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1 **Effect of water depth on muscle activity of dogs when walking on a water treadmill.**

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6 **Abstract**

7 Evidence-informed practice is currently lacking in canine hydrotherapy. This study aimed to
8 investigate if the estimated workload of the gluteus medius (GM) and longissimus dorsi (LD)
9 increased in dogs at different water depths when walking on a water treadmill. Seven dogs
10 were walked for two minutes continuously on a water treadmill at depths of no submersion
11 (depth 1), mid-tarsal (depth 2), between lateral malleolus and lateral epicondyle (depth 3) and
12 between the lateral epicondyle and greater trochanter (depth 4). Continuous electromyographic
13 data from the right and left sides of GM and LD were collected simultaneously during exercise.
14 Friedman's analyses with post-hoc Wilcoxon tests established if significant differences in GM
15 and LD muscle activity occurred between the water depths for mean estimated-workload.
16 Significant differences occurred in estimated-workload in GM and LD between water depths
17 ($P<0.05$). Mean estimated-workload decreased in the right and left GM between depths 2 (mid-
18 tarsal) and 3 (between lateral malleolus and epicondyle) ($P<0.007$) and depths 2 and 4 (between
19 lateral epicondyle and greater trochanter) ($P<0.001$), a pattern which was repeated for left and
20 right LD ($P<0.007$). Right GM mean estimated-workload increased between depth 1 (no
21 submersion) and depth 2 only ($P<0.013$). Water depth influences GM and LD activity in dogs
22 walking on a water treadmill. Increasing knowledge of canine locomotion in water treadmills
23 could be used to inform individualised rehabilitation regimes for dogs undertaking
24 hydrotherapy.

25 Key words: rehabilitation; canine; hydrotherapy; water treadmill; water depth

26

27 **Introduction**

28 Canine rehabilitation is a rapidly developing aspect of veterinary medicine with a growing
29 range of methods and techniques such as manual therapy, therapeutic exercise, physical
30 modalities, massage and hydrotherapy becoming widely available for use in veterinary practice
31 (Tomlinson, 2012). These rehabilitation methods are utilised to restore animals to full health
32 post-operatively, to manage long-term conditions such as osteoarthritis and to maintain general
33 fitness (McGonagle and Taylor, 2004). With scientific research informing changes in industry
34 practice, improvements in training and rehabilitation of non-canine animals have been achieved
35 using evidence-based practice (McGowan et al. 2002). However, many areas such as
36 hydrotherapy still lack an evidence base despite them being widely used in canine rehabilitation
37 (Waining, Young and Williams, 2011; Kirkby and Lewis, 2012).

38 A range of rehabilitation exercises using hydrotherapy exist; swimming and water treadmills
39 (WT; also known as under water treadmills) have been found to be beneficial in the recovery
40 of dogs postoperatively (Monk, Preston and McGowan, 2006). WTs are commonly utilised in
41 hydrotherapy for dogs presenting with hind limb and spinal pathologies as a core component
42 of rehabilitation regimes, and are also used as a fitness and conditioning tool within canine
43 performance training (Davies, 2011). Controlled swimming and WT exercise increase limb
44 flexion and extension and can produce a larger range of motion (ROM) in the limbs when
45 compared to overground walking in dogs (Marsolais, Dvorak and Conzemius, 2002; Marsolais
46 et al. 2003; Monk, Preston and McGowan, 2006). Altering the depth of water during exercise
47 on the WT will also influence kinematics; studies have demonstrated that increased flexion and
48 extension of the stifle and stride lengths (SL) occur with increasing water depth, whilst in
49 contrast stride frequency (SF) decreases as water level height increases (Jackson et al. 2002;
50 Barnicoat and Wills, 2016).

51 Although there is limited research to date into the use of WTs for dogs, the impact of WT
52 exercise on equine kinematics has been more extensively researched and, as a quadruped
53 species, could provide a comparative evidence base for canine WT studies, although more
54 canine-specific studies are needed to confirm this as anatomical differences do exist between
55 the species. In horses, water depths at carpal, tarsal, metacarpophalangeal and
56 metatarsophalangeal joint levels are commonly utilised during rehabilitation (Nankervis et al.,
57 2017). Kinematic evaluation of equine locomotion at different water depths on the WT suggest
58 that if the horse can, it will step out and over the water (Mooij et al., 2013) subsequently

59 increasing flexion and extension in joints above the water level, with the greatest variation in
60 ROM occurring at tarsal height (Mendez-Angulo et al. 2013). Higher water levels correspond
61 to increased buoyancy and reduced ground reaction forces (King, 2016) and when used within
62 a five week rehabilitation regime have been shown to reduce postural sway and to increase
63 limb joint stability in horses (King et al., 2013). Nankervis et al. (2015) have also demonstrated
64 horses adapt their locomotion at higher water levels (stifle and above), resulting in greater
65 cranial thoracic extension and thoracolumbar flexion when compared to walking at lower water
66 depths due to alterations in head position and increased buoyancy. At the same time, at higher
67 depths, increases in flexion and rotation of the back of horses also occur, attributed to increased
68 axial rotation and pelvic flexion (Mooij et al. 2013). However, understanding the influence of
69 water height on kinematics does not provide definitive information on how muscle function
70 adapts to generate the locomotion patterns observed. Joint ROM is influenced by muscle
71 activity, consequently, kinematic studies evaluating joint ROM can provide a broad visual
72 representation of muscle activity during rehabilitation (Kaneda et al. 2007; Agostini et al. 2014;
73 Gommans et al. 2016). Therefore to fully understand the impact of WT treadmill exercise at
74 different water depths in both dogs and horses, further studies evaluating how muscle
75 recruitment and workload varies with changing water heights and speeds are required. The
76 research base within equine WT exercise has developed recommendations for use in practice
77 (Nankervis et al., 2017). A similar approach bringing together kinematic and
78 electromyographic assessment of canine performance on the WT to inform canine
79 rehabilitation protocols is warranted.

80 Surface electromyography is a non-invasive technology, which can be used to assess muscle
81 activity in animals (Williams, 2017). The role of muscles within the axial musculoskeletal
82 system of dogs is not currently well understood despite their functional importance in terms of
83 facilitating postural stability and locomotion (Webster et al., 2014). Schilling and Carrier
84 (2010) identified that the epaxial muscles were involved in stabilisation and sagittal extension
85 of the spine during movement. Similar roles have been established in equine epaxial
86 musculature, where longissimus dorsi (LD) has been demonstrated to ensure stiffness and
87 stabilisation of the vertebral column during locomotion in horses (Licka et al. 2004; Robert et
88 al. 2001; 2002). As horses and dogs utilise comparable gaits, similar roles are expected for LD
89 across species (Robert et al. 2001; Groesel et al. 2010). Ritter et al (2010) and Schilling and
90 Carrier (2010) used EMG to demonstrate LD activity during the trot stride cycle. In the equine
91 a burst of activity is related to push off of the ipsilateral hind limb and a second burst at push

92 off of the contralateral hind limb whilst in the canine a similar biphasic activity is seen but
93 initially during the second half of ipsilateral stance and then again in the second half of the
94 contralateral stance (Ritter et al, 2010), although Schilling and Carrier (2010) report the second
95 burst as during the last third of the ipsilateral hindlimb swing. Therefore in the canine and
96 equine spine it appears that LD acts to counteract the tendency of the trunk to flex and extend
97 in the sagittal plane and therefore provide stiffness of the spine during gait.

98 In dogs, as in horses, movement is initiated in the gluteal and hamstring muscles (Williams et
99 al., 2008; Payne et al., 2005; Wentink, 1976). Few studies have investigated canine caudal
100 musculature to date despite their key contribution to locomotion. The role of gluteus medius
101 (GM) during locomotion has been evaluated, with Deban, Schilling and Carrier (2012)
102 reporting wide involvement of the muscle throughout hind limb movement, propelling the hind
103 limb backwards during retraction and assisting with braking during swing phase. Further
104 understanding the functional remit of canine muscles and how muscles respond during
105 therapeutic modalities and through electromyographic assessment could aid veterinary
106 surgeons, veterinary physiotherapists (UK) and animal rehabilitation therapists globally in
107 designing effective rehabilitation regimes for individual patients.

108 This study aimed to use surface electromyography (sEMG) to measure muscle workload in the
109 GM and LD of sound dogs on the WT at increasing water depths: no submersion (control), mid
110 tarsal, mid stifle and the midpoint between the stifle and the greater trochanter. We
111 hypothesised that as water depth increased, estimated muscle workload measured by integrated
112 EMG (iEMG) in the GM and LD would increase rather than decrease due to increased
113 buoyancy.

114

115 **Materials and Methods**

116 The high level of inter-subject variance for EMG data observed in between subjects' designs
117 combined with differences seen between individuals may preclude reliable comparison of
118 muscle performance between groups (Williams, 2017). Therefore, a repeated measures, within
119 subjects' framework was applied to control for differences in spatial characteristics, and to
120 increase the accuracy and internal validity of the study's outcomes. Within this design dogs
121 also acted as their own controls which further reduced the potential for variation in EMG data
122 recorded due to different physiological factors such as subcutaneous fat levels (De Luca et al.,

123 2010), muscle fibre profile (Nordander et al., 2003; Wijnberg et al., 2003) and health status
124 and fitness level Lopez-Rivero and Letelier, 2000).

125 *Sample selection*

126 A convenience sample of seven dogs of various breed, age (mean age \pm SD: 5.9 ± 3.36 years),
127 weight (mean weight \pm SD: 25.06 ± 6.89 kg) and size (mean forelimb length \pm SD: $40.13 \pm$
128 6.38 cm, mean hind limb length \pm SD: 42.5 ± 6.52 cm) participated in the study (Table 1). Dogs
129 were recruited from staff and students working at the university. All dogs were deemed
130 clinically sound by the referring veterinary surgeon and hydrotherapist (National Association
131 of Registered Canine Hydrotherapists (NARCH) member; BSc (Hons) Bioveterinary Science),
132 had a normal body condition score and had no history of lameness or musculoskeletal
133 pathology (Holler et al. 2010; Breitfuss et al. 2015). Prior to WT sessions, veterinary consent
134 was requested in accordance with the Veterinary Surgeon Act 1966 (Exemptions order 1962)
135 to ensure dogs were physically able to participate. Dogs also underwent a pre-hydrotherapy
136 assessment by a NARCH hydrotherapist. Ethical approval was gained from the Hartpury
137 University Centre Ethics Committee.

138 (Table 1)

139 *Electrode placement*

140 Surface EMG (sEMG) sensors (rectangle dimensions: 41 x 20 x 5mm, with integral double
141 differential 99.9% Ag electrodes fixed at a 10mm inter-electrode distance providing a 10mm²
142 detection area; Delsys EMG system™; USA) were used to measure muscle activity of the GM
143 and LD muscles. Self-adhesive Delsys surface electrodes were attached onto the shaved skin
144 of the GM and LD, over the maximum circumference of the muscle belly and perpendicular to
145 the direction of the muscle fibres (De Luca et al., 2010; Morris and Lawson, 2009; De Luca,
146 1997; Fridlund and Cacioppo, 1986), using the Delsys adhesive sensor patches (Figure 1)
147 (Garcia et al. 2014). Poor adherence of electrodes has been found to reduce the accuracy of
148 EMG recordings and provide misleading results (De Luca et al., 1997; Chowdray et al., 2013).
149 Therefore before each trial, the dog's skin was shaved to remove all hair using grooming
150 clippers followed by disposable razors and then sterilised with alcohol wipes (70% isopropyl
151 alcohol) prior to electrode attachment to improve the impedance of the sensors to the skin in
152 accordance with St George and Williams (2013). Electrode adherence to the skin was further
153 improved through the use of duct tape and vet wrap which was applied over the sensors to
154 reduce movement and prevent loss of adherence (Figure 1) (St George and Williams, 2013).

155 Further duct tape was then loosely applied to protect the EMG sensors from water damage. To
156 improve reliable placement of the electrodes, placement was performed by a single researcher
157 (Hesse and Verheyen, 2010) using the anatomical landmarks specified by Breitfuss et al. (2015)
158 under the guidance of the NARCH registered hydrotherapist prior to each WT session
159 undertaken (Table 2). Due to restraints of the placement of the harness during this study,
160 electrode location for the back was restricted to the lumbar region to ensure sensor connection
161 was not impeded by the harness. Potential interference to the EMG signal due to movement
162 artefacts from the duct tape and vet wrap was assessed subjectively throughout data collection
163 through experimenter observation of live streamed data; runs which displayed interference
164 were excluded from subsequent analysis. However it should be noted that movement artefacts
165 may be present in the data collected due to the presence of the duct tape.

166 (Figure 1a)

167 (Figure 1b)

168 *Kinematic assessment*

169 Two-dimensional circular reflective adhesive markers (radius 7 mm) were produced from
170 silver duct tape and placed on to two pre-defined bony anatomical landmarks on the left side
171 of the dog by the same investigator. This took place whilst the dog was standing squarely with
172 equal weight distribution on all four limbs.

173 A digital video camera (Sony HDR-CX405, 9.2 mega pixels, 60fps interlaced, New York,
174 USA), was situated 58cm from the WT at a height of 1.09m and recorded the left sagittal view
175 of dogs for the entirety of each WT session to facilitate 2D kinematic analysis (Mendez-Angulo
176 et al., 2013). A calibration frame was placed along the side of the water treadmill to allow for
177 the measurement of stride parameters. Data were synchronised via time stamp on both the video
178 and EMG data. Kinematic data were analysed using Dartfish™ (Dartfish Analyser Software,
179 Version 7.0, Fribourg, Switzerland) to enable identification of limb contacts and obtain
180 matched strides between subjects in subsequent EMG data analysis.

181

182 *Data Collection*

183 Research was conducted with the assistance and supervision of a NARCH registered
184 hydrotherapist. A Westcoast canine Hydrotherapy treadmill (Westcoast Hydrotherapy,

185 Norfolk, UK) with internal dimensions of 1.82 m (length)×0.68 m (width)×0.90 m (height) was
186 used for the study. To ensure the safety of participants, water temperature, pH and chlorine
187 levels were measured before each dog entered the WT and were kept within safe parameters.
188 Each dog performed three acclimatisation sessions on the WT prior to data collection; this
189 allowed subjects to become used to walking on the WT and ensured that their gait was
190 repeatable (Scott et al. 2010; Fanchon et al., 2009). During these sessions, individual dogs
191 preferred walking speeds were established and recorded, based on the subjective opinion of the
192 NARCH hydrotherapist, in accordance with normal industry practice. EMG data were
193 collected using the Delsys Trigno™ EMG system (Massachusetts; USA) at a sampling rate of
194 2000Hz, Gain set at 1000 V/V, actual Gain: 1025 and common mode rejection ratio of ≥ 80 dB
195 (Delsys, 2017).

196

197 *Experimental protocol*

198 Dogs were fitted with a standard safety harness and EMG electrodes were secured prior to WT
199 exercise. Dogs then completed a 30 second warm up to allow them to adjust to the activity of
200 the treadmill and to attain their preferred walking speed under the supervision of the
201 hydrotherapist. During this time, the quality of the EMG signal was subjectively assessed
202 through observation of the consistency and visual appearance of the live-streamed EMG data to
203 ensure the electrodes were securely attached; if data signals were intermittent, asynchronous
204 or distorted the contact of the EMG electrode was assessed before continuing. Once the warm
205 up was completed, each dog walked for two minutes continuously on the WT at each water
206 depth: no submersion (depth 1), mid-tarsal (depth 2), between the lateral malleolus and lateral
207 epicondyle (depth 3) and between the lateral epicondyle and greater trochanter (depth 4) in
208 accordance with Barnicoat and Wills (2016) (Figure 2), facilitating simultaneous continuous
209 EMG data collection for the right and left sides of the GM and LD. Water depths followed
210 guidelines recommended by Goddard et al. (2014). Water depths were adapted to the individual
211 conformation of each participant in accordance with industry practice. To control for the
212 potential impact of fatigue during testing the order of completion was randomised; four of the
213 dogs were tested from depth 1 > 2 > 3 > 4 and the remaining three from depth 4 > 3 > 2 > 1
214 (Nankervis et al. 2015). The order of randomisation was set sequentially from high to low or
215 vice versa rather than completely randomised, to ensure data collection could be undertaken
216 within the timeframe of one standard hydrotherapy session to ensure the health and welfare of

217 participants was maintained. Dogs were also rested for 60 seconds after each 2 minute trial,
218 before the next trial commenced, in accordance with the standard practice of the hydrotherapy
219 centre.

220

221

222 (Figure 2)

223 *Data Analysis*

224 Video analysis was used to select visually 10 strides from the middle of each trial at each water
225 depth to ensure uninterrupted, consistent and matched strides were used for analysis for each
226 participant. Gait event detection for the left pelvic limb were visually defined in accordance
227 with the method used by Barnicoat and Wills (2016), with a single stride defined as two
228 successive footfalls of the left hind limb. The first and last 30 seconds of each trial were
229 removed to avoid inaccuracies that may occur when dogs adjusted their locomotion to the new
230 water level.

231 Raw electromyograms were analysed using Delsys EMG works™ analysis version 4.3.1 with
232 an internal band-pass filter applied to remove noise (<20Hz and >450Hz) (De Luca et al., 2010;
233 Zsoldos et al., 2010). Estimated muscle workload was calculated from the internal band-pass
234 filtered EMG data using the iEMG function of Delsys EMG works™ which integrates the
235 facility to remove DC offset from the signal, rectifies the data and analyses the amplitude of
236 the signal. iEMG represents the area under the curve of a rectified EMG trace (Winter, 2009)
237 and provides an approximation of the percentage of work done in muscles for defined exercise
238 periods, enabling comparison across exercise sessions (Richards et al., 2008). In humans,
239 iEMG uses a pre-assessed maximum voluntary contraction (MVC) to provide a baseline value
240 for maximal workload of a defined muscle to facilitate comparison of workload in the same
241 muscle during subsequent tasks (Borghuis et al., 2008; Winter, 2009). MVC cannot be achieved
242 in animals therefore dynamic contraction values are used to normalise data for comparison
243 allowing the work done by a muscle for a defined period to be calculated (Halaki and Ginn,
244 2012). One method of normalizing EMG data which produces high reliability between trials is
245 to utilise the trial anticipated to require the highest muscle workload to obtain the maximum
246 dynamic contraction as a proxy measure of MVC (Halaki and Ginn, 2012). For this study,
247 depth 4 was hypothesised to require the highest muscle activity (Marsolais et al. 2003) and the

248 highest dynamic contraction for each dog across one stride within this trial was selected to
249 normalise EMG data across all trials (Valentin and Zsoldos, 2016). Mean, maximum and
250 minimum iEMG percentage workload for the left and right GM and LD were then calculated
251 for each water depth, for each participant. Mean and standard deviation of the mean, minimum
252 and maximum iEMG at all water depths across the cohort and for each individual dog were
253 calculated.

254 Statistical analysis was undertaken using IBM Statistical Package for the Social Sciences
255 (SPSS) Statistics 23. Kolmogorov-Smirnov analyses determined data were non-parametric
256 therefore a series of Friedman's analyses were used to establish if significant differences in
257 lateral GM and LD muscle activity, considered independently, occurred across the different
258 water depths investigated for mean iEMG percentage values. Significance was set at $P < 0.05$.
259 Subsequent post-hoc Wilcoxon Signed Rank analyses, with a Bonferroni correction applied to
260 adjust for repeated measures (Brown et al, 2015) determined where statistical differences in
261 muscle workload occurred between water depths (revised alpha: $P < 0.01$).

262

263 **Results**

264 iEMG data for a total of 28 trials were analysed with each of the seven dogs that took part in
265 the study completing four water depths.

266

267 *iEMG estimated workload*

268 As expected, a high degree of individual variability was found within iEMG values between
269 participants (Table 2), although this was less in LD than GM. Across the cohort, minima values
270 increased from depth 1 to 2 for GM but showed little change for LD (RGM: +5%; LGM: +10%;
271 RLD: -3%; LLD: 0%). In contrast, maxima contractions and mean estimated workload for GM
272 and LD increased for both GM and LD from depth 1 to 2 (maxima: RGM: +9%; LGM: +3%;
273 RLD: +13%; LLD: +9%; mean: RGM: +11%; LGM: +11%; RLD: +6%; LLD: +1%). This was
274 followed by a trend for all iEMG values to reduce between depths 2 and 3 (minima: RGM: -
275 30%; LGM: -20%; RLD: -22%; LLD: -2%; maxima: RGM: -39%; LGM: -24%; RLD: -27%;
276 LLD: -11%; mean: RGM: -41%; LGM: -20%; RLD: -26%; LLD: -6%). Further reductions in
277 workload were reported from depth 3 to 4 (minima: RGM: -4%; LGM: -3%; RLD: +3%; LLD:

278 -4%; maxima: RGM: -4%; LGM: -16%; RLD: 0%; LLD: -5%; mean: RGM: -8%; LGM: -
279 10%; RLD: -3%; LLD: -3%).

280 (Table 2)

281

282 *Differences between water heights*

283 Significant differences in mean estimated workload (mean iEMG) were found between the
284 water levels for both GM (mean iEMG: RGM: P=0.004; LGM: P=0.002) and LD (mean iEMG:
285 LLD: P=0.002, RLD: P=0.001). Post hoc analyses found significant decreases in mean
286 estimated workload occurred in right and left GM between depths 2 (mid-tarsal) and 3 (between
287 lateral malleolus and lateral epicondyle), and depths 2 and 4 (between the lateral epicondyle
288 and greater trochanter); a pattern which was repeated for left and right LD (Table 3). Only one
289 significant increase was reported for the right GM mean estimated workload between depth 1
290 (no submersion) and depth 2 (mid-tarsal). No significant differences were found between the
291 other water depths for any of the muscles investigated (P>0.01).

292 (Table 3)

293 (Table 4)

294 **Discussion**

295 The results confirm that water depths used within canine WTs can have a significant impact on
296 the mean estimated workload of both GM and LD. Although descriptive increases in estimated
297 workload were observed at depth 2 (mid-tarsal) compared to the dry treadmill (depth 1) in all
298 participants, these were only found to be significant for mean estimated workload in the right
299 GM. Higher water depths reduced mean estimated workload in the GM and LD muscles for
300 participating dogs. This suggests that water levels above the stifle translate to reduced
301 recruitment of GM and LD in dogs undertaking walk exercise on a WT. Therefore, we have to
302 reject the hypothesis that as water depth increases in a WT, estimated muscle workload also
303 increases in the GM and LD.

304

305 *Gluteus medius activity*

306 Descriptive data indicate that for dogs undergoing WT exercise, GM workload increases on
307 average by 11% when water height is set directly above the tarsal joint. However, within this
308 sample, only right GM workload increased significantly from individual dogs' workload on
309 the dry treadmill. Few studies in animals have used EMG to assess the impact of changing
310 water depth on muscle activity in the hind limb. Human research has utilised EMG alongside
311 kinematic gait analysis, and has directly related increased joint ROM in the limb to increases
312 in muscle workload (Kaneda et al. 2007; Agostini et al. 2014; Gommans et al. 2016). Kinematic
313 analysis of quadruped locomotion on the WT has found increased flexion of equine forelimb
314 and pelvic limb joints as horses elevate their limbs to *step out and over* water at tarsus level
315 rather than pushing the limb through it. Adopting this locomotor pattern reduces the effect of
316 water resistance but would require increased GM activity to facilitate this movement (Mendez-
317 Angulo et al., 2013). Similar findings are reported in the dog. Barnicoat and Wills (2016)
318 found the flight arc of canine limbs increased as dogs lifted their limbs above the water level
319 during walk exercise on the WT with water set at tarsal height. In the current study, we
320 observed similar locomotive patterns in the pelvic limb, with dogs lifting the pelvic limb out
321 and above water at depth 1: mid-tarsal height. Conversely at higher water levels, i.e. between
322 lateral malleolous and lateral epicondyle (depth 3) and above, dogs propelled the pelvic limb
323 through the water and did not attempt to step above the water level. Given the small sample
324 size with this study, future kinematic research using more dogs and a wider range of breeds is
325 warranted to confirm these findings.

326 Higher water levels (above the stifle: depths 3 and 4) appear to reduce the estimated workload
327 of GM compared to walking on a dry treadmill (depth 1). Right and left GM estimated
328 workload reduced from depth 1 to depths 3 and 4, by 34% and 40%, and by 11% and 20%,
329 respectively. If as postulated above, dogs adapt their gait to push the hind limb through higher
330 water heights then the activity of GM will be altered. GM propels the pelvic limb backwards
331 during retraction (Deban et al., 2012); this function would be assisted on the WT by the action
332 of the treadmill belt and the dog's mass would be affectively reduced due to the increase in
333 buoyancy associated with higher water levels (King, 2016), thereby reducing GM workload.
334 Another function of GM is to stabilise the pelvic limb during swing (Deban et al., 2012).
335 Barnicott and Wills (2016) reported lengthened swing duration in the pelvic limb in dogs
336 walking at higher water heights. The impact of increased buoyancy at higher water levels is
337 thought to assist the vertical lift in the pelvic limb resulting in a longer flight arc and by

338 association more economical locomotion requiring less GM input to stabilise the limb (Scott
339 et al., 2010; Barnicott and Wills, 2016).

340

341 *Longissimus dorsi activity*

342 A similar pattern to GM estimated workload was found for LD, however differences reported
343 were of a lesser magnitude. This could represent the more general role of LD in stabilising the
344 spine (Groesel et al., 2010). Descriptively LD workload increased from depth 1 (dry) to depth
345 2 (mid-tarsal), but again muscle workload only significantly reduced as water height increased
346 from depth 2 (mid-tarsal) to depth 3 for the left LD (between lateral malleolus and lateral
347 epicondyle), and depth 3 to depth 4 (between the latera malleolus and greater trochanter). Right
348 and left LD estimated workload reduced from depth 1 to depths 3 and 4, by 21% and 23%, and
349 by 4% and 7%, respectively. Limited research has evaluated canine spinal kinematics on the
350 WT. However for horses, Nankervis et al (2015) reported walk exercise with water at the height
351 of the femoropatellar joint (equivalent to depth 4 in this study) produced maximum T10, T13,
352 T18 and L3 vertebra flexion. Whilst, in contrast, water depth at tarsal level (equivalent to depth
353 3 here) resulted in higher extension in T18, L3 and L5 vertebra, accompanied by increased
354 pelvic movement. The increased flexion-extension range of motion observed in the
355 thoracolumbar spine at water heights above the fetlock (equivalent to depth 2 here) suggests
356 that higher water levels could be detrimental within rehabilitation regimes designed to engage
357 equine core and epaxial musculature, unless head and neck position are manipulated to place
358 the back in flexion. The reduced workload found at higher water levels in the current study
359 support a reduced role for LD. Further research incorporating more EMG sensors at a range of
360 loci along LD combined with concurrent spinal kinematic analysis is required to confirm the
361 role water levels have on canine epaxial musculature activity.

362 During testing, dogs were encouraged with either treats or toys to motivate them to walk
363 continuously on the treadmill, which resulted in variable head and neck positioning. Whilst this
364 is normal practice, it has the potential to alter spinal kinematics and muscle function, as dogs
365 lifted their heads up and down in response to handlers' actions. There is a lack of research to
366 show the effect of head and neck position on canine gait, however studies have shown that in
367 horses, having a high head and neck position reduces stride length and disrupts normal gait,
368 whilst flexion and extension of the thoracic and lumbar spinal regions varies with changing
369 head position (Rhodin et al. 2005; Alvarez et al. 2006; Rhodin et al. 2009). This suggests that

370 inconsistent positioning of the head and neck of dogs in this study may have altered their natural
371 gait and the flexion and extension of the spinal muscles, possibly influencing the muscle
372 activity for the GM and LD. Further studies exploring the influence of head and neck position
373 on canine WT kinematics and muscle activity are needed. This study only utilised 2D kinematic
374 analysis to define limb contacts rather than for the quantification of angular or linear kinematic
375 variables, however, water distortion may have resulted in some minor inaccuracies in these
376 measurements. Previous literature has demonstrated that the error in kinematic analysis
377 associated with this type of experimental set-up (water turbulence, light refraction) is minimal,
378 with less than 3° error associated with joint movements (Mendez-Angulo et al, 2013).

379 During data collection, it was also observed that some dogs displayed lateral bending when
380 walking on the WT (Figure 3). A similar phenomenon has been observed in horses; higher
381 water levels above the midline of the shoulder are thought to reduce this occurring (Mooji et
382 al., 2013). Lateral bending during movement in quadrupedal animals is controlled by the
383 epaxial muscles, including LD (Faber et al., 2000; Musienko et al., 2014), therefore lateral
384 bending at lower water levels could be responsible for the increased LD workload found at
385 depth 2. Future research assessing the impact of water depth on lateral bending is warranted to
386 evaluate the optimal water heights to use in WTs during rehabilitation of dogs following spinal
387 surgery.

388 (Figure 3)

389

390 *Implications for practice*

391 The results suggest that WT exercise at higher water levels would be appropriate during the
392 early stages of canine rehabilitation regimes where stability is prioritised as a key goal over
393 strength. As rehabilitation progresses and the challenge to the patient needs to be increased to
394 facilitate greater muscular action, then tarsal water height would be recommended. However,
395 it is important that practitioners consider the clinical history and fitness of individual dogs when
396 designing rehabilitation regimes. The water depth used must be selected with sound clinical
397 reasoning and be altered according to presenting movement patterns and post hydrotherapy
398 response. Therefore post-exercise, re-evaluation of gait and assessment of clinical signs of pain
399 or fatigue should be used to inform progression within rehabilitation regimes.

400 Asymmetric recruitment of GM and LD was found across the dogs used in the study. The
401 reasons for the laterality observed across the cohort examined are not clear. These may be
402 associated with lateral bending or could be due to innate dominant limb laterality (Garcia et
403 al., 2014), may be due to recruitment of additional muscles to compensate for a lack of strength
404 in GM or LD or could be a sign of subclinical pathology. Practitioners should carefully consider
405 the impact of the handler at the front of treadmill including their location (right, left or centre
406 of the patient's visual field) and how the methods they use to encourage movement in the dog
407 and the influence these could have on head and neck position, and therefore on kinematic
408 patterns and muscle recruitment. The length of WT exercise sessions should be considered;
409 short sessions with rest are recommended to prevent fatigue, as anecdotally muscular
410 asymmetry increases with fatigue (Williams et al., 2012). Straightness is a benefit of WT
411 exercise and the unintentional introduction could have a potentially detrimental impact within
412 rehabilitation cases such as post-spinal surgery. We would recommend that one role of the
413 hydrotherapist within the WT should be to control and facilitate straightness in dogs
414 undergoing treatment. Additional training for handlers at the front of the treadmill, particularly
415 if dog owners are used in this capacity, is warranted to ensure appropriate head and neck
416 positioning occurs throughout WT exercise.

417 Water depth has a direct impact on GM and LD muscle activity in dogs undertaking walk
418 exercise on a WT. Walking at a depth directly above the tarsal joint results in increased
419 workload for GM and LD. As water height is increased beyond the stifle joint, GM and LD
420 workload reduced. The findings from this study have relevance to hydrotherapy in practice and
421 could be used to alter rehabilitation regimes and fitness programmes to most suit the individual
422 dog and its specific needs.

423 **Conflict of Interest**

424 No conflicts of interest apply to this work.

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427 willingness to participate in this study.

428

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604 Table 1. Participant Information

Participant	Breed	Age	Gender	Weight (kg)	Forelimb Length (cm)	Hind limb Length (cm)
Participant 1	Springer Spaniel	3	M	15.5	33	35
Participant 2	Golden Retriever	7	F	29.3	43	47
Participant 3	Weimaraner	6	F	36.2	53	55
Participant 4	Labrador	12	F	30.7	38	40
Participant 5	Labrador	2	F	22.0	39	42
Participant 6	Labrador	4	F	25.0	40	42
Participant 7	Cocker Spaniel	4	M	24.3	42	44

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608 Table 2. Minima (min), maxima (max) and mean with standard deviation (SD) for normalised
 609 iEMG estimated workload, reported to 2 decimal places, for gluteus medius (GM) and
 610 longissimus dorsi (LD) across all water depths for the cohort

iEMG (% of dry maximum dynamic contraction)		Value	Water Depth			
			1: No submersion	2: Mid-tarsal	3: Between LM and LE	4: Between LE and GT
Gluteus medius	Right	Min iEMG±SD	4.49±7.04%	4.71±5.68%	2.82±3.89%	2.72±2.25%
		Max iEMG±SD	60.16±77.69%	65.46±70.97%	40.07±39.31%	38.63±36.6%
		Mean iEMG±SD	19.88±24.95%	21.97±24.96%	13.06±15.43%	11.99±15.19%
	Left	Min iEMG±SD	18.86±11.58%	20.71±11.78%	16.65±11.7%	16.23±11.29%
		Max iEMG±SD	80.60±42.29%	83.23±29.86%	63.21±24.03%	52.93±27.8%
		Mean iEMG±SD	39.36±14.63%	43.74±12.14%	34.98±14.91%	31.61±15.89%
Longissimus dorsi	Right	Min iEMG±SD	33.17±31.24%	32.08±26.33%	25.01±22.67%	25.99±22.55%
		Max iEMG±SD	40.53±31.25%	45.94±24.7%	33.44±22.29%	33.32±21.57%
		Mean iEMG±SD	35.19±31.42%	37.35±25.7%	27.77±22.27%	26.96±22.25%
	Left	Min iEMG±SD	30.17±20.67%	30.22±21.07%	30.77±20.36%	29.48±20.7%
		Max iEMG±SD	36.31±23.15%	39.49±22.52%	35.04±21.99%	33.19±21.41%
		Mean iEMG±SD	33.61±23.43%	34.09±22.39%	32.16±22.02%	31.41±21.41%

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612

613 Table 3. Post hoc Wilcoxon Signed Rank results for mean iEMG percentages between water
 614 levels for GM and LD (* denotes significant result; revised Bonferroni adjusted alpha:
 615 $p < 0.01$). iEMG: integrated electromyography; GM: gluteus medius; LD: longissimus dorsi.

Muscle	Depth 1 - Depth 2	Depth 1 - Depth 3	Depth 1 - Depth 4	Depth 2 - Depth 3	Depth 2 - Depth 4	Depth 3 - Depth
Right GM	P=0.013*	P=0.679	P=0.408	P=0.004*	P=0.001*	P=0.679
Left GM	P=0.23	P=0.679	P=0.147	P=0.007*	P=0.0001*	P=0.301
Left LD	P=0.147	P=0.147	P=0.38	P=0.004*	P=0.0001*	P=0.535
Right LD	P=0.147	P=0.214	P=0.023	P=0.007*	P=0.0001*	P=0.301

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622 Table 4. Post hoc Wilcoxon Signed Rank Test for mean MUAP for GM and LD (* denotes
 623 significant result; revised Bonferroni adjusted alpha: $p < 0.01$). iEMG: integrated
 624 electromyography; GM: gluteus medius; LD: longissimus dorsi

Muscle	Depth 1 -	Depth 1 -	Depth 1 -	Depth 2 -	Depth 2 -	Depth 3 -
	Depth 2	Depth 3	Depth 4	Depth 3	Depth 4	Depth 4
Right GM	P=0.23	P=0.535	P=0.214	P=0.004*	P=0.0001*	P=0.535
Left GM	P=0.062	P=0.301	P=0.038	P=0.004*	P=0.0001*	P=0.301
Left LD	P=0.098	P=0.0147	P=0.023	P=0.002*	P=0.0001*	P=0.408
Right LD	P=0.098	P=0.408	P=0.038	P=0.013	P=0.0001*	P=0.214

625

626 Figure Legends

627 Figure 1: A: Patient preparation pre-hydrotherapy and B: Sensor locations.

628 *Sensors were applied over the muscle belly of the gluteus medius (GM) and longissimus dorsi (LD) and were*
 629 *secured with duct tape and vet wrap to prevent erroneous movement. GM electrodes were positioned at the*
 630 *midpoint of between the iliac crest and greater trochanter on the left and right side (Breitfuss et al. 2015). LD*
 631 *electrodes were located to the left and right side of L3 vertebrae on the sagittal plane.*

632

633 Figure 2. Water depths used during study. 1) no submersion (depth 1), 2) mid-tarsal (depth 2),
 634 3) between the lateral malleolus and lateral epicondyle (depth 3) and 4) between the lateral
 635 epicondyle and greater trochanter (depth 4). Red line represents the water level.

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637 Figure 3: Lateral bending of the spine of dogs during walking on the WT. Red lines show
 638 estimated spinal position based on subjective observations.

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641 Figure 1a

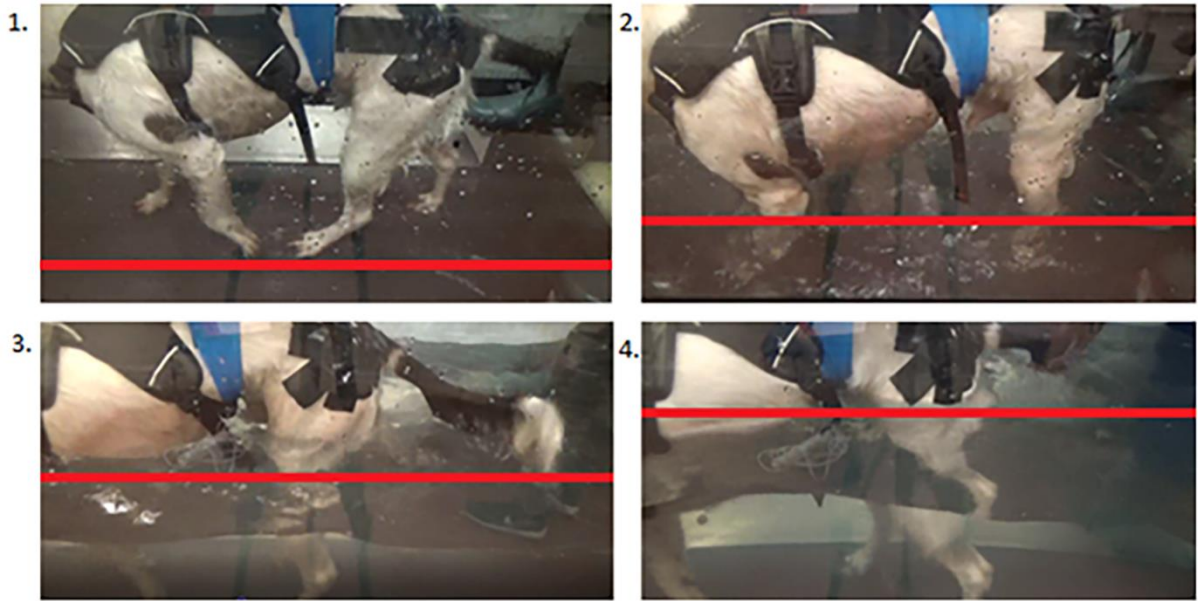


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643 Figure 1b

644

Running header: Effect of water depth on muscle activity of dogs on WT



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646

Figure 2

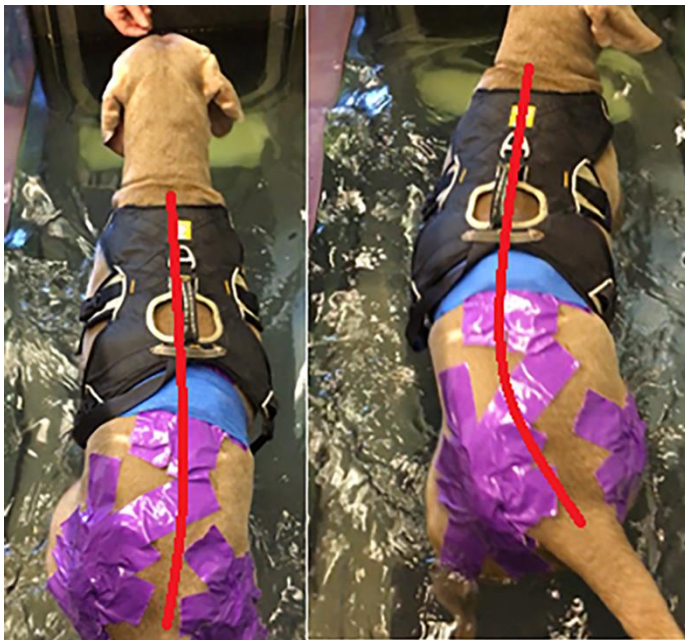


Figure 3