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Four weeks of incline water treadmill exercise can contribute to increase epaxial muscle profile in horses.

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Abstract

Background: Water treadmill (WT) exercise is a popular modality for the training and rehabilitation of horses. However, evidence-based literature regarding the use of WT exercise, particularly using inclines, is lacking.

Objectives: The aim of this study was to assess the effect of recurring inclined WT sessions on equine epaxial muscles development.

Methods: Six horses completed 24 sessions of 15 minutes of WT activity over four weeks. Horses walked with water at mid-cannon level at a treadmill incline of 4%. Back traces were measured at three and seven centimetres ventral to the dorsal midline at T5, T9, T14 and T18, prior to the first session (W0) and weekly for 4 weeks (W1-4).

Results: Overall the back traces demonstrated progressive increases in muscle development ($p < 0.05$), starting at W2 up to W4. At three centimetres ventral to the dorsal midline the most to least significant increases in gross muscle development were at T18, T5, T9 and T14, respectively and when measured at seven centimetres ventrally, the most to least significant increases were demonstrated at T5, T18 and T14. It was noted that increases in thoracic back profile musculature were mainly observed within two to four weeks of the WT intervention.

Conclusions: It has been concluded that repeated WT exercise on an incline setting has a significant effect on the rate and size of growth of equine thoracic back profile musculature. Muscle hypertrophy due to resistance training in the WT starts at 2 weeks within the programme, and it progresses as exercise continues to be performed.

Key words: Biomechanics, Horse, Hydrotherapy, Kinematics, Spine

A preprint has previously been published[1].

1. Introduction

Water treadmill (WT) is a popular exercise for training and rehabilitation of horses[2]. Most of the research on equine WT are focused on the effects of the exercise during its practice, not on the long-term effects that can be accumulated. It has been well established that walking on a WT at different water levels will elicit different kinematics on limbs and back [3–8]. Although WT are used for rehabilitation of limbs and back injuries [2,9]there is currently a paucity of data regarding the long-term effect of WT exercise, particularly whether the use of water treadmill (WT) exercise on an incline setting causes an increase in musculature over the back of horses.. It is however understood that due to the unnatural movement of the treadmill belt causing the horse to become unbalanced they then chose to employ the neck and back muscles in order to re-establish their natural stride pattern. Once accustomed to the UWTM the horse can freely engage the muscles along their back without the added weight of a rider or saddle on top; the weight of the saddle and rider combined has previously been found to have a significant negative impact on the thoracolumbar posture [9]. It has also been observed that as the water level increases from the baseline there is a greater amount of flexion caused over the lumbar region of the back [4,5]. Interestingly, the velocity of the treadmill belt is also said to have an influence on the flexion and extension of the spine; as the velocity of the treadmill belt increases the horses body recruits the main muscles known for stabilising the vertebral column (*m. longissimus dorsi* and *m. rectus abdominis*)[10] . The *m. rectus abdominis* works as an antagonist muscle to the *m. longissimus dorsi* for stabilisation in the horse and so, with increasing water level this muscle works to flex the thoracolumbar spine; it could be hypothesised that this will in turn have a cumulative effect on muscle growth over the back-profile of the horse [11]. The bow and string theory can be used to help visualise the flexion and extension of the horses back; there are three main biomechanical observations of the equine back during locomotion known as the planes of movement: pelvic flexion (PF), lateral bending (LB) and axial rotation (AR) [6]. The bow and string theory relates with the flexion and extension of the thoracolumbar spine caused by the stabilisation muscles *m. longissimus dorsi* and *m. rectus abdominis* working antagonistic to one another. The *m. longissimus dorsi* eccentric and concentric contractions are synchronised with the opposing contractions of the *m. rectus abdominis* to maintain the centre of mass over the base of support[12] in order to counteract the destabilising forces caused by the internal visceral mass during ambulation [13,14]. However, it is important to remember that every case is different, an increase in thoracolumbar flexion is very much dependent on the horse's spinal conformation and range of motion (ROM) of their spinal joints [9]. Previous studies carried out on ventral spinal flexion and extension in walk and trot have shown a linear increase in muscular electromyographic activity of both the *m. longissimus dorsi* and *m. rectus abdominis* when changed from an incline of 0% to 6%[14,15]. It could be therefore postulated that the recruitment of the stabilisation muscles during walking on an UWTM whilst on an incline setting should in fact show an increase in facilitation and development of muscle across the thoracolumbar back profile more than walking on a level surface would. Remarkably, after just 10 days, UWTM training was shown to effect equine back ROM[6]There are however, little to no studies which have been carried out that describe or in fact, assess the effect that UWTM training has on the equine back profile musculature; further research such as the current study are needed in order to provide an insight into this gap in knowledge. Therefore, the aim of this study was therefore to assess the effect of recurring inclined WT sessions on equine epaxial muscles development. We hypothesised that inclined WT therapy would increase muscle mass in the epaxial musculature.

45 2. Materials and Methods

46

47 The material in this manuscript has been acquired according to guidelines set by The Animal (Scientific
48 Procedures) Act 1986 and the Declaration of Helsinki and has been approved by the Animal Welfare
49 and Ethics Committee of Writtle University College. The approval number is 98360534/2019. A written
50 informed consent was obtained from the owners of the participants of the study. Veterinary surgeon
51 consent was obtained

52

53 2.1. Horses

54 This study involved six healthy, clinically sound and WT habituated horses, with a mean \pm standard
55 deviation (SD) age of 9.5 ± 3.73 years old and mean height was 169 ± 22 cm. The six subjects
56 encompassed four breed types: Hanoverian (n=1) Thoroughbred (n=1) Irish Sport Horse (n=3) and
57 Dutch Warmblood (n=1). All six were sourced in Northern Ireland from The Irish Equine
58 Rehabilitation and Fitness Centre. Horses were training and competing at varying levels mainly for
59 dressage and showjumping. Horses were stabled at The Irish Equine Rehabilitation and Fitness Centre
60 and each were fed their own standard diets for the duration of the trial. Besides of the WT exercise,
61 horses had a walk in-hand exercise, ranging from 5 to 10 minutes a day at the start of the trial, reaching
62 15 to 20 minutes a day by the end of the trial. To ensure for validity and reliability of the research, the
63 same experienced handler, prepared, loaded, and secured each horse in the treadmill, and operated the
64 individual treadmill programmes. Sample size was calculated using the resource equation approach [16],
65 based on published studies assessing back musculature increase as cross-section area with different
66 therapies, adequate statistical discrimination has been achieved with between 3 to 8 horses per
67 group [17,18]. Assuming the methods differ in their intervention and outcomes, estimates by 10%, for
68 Type I and II errors of 0.05 and 0.20, respectively, using the Bland-Altman Test we estimated a sample
69 size of between 5 to 11 horses would be needed (MedCalc® Statistical Software version 20.115).

70

71 2.2. Water treadmill (WT) exercise

72 The WT (ECB Aqua Treadmill, ECB Equine, UK) sessions were standardised for all horses and weeks
73 and consisted of 24 sessions of 15-minute each over four weeks, with 6 sessions being undertaken
74 weekly. The before measurements were called W0, the measurements after the first week was named
75 W1 and so on until the measurements after the fourth week of exercise, week 4, which was named W4.
76 The water height, on every session, was middle of third metacarpal bone. The WT belt speed was
77 determined by selecting a safe but also effective walking velocity that was comfortable for each horse,
78 as assessed by the therapist, which was then maintained throughout the entirety of the data collection.
79 Speed ranged from 3.5-3.8 km/h with a mean of 3.63 ± 0.14 km/h. Angle of incline for the WT belt was
80 set at 4% to ensure all subjects could cope with the same inclination intensity.

81

82 2.3. Back Profile Dimension Measurements

83 Prior to the first WT treadmill session, fifth (T5), ninth (T9), fourteenth (T14) and eighteenth (T18)
84 thoracic vertebrae were identified by palpation. Measurements were taken at each location using a
85 saddle fitting kit (The Perfect Fit, San Francisco, USA) which includes a 50 cm flexible curve ruler
86 (pliable metal encompassed by rubber) to shape around the dorsal surface of the back, following the
87 contours of the body (Figure 1). A flexible curve ruler can be moulded to the contours of the spine and
88 has been validated for assessment of transverse muscular profile [19].

89 The levels identified for the relevant vertebrae was marked with clipping a small patch of hair, to
90 ensure the measurements were repeated at the same landmarks. The subsequent outline was then
91 mapped out on A3 graph paper (Figure 1) [19], each timepoint on a separated sheet to denote the
92 different measurements and avoid bias. For one single horse, besides of tracing the back profiles of
93 each timepoint on separated sheets, the profiles were also traced on a common sheet to allow the
94 visualisation of all timepoints on single sheet, only for purposes of illustration of results. The same
95 researcher (NF) repeated each measurement throughout the study. Before taking measurements, the

96 assessor ensured the horse was standing squarely, with its weight evenly distributed over all four limbs,
97 and the head and neck position was maintained in a neutral posture with the horse's mouth level with
98 the point of the shoulder by an assistant. All measurements were taken in a concrete flat surface
99 adjacent to the WT. The thoracic back profile dimension was measured immediately before the first
100 WT session (W0) and after each 6 sessions (weekly W1-W4), totalling 5 measurements during the 4
101 weeks of data collection. The measurements after sessions was done 20 minutes after the exercises to
102 ensure cool down, as it has been reported that immediately after exercise muscle cross-sectional area
103 may change[20]. The thoracolumbar dimensions were defined as the widths, in cm, at two levels: 3
104 cm and 7 cm ventral to the dorsal midline (Figure 2).

105



106

107 Figure 1. Representation of back profile dimensions measurements at T5 level. On the left the flexible
108 ruler was used at T5 level, over the spine, to shape the back profile. On the right, the back profile was
109 transferred to a graph paper.

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111 2.4. Statistical Analysis

112 Once all measurements were taken a standard ruler was used to measure the width of the back traces,
113 in centimetres, at three and seven centimetres below the dorsal line, using each of the A3 traces
114 collected, with data recorded in excel, and subsequently transferred to SPSS v26.0.

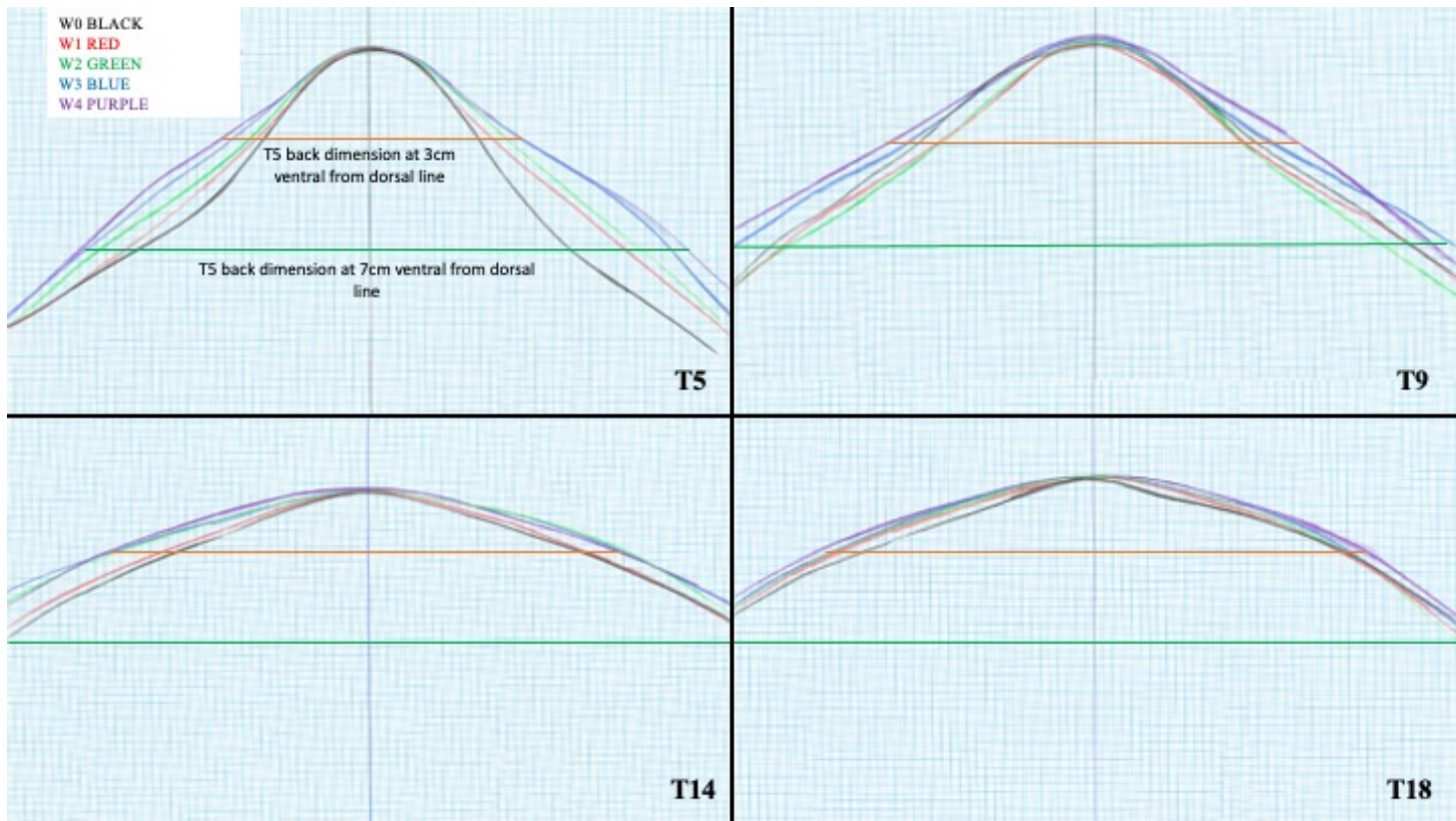
115 The mean \pm SD of the back traces at each timepoint was calculated and subjected to a Shapiro-Wilk test
116 for normality which showed parametric data. Following this, all data underwent Mauchley's test of
117 sphericity to identify whether the assumption of sphericity had been violated, if this was the case, the
118 Greenhouse-Geisser correction was applied to the within subjects' effects. From here, it was ascertained
119 as to whether the back traces exhibited statistically significant increases ($p < 0.05$) between different
120 timepoints. As all the data were parametric ($p > 0.05$) (apart from W7, M5 on T14, however there was no
121 outliers as assessed by inspection of the boxplots), they all underwent assessment by the means of one-
122 way repeated measures analysis of variance (ANOVA). A post hoc analysis was then conducted with
123 Bonferroni correction (95% confidence interval (CI)) assessments in order to determine where the
124 statistically significant differences of the back traces lay, and between which timepoints. The results
125 report SPSS Bonferroni adjusted p-values.

126

127 3. Results

128 An example of back width measurements taken over the period of four weeks can be seen in a
129 representative image of the back profile dimensions measurement is presented in figure 2 (note that
130 this is just an illustrative figure, and the measurements were taken on timepoints traced in separated
131 sheets to avoid bias).

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Figure 2: Example graphs illustrating the changes in back dimension before the water treadmill programme (W0) and after each week of treatment (W1-W4) of water treadmill programme at fifth (T5), ninth (T9), fourteenth (T14) and eighteenth (T18) thoracic vertebrae before. The orange lines shows the levels of measurement at 3cm (orange line) and 7cm (green line) ventral from the dorsal line.

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3.1 T5

141 The width of the T5 back traces at three centimetres below the dorsal line were statistically significant
142 different at timepoints ($F(4, 20) = 5.443, p = 0.004$). The width of the T5 back traces at three centimetres
143 below the dorsal line had statistically significantly increased from measurement W0 (before any WT
144 activity) when compared with W3 (1.067 (95% CI, 0.108 to 2.025) cm, $p = 0.035$), and with W4
145 (1.383 (95% CI, 0.339 to 2.427) cm, $p = 0.019$). Statistically significant increases were also noted from W1
146 when compared with W4 (1.133 (95% CI, 0.404 to 1.862) cm, $p = 0.010$), and from W2 compared to W4
147 (0.933 (95% CI, 0.141 to 1.726) cm, $p = 0.029$) as illustrated in figure 3. The other pairwise
148 comparisons were not significant ($p > 0.05$).

149 The width at T5 level at seven centimetres below the dorsal line were also statistically significant at
150 different timepoints ($F(1.461, 7.303) = 7.271, p = 0.023$). Test revealed a statistically significant increase
151 from measurement W0 when compared with W3 (2.717 (95% CI, 0.125 to 5.308) cm, $p = 0.043$), and
152 with W4 (3.150 (95% CI, 0.063 to 6.237) cm, $p = 0.047$). Statistically significant increases were also
153 noted from W1 when compared with W3 (2.050 (95% CI, 0.661 to 3.439) cm, $p = 0.013$), and with W4
154 (2.483 (95% CI, 0.977 to 3.990) cm, $p = 0.008$), and from W2 when compared with W3 (1.717 (95% CI,
155 0.147 to 3.286) cm, $p = 0.038$), and with W4 (2.150 (95% CI, 0.537 to 3.763) cm, $p = 0.019$) as illustrated
156 in figure 3.

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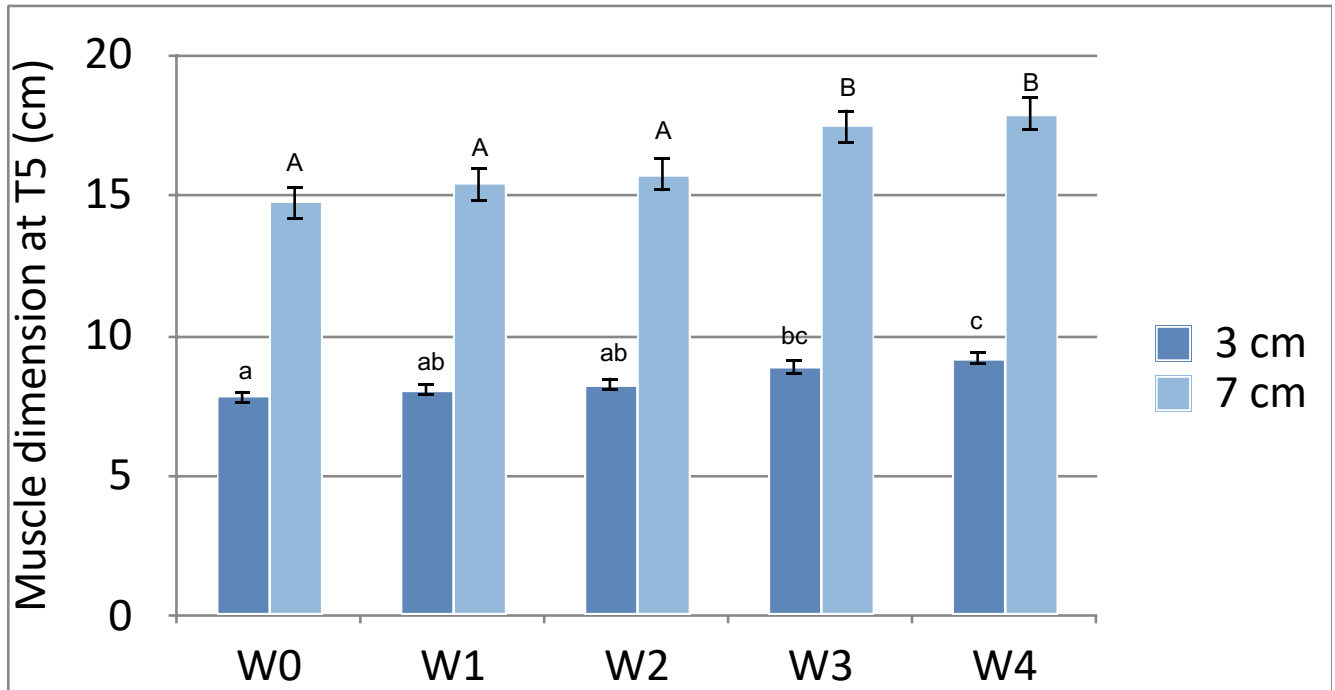


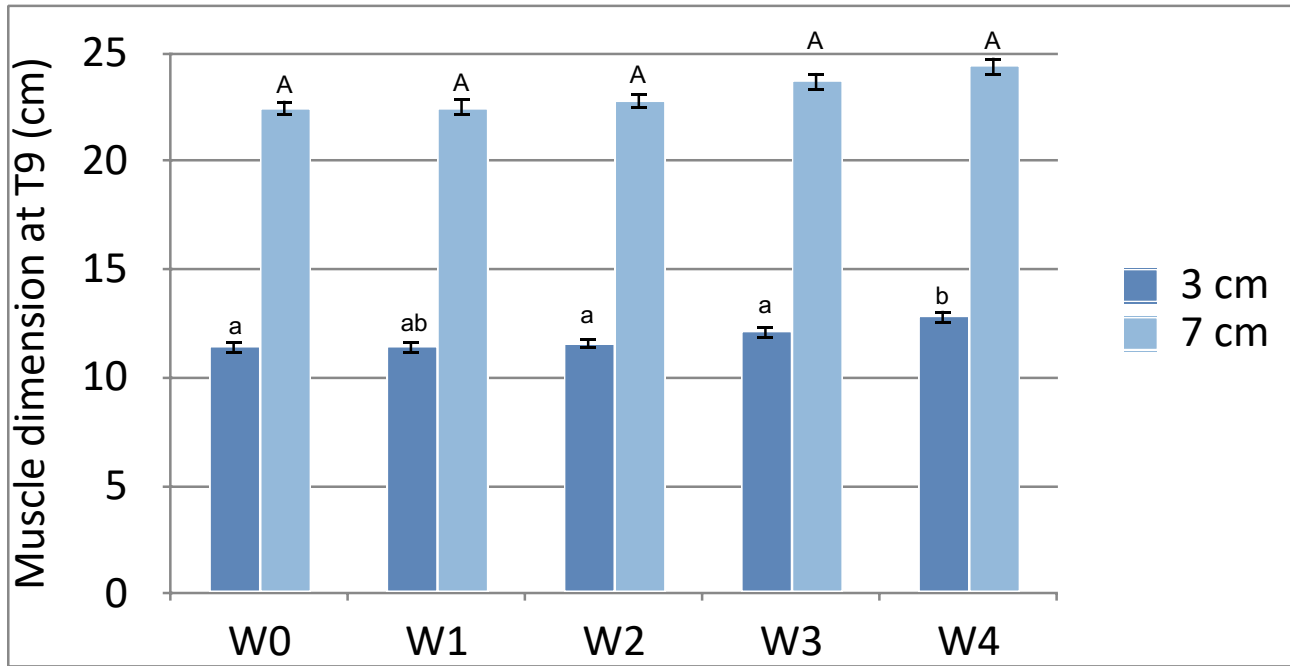
Figure 3: Muscle dimensions at T5 before the water treadmill programme (W0) and after each week of treatment (W1-W4) (n=6). Standard error is shown. Statistically significant differences, by repeated measures ANOVA, are indicated by different letters. Lower case letters are used for measurements 3 cm ventral to the dorsal line. Capital letters are used for 7 cm ventral to the dorsal line.

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3.2. T9

166 The width of the T9 back traces at three centimetres below the dorsal line were statistically significant at
167 different timepoints ($F(4,20) = 3.805, p=0.019$). Post hoc analysis with Bonferroni correction revealed
168 that the width of the T9 back traces at three centimetres below the dorsal line had statistically
169 significantly increased from W0 (before any WT activity) when compared with W4 (1.400 (95% CI,
170 0.091 to 2.709) cm, $p = 0.040$), and from W2 when compared with W4 (1.250 (95% CI, 0.136 to 2.364)
171 cm, $p = 0.034$), and from W3 when compared with W4 (0.717 (95% CI, 0.118 to 1.316) cm, $p = 0.028$)
172 as illustrated in figure 4. The other pairwise comparisons were not significant ($p>0.05$).

173 Traces of T9 profile at seven centimetres below the dorsal line at different timepoints were not
174 statistically significant ($p>0.05$) (figure 4).



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176 **Figure 4:** Muscle dimensions at T9 before the water treadmill programme (W0) and after each week of treatment (W1-
 177 W4) (n=6). Standard error is shown. Statistically significant differences, by repeated measures ANOVA, are indicated by
 178 different letters. Lower case letters are used for measurements 3 cm ventral to the dorsal line. Capital letters are used for 7
 179 cm ventral to the dorsal line.

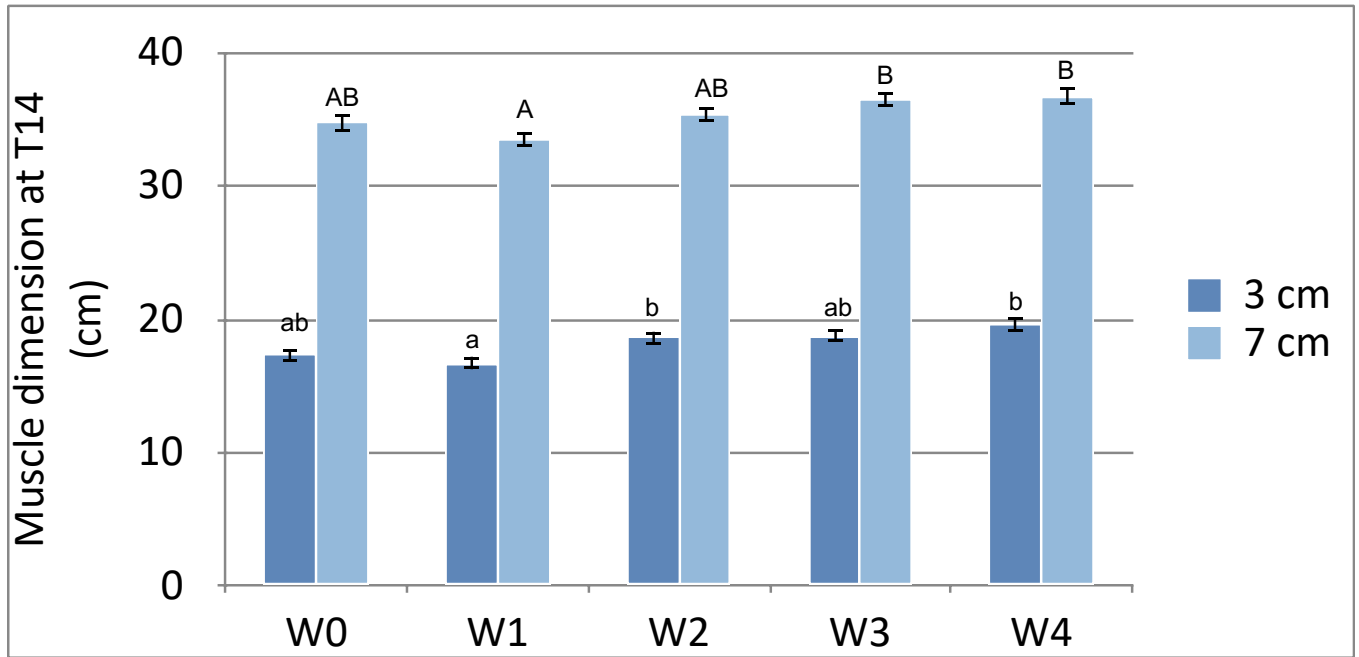
180

181 *3.3. T14*

182 At three centimetres below the dorsal line back traces at T14 were statistically significant different between
 183 timepoints ($F(4,20) = 3.849, p=0.018$). The width of the T14 back traces at three centimetres below the
 184 dorsal line have statistically significantly increased from W1 when compared with W2 (1.9 (95%CI,
 185 0.103 to 3.697) cm, $p=0.042$) as well as when compared to W4 (2.867 (95% CI, 0.167 to 5.566) cm, p
 186 $= 0.004$), as illustrated in figure 5.

187 Traces of T14 at seven centimetres were also statistically significant different at timepoints ($F(4,20) =$
 188 $3.108, p=0.038$). The width of the T14 back traces at seven centimetres below the dorsal line had
 189 statistically significantly increased from W1 when compared with W3 (2.933 (95% CI, 0.060 to 5.807)
 190 cm, $p = 0.047$) and with W4 (3.233 (95% CI, 1.037 to 5.429) cm, $p = 0.013$) as illustrated in figure 5.

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193 **Figure 5:** Muscle dimensions at T14 before the water treadmill programme (W0) and after each week of treatment (W1-
 194 W4) (n=6). Standard error is shown. Statistically significant differences, by repeated measures ANOVA, are indicated by
 195 different letters. Lower case letters are used for measurements 3 cm ventral to the dorsal line. Capital letters are used for 7
 196 cm ventral to the dorsal line.

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198 *3.4 T18*

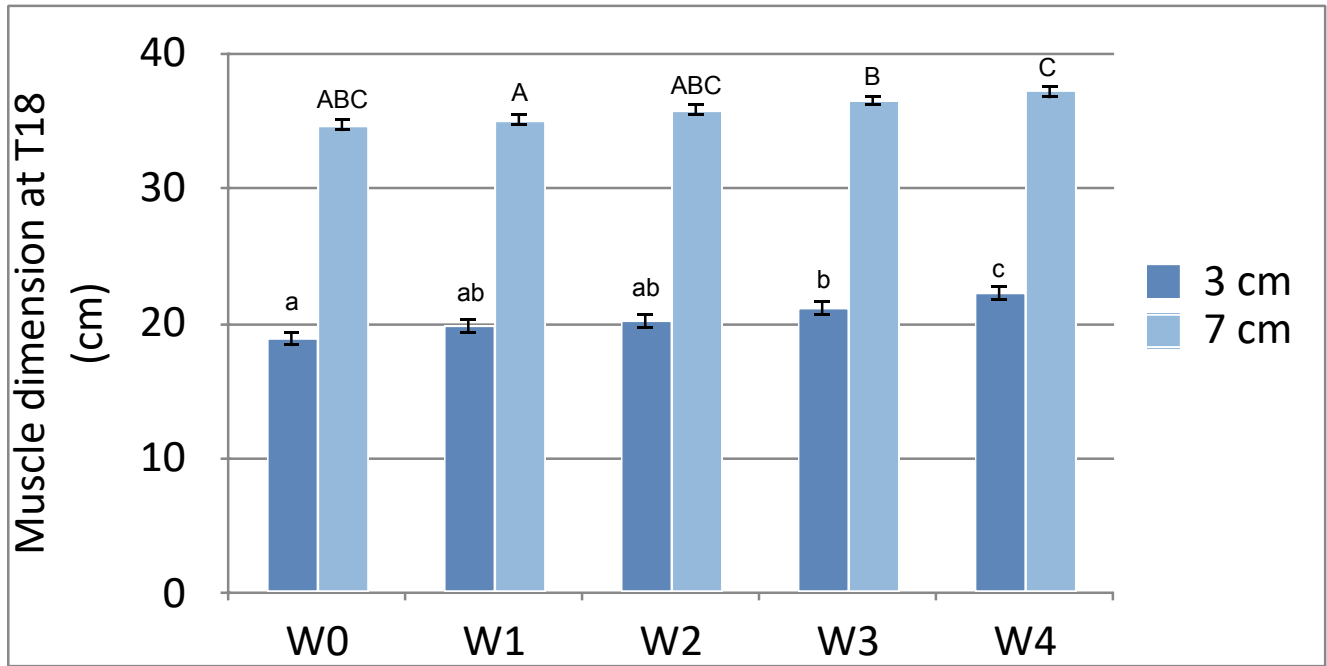
199 Statistically significant differences at the 3cm deep measures were observed at different timepoints
 200 ($F(2,150, 10.751) = 5.937, p=0.003$). T18 back traces at three centimetres below the dorsal line have
 201 statistically significantly increased from W0 when compared with W3 (2.283 (95% CI, 0.201 to 4.366)
 202 cm, $p = 0.037$), and with W4 (3.300 (95% CI, 1.252 to 5.348) cm, $p = 0.009$). Statistically significant
 203 increases were also noted from W1 when compared with W4 (2.283 (95% CI, 1.008 to 3.559) cm, $p =$
 204 0.006), and from W2 when compared with W4 (2.017 (95% CI, 0.320 to 3.713) cm, $p = 0.028$), and from
 205 W3 when compared with W4 (1.017 (95% CI, 0.123 to 1.911) cm, $p = 0.033$) as illustrated in figure 6.

206 At seven centimetres trace widths at T18 were statistically significant at different timepoints ($F(4,20) =$
 207 3.551, $p=0.024$). T18 back traces at seven centimetres below the dorsal line have statistically
 208 significantly increased from W1 when compared with W3 (1.417 (95% CI, 0.092 to 2.741) cm, $p =$
 209 0.040), and with W4 (2.100 (95% CI, 0.813 to 3.387) cm, $p = 0.009$), and from W3 when compared with
 210 W4 (0.683 (95% CI, 0.059 to 1.307) cm, $p = 0.037$) as illustrated in figure 6.

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215 **Figure 6:** Muscle dimensions at T18 before the water treadmill programme (W0) and after each week of treatment (W1-
 216 W4) (n=6). Standard error is shown. Statistically significant differences, by repeated measures ANOVA, are indicated by
 217 different letters. Lower case letters are used for measurements 3 cm ventral to the dorsal line. Capital letters are used for 7
 218 cm ventral to the dorsal line.

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241 4. Discussion

242 The present study was conducted with the purpose of investigating the efficacy of recurring WT
243 exercise with incline resistance to cause increases in thoracic epaxial muscles of horses. Overall,
244 significant increases in epaxial muscle profile have started to be shown at W3 of the WT therapy, with
245 usually a further increase at W4. The most significant changes were seen at T5 and T18 level, although
246 all back points assessed have shown some increase in epaxial muscle profile. Our results, as a whole
247 have supported the hypothesis that WT therapy can help on the development of the epaxial muscles.
248 To the authors' knowledge, the present study is the first of its kind to periodically measure the difference
249 in equine back profile musculature over a four-week period, using back traces at four different points
250 along the thoracic vertebrae. Current literature regarding the use of the WT as a rehabilitation tool is limited
251 with respect to its efficacy in activating and building the paraspinal musculature in the thoracic region.
252 This study demonstrates statistically significant increases in muscle development at each of the
253 anatomical reference points (T5, T9, T14 and T18) at three centimetres ventral to the dorsal midline,
254 with the most significant increases in muscle development noted at T18. Although, during UWT work,
255 the thoracolumbar joint (T18/L1) sees a decrease in flexion-extension in comparison with more cranial
256 thoracic areas[21], we could infer that deeper stabilising muscles may have been activated. It is well
257 known that incline of treadmill plays a role in epaxial muscles activation, particularly *longissimus dorsi*[14].
258 When treadmill inclination changed from 0 to 6%, EMG activity of the *longissimus dorsi* began and ended
259 later; therefore, a longer activity duration was noted[14]. Lower inclinations as 3% has also been reported to
260 increase EMG values for the *longissimus dorsi*[22] and this effect of the incline has contributed towards the
261 increased epaxial profile reported in our study, as we have used an incline of 4%.

262 The next most statistically significant increases in muscle growth were found at T5, noted as the highest
263 point of the withers, and as such is subject to the greatest level of shear forces during ridden exercise due
264 to direct transmission of rider weight through the stirrups, which may affect the muscles responsible for
265 protraction and retraction of the forelimbs[23]. It could be suggested that the muscle development in
266 this region may be due to the horse being able to freely engage the muscles in this area without the
267 negative influence of a rider or saddle during the WT exercise[5]. Additionally, it could be postulated that
268 the increases in size of the musculature was facilitated by the water level being set low enough to allow
269 the horses to lower their head and neck, causing traction on the withers from the nuchal and supraspinous
270 ligaments[24,25]. This creates cranial thoracic flexion [26] and activates the vertebral stabilisation
271 muscles. Furthermore, research has reported that WT exercise increases considerably activation of *m.*
272 *splenius*[22], and as this muscle inserts at T5, its activations during WT exercises could have
273 contributed towards the muscle profile observed at this level in our study.

274 Statistical significance was also apparent at T9 between different timepoints, which may be associated
275 with the specific anatomical characteristics of the vertebrae involved in the adjacent articulations, which
276 determines the degree of movement at each joint complex[27]. The body of vertebral segments T2-T9 are
277 shorter but provide larger points of attachment for the nuchal and supraspinous ligaments as well as for
278 the relevant muscles which produce the movements of the back[28]. Therefore, possessing the capacity
279 for greater amounts of intervertebral movement, displacement, and axial rotation between these joint
280 complexes, due to the elasticity of the ligaments[25,27,28]. The aforementioned biomechanical
281 characteristics of the ninth thoracic vertebrae may have contributed to muscle build by allowing
282 increased spinal ROM, stimulating antagonistic epaxial and hypaxial muscle contractions to resist
283 excessive displacement of the vertebral column[29]. Furthermore, it has been discovered that movements
284 of the back, limbs, head and neck are closely associated[27,30], therefore it can be assumed that
285 exercising on an incline will require synergistic contractions of both the back and hindlimb muscles to
286 produce propulsion in the sagittal plane[13].

287 We therefore hypothesise that the benefits of the incline associated with the benefits of water at mid cannon
288 bone, have created a synergic benefit for the muscular development in our study.

289 The vertebral segment which revealed the least differences in muscle build between the various timepoints
290 was T14, only demonstrating a statistically significant average increase of 2.9 cm between week one and
291 four of WT intervention. Upon closer inspection of the results, apart from an initial decrease following

292 the first week of WT exercise, it is clear that a smaller cumulative increase in gross muscle size occurred
293 each week, however these may have been too minimal to reach statistical significance. The statistically
294 significant increase in muscle mass between week one and four may have occurred as the greatest
295 amounts of axial rotation and lateral bending occur at the vertebral level of T14[28,31] due to the
296 presence of asternal ribs, which are indirectly attached to the sternum[28] Hence, the increased axial
297 rotation and lateral bending experienced in this vertebral region, along with the increased dorsoventral
298 flexion-extension caused by exercising on an incline[13,15,32] are both expected to have influenced
299 the facilitation and therefore also the gradual increase in mass of the *m. longissimus dorsi* and *m. rectus*
300 *abdominis* in the current study as they are required for vertebral stabilisation in the horse[32]. What is
301 worthy of note is that the timing of increases correlate with the effect of resistance training and the
302 process of hypertrophy, whereby a minimum of two to four weeks of resistance training are required
303 in humans to facilitate skeletal muscle hypertrophy[33].

304 At 7cm, our results indicated that the most significant increases in overall thoracic musculature
305 development occurred at T5 and T18. A possible explanation for the differences noted between the back
306 traces at three and seven cm could be attributable to the function of the hypaxial muscles which are
307 situated ventrally to the transverse processes of the spine. The hypaxial muscles mainly function to
308 produce flexion of the cranial thoracic spine while the *m. rectus femoris* is activated to counter spinal
309 extension[34]. Furthermore, as the *m. rectus abdominis* inserts onto the head of the femur it could be
310 postulated that this muscle plays a role in the synergistic contractions of the back and hindlimbs during
311 forward propulsion[13] possibly explaining the significantly increased muscle mass in the region.
312 Comparatively, the next most statistically significant increases in thoracic muscle development was
313 observed at T18. It could be inferred that the increased muscle growth observed when measured further
314 ventrally, could be linked to the slightly more lateral and ventral origin of the *m. obliquus abdominis*
315 *externus* on the lateral surface of ribs 4- 18 and the thoracolumbar fascia. Likewise, the *m. rectus*
316 *abdominis* is recruited during the second half of the stride in the region of T18 once the forelimb is
317 protracted and ipsilateral hindlimb is retracted to counter the excessive thoracolumbar extension caused
318 by the downward movement of the internal visceral mass[29], possibly further explaining the increase
319 in muscle development when exercising against increased resistance from both the water[8] and
320 inclination[14,15]. Statistical significance was also evident at T14, although not to the same extent as
321 T5 and T18 as only two of the timepoints demonstrated increases in gross muscle development. This
322 could also be attributable to the more controlled exercise in the sagittal plane without the added weight of
323 a saddle or rider[5,35] which may allow for more symmetrical muscle development than when
324 exercising on land[15]. As for the significant increases in thoracic musculature observed at T14, the
325 greatest amounts of axial rotation and lateral bending occur in this region during locomotion[28,31].
326 Moreover, the horse also shows increases in hindlimb ROM when walking in water[8]. Research by
327 Mooij[6] has also suggested in order to produce hindlimb movement in the water, there must first be a
328 further increase in axial rotation of the caudal thoracic spine, which may explain the resulting increased
329 muscle development at the level of T14.

330 No statistically significant increases in thoracic back profile musculature, were observed at T9 when
331 measured at 7 cm, however there was evidence of cumulative weekly increases in the back trace widths
332 when compared to the individual means.

333 Overall, our research agrees with a study recently presented, which performed a semi-qualitative
334 analysis of muscle development in horses undergoing WT work and has concluded that WT can
335 increase thoracic epaxial muscle development, after 20-weeks of exercise[36]. However, our study has
336 found earlier improvements at muscle development at thoracic epaxial musculature. We attribute this
337 earlier muscle development due to the fact we have used an incline on the WT, while the previous
338 study[36] did not mention incline so was probably performed under flat WT. As mentioned before,
339 both water and incline have an influence on epaxial musculature activation and, therefore, growth.
340 Hence, we believe that the combination of these two elements have contributed to the earlier positive
341 results observed in our study.

342 That said, our study also had its limitations. The first could be small sample size, which could lead to

343 the assumption that our results may lack ecological value; that being said, the majority of our results
344 demonstrated statistical significance between different timepoints, which warrants further investigation
345 with a larger sample size in the future to more precisely evaluate the effects of the intervention, to allow
346 more definitive conclusions to be drawn. A potential limitation concerning the repeated measures study
347 design is the effect that the treatment intervention has on subsequent treatments, the lack of control group
348 and not having flat UWT and inclined dry treadmill groups to ascertain which independent variable
349 (water or incline) was more relevant in eliciting the observed changes, or if both interventions will
350 contribute towards muscle hypertrophy. As this study utilised a longitudinal layout over the course of
351 four weeks, it could be said that biased estimates of the treatment efficacy may be made due to the carry-
352 over effect of the treatment modality. However, this study design emphasised comparisons within each
353 horse over time, and as horses had a similar trend on muscular growth, we can attribute this to the
354 exercise on the inclined WT with a good certainty, although we cannot attribute the effects to the water
355 or incline separated. Despite all limitations, it is our belief that the current study presents an insightful
356 account into the rate and size of paraspinal muscle growth in the thoracic region through the
357 implementation of repeated WT exercise with incline resistance. Another limitation is that as a result
358 of being reshaped repeatedly the flexible curve ruler is susceptible to deterioration over time, therefore
359 it is advised to frequently replace the flexible curve ruler used for data collection to ensure the results
360 remain consistent. We have used the same piece of equipment all throughout the measurements and
361 we have not noticed any significant misshapen of the ruler. We also acknowledge, that there are other
362 more objective means to measure epaxial muscle changes in size as evaluation of cross-sectional area
363 with ultrasound scans as described in the literature [37,38]. However, our choice to use the flexible
364 curve ruler was due to equipment availability and the fact there is a validation for the use of this tool⁹.

366 **5. Conclusions**

367 The results of this study support the original hypothesis, revealing that repeated WT exercise on an incline
368 setting does in fact have a significant effect on the rate and size of growth of the equine thoracic back
369 profile musculature, although further research should be conducted to determine which intervention is
370 more relevant, incline or water, and if both interventions have a synergic effect, as well as which specific
371 muscles are recruited during WT intervention with incline resistance and to establish the increases in
372 cross-sectional area of the individual muscles with ultrasound scans rather than just the total increases
373 in overall muscle development.

374
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379 **Declarations**

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383 ***Conflicts of Interest***

384 The authors declare that there are no conflicts of interest

386 ***Data Availability***

387 The data that support the findings of this study are available from the corresponding author, RFG,
388 upon reasonable request.

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