellular level has been to reduce experimentally induced inflammation by between 20–30% and to encourage macrophage-based stimulation of fibroblast proliferation [21].

Over the past few years, advancements in acoustic myography (AMG) equipment has made it possible to objectively monitor musculoskeletal function and health in real-time for both human and animal subjects alike [22]. Acoustic myography is a technique that measures the pressure waves generated by physically active muscles as they contract, it registers these signals in terms of their amplitude (spatial summation), their frequency (temporal summation), and their degree of coordination (time active relative to a recording frame) and displays these values in real-time. Acoustic myography signals have not only been found to be very repeatable but also highly correlated with muscle force [23,24]. Unlike electromyography, AMG measurements are not affected by the skin/sensor interface, motor endplate signals, or other physical parameters [22]. Acoustic myography uses the components of temporal and spatial summation to assess muscular performance as proposed by Harrison et al. [22] who suggest that AMG provides valuable information relating to muscle pain by accurately detecting and monitoring changes in temporal summation.

While PA evaluation has been linked with various therapeutic interventions and used in trials by researchers such as De Heus et al. [8], Varcoe-Cocks et al. [7] and Haussler and et al. [25], to the best of the authors knowledge, evaluation of muscle function using an AMG after cold LLLT has yet to be tested in terms of its ability to treat back pain in horses. The overall aim of this study was therefore to investigate muscle activity within the *M. longissimus* and *M. gluteus medius* and to evaluate tissue response in the epaxial muscles to photobiomodulation (low-level light laser) intervention in horses. To which end, the present study has tested the hypothesis that PBMT can effectively increase mechanical nociceptive thresholds and that beneficial changes may be objectively assessed using pressure algometry and AMG.

2. Materials and Methods

2.1. Animals

To better reflect the variety of the equine population, horses of various breeds and sizes were selected from a population of fifteen general riding horses and they fell between the ages of 9–26 years, with an average height of 1.5 ± 0.1 meter. All horses receive regular veterinary evaluation were deemed clinically sound and were in regular ridden work of approximately 5 hours per week (for details of the horses included in this study see Table 1). Initially, all horses were evaluated through manual palpation of the epaxial muscle region by a veterinary physiotherapist and any horses exhibiting severe pain responses in accordance with the grimace scale [26] were excluded from the study. Of the eight horses selected, none had any previous history nor were exhibiting responses associated with any clinical or subclinical pathologies and were deemed suitable for evaluation using PA of the *M. longissimus* and *M. gluteus medius* region and were accepted into the trial if they fell into one of three categories of sensitivity, as adapted from De Heus et al. [8]. The owners of the horses were informed of the trial design and gave their full consent, moreover full ethical approval was gained from Writtle University College before the start of the trial. All measurements were undertaken in the morning, during the month of June 2019, at Writtle College University, UK.

All horses in this trial were exposed to the same PBMT, harness equipment, and exercise protocol. To blind the trial and reduce any bias, where possible, independent assessors were used to perform any evaluations or interventions. Epaxial responses to the PA were scrutinized and graded and agreed on by two experienced veterinary physiotherapists, moreover the application of the low-level light therapy was undertaken by an experienced veterinary physiotherapist familiar with the equipment while following product treatment guidelines. The AMG measurements were measured blinded by AH, who had no idea which horse or treatment he was analyzing until after all data had been examined.

2.2. Equipment

Low level laser: Photobiomodulation treatment was administered using a low-level laser (MR4 ACTIVet PRO, Multi Radiance Medical; California, USA). This device is capable of delivering 50 W of super-pulsed laser power with a laser radiation of 905 nm and a pulse duration of 110 ± 20 ns. The treatment protocol adopted was as recommended by the manufacturer for the treatment of back pain and comprised setting ACTIVet PRO (1000 Hz for a duration of 1 minute per site) using the contact probe. The manufacturer

Table 1
The equine population used in this study included only experienced working horses in consistent and moderate work.

Horse	Age (y)	Breed	Height (hands)	Gender	Color
1	17	KWPN Dutch Warm blood	15.2	Mare	Bay
2	26	KWPN Dutch Warm blood	16.2	Gelding	Bay
3	9	Warm blood × Connemara	16.1	Mare	Chestnut
4	11	KWPN Dutch Warm blood	17.2	Gelding	Bay
5	14	Welsh C	14.1	Mare	Bay
6	25	Arab cross	14.3	Mare	Dun
7	20	Welsh B cross	14.2	Gelding	Dark bay
8	15	Irish Sports Horse	16.0	Gelding	Dark bay

guidelines for use of the laser were followed for the treatment of the location sites (see Table 2). In brief, the laser head was placed a few mm's above, but not in direct contact with the location site to be treated, the low-level laser was then activated, and the head of the laser was slowly scanned over the site for the duration of the treatment.

Algometer: A pressure algometer with a capacity of 500 × 0.5 N was used to evaluate the MNT's in this study (Wagner FDX 100 Force Gauge; accuracy ± 0.3%; Greenwich, CT, USA). The pressure algometer was set to Newtons and rounded to the nearest 0.1 N. The pressure algometry points used in this study can be found in Table 2. The algometer used in the present study was not only intended for hand-held force testing, it had a high-speed sampling rate of 1000 Hz to optimize measurement accuracy.

Acoustic Myography: An AMG unit (CURO-Diagnostics ApS, Bagsværd, Denmark) [22,27] was used to measure the effects of the cold laser therapy on muscle function. The measurements from the muscles at each gait were analyzed using CURO-Diagnostics ApS Software in terms of signal firing frequency (T-score).

In brief, 2.5 cm diameter AMG sensors were positioned on the horses over the muscles of interest. The hair between the sensor and skin was not cut, instead acoustic gel (Ekkomarine Medico A/S, Holstebro, Denmark) was placed between the skin and the sensors. The sensors were then fastened in place using adhesive bandage (Snøgg AS, Kristiansand, Norway), and the sensors were subsequently connected to the CURO device. The muscle signal was transmitted via Wi-Fi from the CURO device to an iPad 2 (Apple Inc, California, USA), and measurements were followed in real-time to ensure proper transmission of recordings [23,27,28] (see Fig. 1).

2.3. Selected Muscles

The muscles chosen for measurements in this particular study were selected for both anatomical and functional reasons. *M. longissimus* and *M. gluteus medius* are not only among the largest muscles in the equine back and hind limb, respectively, and they also play an important role in equine posture and performance and are often injured during exercise and competition.

M. longissimus and *M. gluteus medius* were evaluated for MNT responses before and after intervention, for the purpose of measuring associated muscle function. Both muscles were evaluated using AMG to correlate the synergistic response of treatment preintervention versus postintervention.

2.4. Pressure Algometry Measurement Sites

Pressure algometry measurement details can be found in Table 2, which specifies the optimal locations available for repeatability and accuracy as adapted from the work of Haussler and Erb [5], as well as that of Varcoe-Cocks et al [7]. It should be noted that pressure points 3−5 in the present study are the same as points 1−3 cited in the study by Long et al. [6], who studied caudal traction and MNTs in a group of horses with back pain.

Table 2
Details of the location sites for the pressure algometry measurements and the low level laser treatment.

Site	Location
1	*M. longissimus thoracis*, at the level of T13, 2 cm lateral to the dorsal midline
2	*M. longissimus thoracis*, at the level of T13, 10 cm lateral to the dorsal midline
3	*M. longissimus thoracis*, at the level of T18, 2 cm lateral to the dorsal midline
4	*M. longissimus thoracis*, at the level of T18, 10 cm lateral to the dorsal midline
5	Mid portion of *M. gluteus medius*, at the level of L3, 10 cm from midline
6	Midpoint between cranial aspect of tuber sacrale and coxae (*M. gluteus medius*)

Note that L denotes lumbar vertebrae, S denotes sacral vertebrae, and T denotes thoracic vertebrae. The corresponding number relates to the specific vertebrae in that region

2.5. Exercise Protocol

Once the equipment had been secured on the horse, a regulated warm up routine was followed, thereafter, a set exercise protocol was adopted for this trial, as follows.

A controlled exercise routine was undertaken on the longe using a cavesson and longe line. This exercise protocol enabled the evaluation of not only synergistic muscle function of *M. longissimus* and *M. gluteus medius* but also any effects of postintervention compared with preintervention values. Each horse was exercised on a soft surface, by the same experienced handler. After a regulated exercise routine of 5 minutes of walk and trot incorporating both reins, a 3-min period of controlled exercise on the longe was adopted, and this included a period of walk, trot, and canter undertaken on a 20 m circle on each rein, with continuous AMG recordings.

2.6. Acoustic Myography Measurements

The measurements from the selected muscles and all three gaits were analyzed using CURO-Diagnostics ApS Software in terms of the signal T-score, that is to say the firing frequency (T for temporal summation). For example, a signal frequency of 53 Hz (16 spikes per 300 mSec) in relation to a maximum level of 120 Hz, would be calculated thus: $T = \frac{120-53}{120}*10$ giving a T-score of 5.6.

2.7. Statistical Analysis

The data were initially tested for a normal distribution using a Kolmogorov-Smirnov (K-S) test run as part of the analysis undertaken using GraphPad InStat 3 software for Mac (Version 3.0 b, 2003; GraphPad Inc., La Jolla, CA). Differences between means were tested using the same software for statistical significance using an ANOVA. Differences between means with a P-value < .05 were considered significant. Values are presented as the mean ± SD for AMG and mean ± SEM for PA measurements.

Fig. 1. A photo of one of the horses measured in this study equipped with AMG sensors and a CURO recording unit attached to an elastic girth, taken while participating in the exercise protocol outlined in the Materials and Methods section.

3. Results

The horses in this study did not show adverse effects to the cold laser therapy, as well as the pressure algometry and AMG measurements. All eight horses remained in the study until its completion.

3.1. Pressure Algometry

It was found that the pressure range for the horses in this study varied from as little as 10 N to as much as 50 N, and as a result of such variability in response to the pressure algometer, no clear difference was noted for the pressure measurements when compared for the left and right sides of the animals, between individual sites and even when comparing pretherapy values with those taken 1 and 24 hours after cold laser therapy (overall $P > .99$) (see Fig. 2).

3.2. Acoustic Myography

For *M. longissimus,* it was noted that the cold laser therapy had a significant effect on the firing frequency when measured 24 hours after treatment compared with baseline values taken pretreatment (see Fig. 3). There was a significant improvement in the T-score when horses walked in a straight line from 4.27 ± 1.35 (equivalent to 80 Hz) pretreatment to 6.84 ± 1.31 (equivalent to 45 Hz) 24 hours after treatment ($P = .005$). Likewise, significant improvements in the T-score were noted for horses walking ($P = .037$), trotting ($P = .050$), and cantering ($P = .032$) on a left-hand circle and walking ($P = .005$) and trotting ($P = .004$) on a right-hand circle (see Fig. 3).

For *M. gluteus medius,* it was noted that the cold laser therapy had a significant effect on the firing frequency when measured 24 hours after treatment compared with baseline values taken pretreatment (see Fig. 4). There was a significant increase in the T-score when horses walked in a straight line from 3.36 ± 1.19 (equivalent to 94 Hz) pretreatment to 5.86 ± 0.97 (equivalent to 59 Hz) 24 hours after treatment ($P = .001$). Likewise, significant increases in the T-score were noted for horses walking ($P = .008$), trotting ($P = .009$), and cantering ($P = .045$) on a left-hand circle, and walking ($P = .006$) and trotting ($P = .050$) on a right-hand circle (see Fig. 4).

4. Discussion

The principle aim, which was to investigate muscle activity within the *M. longissimus* and *M. gluteus medius* of horses, was achieved, as was the response of these muscles to photobiomodulation with the aid of AMG. Moreover, the hypothesis tested has been partially accepted in that data support an analgesic effect of photobiomodulation on the measured muscles in terms of AMG measurements but not those obtained using pressure algometry.

This study, which has sought to examine whether photobiomodulation has an analgesic effect in horse muscles, has not only demonstrated that cold laser therapy has an immediate effect (within 24 hours of treatment) on two muscles of functional importance for the horse, it has also revealed a change in the firing frequency of active muscles, detected using AMG (T-score), a change that is commensurate with what one might expect to occur as the result of pain relief.

There are many analogies to describe the desirable “position of the horse” and one that is commonly used is referred to as the “bow and string theory” because it describes the engagement and activation of the epaxial and abdominal muscle groups to encourage core strength, spinal flexion, and stability, as described by Heuschmann [29]. Researchers such as Greve and Dyson [30] have raised awareness about the importance of strengthening specific muscles, and have strongly advocated the training of *M. longissimus* and the *M. gluteus medius*, respectively, principally because these muscles play a key role in thoracolumbar support. Moreover, as mentioned previously, thoracolumbar pain in horses is common, and treatment by physiotherapists and veterinary practitioners can be diverse [1].

Photobiomodulation has been shown to relieve pain caused by a variety of etiologies including lower back pain [31]. Although there is significant evidence to suggest that photobiomodulation can provide analgesia for both acute and chronic pain in humans and many other species [32,33], there is minimal photobiomodulation research on horses, particularly in relation to optimal parameters

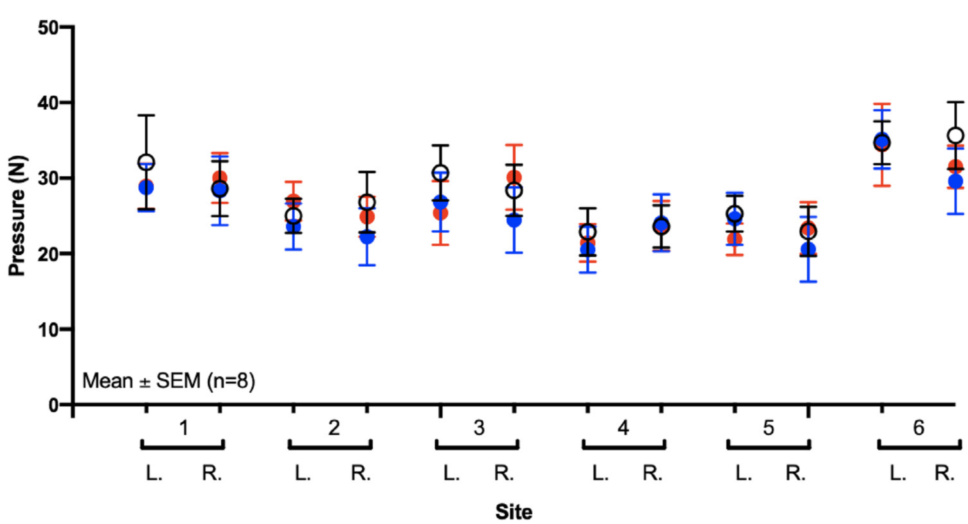

Fig. 2. The results for repeated pressure algometry measurements for six anatomical sites along the back of the horses (for details see Materials and Methods) taken both before laser treatment (PRE; open black symbol), 1 (1 POST; red circle), and 24 hours after treatment (24 POST; blue circle). Values are the mean ± SEM of 8 horses, and an assessment of statistical significance produced a *P* value of NS (>0.999), suggesting no significant difference between means. Numbers 1–6 represent the six anatomical sites along the back, while L and R denote the left and right sides of the back, respectively.

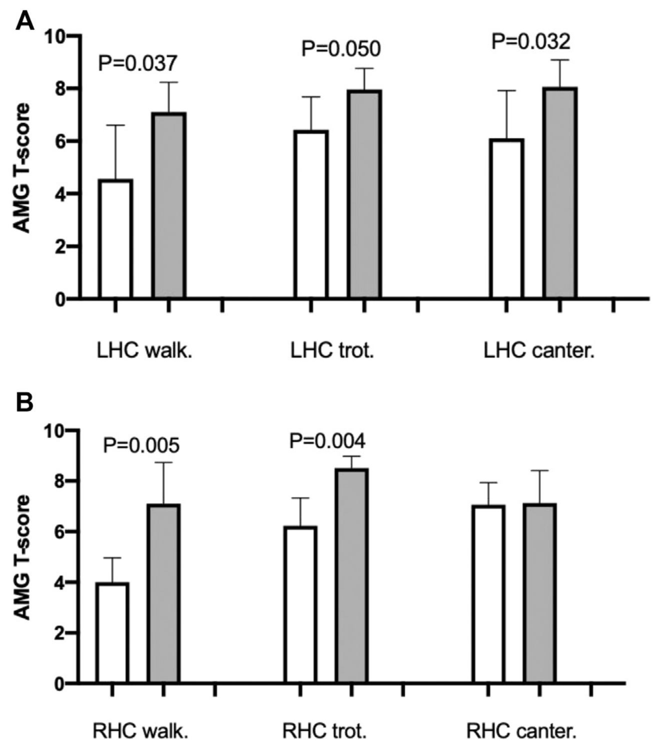

Fig. 3. The results for acoustic myography T-score recordings of muscle function for *M. longissimus* for both a left-hand circle (LHC; A) and a right-hand circle (RHC; B) at three different gaits. Values are the mean ± SD of 8 horses, and statistical significance between the baseline pretreatment measurement and one made 24 hours after laser treatment are shown. Note that in general, there is an increase in the T-score after laser treatment, a change that represents a lower muscle firing frequency. Open columns represent baseline values, while solid columns are values taken 24 hours after cold laser therapy.

and doses, interspecies differences in skin thickness, skin pigmentation, and hair thickness, all of which make comparison of results from humans and small mammals difficult to apply directly to horses [34–36]. Duesterdieck-Zellmer et al [37] reported that less light energy penetrates darker hair and skin colors as well as thicker skin subjects. The effects of different laser parameters on penetration has also been investigated [13,38] with findings showing that significantly greater penetration of light energy occurs using a super-pulsed (frequency = 6 kHz 904 nm, pulse width = 100 ns) laser than a continuous 810 nm laser based on rat and human models, respectively. It is for this very reason that the setting of ACTIVet PRO was used with the MR4 low-level laser in the present study, delivering the optimal wavelength and pulse width.

De Heus et al [8] advocated the application of a pressure algometer (PA) to assess muscle sensitivity objectively, however, PA measurements can vary significantly depending on the individual's age and body mass, the size/shape of the algometer probe, the ambient temperature, the day/week of measurement, the time of day, and the rate of pressure applied [3,4,39–43]. It is therefore critical to control all possible confounding variables when using PA in trials, and although every care was taken in this study, we are not able to exclude the possibility that findings were confounded by learned apprehension over time, with animals in this study reacting to a lower threshold due to the anticipation of pain [39,44]. The pressure algometer recordings made in this study showed a considerable degree of variability between subjects when comparing the efficacy of cold laser therapy between the left- and right-hand sides of the subjects, as well as between the six treatment sites along the back, all of which were nonsignificant (see Fig. 2). The findings of the present study are, however, in contrast with recently published work. Long et al. [6] determined whether caudal traction affected MNTs in a group of horses with clinical signs of back pain. Their study relied on algometry to measure MNTs from five bilateral anatomical sites in the epaxial and pelvic musculature of eleven horses [6]. These authors found a significant difference ($P \leq .05$) between MNT values before and after caudal traction in terms of the algometry measurements at all sites they measured [6]. In the present study, photobiomodulation was not found to have an effect on the algometer readings and by inference the MNTs. However, it has been established that there is a direct muscular attachment from the lumbar vertebrae in the horse to the caudal vertebrae of the tail [6]. Moreover, it would seem that caudal traction (a tail pull) may be a more advantageous therapeutic technique for horses with signs of back pain, based on the commonly used human manual therapy technique of traction, rather than photobiomodulation.

In terms of the other technique applied in the present study, AMG has been reported as being a useful assessment tool of muscle function [22] as confirmed by the study of Millares et al [45] who showed AMG capable of recording levels of muscle activity and adaptations subsequent to acupuncture intervention. Indeed, in the present study, AMG recordings revealed a significant effect of cold and low-level laser therapy within a 24 hour window of treatment. This improvement in the T-score (firing frequency of the muscle) was noted for not only *M. longissimus*, but also *M. gluteus medius* and was found for both left- and right-hand circles, and in most cases for the three gaits tested: walk, trot, and canter (see Figs. 3

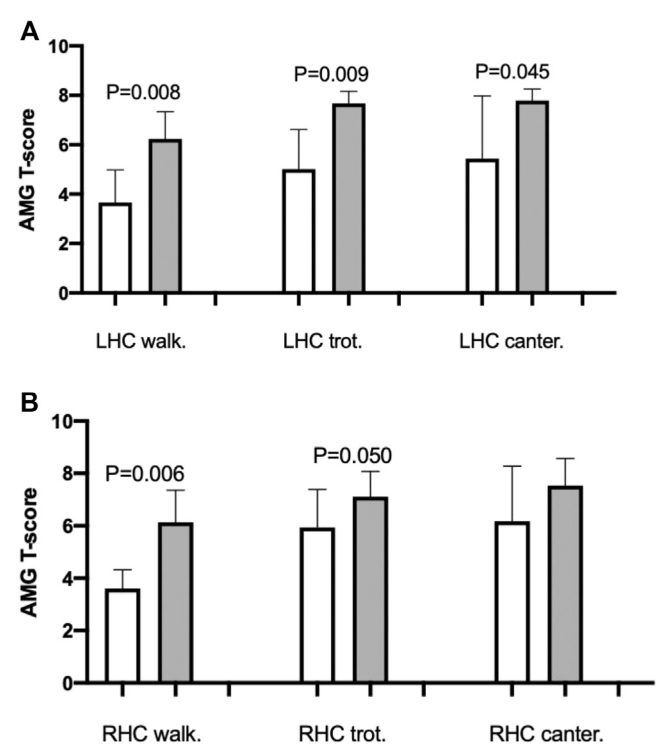

Fig. 4. The results for acoustic myography T-score recordings of muscle function for *M. gluteus medius* for both a left-hand circle (LHC; A) and a right-hand circle (RHC; B) at three different gaits. Values are the mean ± SD of 8 horses, and statistical significance between the baseline pretreatment measurement and one made 24 hours after laser treatment are shown. Note that in general, there is an increase in the T-score after laser treatment, a change that represents a lower muscle firing frequency. Open columns represent baseline values, while solid columns are values taken 24 hours after cold laser therapy.

and 4). It should be noted that an increase in the AMG T-score in fact represents a reduction in the firing frequency of the muscle because the T-score is inverted (0 = a high firing frequency and 10 = a low firing frequency).

Muscles located close to a site of pain often exhibit a high firing rate [22], typically remaining in a state of contraction and thereby helping to stabilize, for example, a painful joint. Likewise, the use of compounds such as ketamine on human fibromyalgia subjects, where muscle pain is often a daily issue, has been shown to reduce the temporal summation (firing frequency) of muscle contractions [46]. The present study confirms the work of Graven-Nielsen et al [46] by showing that therapeutic treatment for back pain results in a change in the T-score (temporal summation) for the AMG signal for the two muscles measured, which represents a reduction in the firing frequency of these muscles over three different gaits and two different circles. Moreover, the present findings support those recently published by Haussler and et al [25], who reported that low-level laser therapy produced a significant reduction in back pain in sixty one Quarter horses. These authors furthermore published evidence that chiropractic treatment by itself did not produce any significant change in back pain, rather combined laser therapy and chiropractic care was found to be additive in treating back pain and trunk stiffness [25]. These results also confirm a study involving human subjects with myofascial pain syndrome, in which both low-level laser therapy and shockwave therapy were found to be effective, although these authors found shockwave therapy to be somewhat more beneficial [47]. Furthermore, the present findings support those of a detailed meta-analysis of human patient effects of low-level laser therapy as an effective method for relieving pain [17].

5. Conclusions

It is concluded that cold and LLLT causes a decrease in the firing frequency of horse muscles, a change that is similar to the effects of analgesia on muscles of subjects experiencing pain. However, for the first time, the present study reveals evidence that supports an effect of LLLT on muscle function. Further studies are now needed to carefully explore the effects of cold and LLLT on other muscles in horses in synergy (e.g., *M. rectus abdominis* and *M. longissimus*), to explore the effects of photobiomodulation on muscles associated with the “bow string” mechanism. Moreover, new studies are now needed to measure the effects of photobiomodulation on muscles functionally related to balance and the spine, and to explore this technique’s apparent ability to alleviate the symptoms of pain.

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