The immediate effect of water treadmill walking exercise on overground in-hand walking locomotion in the horse.

Short title: Changes in overground in-hand locomotion after water treadmill exercise

CA Tranquille[1*], JB Tacey[2], VA Walker[1], R Mackechnie-Guire[a], J Ellis[b,3], KJ Nankervis[c], R Newton[4], RC Murray[5]

Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU, UK; [a]Centaur Biomechanics, Dunstaffanage House, Moreton Morrell, Warwickshire CV35 9BD, UK; [b]Warwickshire College, Moreton Morrell, Warwickshire CV35 9BL, UK; [c]Hartpury University, Hartpury, Gloucestershire GL19 3BE, UK.

*Corresponding author: carolyne.tranquille@outlook.com

Current addresses: [1]Hartpury University, Hartpury, Gloucestershire GL19 3BE, UK; [2]Granby Street, Newmarket, Suffolk CB8 8HB, UK; [3]Arden University, Middlemarch Park, Coventry CV3 4FJ, UK; [4]Cambridge Veterinary School, Madingley Road, Cambridge, Cambridgeshire CB3 0ES, UK; [5]Rossdales Diagnostic Centre, Cotton End Lane, Exning, Suffolk CB8 7NN UK.

Abstract

Water treadmill (WT) exercise is frequently used for training/rehabilitation of horses but the effect of WT exercise on short-term movement patterns is yet to be investigated. The objective of this study was to determine the immediate effect of WT exercise on overground limb and back kinematics. Six horses (mean±S.D., age 15±6.5years, height 164±2cm and weight 539±37kg) walked twice in a straight line, led from both sides, before and after a standardised WT exercise session (19 minutes duration; speed 1.6m/s; water depths: 0.0/7.5/21.0/32.0/47.0cm) on a flat concrete surface. Horses wore five inertial-measurement-units to determine poll/wither/pelvic displacement, and 10 anatomical markers to determine fetlock/carpal/tarsal joint angles at specific stride points. Degree of mediolateral tarsal oscillation during stance was graded. Wilcoxon-signed-rank tests were used to investigate differences between pre and post-WT exercise for each variable. Post-WT exercise, there was a significant decrease in hindlimb fetlock extension at mid-stance compared with pre-WT exercise. No significant changes in movement patterns of the poll/withers/pelvis were detected post-WT exercise. In all horses there was greater mediolateral tarsal oscillation during the stance phase of the stride post-WT exercise, which could relate to muscle fatigue. The results suggest that a 19 minute WT session has an effect on immediately-following overground in-hand walking locomotion patterns. Further work is required to determine the duration of this effect, and how different WT speeds and water depths affect locomotion patterns.

Keywords: equine; hydrotherapy; overground; kinematics.

Conflict of interest: None to declare.

1. Introduction

Water treadmill (WT) exercise is frequently used for rehabilitation in injured humans, dogs and, more recently, horses (Tranquille *et al.*, 2017). Water treadmill exercise is generally accepted to be aerobic exercise, performed at relatively low heart rates (Tranquille *et al.*, 2017). Several studies have investigated the kinematics of the horse during WT exercise, demonstrating an increase in carpal/tarsal flexion at mid-swing (Mendez-Angulo *et al.*, 2013; Tranquille *et al.*, 2022) and an increase in the flexion-extension range of motion (ROM) of the thoracolumbar spine as water depth increases (Nankervis *et al.*, 2016; Tranquille *et al.*, 2022). The characteristic change in gait pattern observed as water depth increases from fetlock to carpal depth, of a longer stride length and a lower stride frequency (Scott *et al.*, 2010) are suggested as useful for training of horses, since an increase in stride length and a decrease in stride frequency are considered desirable in dressage horses (Clayton and van Weeren, 2013).

It is not yet fully established whether stride characteristics observed whilst water walking induce physiological changes that influence overground gait, either in the short or longer term, and whether any changes would provide beneficial effects within training or rehabilitation programmes. Two studies describing overground locomotion patterns at trot in a small number of horses (Clegg and Welford, 2014; Bowen and Paddison 2017) have reported that overground stride length and flexion-extension ROM of the cervical and thoracolumbar spine did not significantly change between six and nine sessions of walking and trotting on a WT. There is currently no evidence regarding the immediate effects of WT exercise on overground gait.

The biomechanical effects of walking in water suggest that specific muscle groups will be recruited. A previous study reported that owners perceived that regular use of WTs within sport horse training programmes led to general improvement in 'gait, strength and muscle development, including the core muscles' (Tranquille *et al*., 2018). Although there may be a long term effect of regular WT use, it is also possible that WT exercise could provide changes in biomechanics post session due to recruitment and/or activation of certain muscle groups or localised muscle fatigue. Muscle recruitment or activation could potentially improve stability, reducing pelvic or wither movement, while muscle fatigue could be associated with pelvic or limb instability. Increased tarsal instability/oscillation has previously been reported with poor muscle development (Dyson *et al*., 2018). Reduced propulsion secondary to fatigue or altered muscle recruitment could be manifest as hindlimb toe drag (representative of reduced joint flexion during swing), or reduced fore- and hindlimb fetlock extension during stance (Johnston *et al.*, 1999; Riber *et al.*, 2006; Wickler *et al.*, 2006; Dyson *et al.*, 2018; Pugliese *et al*., 2020). There is a need to understand the immediate impact of WT exercise on the horse and whether typical industry protocols induce fatigue in horses accustomed to exercising on a water treadmill.

The objective of the study was to determine the effect of a standardized WT protocol, similar to that being used in practice (Tranquille *et al.*, 2018), on overground in-hand walking limb kinematics and wither and pelvic displacement by comparison between overground measurements before and immediately after a WT exercise session in a group of six horses. It was hypothesized that immediately after WT exercise there would be evidence of fatigue, demonstrated by 1. decreased peak carpal and tarsal flexion during swing; 2. altered pelvic displacement in dorsoventral, craniocaudal and mediolateral planes; 3. increased tarsal oscillation during stance compared to pre-WT exercise.

2. Materials & Methods

This project was approved by the Ethical Review Committee of the Animal Health Trust (project number: AHT 09-2016).

Six horses (mean±standard deviation, 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 equipped with and regularly using an equine WT (Aqua Icelander, Formax). Horses were included based on routine WT use and familiarity. Horses were in regular work for general purpose exercise at the college, deemed fit for purpose, and had been exercising on the WT twice a week for between 6 and 24 months. On the study day, horses underwent a gait evaluation by an orthopaedic specialist (RCM) and were deemed fit to take part in the study based on an International Equestrian Federation pre-competition veterinary assessment.

Study Protocol

Horses were walked in-hand for five minutes on a firm surface, in both a left and right direction, to warm-up.

For the purpose of pre-WT exercise overground data collection, horses were walked twice in a straight line, on a flat, level concrete surface that was 20 m long, with one handler leading from each side of the horse. Horses walked at their own comfortable pace which was standardised within horse. Handlers were advised to walk at the same tempo and to stay level with the horse's head to avoid obscuring the limb markers.

Following pre-WT exercise overground data collection, each horse then moved immediately to the WT. Details of the WT session can be found elsewhere (Tranquille *et al.*, 2022) but in brief horses walked at 1.6 m/s throughout the session for three minutes at five water depths (0.0, 7.5, 21.0, 32.0 and 47.0cm), and four minutes while emptying. The total duration of the WT session was 19 minutes. Directly after completing the WT session, marker position was visually inspected and then the horses were walked immediately to the experimental track. Horses followed the identical overground walk protocol as pre-WT exercise, during which post-WT data collection was performed.

Measuring systems

A waterproof battery powered light-emitting diode (LED) (diameter: 2.9 cm, weight: 15 g) was glued to a thin layer of adhesive bandage with double-sided tape around the limb at previously published locations (Tranquille *et al.*, 2022) to determine limb kinematics. A neoprene pastern wrap, with an LED attached, was used as a coronary band marker. The same researcher applied the markers throughout the study (VAW).

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.

Five motion tracker wireless inertial measurement units (IMU) (Xsens) were attached over the poll, withers, sacrum, and left and right tubera coxae, using custom built pouches and double-sided tape with the horse standing square, using a validated sensor-based system (Pfau *et al.,* 2005; Warner *et al.,* 2010). The same researcher applied each sensor throughout the study (RMG).

Data collection

Limb kinematics were quantified using two-dimensional high-speed videography. Data were collected from the left side of the horse at 240 Hz using a 6.90 m field of view. The camera was positioned 2.08 m from the centre of the field of view.

The IMUs collected triaxial sensor data calculating 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), to a receiver station (Awinda, Xsens) connected to a laptop computer running MTManager (Xsens) software. Details on IMU specifications can be found elsewhere (Pfau *et al.,* 2005; Mackechnie-Guire *et al.,* 2018, 2021a, 2021b). IMU data were processed following published protocols (Pfau *et al.,* 2005).

Videography and IMU data collection were synchronised.

Tarsal oscillation

Degree of mediolateral oscillation of the left and right tarsi during stance was assessed from behind with the horse walking overground away from the camera pre and post WT exercise, using published methods (Dyson *et al.,* 2018). The horses turned behind the camera, the first stride in the field of view was excluded and the subsequent three strides were assessed for tarsal oscillation with each stride being assessed individually and the median score for the three strides recorded. This was graded on an ordinal scale of 0 to 2; with 0 representing no oscillation, 1 representing mild (slight increase of mediolateral range of motion of the metatarsus during stance and no outwards rotation of the limb) and 2 representing severe (significant increase of mediolateral ROM of the metatarsus during stance and outward rotation of the tarsus with greater visibility of the medial aspect of the limb). Tarsal oscillation was assessed by an experienced researcher (CAT) from the videos after data collection.

Data Analysis

Carpal angle was measured on the palmar aspect of the limb using markers 1, 2 and 3, and was measured at forelimb mid-stance, peak carpal flexion and peak carpal extension during swing. Forelimb fetlock angle was measured on the palmar aspect of the limb using markers 3, 4 and 5, and was measured at forelimb mid-stance and peak fetlock flexion during swing. Tarsal angle was measured on the dorsal aspect of the limb using markers 6, 7 and 8, and was measured at hindlimb mid-stance, peak tarsal flexion and peak tarsal extension during swing. Hindlimb fetlock angle was measured on the plantar aspect of the limb using markers 8, 9 and 10, and was measured at hindlimb mid-stance and peak fetlock flexion during swing. Mid-stance was defined as the stride point where the third metacarpal/metatarsal bone was vertical to the ground, peak flexion was defined as the smallest carpal, tarsal or fetlock angle and peak carpal extension or tarsal extension defined as the largest measurement of the angle during swing. Maximum protraction, i.e. the frame in which the measured limb was maximally extended cranially, and maximum retraction, the frame in which the measured limb was maximally extended caudally, of the forelimb and hindlimb was also measured. Maximum protraction and retraction were expressed relative to the vertical using markers 1 and 4 in the forelimb and 6 and 9 in the hindlimb. 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, Xcitex, USA).

IMU data from 20 consecutive walk strides were analysed. Outcome parameters were ROM in a dorsoventral, craniocaudal and mediolateral direction for each IMU sensors

Statistical Analysis
Limb angles and IMU data were pooled, split into condition (pre vs post WT exercise) and analysed descriptively. A Wilcoxon signed rank test was used to determine differences between pre and post WT exercise for each variable.

All analyses were conducted using statistical analysis software (Stata 15.0) with a significance level of $P<0.05$.

3. Results

No difference in stride duration was observed from the IMU data.

There were no significant changes in carpal or forelimb fetlock angles at any stride point, nor in forelimb protraction and retraction angles after WT exercise (Table 1).

Table 1: Mean and standard deviation of the carpus and forelimb fetlock angles at mid-stance, peak carpal flexion (PCF), peak carpal extension (PCE) and peak fetlock flexion (PFF) during the swing phase, and forelimb protraction and retraction angles in a group of six horses before (pre) and after (post) water treadmill exercise. °=degrees, MCPJ= metacarpophalangeal joint.

Joint	**Stride Phase**	**Pre (˚)**	**Post (˚)**	***P*-value**
Carpus	Mid-stance	179.7±4.9	180.2±4.4	0.92
	PCF	132.4±5.0	132.8±4.4	0.92
	PCE	183.3±5.0	183.8±5.0	0.35
MCPJ	Mid-stance	214.7 ±12.4	205.3±12.0	0.17
	PFF	180.7±10.5	175.3±11.9	0.46
Protraction		26.1±2.4	25.6±2.4	0.59
Retraction		28.7±1.8	28.0±2.9	0.27

There were no significant changes in hindlimb protraction and retraction angles, or tarsal angle at mid-stance, peak tarsal flexion or maximum tarsal extension after WT exercise. A significant decrease in fetlock angle at all stride points, representing less extension, was detected post-WT exercise (Table 2).

Table 2: Mean and standard deviation of the tarsus and hindlimb fetlock angles at mid-stance, peak tarsal flexion (PTF), peak tarsal extension (PTE) and peak fetlock flexion (PFF) during the swing phase, and hindlimb protraction and retraction angles in a group

of six horses before (pre) and after (post) water treadmill exercise. °=degrees; *=significant difference; MTPJ= metatarsophalangeal joint.

Joint	Stride Phase	Pre (˚)	Post (˚)	***P*-value**
Tarsus	Mid-stance	161.7±6.9	163.4±6. 9	0.35
	PTF	137.3±8.1	138.4±8.5	0.35
	PTE	167.2±7.0	169.1±7.1	0.17
MTPJ	Mid-stance	203.4±11.6	192.0±20.1	0.046*
	PFF	171.0±10.4	160.3±15.1	0.028*
Protraction		26.8±2.6	26.0±2.4	0.34
Retraction		29.7±3	29.1±3.9	0.53

No significant changes in craniocaudal, mediolateral or dorsoventral ROM were detected after WT exercise for any sensor location (Table 3).

Table 3: Mean and standard deviation of the inertial measurement unit data collected for the poll, withers, sacrum and tubera coxae range of motion in craniocaudal, mediolateral and dorsoventral planes in a group of six horses before (pre) and after (post) water treadmill exercise. LTC = left tuber coxae; RTC = right tuber coxae; mm = millimeters.

Site	Plane	Pre (mm)	Post (mm)	***P*-value**
Poll	craniocaudal	67.8±14.9	70.6±14.3	0.81
	mediolateral	40.7±9.2	45.0±11.0	0.44
	dorsoventral	99.7±35.9	117.8±18.1	0.09
Withers	craniocaudal	57.2±9.5	55.3±6.2	0.31
	mediolateral	36.0±7.9	43.2±10.6	0.56
	dorsoventral	44.2±6.1	45.2±11.1	0.84
Sacrum	craniocaudal	53.8±8.0	50.8±3.6	0.56
	mediolateral	49.3±9.1	47.6±8.0	0.26
	dorsoventral	89.8±8.9	92.8±10.5	0.84
LTC	craniocaudal	62.8±7.4	60.7±9.1	0.31
	mediolateral	55.8±7.4	50.2±7.0	0.44
	dorsoventral	94.2±19.4	99.8±6.8	1
RTC	craniocaudal	67.7±6.6	64.0±5.9	0.69
	mediolateral	55.2±7.4	49.0±7.0	0.19
	dorsoventral	96.5±20.8	101.3±10.8	1

Tarsal oscillation

There was significantly greater mediolateral oscillation of the left and right tarsi during the stance phase of the stride after WT exercise (P=0.031). An increase in oscillation magnitude and greater visibility of the medial aspect of the hindlimbs was observed in all horses. Pre WT exercise the median score was 1 and Post WT exercise the median score increased to 2.

4. Discussion

The objective of the study was to determine the effect of a standardized WT exercise protocol on overground in-hand walking limb kinematics and wither and pelvic displacement patterns before and immediately after WT exercise. The results indicate that WT exercise had some immediate effects on overground locomotion patterns. The results from this study support hypothesis 3, but do not support hypotheses 1 and 2.

An increase in tarsal oscillation after WT exercise was observed in all horses suggesting that after the protocol used in this study horses had reduced stability of the hindlimbs. It has previously been suggested that tarsal oscillation may reflect lack of muscular strength (Dyson *et al.,* 2018). Our findings could be consistent with local muscular fatigue of the hindlimb stabilising musculature, notably *quadriceps* and *biceps femoris.* Despite this sample of horses using the WT on a regular basis and being in regular work, it could be that these horses did not have sufficient muscular strength and endurance to maintain the same overground locomotion patterns after the specific WT session selected for the study suggesting potential individual muscle group fatigue. In this study the horses walked at the same speed throughout the WT test but in four different water depths. Although the WT test protocol simulated that being used in practice (Tranquille *et al.*, 2018), it was not the same as the WT training that these horses had previously been undertaking, so it is possible that the horses in this study had local muscular fatigue.

A previous study (Bowen and Paddison, 2017) showed that overground thoracic and lumbosacral ROM, determined by two-dimensional videography, was not different in a group of horses incorporating land/dry treadmill exercise versus a group of horses incorporating WT exercise in their training program during a two week period. Despite using different data collection methods, this is in accordance with findings from the current study where it was shown that overground ROM of the poll, withers, sacrum and left and right tubera coxae, determined with IMU's, were not significantly different after WT exercise. Mooij *et al.* (2013) found no changes in pelvic flexion whilst walking on a WT after 10 days of daily WT exercise. It is possible that to induce the changes to overground movement patterns of the pelvis the horse has to include WT exercise in its training program for a longer duration.

The results of this study provide novel information on the effect of a standardized WT exercise session on immediately-following overground in-hand walking locomotion patterns in the horse. However, areas warranting further work include: investigating the effect of different WT exercise programs, including duration, water depth and speed, and whether WT exercise induces long-term changes in overground locomotion.

Limitations

The main limitation of this study is the small sample size, but patterns were repeatable between horses. Video analysis was limited to two-dimensions. The mean age of the horses in the current study was 15 years, and therefore would be considered old/geriatric (Ireland *et al.,* 2011). Different results could potentially have been seen in a younger or fitter group of horses.

5. Conclusions

The results suggest that a 19-minute, mixed water depth walking WT training session at 1.6m/s had a significant immediate effect on overground in-hand walking locomotion patterns. The significance of these changes requires further investigation to ensure optimal WT protocols are used.

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