Original Article

Whole body vibration affects the cross-sectional area and symmetry of the *m. multifidus* of the thoracolumbar spine in the horse

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Summary

Whole body vibration (WBV) has been used as an adjunctive therapy to improve the strength and size of paraspinal muscles as well as postural control in people with lower back pain. It has been proposed that activation of the *m. multifidus* plays a key role. As the function and anatomy of the *m. multifidus* in the horse is comparable to that in man, the authors investigated whether WBV might also be a valuable physiotherapeutic modality in horses. The effects of WBV on the cross-sectional area (CSA) and left to right symmetry of the *m. multifidus* at various locations of the thoracolumbar spine of the horse was evaluated in a single-subject quasi-experimental time-series design with repeated measure. Nine horses were subject to WBV, 30 min, twice daily, 5 days a week, for 60 days in addition to their regular exercise routine. The CSA of the left and right *m. multifidus* was measured ultrasonographically at four levels (T15–T16, T16–T17, T18–L1 and L1–L2) along the thoracolumbar spine at Days −30, 0, 30 and 60 of the study. Changes in the CSA and CSA symmetry (left to right) of the *m. multifidus* were analysed using nonparametric, repeated measures, comparison of mean ranks with post-hoc analysis as necessary. A significant increase ($P<0.05$) in *m. multifidus* CSA was found at all spinal levels after 30 and 60 days of WBV and a statistically significant improvement in *m. multifidus* symmetry (becoming more symmetrical) was found after 60 days of WBV, indicating that WBV may be a valuable alternative to dynamic mobilisation exercises when an increase in size and improvement in left to right symmetry of the *m. multifidus* is sought.

Introduction

Dynamic mobilisation exercises in horses, targeting preparative activation and strengthening of the *m. multifidus* and other core muscles, can increase cross-sectional area (CSA) and improve symmetry of *m. multifidus* (Stubbs *et al.* 2011). Based on these results and research in man on the role of the *m. multifidus* in spine stability (Freeman *et al.* 2010), Stubbs *et al.* (2011) suggest that these exercises might be potentially beneficial in improving or restoring spinal function and stability leading to reduced back pain; however, these exercises are labour intensive, time-consuming and require some practice to ensure they are performed correctly.

Whole body vibration (WBV), by contrast, is an alternative method of neuromuscular training that exposes the whole body to vertical mechanical sinusoidal oscillations in a controlled manner by the selection of preset intensities, amplitudes and frequencies. Research in man indicates that WBV is capable of increasing muscle activity (Marín *et al.* 2015), muscle strength (Machado *et al.* 2009; Osawa and Oguma 2013), inducing muscle hypertrophy (Gilsanz *et al.* 2006), improving postural balance and reducing chronic back pain (del Pozo-Cruz *et al.* 2011).

The aim of this study was to conduct a preliminary exploration of the effects of WBV on the *m. multifidus*. The authors hypothesised that daily WBV over a 60-day period will result in an overall increase CSA and improved symmetry (left to right) of the *m. multifidus* in the horse.

Materials and methods

Study design

The study was a single-subject quasi-experimental time-series design with repeated measure. Attribute independent variables included breed, age, gender, primary use, exercise level and AAEP lameness score; the active independent variable was WBV treatment. Of the nine participants, five (B1, B2, B3, B4 and B5; **Table 1**) were selected to assess the stability of the CSA of the *m. multifidus* prior to WBV treatment (from Days −30 to 0). All participants received WBV treatment from Days 0 to 60 and the dependent variable consisted of *m. multifidus* CSA measurements taken at Days 0, 30 and 60. All inferential statistics were evaluated within subject differences.

Participants

Of the theoretical population horses with clinical signs for back pain and associated lameness, nine horses (age 10.2 ± 4.2 years) (**Table 1**) were selected via convenience sampling from an accessible population of 1651 horses owned by clients of Peninsula Equine Medical Center, Menlo Park, California. Inclusion criteria were as follows: a gradable lameness before the start of the study not exceeding a lameness score of 4/5 on an AAEP scale (Stashak 2002), a previous lameness history indicative of a chronic or recurring lameness issue, no treatments or any other therapies within the last 6 months prior and during the study and signed informed client consent form before the start of the study. All horses (n = 9) were required to be housed in a stall (4×4 m) with daily turnout in a small paddock (8×16 m) and exercised 6 days a week. A specific type and duration of

TABLE 1: Characteristics of horses included in the study

Horse ID	Breed	Age (years)	Gender	Discipline	Exercise/performance level	AAEP lameness score (0–5) (Day 0)
A1	WB	9	G	Dressage	Third	3
A2	WB	15	G	Dressage	Second	1
A3	WB	19	G	Dressage	Second	2
A4	WB	8	G	Dressage	Laid-up	3
B1	TB	8	M	Eventing	Training	1
B2	ISH	5	G	Eventing	Training	3
B3	ISH	10	G	Eventing	Training	1
B4	TB	8	G	Eventing	Training	1
B5	WB	10	G	Eventing	Laid-up	4

WB, Warmblood; TB, Thoroughbred; ISH, Irish sport horse; G, Gelding; M, Mare; Training, performance/competition level for each discipline as defined by the United States Equestrian Federation; Laid-up, stall rest with 15 min of hand walking.

exercise, as well as diet was not a prerequisite, but for participation in the study, rider, exercise regime and diet for that individual horse were required to remain the same from 3 months prior to the start of WBV throughout the 2 months' duration of the study.

Lameness assessment

Horses were examined by a veterinarian (primary author) experienced in lameness diagnosis and ultrasound imaging. All horses demonstrated lameness, ranging from grades 1–4 on the AAEP lameness scale (Stashak 2002), before the start of WBV treatment (**Table 1**). Back pathology was present in six of the nine horses. Horse (A1) had grade 2 kissing spines (overriding dorsal spinous processes); three horses (B2, B3 and B5) had grade 1 kissing spines and the other two horses (B1 and B4) had grade 3 kissing spines, as well as thoracolumbar facet joint arthritis.

Whole body vibration and exercise

All horses (n = 9) underwent WBV 5 days a week (Tuesday, Wednesday, Thursday, Saturday, Sunday), twice daily (morning and afternoon) for 30 min at a frequency of 40 Hz, amplitude of 0.8 mm and an acceleration of 4.9 m/s^2 (0.5 g) for a total of 60 days using a mobile linear (vertical) type vibrating platform (Vitafloor VM0)[1], producing an indirect vertical sinusoidal vibration applied to the feet. This was added to their normal exercise routine. The regimen of WBV and *m. multifidus* CSA measurement is shown in **Figure 1**.

Ultrasound image capture of the m. multifidus

Procedures for ultrasound image capture of the *m. multifidus* have been previously described (Stubbs *et al.* 2011). All ultrasonography and image capture was performed by the primary author with the horse restrained in cross ties and light sedation (5 mg detomidine [Dormosedan][2] i.v.). After standard skin preparation, ultrasound images (Toshiba Viamo)[3] were captured at the levels of T15–T16, T16–T17, T18–L1 and L1–L2 articular facet joints, using a curvilinear probe (Toshiba Viamo probe 6C1, convex, T4.0 Hz)[3], when the bone margin of the dorsal spinous process, articular processes, mammillary process and lateral fascial border between the *m. multifidus* and *m. erector spinae* was clearly visible (**Fig 2**).

The anatomical positions of the articular facet joints were confirmed each time ultrasonographically, by first distinguishing the last (18th) rib, which is convex, from the transverse process of the first lumbar vertebrae, which is flat. Subsequently, the T18–L1 articular facet joint was visualised in between the last rib and transverse process of the first lumbar vertebrae. The T18–L1 facet joint was then used as the landmark to count cranially or caudally to identify the other articular facet joints L1–L2, T16–T17 and T15–T16, respectively. The primary author selected a single image at the level of the articular facet joints instead of between articular facet joints, as described by Stubbs *et al.* (2011), because he felt that landmarks at the level of the articular facet joints were easier and more reliably imaged.

The reliability *m. multifidus* CSA measurement was quantified by calculating the standard error of measurement (SEM). The total combined SEM of ultrasonographic image capture and the measurement of the CSA via imaging processing software (ImageJ)[4] ranged from 0.012 to 0.014. Complete validation information is available in **Supplementary Item 1**.

Cross-sectional area measurement

The procedure for the measurement of the CSA of the *m. multifidus* has been previously described (Stubbs *et al.* 2010). The *m. multifidus* CSA measurements were completed by the primary author in a nonblinded, random order and all images were measured five times. The average of the five measures was used for all statistical analysis. Imaging processing software (ImageJ)[4] was used to measure the CSA
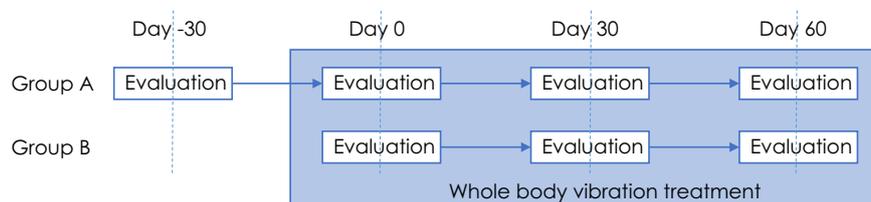

Fig 1: *The two groups of horses and the timing of whole body vibration (WBV) treatment and* **m. multifidus** *CSA determination.*
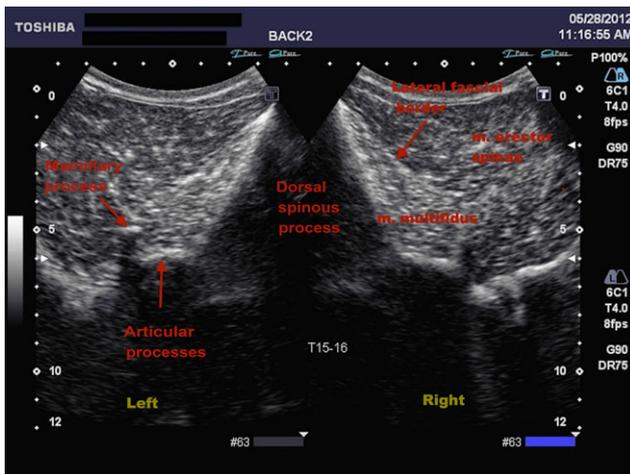
Fig 2: The ultrasound image is of the left and right m. **multifidus** *at the level of the T15–T16 articular facet joints and is representative of the images collected for the study. The ultrasonographic landmarks (dorsal spinous process, articular processes, mammillary process and lateral fascial border) can be seen between the* m. **multifidus** *and* m. **erector spinae.**

(cm^2) of the left and right *m. multifidus*. The SEM for the measuring of the CSA via imaging processing software (ImageJ)[4] only ranged from 0.009 to 0.042. Complete validation information is available in **Supplementary Item 1**.

The symmetry ratio of the *m. multifidus* CSA was calculated according to the linear response formula of Reeves *et al.* (2006).

$$Ratio = \frac{right\ m.multifidus\ CSA}{left\ m.multifidus\ CSA}$$

$$If\ ratio \geq 1, symmetry = \{ratio - 1\} \times 100\%$$

$$If\ ratio < 1, symmetry = -\left(\frac{1}{ratio}\right) \times 100\%$$

Statistical analysis

All data analysis was completed using IBM SPSS Statistics for Macintosh Version 22.0.[5] Unless otherwise stated, the alpha level of 0.05 (α = 0.05) used for inferential statistical and all tests were 2-tailed.

Results

Mean and standard deviation (s.d.) for all *m. multifidus* CSA measurements are available in **Supplementary Item 2**. One horse (B3) was sold and as such lost for the Day 60 *m. multifidus* CSA assessment follow-up. Due to poorer image quality in some horses (A1, A2, A3 and A4) related to the thickness of the skin and fat, not all borders of the *m. multifidus* were easily identifiable on ultrasound images at some levels (T16–T17 and L1–L2) and as such were not further analysed in those four horses.

M. multifidus CSA stability prior to WBV

The stability assessment data (Days −30 and 0) are repeated measures and not normally distributed; thus, a Wilcoxon

Signed Ranks test with listwise exclusion was performed to determine if there were differences among the mean ranks (means) of the CSA of the *m. multifidus* at each spinal region. The grouping provided two values (left and right) for each participant (n = 5) at each spinal region; thus 10 paired values for analysis (n = 10) at each spinal region. No statistical difference was found (P<0.05) for each spinal region when contrasting Days −30 and 0. A Wilcoxon Signed Ranks test with listwise exclusion was performed to determine if there were differences among the mean ranks (means) of the CSA mean symmetry score of the *multifidus* muscle of each participant. The grouping provided 4 values (T15–T16, T16–T17, T18–L1 and L1–L2) for each participant (P = 5); thus 20 paired values for analysis (n = 20). No statistical difference was found (P<0.05) when contrasting Days −30 and 0. Thus there was no change in *m. multifidus* CSA or CSA symmetry ratio without WBV treatment.

M. multifidus CSA with WBV

For all analyses of *m. multifidus* CSA, data were grouped by spinal region. The grouping provided two values (left and right) for each participant (n = 9) at each spinal region; thus, a potential for 18 paired values for analysis (n = 18) at each spinal region. The n is provided for each analysis and those that are different than 18 are the result of missing data.

The data are three repeated measures (Days 0, 30 and 60) and not normally distributed; thus a Freidman test with listwise exclusion was performed to determine if there were differences in mean ranks (means) of *m. multifidus* CSA at Days 0, 30 and 60 of WBV at each spinal region. A statistical difference was found at spinal region T15–T16 ($\mathcal{X}^2$ (2, n = 16) = 22.88, (P<0.01); T16–T17 ($\mathcal{X}^2$ (2, n = 8) = 16.00, (P<0.01); T18–L1 ($\mathcal{X}^2$ (2, n = 16) = 30.13, (P<0.01) and L1–L2 ($\mathcal{X}^2$ (2, n = 8) = 13.00, (P<0.01). This indicates that there was a difference among the three mean ranks (means) at Days 0, 30 and 60 at each of the spinal regions. The three orthogonal contrasts were performed using a Wilcoxon signed rank test with listwise exclusion. To hold the experiment wise type 1 error to less than 0.05, Bonferroni correction (comparison-wise alpha = 0.01) was used. The contrast between Days 0 and 30 at T15–T16 (n = 18, z = −3.201, (P<0.01, r = −0.75), T16–T17 (n = 10, z = −2.803, (P<0.01, r = −0.89), T18–L1 (n = 18, z = −3.724, (P<0.01, r = −0.88); and L1–L2 (n = 10, z = −2.497, (P<0.01, r = −0.79) were found to be statistically significant at each spinal region. The contrast between Days 30 and 60 at T15–T16 (n = 16, z = −3.154, (P<0.01, r = −0.79), T16–T17 (n = 8, z = −2.521, P<0.01, r = −0.89), T18–L1 (n = 16, z = −3.464, (P<0.01, r = −0.87); and L1–L2 (n = 8, z = −2.521, (P<0.01, r = −0.89) were found to be statistically significant at each spinal region. The contrast between Days 0 and 60 at T15–T16 (n = 16, z = −3.516, (P<0.01, r = −0.88) T16–T17 (n = 8, z = −2.521, (P<0.01, r = −0.89) T18–L1 (n = 16, z = −3.516, (P<0.01, r = −0.88) and L1–L2 (n = 8, z = −2.521, (P<0.01, r = −0.89) were found to be statistically significant at each spinal region. Additionally, the r effect size for each is greater than the absolute value of 0.75. Although there is minimal to no information regarding effect size in the literature in this field, the r effect size is considered much larger than typical in many other fields (Cohen 1988; Nakagawa and Cuthill 2007); thus the authors feel this is a clinically relevant increase in *m. multifidus* CSA. In all cases, the statistically significant contrasts indicated that as the duration of WBV increased the

TABLE 2: Mean and standard deviation (s.d.) for cross-sectional area (CSA) of *m. multifidus* muscle at four spinal levels at Days 0, 30 and 60 of whole body vibration treatment

Spinal region	Day 0 CSA (n = 9)		Day 30 CSA (n = 9)		Day 60 CSA (n = 8)	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
T15–16	8.431*	0.678	8.782*	0.471	9.243*	0.713
T16–17	9.111[†]	0.673	9.387[†]	0.527	9.956[†]	0.693
T18–L1	8.539[‡]	0.771	8.953[‡]	0.717	9.471[‡]	0.829
L1–2	8.162[§]	1.289	8.456[§]	1.167	8.812[§]	1.511

T, thoracic vertebra; L, lumbar vertebra; CSA, cross-sectional area in cm^2.
[*,†,‡,§]All values are statistically significantly different (P<0.01).

CSA of the *m. multifidus* increased and this is biologically relevant (**Table 2**).

M. multifidus CSA symmetry with WBV

For all analyses of *m. multifidus* CSA symmetry, data were grouped by participant. The grouping provided four values (T15–T16, T16–T17, T18–L1 and L1–L2) for each participant (n = 9); thus 36 paired values for analysis (n = 36). The n is provided for each analysis and those that are different than 36 are the result of missing data.

The data are three repeated measures (Days 0, 30 and 60) and not normally distributed; thus a Freidman test with listwise exclusion was performed to determine if there were differences in mean ranks (means) of the symmetry score of the *m. multifidus* CSA at Days 0, 30 and 60 of WBV. A statistical difference was found ($\mathcal{X}^2$ (2, n = 24) = 6.083, P = 0.05). This indicates that there was a difference among the three mean ranks (means) for Days 0, 30 and 60. The three orthogonal contrasts were performed using a Wilcoxon signed rank test with listwise exclusion. To hold the experiment-wise type 1 error to less than 0.05, Bonferroni correction (comparison-wise alpha = 0.013) was used. The contrast between Days 0 and 60 (n = 24, z = −2.657, (P<0.01, r = −0.54)) s found to be statistically significant, whereas contrast between Days 0 and 30 and Days 30 and 60 was not statistically significant. The r effect size is greater than the absolute value of 0.54 and this is considered larger than typical in many other fields (Cohen 1988; Nakagawa and Cuthill 2007); thus the authors feel this is a clinically relevant improvement in left to right symmetry of the *m. multifidus* CSA. The statistically significant contrast indicates that as the duration of WBV increased the symmetry score in the CSA of

TABLE 3: Mean, minimum, maximum and standard deviation (s.d.) of symmetry score for cross-sectional area (CSA) of *m. multifidus* muscle at Days 0, 30 and 60 of whole body vibration treatment

Days of treatment	Symmetry score (n = 9)			
	Minimum	Maximum	Mean	s.d.
Day 0	0.41	26.29	6.39*	7.81
Day 30	0.28	19.79	5.26	6.21
Day 60	0.15	22.36	4.25*	6.39

*All values are statistically significantly different (P<0.01).

the *m. multifidus* decreases, meaning that the CSA of the *m. multifidus* from left and right became more symmetrical and this is biologically relevant (**Table 3**).

Discussion

This study shows that WBV is capable of increasing the total (left and right) CSA at each spinal level in the horse (**Table 2**, **Figs 3** and **4**), an indicator of *m. multifidus* hypertrophy. Furthermore, an improvement in *m. multifidus* symmetry takes place over the 60 days of WBV, seen by the CSA symmetry ratio moving towards zero (**Table 3**). The ability of WBV to increase CSA of the paraspinal musculature (Gilsanz *et al.* 2006), as well as minimise *m. multifidus* atrophy during bed rest induced deconditioning (Belavý *et al.* 2008) has previously been described in man, but to the best of the authors' knowledge, has never been investigated in the horse. No significant change in *m. multifidus* CSA was seen over time without WBV treatment, indicating that the horse's current environment, diet and exercise programme had no significant impact on the *m. multifidus* size over time. In contrast, addition of WBV to each horse individual exercise programme was able to induce hypertrophy of the *m. multifidus* in as early as 30 days and *m. multifidus* hypertrophy continued during the second 30 days of WBV.

The muscle response to training will depend on the primary function of the muscle and whether or not the training stimulus is appropriate for that particular muscle function and composition. In horses, the *m. multifidus* is comprised of approximately equal muscle fibre type (MFT–I) (slow twitch) and MFT-II (fast twitch) fibres indicating a dual postural and locomotor role (Hyytiäinen *et al.* 2014). The functional anatomy of the *m. multifidus* is comparable to that in man comprising a series of overlapping fascicles running caudo-laterally, spanning one to five intervertebral joints. There are superficial and deep fascicles with the superficial fascicles being longer (Stubbs *et al.* 2006).

In man, it appears that dynamic static resistance training (an exercise combining movement with a static holding pattern) is most effective in increasing CSA of the *multifidus* muscle (Danneels *et al.* 2001). This can be explained by the recruitment of a larger number of motor units, including both the low threshold motor units (MFT-I) and high threshold motor units (MFT-II) (Goldspink and Harridge 2002). In horses, the same principle regarding motor unit recruitment is true. It is, a larger number of motor units will be recruited as intensity, duration and speed of exercise increases (Rivero and Piercy 2008).

Horses standing quietly on a WBV platform perform, strictly speaking, a static rather than a dynamic static exercise such as dynamic mobilisation exercises described by Stubbs *et al.* (2011). However, WBV on itself appears to be similar to a dynamic static exercise. This seems to be confirmed by at least one research paper, showing that WBV training was superior to a low intensity resistance training in improving static and dynamic muscle strength in man (Verschueren *et al.* 2004). This idea is further supported by research findings in mice and rats using similar type WBV platforms as used in this study that show that WBV is able to induce both changes in MFT-I and MFT-II fibres (Xie *et al.* 2008; Lochyński *et al.* 2013), indicative for high muscle activation as seen with dynamic static exercises and resistance training. This high
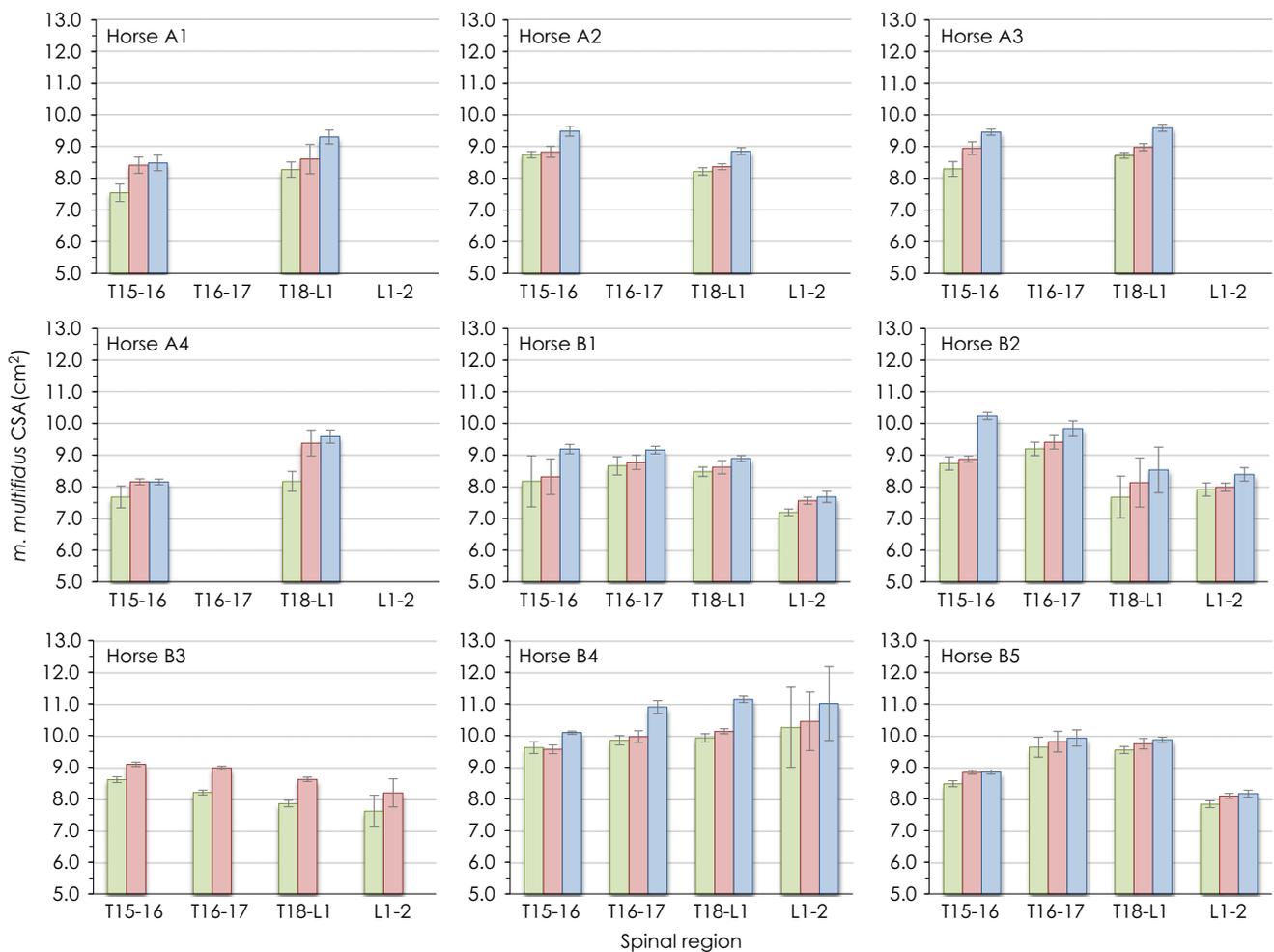
Fig 3: *The bar graphs display the* **m. multifidus** *CSA (cm^2) of each participant at each individual spinal regions for each of the three assessment periods (Days 0, 30 and 60). Each bar represents the average cross-sectional areas of left and right* **m. multifidus** *(mean ± s.d.) at four thoracolumbar levels in nine horses on Days 0, 30 and 60 of whole body vibration (WBV) treatment.*

muscle activation by WBV seems to be frequency dependent, with maximum activation of MFT-I fibres occurring at lower frequencies than for MFT-II fibres (Lochyński *et al.* 2013).

We assumed that a 60-day WBV protocol would be long enough for a muscle change to occur. This was based on the fact that in man 8–10 week WBV protocols are able to improve muscle CSA (Belavý *et al.* 2008; Machado *et al.* 2009) and in mice as little as 6 weeks (Xie *et al.* 2008). Whole body vibration training has been reported as being similar to resistance training (Bosco *et al.* 1998), with protein synthesis, a precursor for muscle hypertrophy, occurring after one resistance training session and changes in muscle size occurring after 4–6 weeks of resistance training (Gabriel *et al.* 2006). In horses specifically, significant muscle hypertrophy has been observed after a 2-month progressive training programme in unconditioned horses and changes in muscle fibre composition was noticed after short-term (3 weeks) specific training protocols in pretrained horses (Rivero *et al.* 2007).

The increase in the *m. multifidus* CSA seen after only 30 days is relatively fast. This could be related to the

immediate increase in muscle CSA seen post-exercise, which is well established in man and further supported by a more recent study in horses showing that a transient exercise-induced increase in back dimensions takes place (Greve *et al.* 2015). Although the mechanism of action responsible for a post exercise-induced increase in muscle CSA is not clear, one possible explanation is that the initial increase in muscle CSA is related to an increase in muscle fluid content rather than true hypertrophy secondary to increase in size and/or numbers of the contractile proteins (actin and myosin). This is supported by research in man, showing that an increase in muscle CSA is seen post-exercise secondary to extravascular fluid accumulation linked to muscle activity and perfusion (Nygren and Kaijser 2002). However, it is possible that the increase in CSA is related to a true muscle hypertrophy. A change in muscle fibre composition is readily seen after 3 weeks of specific training in pretrained horses (Rivero *et al.* 2007); the horses used in this study were pretrained and thus are likely to have been able to demonstrate a change in muscle fibre composition. Alteration in fibre size is easier achieved than a transformation (shift) from one muscle fibre type to another (Goldspink 1985),
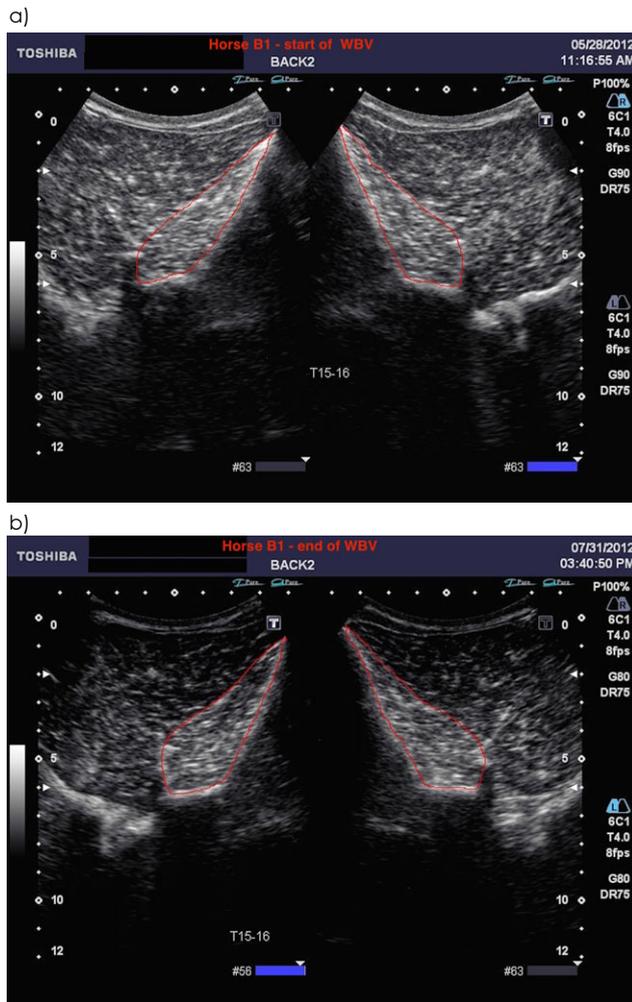
a)

b)

Fig 4: The m. multifidus *cross-sectional area (CSA) at the level of the T15–16 articular facet joints of Horse B1 is outlined in red. a) start of the whole body vibration (WBV) study. b) end of the WBV study. Note the difference in CSA between the assessment periods.*

meaning that whenever a shift in fibre type is seen, it is most likely proceeded by hypertrophy. This would tend to support the assertion that the changes noted in this study were predominantly due to muscle hypertrophy. Furthermore, we chose a high volume (two sets of 30 min twice daily), low intensity (0.5 g) WBV protocol, based on research in man. Researchers have speculated that longer duration of vibration may be necessary to elicit WBV effects on the neuromuscular systems (Osawa and Oguma 2013) and that low-load high volume resistance exercise is more effective at inducing acute muscle anabolism than high-load low volume resistance exercise due to full motor unit activation (Burd *et al.* 2010). Therefore, the combination of high volume WBV and the ability of WBV to increase recruitment of motor units is another explanation for the relatively fast increase in CSA seen in this study.

Although a trend was noted from Days 0 to 30 and Days 30 to 60, a statistically significant difference in left and right muscle symmetry was not seen until 60 days of WBV

treatment (**Table 3**). The reason for this is unclear. In man, transmissibility is influenced by posture (Rubin *et al.* 2003). As such, it is possible that because horses were allowed to stand in their preferred posture, resting a particular leg, that transmissibility was likely less uniform; thus affected one side (left or right *m. multifidus*) to a greater degree. The use of a stall unit with tilting platform might yield better outcome in that respect as it forces the horse to reposition itself at specific time intervals. Nevertheless, a significant improvement in muscle symmetry occurred and this improvement has been postulated to be a sign that the muscle is returning to a more normal physiological state (Stubbs *et al.* 2011).

The limitations of this study are the relative small sample size, a nonstandardised exercise programme and the fact that the CSA measurements were performed nonblinded. Nevertheless, we can conclude that WBV appears to be a viable alternative to dynamic mobilisation exercises to increase CSA and improve symmetry of *m. multifidus*. Further research will need to be performed to determine whether or not this increase in *m. multifidus* CSA has the potential to reduce back pain through improvement of the spinal function and stability.

Authors' declaration of interest

No conflicts of interest have been declared.

Ethical animal research

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Cross-sectional area measurement validation.

Supplementary Item 2: Mean and standard deviation (s.d.) of all cross-sectional area measurements.