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Effect of water depth on limb and back kinematics in horses walking on a water treadmill.

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Abstract

Water treadmill (WT) exercise is frequently used for training/rehabilitation of horses. There is limited study into the effect of water depth on limb/back kinematics warranting investigation. The objective was to determine the effect of walking in different water depths, at the same speed, on limb/back kinematics measured simultaneously in a group of horses. Six horses (age:15±6.5 years) completed a standardised WT exercise session (19min duration; speed:1.6m/s; water depths:0.0/7.5/21.0/32.0/47.0cm). Ten waterproof light-emitting-diode tea-light-markers and reflective-spheres were affixed to the skin at predetermined locations; inertial-measurement-units were fixed to the poll/withers/left and right tubera coxae (TC)/sacrum to determine range-of-motion (ROM) changes of these locations. Univariable-mixed-effects-linear-regression-analyses were carried out, with a significance value of $P \leq 0.05$. At maximum carpal/tarsal flexion during swing, regression analyses showed a clear and consistent non-linear increase in carpal and tarsal flexion at increasing water depths ($P<0.0001$ for both variables). As water depth increased there was a significant increase in thoracic spine flexion-extension ROM ($P<0.0001$ at all thoracic sites) and increased dorsoventral and mediolateral ROM of the sacrum/left and right TC ($P<0.001$ for all variables) as water depth increased. Results suggest that horses responded to an increase in water depth until a threshold depth was reached when the biomechanical response levelled off, and there was increased pelvic roll. In conclusion, changes in limb kinematics brought about by relatively modest increases in water depth at walking speed of 1.6m/s are sufficient to induce significant changes in back/pelvic movement highlighting key issues with relevance for WT programme design.

Keywords: equine; hydrotherapy; gait analysis; thoracolumbar.

1. Introduction

Water treadmill (WT) exercise is frequently used for training of athletes and rehabilitation of injured humans, dogs and horses [1]. Studies investigating the effect of walking on a WT on equine kinematics have shown that water depth has a significant effect on movement patterns of the limbs [2-5], back [6] and pelvis [7]. These studies used a variety of water depths (fetlock, carpal/tarsal or shoulder/stifle depth), and speeds which varied from 0.8 m/sec [4-7], 0.9 m/sec [3] and 1.3 and 1.5 m/sec [2]. A recent questionnaire based study [8] showed that in practice a wide range of water depths are used and the mean walk speed used was 1.6 m/sec (range: 0.7-3) with no apparent correlation between water depth and speed used. The wide variety of water depths and speeds used in practice is a concern especially when horses are undertaking WT exercise as part of a rehabilitation programme with a variety of musculoskeletal conditions. Currently, WT venues apply a wide range of depths and speed combinations which are based on experience and not supported by any quantitative data [8].

In the horse, it has been shown that back movement alters after inducing a reversible unilateral hind limb (HL) lameness [9] and that improvement in HL lameness by diagnostic analgesia resulted in increased thoracolumbosacral range of motion (ROM) [10]. This suggests that changes in limb kinematics may simultaneously alter back kinematics. However simultaneous changes in limb and back kinematics during WT exercise have not yet been quantified, therefore warranting investigation. It is important to understand how the locomotor apparatus of the horse responds to walking on a WT with varying depths of water. This evidence will further our understanding of the effect that WT exercise has on equine biomechanics and aid the designing of optimum WT protocols.

As a result of buoyancy, water provides an upthrust which reduces vertical ground reaction forces as water depth increases. The impact of buoyancy on weight bearing has been estimated via study of horses in flotation tanks [11]. At the level of the ulna weight reduction was estimated to be 30%, therefore it could be expected that as water depth increases fetlock extension would decrease, because fetlock extension is an indicator of weight bearing [12-13], but this has yet to be reported. Two-dimensional videography has been used to quantify limb kinematics during WT exercise. In the horse it has been shown that as water depth increases so does limb joint ROM in swing [2-3]. In the fore limb (FL), greatest flexion of fetlock and carpal joints occurred in fetlock and tarsal depth water respectively, and in the HL the greatest tarsal flexion occurred in tarsal and stifle depth water [3]. However, it could be expected that maximum joint flexion would be reached at different water depths depending on the inherent ability of the horse to flex the distal limb joints, which in turn will be influenced by factors such as conformation, breed and orthopaedic health.

A study of FL and HL protraction and retraction ROM in WT walking at 0.8 m/s showed that FL and HL gait patterns were influenced by water depth, resulting in gait patterns that were significantly different to those seen on a dry treadmill at 1.6 m/s [4]. In this study, individual differences were observed in the ability of horses to protract the HL in response to higher water depths. Mooij *et al.* [7] described different strategies employed by horses walking in water, i.e. lifting the limb above the water at lower water depths, switching to greater reliance on pelvic flexion as water depth increased. Nankervis *et al.* [6] also observed increases in lumbar flexion as water depth increased. The point at which a horse transitions from one strategy to another is therefore likely to be dependent on the ability of the horse not only to flex the distal limb joints, but on its ability to flex more proximal limb joints and the lumbosacral spine.

The objective of the study was to determine the effect of walking in different water depths, at the same speed, on limb and back kinematics measured simultaneously in a group of six horses. It was hypothesised that: 1. at maximum carpal/tarsal flexion during swing, metacarpophalangeal/metatarsophalangeal joint, carpal and tarsal flexion angles will plateau once the water reaches a depth of the proximal metacarpal region; 2. at mid-stance metacarpophalangeal/metatarsophalangeal joint extension angle will decrease as water depth increases; 3. FL and HL retraction angles will increase as water depth increases; 4. Flexion-extension ROM of the thoracic and lumbar spine will increase as water depth increases; and 5. The movement of the poll, withers, sacrum and tubera coxae in the dorsoventral, mediolateral and craniocaudal planes will increase as water depth increases.

2. Materials & Methods

2.1: Validation of limb marker placement and attachment

Although zinc oxide cream has previously been used as a limb marker [2-3] for the current study pilot work indicated that marker visibility deteriorated with submersion in water, so a new method of marker placement had to be developed focussing on limiting marker displacement when submerged. A thin layer of adhesive double-sided tape bandage was placed around the limb at marker locations. A waterproof battery powered light-emitting diode (LED) tealight (diameter: 2.9 cm, weight: 15 g) was glued to the adhesive bandage. A neoprene pastern wrap with a LED attached, was used as a coronary band marker. This method successfully tracked movement even when water became increasingly opaque and turbulent throughout the course of data collection. In order to confirm that the gait pattern of horses with markers attached with adhesive bandage was not altered, movement patterns were compared to the movement patterns with markers attached directly to the skin [14-19] using the following method.

2.1.1 Horses

Six horses (mean±standard deviation age: 11 yrs±1.5, height: 163 cm±9), deemed fit for purpose by their owners, were recruited as a convenience sample based at a single local livery yard. Horses wore 30 mm dome-shaped markers (n=10) affixed at pre-determined locations, either directly to the skin (method 1) or using neoprene pastern wraps and adhesive bandage with LED lights, that were stuck to the skin with double-sided tape (method 2) (Figure 1). Joint angles at specific stride points of the horses walking overground were compared between methods 1 and 2, in a cross-over design.

2.1.2 Data collection

Two-dimensional high-speed videography (Casio[a]) data were collected from the left side of the horse at 240 Hz using a 6.90 m field of view. Data collection was performed on a firm level surface with the horse walking in a straight line for 10 m. Two passes were performed by each horse. The handler was advised to walk at the same tempo and to stay level with the horse's head to avoid obscuring markers.

2.1.3 Data Analysis

Mid-stance was defined as the stride point where the third metacarpal/metatarsal bone was vertical to the ground, maximum carpal or tarsal flexion was defined as the smallest carpal or tarsal angle during swing and maximum carpal or tarsal extension defined as the largest measurement of the angle during swing. Carpal angle was measured on the palmar aspect of the limb using markers 1, 2 and 3 (see Fig 1), and was measured at FL mid-stance, maximum carpal flexion and maximum carpal extension during swing. Metacarpophalangeal joint angle was measured on the palmar aspect of the limb using markers 3, 4 and 5, and was measured at FL mid-stance and maximum carpal flexion. Tarsal angle was measured on the dorsal aspect of the limb using markers 6, 7 and 8, and was measured at HL mid-stance, maximum tarsal flexion and maximum tarsal extension during swing. Metatarsophalangeal joint angle was measured on the plantar aspect of the limb using markers 8, 9 and 10, and was measured at HL mid-stance and maximum tarsal flexion. Peak protraction, i.e. the frame in which the measured limb was maximally extended cranially, and peak retraction, the frame in which the measured limb was maximally extended caudally, of the forelimb and hind limb was also measured. Maximum protraction and retraction were expressed relative to the vertical using markers 1 and 4 in the FL and 6 and 9 in the HL. Four strides were measured when the limb was in the centre of the field of view for each horse in each condition.

All measurements were acquired using digital image analysis software (Pro Analyst Professional edition, Xcitex[b]). Markers were tracked automatically. Repeatability of marker tracking was determined by tracking all markers and deriving angles five times in three horses. A coefficient of variance of <3% was calculated and deemed acceptable based on previous studies [14-19].

2.2: Effect of water depth on kinematics during water treadmill exercise

2.2.1 Horses

Six horses (mean±S.D., age 15±6.5 years, height 164±2 cm and weight 539± 37 kg) were selected for the study, as a convenience sample based at a single equestrian college. Horses were included on the basis of previous acclimation to the WT [5, 20] and regular WT use. They were deemed fit to participate after undergoing a gait evaluation on the basis of an International Equestrian Federation veterinary pre-competition assessment, by an orthopaedic specialist (RCM).

2.2.2 Measuring systems

2.2.2.1 High-speed videography

Waterproof light emitting diode tea lights were applied at standardised anatomical sites using the method described in Part 1. The same researcher applied the markers throughout the study.

2.2.2.2 High-speed motion-capture

Reflective spheres (19 mm in diameter) were affixed to the skin on the 6th, 10th, 13th and 18th thoracic (T) vertebrae (T6, T10, T13 and T18 respectively), the 3rd and 5th lumbar (L) vertebrae (L3 and L5 respectively) and the 3rd sacral vertebrae. Markers were placed by the same person each time (KN) based on palpation of the spinous processes.

2.2.2.3 Inertial measurement units

Horses wore five MTw inertial measurement units (IMU) (Xsens[c]) within a validated sensor-based system [21-22]. These were attached to the poll, withers, sacrum, and left and right tubera coxae (TC), using custom built pouches and double-sided tape with the horse standing square. The same researcher applied each sensor throughout the study. Repeatability of IMU placement using this method has previously been shown [23-24].

2.2.3 Study Protocol

Horses were walked in-hand overground for five minutes on a firm surface to warm-up and acclimatise. The horses were exercised in an Aqua Icelander WT (Formax[d]). The WT session consisted of five consecutive steps: Step 1 – walking on a dry belt; Step 2 – walking in 7.5 cm water (in the region of the coronary band); Step 3 – walking in 21 cm water (in the region of the fetlock); Step 4 – walking in 34 cm water (in the region of the proximal metacarpal); Step 5 – walking in 47 cm water (in the region of the distal radius). For the duration of the session horses walked at 1.6 m/s for three minutes at each step, walking for a further four minutes while the chamber emptied. These water depths and speed were selected based on information from a previous study as the most frequently used WT protocols [8]. The total duration of the WT session was 19 minutes. A handler was positioned either side of the WT and maintained a light contact with the bit to ensure straightness of the head and neck. At the front of the WT chamber there was a buffer bar.

2.2.4 Data Collection

Limb kinematics were quantified using two-dimensional high-speed videography (Casio[a]). Data were collected from the left side at 240 Hz using a 4 m field of view. The cameras were positioned 190 cm, for the forelimb, and 195 cm, for the hindlimb, from the centre of the field of view (Figure 2). Both cameras were perpendicular to the side of the WT and 90 cm from the ground.

Six ProReflex opto-electronic cameras (Qualisys Medical AB[e]) sampling at 240 Hz were positioned around the front, right hand side and behind the WT at a height of 200 cm. Data were collected for 20 sec at each water depth for each horse.

The IMUs collected triaxial accelerometer data allowing calculation of the displacement of the sensors in the dorsoventral, craniocaudal and mediolateral planes of all five units. Sensor data were collected at 60 Hz per individual sensor channel and transmitted via proprietary wireless data transmission protocol (Xsens[c]), to a receiver station (Awinda[f], Xsens) connected to a laptop computer running MTManager (Xsens[c]) software. Details on IMU specifications can be found elsewhere [21, 25]. IMU data were processed following published protocols [25].

In order to capture the same strides for all modalities at each test, videography, high-speed motion-capture and IMU data collection were synchronised manually in response to a verbal countdown.

2.2.5 Data Analysis

2.2.5.1 Limb kinematics

Limb angles were measured for four strides using the same techniques and software as Part 1. The centres of the LED lights were tracked automatically by the software or manually when it was not correctly selected by the software at the appropriate point of the stride.

2.2.5.2 Back flexion-extension angles

Flexion-extension angular motion patterns around T10, T13, T18, L3 and L5 were measured using the method of Faber *et al*. [26].

2.2.5.3 Inertial measurement unit range of motion

IMU data from repeated walk strides (mean±SD) (dry belt (23±2); 7.5 cm water (22±2); 21 cm water (20±2); 34 cm water (15±3); 47 cm water (10±2)) was used for the analysis. Outcome parameters were range of motion in a dorsoventral, craniocaudal and mediolateral direction for each of the five IMU sensors.

2.3 Statistical Analysis

2.3.1 Part 1

A Wilcoxon signed rank test was used to determine differences between the two methods of marker attachment for each variable using statistical analysis software (Analyse-It for Excel Microsoft version 1.73). A significance value of $P \leq 0.05$ applied in all analyses.

2.3.2 Part 2

Descriptive statistics were carried out for each variable at each condition using statistical analysis software (Analyse-It for Excel Microsoft version 1.73). Normality of data distribution was assessed using manual graphical methods that included kernel density and quantile-quantile plots to ensure that the regression methods being adopted were suitable. Scatter plots of individual horse data were created for all variables and reviewed visually for consistency of patterns between all horses. Univariable mixed effects linear and polynomial (quadratic terms) regression analyses were used to examine the relationship between outcome variable measurements and water depth (cms), with horse identity used as a random effect term in order to control for the multiple ('clustered') measures that were taken from each of the six horses (using Stata 15.0). A significance value of $P \leq 0.05$ applied in all analyses.

3. Results

0.0 *Part 1*

No statistically significant difference was observed for any variable between the two methods of marker attachment to the horse (Table 1).

3.2 Part 2

3.2.1 Limb kinematics

In the FL (Table 2 and 6), no relationship between water depth and fetlock angle at mid-stance was observed. Maximum carpal flexion angle significantly decreased (became more flexed) with increasing water depth ($P<0.0001$). Peak FL retraction significantly increased as water depth increased ($P=0.004$). FL protraction could not be measured due to the hoof/belt impact being inconsistently cranial to the field of view.

In the HL (Table 3 and 6), the fetlock became less extended at mid-stance and significantly more flexed at maximum tarsal flexion as water depth increased ($P=0.036$). Tarsal angle at mid-stance and at maximum extension did not change significantly, but maximum tarsal flexion angle significantly decreased (became more flexed) as water depth increased ($P<0.0001$). HL protraction did not change significantly with increasing water depth but peak retraction angle significantly increased with an increase in water depth (P=0.004).

Figures 3 and 4 present the maximum tarsal flexion angles for all horses, individually and aggregated, on a dry WT and at increasing water depths. Figures 5 and 6 present the maximum carpal flexion angles for all horses, individually and aggregated, on a dry WT and at increasing water depths. A clear and consistent statistically significant, non-linear declining pattern can be seen for all horses, which was best represented by a quadratic function (fitted value line in Figures 4 and 6; both P<0.0001). No other variables demonstrated clear and consistent patterns of non-linear distribution over the increasing water levels.

3.2.2 Thoracolumbar kinematics (Table 4 and 6)

Flexion-extension ROM of the 10th, 13th and 18th thoracic vertebrae and the 3rd lumbar vertebra significantly increased as water depth increased (P<0.0001 for all variables). Flexion-extension ROM of the 5th lumbar vertebra significantly decreased with an increase in water depth (P=0.002).

3.2.3 Inertial measurement units (Table 5 and 6)

The IMU data indicated that stride duration increased as water depth increased on the WT (P<0.0001).

The dorsoventral displacement of the poll decreased in 7.5cm of water compared to no water and then increased as water depth increased. The mediolateral displacement of the poll increased in 7.5cm of water compared to no water; no pattern was observed as water depth increased. There was increased craniocaudal displacement of the poll as water depth increased. These changes were not significant.

There was a decrease in dorsoventral, mediolateral and craniocaudal displacement of the withers in 7.5cm of water compared to no water. Dorsoventral and mediolateral displacement increased with increasing water depth, craniocaudal displacement did not significantly change as water depth increased. Dorsoventral displacement was the only variable to significantly change with water depth (P=0.031).

The sacrum, and left and right tuber coxae had similar movement patterns. As water depth increased there was a significant increase in dorsoventral displacement and mediolateral movement (P<0.0001 for all variables) but a decrease in craniocaudal movement was observed. The decrease in craniocaudal movement was significant for the sacrum and RTC (P<0.0001 for both variables).

4. Discussion

The objective of the study was to determine the effect of walking in different water depths, at the same speed, during a single WT exercise session on limb and back kinematics in a group of six horses. No significant differences in overground limb kinematics were observed between a standard method of marker attachment compared to the new method devised for this study. The results indicate that increasing water depth affected limb and back kinematics in the horse and partially support the hypotheses.

0.0 *Limb kinematics*

We observed an increase in limb joint ROM and flexion at maximum carpal/tarsal flexion angles and a decrease in fetlock extension at mid-stance as water depth increased, supporting hypotheses 1 and 2. We also observed an increase in stride duration in accordance with previous research [5]. Despite the similarities between results, direct comparison between our results and those from other studies is difficult due to differences in methods. Mendez-Angulo *et al*. [3] collected data for the fore and hind limbs on two separate days whereas in the current study videos from the fore and hind limbs were collected simultaneously which allowed a more specific comparison. Our study differed in speed and water depth from previous studies [2-7]. The combination of speed and depths was based on a previous study of industry practice to more closely explore the simultaneous back and limb kinematics within protocols that replicated the mean speed and depths used within training [8]. The speed used in this study may have increased the musculoskeletal challenge with increasing water depth as guidelines suggest that as water depth increases, speed should decrease [27].

Assignment of water depth was based on a specific measured level in the current study, while previous studies assigned water depths based on the level of the individual horse joints, making the standardisation different. We also observed an increase in FL retraction and HL retraction with increasing water depth but no change in HL protraction, supporting hypothesis 3. The significant increase in peak HL retraction is similar to previous findings at lower speed (0.8 m/s) but greater depth (stifle) [4], however, the significant increase in FL retraction is in contrast to findings at lower speed (0.8 m/s) and greater water depth. Exercise on a treadmill belt was found to increase FL and HL retraction in comparison with overground trotting [28] The combination of belt speed and water increasing drag forces on the FLs may explain the difference in FL movements patterns seen between this and the previous study [4].

A novel finding of the current study which was not reported by previous studies is that maximum carpal and tarsal flexion plateaued once a certain water depth was reached, either just below or above the tarsus, and this level appeared to be horse specific. This was especially highlighted with the use of mixed effects linear regression analyses that allowed for the differential effects of both within- (accounted for by water depth as a fixed effect) and between-horse variation (represented by a random effects term) to be accounted for. Such horse-level effects are likely to be related to individual horse conformation, flexibility, and differences in muscular strength available to step over the water. It could be that in this population, some horses were less able to flex their HLs as water depth increased, resulting in the need to change their movement strategy at a relatively lower water depth.

4.2 Thoracolumbar kinematics and pelvic displacement

Hypothesis 4 was partially accepted as there was an increase in flexion-extension ROM of the thoracic spine and the cranial lumbar region. These changes were similar to changes in back kinematics seen at 0.8 m/s in water increased up to stifle depth [6]. However a decrease in flexion-extension ROM in the more caudal lumbar spine (L5) was in contrast to previous studies [6-7] of back kinematics at 0.8 m/s in water increased up to stifle depth [6]. The findings being presented here differ from those of Mooij et al., [7] where an increase in pelvic flexion was reported. Although a decrease in FE ROM at L5 was found in the current study, this should be interpreted with caution as it is possible that the method applied here [26], may be influenced by a concurrent dorsal displacement of the sacrum as observed in the current study.

Whilst pelvic roll was not measured directly, the similar magnitudes of increase in LTC/RTC and sacrum vertical displacement, coupled with increased mediolateral displacement of the pelvis and increased retraction of the HLs, observed in the current study suggests that horses may have increased pelvic roll as water depth increases. This biomechanical adaptation could be interpreted as a compensatory movement pattern, as a function of the horses being limited by their maximal tarsal flexion. This information adds to the findings regarding pelvic movement changes from previous studies of horses walking and trotting on a WT [7, 29].

4.3 Clinical application of results

This study is the first to describe the effect of water depth on limb and back kinematics simultaneously. The results have highlighted a number of factors that should be taken into account when designing WT exercise protocols.

For horses where HL flexion would be of value in a rehabilitation programme, for example for targeted muscle development [30], our data suggests the use of WT exercise as part of a rehabilitation programme could be beneficial. We showed that maximum tarsal flexion did not change between 34 and 47 cm depth (just below and just above the tarsus), supporting the findings of Mendez-Angulo *et al.* [3] who showed that peak tarsal flexion did not significantly change between tarsal and stifle depth water, suggesting that deep water may not be essential for all horses unless a reduction of impact or limb load was warranted which is best achieved in deep water [2, 11]. However, the results indicated that there was individual variation in the water depth at which the response plateaued which suggested that the water depth at which the maximum tarsal flexion peaked could be lower than that reported by Mendez-Angulo *et al.* [3] in some individuals, which may be explained by differences in horse age and breed between the two studies.

Once the horses were not able to lift their limbs over the water, it is proposed that their locomotor response was altered in response to increased drag as described by Mooij *et al.* [7].This may be explained by the individual horses ability to lift their limbs over the water which may be influenced by their own conformation, flexibility and muscular strength highlighting a concern raised by Nankervis *et al.* [31] on the suitability of a single WT protocol design for individuals. It appears that pelvic roll may be an indicator that the horse has reached it capacity for maximal tarsal flexion. This supports recommendations based on expert consensus [27] that a WT programme should be designed on an individual basis, and monitored as a horse continues through a WT exercise programme.

In horses with concurrent back and limb injuries it is important to design a protocol that will aid in the recovery of both sites, and to promote optimum posture to avoid inducing negative and detrimental movement patterns. In the absence of evidence based protocols for the incorporation of WT exercise into the programme of a horse being rehabilitated following a known and specified injury, best practice must include monitoring of the individual horse's responses to WT exercise. This study has shown that depth at which a horse becomes unable to further flex and lift the distal limb over the water is individual. So whilst we continue to increase our understanding of generic responses to WT exercise, we must be cognisant of individual variation when attempting to apply our findings to clinical cases. If a protocol focuses on one injury, this could then potentially compromise the recovery of the other injury [30]. Based on clinical experience (Murray, personal communication), for example the ideal water depth for a horse with HL proximal suspensory desmopathy could be stifle depth and if a horse were recovering from impinging DSPs the ideal water depth would be no more than tarsal depth water. For a horse that was undergoing rehabilitation from both conditions a compromise on water depth would need to be reached depending on the individual horse response. This could potentially be using different depths in one session to promote recovery of both sites, or using one depth that was not detrimental to either site. However, further work in this area is warranted. The effect of speed also needs to be considered when interpreting the findings being presented here, and should be considered for future studies.

4.4 Limitations

Due to the small sample size the number of observations restricts the power of the study. It is possible that refraction of light emitted from the immersed limb markers may have limited the accuracy of the absolute values. Water heights were based on set measurements rather than being set to the anatomical regions of the horse, which does provide some variation in terms of the exact depth of the water relative to the horse, although horses were of similar heights. Previous studies have used proximal anatomical locations to measure protraction and retraction angles. Due to the design of the WT different locations had to be used and different results may have been observed if the same more proximal locations had been used. The mean age of the horses was 15 years, and therefore our sample of horses could be considered old/geriatric [32]. Different results may be observed if each horse walked at their preferred speed at the different water depths or in a younger group of horses.

5. Conclusions

Horses responded to an increase in water depth by increasing distal limb flexion until a threshold water depth was reached, when the response levelled off. The threshold was either below the carpus or above the carpus depending on the individual horse. Our data shows that changes in limb kinematics brought about by relatively modest increases in water depth at 1.6 m/s are sufficient to induce significant changes in back and pelvic movement. This pilot study highlights key issues with relevance for the design of WT programmes.

Manufacturers' Addresses

[a]Casio, London, UK
[b]Xcitex, Pro Analyst, Cambridge, MA, USA
[c]Xsens, Enschede, The Netherlands
[d]Formax, Selfoss, Iceland
[e]Qualysis AB, Gothenburg, Sweden
[f]Awinda. Xsens, Enshede, The Netherlands

Tables

Joint	**Stride Phase**	**Method 1 (˚)**	**Method 2 (˚)**	***P*-value**
Carpus	Mid-stance	181±5	182±4	0.96
	Max flexion	139±6	136±12	0.09
	Max extension	184±6	183±5	0.43
MCPJ	Mid-stance	222±11	225±8	0.11
	Max flexion	185±11	184±7	0.49
Tarsus	Mid-stance	174±5	172±3	0.11
	Max flexion	151±7	152±7	0.61
	Max extension	180±6	178±3	0.45
MTPJ	Mid-stance	218±10	215±8	0.17
	Max flexion	175±8	171±6	0.06

Table 1: Mean and standard deviation of limb joint angles at mid-stance, maximum carpal/tarsal flexion and maximum carpal/tarsal extension during the swing phase, in a group of six horses using two different methods of marker attachment. Method 1: Dome-shaped markers attached directly to the skin. Method 2: Neoprene pastern wraps and adhesive bandage, with LED lights, attached to the skin with double-sided tape. MCPJ=metacarpophalangeal joint; MTPJ=metatarsophalangeal joint; °=degrees.

Variable		Water depth (cm)				
		0.0 (n=)	7.5 (n=)	21.0 (n=)	34.0 (n=)	47.0 (n=)
MCPJ	Mid-stance (°)	208±11 (6)	206±13 (6)	212±11 (3)	205±8 (3)	M
	Max flexion (°)	175±13 (6)	170±12 (6)	159±12 (6)	151±17 (6)	154±16 (4)
Carpus	Mid-stance (°)	177±4 (6)	178±4 (6)	175±2 (3)	180±4 (3)	M
	Max flexion (°)	134±5 (6)	129±5 (6)	121±6 (6)	120±6 (6)	117±7 (4)
	Max extension (°)	180 ±5 (6)	180±4 (6)	181±4 (6)	184±4 (6)	184±2 (4)
Peak Protraction (°)		M	M	M	M	M
Peak Retraction (°)		29±4 (6)	30±5 (6)	30±4 (6)	32±6 (6)	28±3 (4)

Table 2. Mean and standard deviation for the carpus and forelimb fetlock angles at mid-stance, maximum carpal flexion, maximum carpal extension during the swing phase, and forelimb protraction and retraction in a group of six horses that walked on a water treadmill. n = number of observations; MCPJ = metacarpophalangeal joint; M = missing data; ° = degrees.

Variable		Water depth (cm)				
		0.0 (n=)	7.5 (n=)	21.0 (n=)	34.0 (n=)	47.0 (n=)
MTPJ	Mid-stance (°)	201±9 (6)	200±11 (6)	197±11 (5)	195±12 (4)	194±12 (5)
	Max flexion (°)	172±6 (6)	166±7 (6)	157±7 (6)	155±11 (6)	150±18 (6)
Tarsus	Mid-stance (°)	163±3 (6)	165±7 (6)	166±5 (5)	164±7 (4)	163±8 (5)
	Max flexion (°)	135±7 (6)	123±9 (6)	110±9 (6)	107±7 (6)	107±8 (6)
	Max extension (°)	166±5 (6)	168±7 (6)	168±7 (6)	167±7 (6)	167±8 (5)
Peak Protraction (°)		16±1 (6)	16±1 (6)	16±2 (6)	16±3 (4)	16±2 (4)
Peak Retraction (°)		36±4 (4)	36±3 (5)	37±4 (6)	37±6 (3)	37±5 (4)

Table 3. Mean and standard deviation for the tarsus and hindlimb fetlock angles, in degrees, at mid-stance, maximum tarsal flexion and maximum tarsal extension during the swing phase, and hindlimb protraction and retraction angles in a group of six horses that walked on a water treadmill. n = number of observations; MTPJ = metatarsophalangeal joint; ° = degrees.

Plane	Location	Water depth (cm)				
		0.0	7.5	21.0	34.0	47.0
Flexion-extension (°)	T10	8.0±2	9.5±2	10.6±2	10.4±2	11.6±2
	T13	8.0±1	9.3±1	10.5±2	12.0±2	11.6±2
	T18	7.7±1	8.6±2	9.8±2	9.8±2	10.6±2
	L3	7.3±1	7.8±2	8.5±2	8.5±2	8.7±2
	L5	6.2± 1	6.6±1	6.4±2	6.1±2	5.6±1

Table 4. Mean and standard deviation of the flexion-extension range of movement for all data pooled, at specific locations on the spine in a group of six horses that walked on a water treadmill. T = thoracic; L = lumbar; ° = degrees.

Variable		Water depth (cm)				
		0.0	7.5	21.0	34.0	47.0
Stride Duration (ms)		1277±53	1316±95	1343±86	1364±74	1403±91
Poll	Dorsoventral (mm)	76±38	60±28	73±33	93±44	89±33
	Mediolateral (mm)	52±30	58±10	54±15	57±15	64±15
	Craniocaudal (mm)	98±56	121±33	140±37	164±49	171±22
Withers	Dorsoventral (mm)	41±8	39±12	42±11	43±13	46±14
	Mediolateral (mm)	55±27	38±19	39±9	48±21	56±20
	Craniocaudal (mm)	51±8	45±9	47±8	46±6	47±6
Sacrum	Dorsoventral (mm)	74±9	77±15	85±13	91±13	97±15
	Mediolateral (mm)	55±21	51±9	58±13	70±20	79±22
	Craniocaudal (mm)	50±9	47±10	47±11	42±12	39±10
LTC	Dorsoventral (mm)	81±10	85±12	90±8	100±7	108±10
	Mediolateral (mm)	62±20	59±11	64±10	75±16	84±17
	Craniocaudal (mm)	54±13	53±9	54±13	55±11	52±11
RTC	Dorsoventral (mm)	76±7	83±17	91±15	96±14	103±16
	Mediolateral (mm)	59±18	57±11	63±13	77±18	86±20
	Craniocaudal (mm)	60±13	57±11	58±12	49±12	49±10

Table 5. Mean and standard deviation of the IMU data collected for stride duration and the poll, withers, sacrum and left and right tubera coxae range of motion in the dorsoventral, mediolateral and craniocaudal planes in a group of six horses that walked on a water treadmill. ms = millisecond; LTC = left tubera coxae; RTC = right tubera coxae; mm = 17illimetres.

LINEAR MODELS						
Outcome measure	**Intercept (baseline)**	**Water depth regression coef.**	**Coef. SE**	**Coef. 95% CI**		**P-value**
MCPJ mid-stance	208.5	-0.204	0.086	-0.372	-0.035	0.018
Forelimb retraction	29.2	0.048	0.017	0.016	0.81	0.004
MTPJ mid-stance	198.5	-0.314	0.057	-0.427	-0.202	<0.001
Tarsal mid-stance	163.9	-0.039	0.025	-0.088	0.011	0.126
Tarsal max extension	167.2	-0.006	0.023	-0.051	0.039	0.79
Hindlimb protraction	16.2	-0.009	0.018	-0.045	0.026	0.607
Hindlimb retraction	36.2	0.047	0.016	0.016	0.080	0.003
Withers DV-ROM	39.5	0.130	0.060	0.012	0.246	0.031
Withers ML-ROM	44.3	0.126	0.177	-0.221	0.473	0.477
Withers CC-ROM	48.5	-0.051	0.051	-0.164	0.063	0.382
Sacrum DV-ROM	73.9	0.499	0.094	0.316	0.682	<0.001
Sacrum ML-ROM	50.1	0.575	0.128	0.325	0.825	<0.001
Sacrum CC-ROM	49.9	-0.223	0.051	-0.323	-0.124	<0.001
LTC DV-ROM	77.4	0.657	0.070	0.520	0.794	<0.001
LTC ML-ROM	55.4	0.572	0.091	0.394	0.750	<0.001
LTC CC-ROM	53.7	0.005	0.064	-0.120	0.130	0.936
RTC DV-ROM	75.8	0.601	0.081	0.441	0.761	<0.001
RTC ML-ROM	52.7	0.688	0.091	0.509	0.866	<0.001
RTC CC-ROM	60.1	-0.254	0.054	-0.360	-0.149	<0.001
T10 FE-ROM	8.6	0.067	0.012	0.044	0.090	<0.001
T13 FE-ROM	8.5	0.072	0.009	0.055	0.089	<0.001
T18 FE-ROM	8.1	0.057	0.008	0.041	0.073	<0.001
L3 FE-ROM	7.5	0.029	0.006	0.016	0.041	<0.001
L5 FE-ROM	6.5	-0.016	0.005	-0.0260	-0.006	0.002
QUADRATIC MODELS						
Outcome measure/	**Intercept (baseline)**	**Regression Coef.**	**Coef. SE**	**Coef. 95% CI**		**P-value**
predictor variable						
Maximum tarsal flexion angle during swing	134.5					
Water depth		-1.57	0.117	-1.8	-1.34	<0.001
Water depth sq.		0.021	0.002	0.017	0.026	<0.001
Maximum carpal flexion angle during swing	133.7					
Water depth		-0.72	0.125	-0.96	-0.47	<0.001
Water depth sq.		0.009	0.003	0.003	0.014	0.002

Table 6. Summary of mixed effects linear/quadratic regression analyses of limb and back kinematic measurements with variation in water treadmill water depth (cm), with horse (n=6) included as a statistically significant (P<0.001) random effect variable in each

model**.** MCPJ = metacarpophalangeal joint; MTPJ = metatarsophalangeal joint; LTC = left tubera coxae; RTC = right tubera coxae; DV-ROM = dorsoventral range of motion; ML-ROM = mediolateral range of motion; CC-ROM = craniocaudal range of motion; T = thoracic; L = lumbar; FE-ROM = flexion-extension range of motion.

Figure Legends

Figure 1. Limb marker placement for data collection. 1: Lateral epicondyle of the humerus, 2: Lateral styloid process of the ulna, 3: Lateral proximal aspect of the third metacarpus, 4: Lateral distal aspect of the third metacarpus, 5: Lateral aspect of the mid proximal phalanx of the forelimb, 6: Head of the fibula, 7: Lateral malleolus of the fibula, 8: Lateral proximal aspect of the third metatarsus, 9: Lateral distal aspect of the third metatarsus, 10: Lateral aspect of the mid proximal phalanx of the hind limb.

Figure 2: Camera set-up for data collection. The camera focussing on the forelimb was 1.9m away from the centre of the field of view and the camera focussing on the hindlimb was 1.95m away from the centre of the field of view. Videos were captured of the left side of the horse. The red stars represent the location of the Qualysis cameras.

Fig 3. Scatterplot of individual horse data showing maximum tarsal flexion angle during swing on a dry treadmill and four water depths (7.5, 21, 34 and 47 cm).

Fig 4. Graph of aggregated data with fitted line showing the non-linear (quadratic) relationship between maximum tarsal flexion angle during swing and water depth (WD). (Tarsal angle = 134.5 – [1.57xWD] + [0.02xWD^2]; R^2 value = 96%.)

Fig 5. Scatterplot of individual horse data showing maximum carpal flexion angle during swing on a dry treadmill and four water depths (7.5, 21, 34 and 47 cm).

Fig 6. Graph of aggregated data with fitted line showing the non-linear (quadratic) relationship between maximum carpal flexion angle during swing and water depth. (Carpal angle = 133.7 – [0.72xWD] + [0.0085xWD^2]; R^2 value = 88%.)

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