Kinematics of the equine axial skeleton during aqua-treadmill exercise

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Abstract

Equine aqua-treadmills are increasingly applied within the industry for rehabilitation from injury and training. Research into aqua-treadmill exercise has been increasing yet there are still opportunities to further quantify the effect of water depth on locomotion in order to optimise aqua-treadmill protocols to see improved rehabilitation from injury or to exercise horses more effectively for their chosen discipline. Much of the current aqua-treadmill literature focusses on the effects of water on locomotory parameters in walk, thus providing an opportunity for investigation into the impact of water at trot. Trot is the favoured gait for effective quantification of lameness and symmetry studies so it was anticipated that there may be opportunities to make comparisons to previously published overground data. Effective schooling of horses overground includes training aids, such as side reins, to constructively develop a horse's way of going and often to assist the horse in maintaining concentration. This provided a further opportunity for investigation into the use of side reins during aqua-treadmill exercise. This project therefore, aimed to quantify the effect of increasing water depths on pelvic and withers movement of horses trotting on an aqua-treadmill and to analyse the impact the use of side reins has on these movements.

Seventeen sound horses were habituated to aqua-treadmill exercise and subjected to one of two exercise protocols where data were collected either by optical motion capture (Qualisys©) or an inertial sensor system (Xsens©). The exercise protocol involved trotting on the aqua-treadmill at four increasing water depths, that of the third phalanx (P3), mid fetlock, mid third metacarpal (MC3), and mid carpus. Markers for optical motion capture were located on the poll, withers (T4/T5), mid thorax (T13), *tuber sacrale*, left and right *tuber coxae*, left and right *tuber ischia*. Inertial sensors were located on the poll, withers (T4/T5), mid thorax (T13), lumbar vertebrae (L4), *tuber sacrale*, left and right *tuber coxae*, and top of the tail (1st coccygeal vertebrae). Data were cut into strides with accelerations double integrated to generate displacement amplitudes, both vertical and mediolateral, for statistical testing. Pitch and roll data from the inertial sensors was also extracted and processed for analysis. Data were processed using custom written scripts (Matlab®) and repeated measures ANOVAs were performed throughout to test for significance with *post hoc* analysis where appropriate.

Water depth was found to have a significant effect on vertical displacement amplitudes of the pelvis and withers with vertical displacements increasing with increasing water depth, and a greater displacement in the pelvis than the withers. Minimum and maximum positions of the pelvis and withers were found to decrease and increase accordingly with increasing water depth, with minimum values decreasing significantly indicating an increase in limb compression during stance. Maximum vertical positions also increased significantly indicating greater maximum lift out of the water as a result of the increased compression. Water depth had no effect on symmetry of horses trotting on an aqua-treadmill and no effect on pitch amplitudes. Vertical displacements, pitch and symmetry were not altered with the addition of side reins, suggesting that the adoption of a different head and neck position whilst reaching comparable displacement amplitudes encourages

further engagement of back muscles possibly providing stimulus for building greater strength through muscular development.

Water depth was found to have no effect on mediolateral displacements of the pelvis or withers but with the withers exhibiting larger mediolateral displacements than the pelvis at lower water depths but reducing to an amount comparable to the pelvis at deeper depths suggesting that deeper water provides a stabilising effect on the front end of the horse. Side reins had no effect on mediolateral displacement amplitudes or on roll amplitudes. Mediolateral flexions of the spine were not affected by water depth or side reins, suggesting that the horse can be worked harder at greater water depths without over stressing the mediolateral capabilities of the spine.

Vertical displacements of the pelvis were significantly increased when trotting on the aqua-treadmill in a very low depth of water compared to measurements overground but this effect was not seen in the withers suggesting the front end of the horse can efficiently compensate for water depth by flexing at the carpus, although larger pitch amplitudes were reported at the withers suggesting a change in head and neck position to create a 'jump up' over the water. Side reins were found to decrease vertical displacement amplitudes in the withers overground but trotting on the aqua-treadmill in a small amount of water counteracted this effect suggesting that the addition of water may counteract a 'downhill' effect seen in horses wearing side reins overground.

This project suggests that the aqua-treadmill is beneficial at increasing the workload for the horse that may possibly have a corresponding effect of increasing muscle mass, strength and condition, but without detrimental effects to cranial-caudal or mediolateral symmetry patterns and that side reins have a potential benefit in supporting these locomotory patterns. Knowledge of this primary scientific data will better assist professionals working with aqua-treadmills to more effectively benefit the horses with which they work. There is, however, an opportunity for further longitudinal research to further support the effective application of the aqua-treadmill as a tool for rehabilitation and training.

Publications and Conferences

Publications:

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Abbreviations List

ANOVA	analysis of variance
ATM	aqua-treadmill
ATMP3	aqua-treadmill at a water depth of mid P3
bpm	beats per minute
C1	cervical vertebrae 1 *etc.*
c.	circa
DIP	distal interphalangeal joint (coffin joint)
EMG	electromyography
Hz	Hertz
IMU	inertial measurement unit
km/h	kilometres per hour
L1	lumbar vertebrae 1 *etc.*
L or R	left or right diagonal stride pair (measured from left or right hind stance)
MC3	third metacarpal (cannon bone)
mm	millimetres
m/s	metres per second
MTP	metatarsophalangeal (fetlock)
OG	overground
P3	third phalanx
PIP	proximal interphalangeal (pastern)
P	pelvis
S1	sacral vertebrae 1 *etc.*
SD	standard deviation
SEM	standard error of the mean
SHJ	scapulohumeral joint (shoulder)
T1	thoracic vertebrae 1 *etc.*
Ts	*Tuber sacrale* (*os sacrum*) (croup)
T4 / T5	thoracic vertebrae 4 and 5 (withers)
W	withers

CHAPTER 1: Introduction

1.0 Introduction

Equine aqua-treadmill exercise has become increasingly popular as a mode of rehabilitation and training for horses due to the ability to standardise and monitor many more variables than traditional overground training, thereby potentially being able to deduce the exercise load. Aqua-treadmills for horses in a crude form were first described in the literature by Auer in 1989, where the idea of the combined use of a treadmill and a whirlpool with jets of water positioned at strategic locations was proposed to be the ideal system for rehabilitation of a previously injured horse (Auer, 1989). Current day demands on all sport horses require them to run faster, jump higher and have more expression, all of which have implications on how horses are bred and trained for specific optimal characteristics for different disciplines. Horses are also required to remain competitive for longer putting further strain on body systems. However, equine aqua-treadmills as a tool for both rehabilitating and training horses with much positive anecdotal evidence of the influences on biomechanics and subsequent performance, are still relatively understudied in the literature to provide quantitative evidence to support these positive anecdotal claims. The equine aqua-treadmill may be a useful tool in helping to fulfil current day demands.

1.1 Equine gait

The gait of the modern equid is not reported to have undergone significant changes since the first documentation of *Equus caballus* (Linnaeus, 1758) and the first time that equine gait was measured and recorded by Eadweard Muybridge in the 1870s. Equines, as terrestrial prey mammals, have a terrestrial quadrupedal gait that has evolved to enable them to flee from predators at speed. The major evolutionary changes from *Eohippus* through to *Equus* in relation to the movement of the horse resulted in an increased length of leg, particularly the lower limb with no muscle mass below the carpus or tarsus (Goody and Goody, 2000). This resulted in long, lightweight limbs with a heavy hoof that acts as a pendulum to increase the swing of the leg increasing the length of the stride.

The equine is not unusual as a quadrupedal mammal in terms of its biomechanics in that it can alter its gait according to the terrain. There are four main gaits associated with the horse with increasing speed and decreasing duty factor (see Table 1.1) from walk through trot, canter and gallop, where each gait can be described quite specifically according to the fundamentally different mechanisms, foot fall sequence, forces, powers and metabolic costs (Minetti, 1998). There are also a couple of rarer and more unusual gaits in the horse of the tolt and pace that are seen in the gaited horse breeds such as the Icelandic Pony and Tennessee Walking Horse. The gait of the equine can be broken down into specific parameters such as displacements and the duty factor in order to describe the linear and temporal gait characteristics. Recent application of accelerometers has been used to identify footfall sequence and timings to quantify equine gaits further to establish whether gaits should be considered as discrete entities or a continuum (Robilliard *et al.*, 2007).

1.1.1 Gait parameters

In order to describe, quantify and investigate equine gait, clear parameters have been defined that necessitate consistent use to enable comparative, objective studies between subjects, environments and observers. Common parameters are defined in Table 1.1.

1.1.2 Gaits

Walk is the slowest, and possibly the most complex of the equine gaits with 4 beats that have large overlap times between the stance phases of the limbs and no period of suspension (Back and Clayton, 2007). Walk speeds in dressage horses have been reported as 1.37 m/s in collected walk to 1.82 m/s in extended walk with the speed change attributed to a lengthened stride (Clayton, 1995). In walk, each individual limb has a longer stance phase than swing phase i.e. a duty factor greater than 0.5 (Biewener, 1983; Hoyt *et al.,* 2006) which is possible due to the periods of triple support which describes the part of the stride where three limbs are in simultaneous contact with the ground.

Table 1.1: Common parameters used in gait analysis and their definitions.

PARAMETER	DEFINITION
Stance Phase	The portion of the limb motion cycle when the limb is in contact with the ground (Back and Clayton, 2007).
Swing Phase	The portion of the limb motion cycle when the limb is free from contact with the ground (Back and Clayton, 2007).
Stride	A complete cycle of the repetitive series of limb movements that characterize a particular gait (Back and Clayton, 2007).
Stride Duration	The time required to complete one stride (Back and Clayton, 2007).
Duty Factor	The duration of the stance phase of a specified limb as a proportion of the total limb cycle duration or stride duration (Back and Clayton, 2007). Stride duration, stance duration and protraction duration together determine the duty factor (the fraction of the stride for which the limb maintains contact with the ground surface) from which the peak vertical force can be estimated (Witte *et al.*, 2004).
Ground Reaction Forces (GRF) (Kinetic data)	The force of the ground against the limb that acts in opposition to the force exerted by the limb against the ground (Back and Clayton, 2007). A method for evaluating the accuracy of predicting peak ground reaction force from the duty factor using hoof mounted accelerometers to detect foot on and foot off has been identified as being accurate in the trot and symmetrical gaits while a correction factor is required to compensate for the difference between the lead and non-lead limbs of a pair in asymmetrical gaits (Witte *et al*., 2004). Vertical GRF is of the greatest magnitude most directly measuring specific limb weightbearing and sensitivity in grading lameness (Weishaupt, 2008). Craniocaudal GRF quantitates forces affecting forward progression—braking (deceleration) and propulsion (acceleration). Mediolateral GRF has the smallest amplitude, so few studies have used this variable (Hodgson *et al.*, 2014).

Trot is a symmetrical contralateral two-beat gait which is the equivalent to running or hopping in bipeds. In trot, the horse moves the limbs in diagonal pairs. Footfall timings have been quantified as equal between left and right hind limbs so the trot can be considered as symmetrical (Robilliard *et al.*, 2007). The trot has a shorter stance phase, a longer swing phase and the addition of an aerial phase. Trotting is generally faster than walk with ridden trot speeds ranging from 3.2m/s in collected trot, 3.6m/s in working trot, 4.5m/s in medium trot and 4.9m/s in extended trot (Clayton, 1994a) while trotting in hand has been reported to be approximately 3.9m/s (Galisteo *et al.*, 1998) plus with the addition of the aerial phase and reduction in stance phase, results in a duty factor of less than 0.5 (Biewener, 1983; Hoyt *et al.*, 2006).

Canter is the term used for a slow, collected gallop (Hildebrand, 1977). Canter and gallop refer to the same asymmetrical gait at different speeds with canter being a slower three-beat gait and gallop being a four-beat gait performed at the fastest speeds (Back and Clayton, 2007). The footfall pattern of a horse at left lead canter, is as follows: first the right hindlimb, then the left hind and right forelimbs as a diagonal pair, and finally the left forelimb followed by a short suspension phase with all limbs of the ground (Back *et al.*, 1997). Canter has been described at speeds ranging from 3-11 m/s from a collected canter of a mounted horse overground to an extended canter of an unmounted horse on a treadmill (Deuel and Park, 1990; Clayton, 1994b; Corley and Goodship, 1994), whereas gallop has been measured over a range of speeds from 9 to 17 m/s in field conditions (Witte *et al.*, 2006).

1.1.3 Symmetry and lameness

The symmetrical gaits of walk and trot (where movement patterns are the same on both sides of the horse) are difficult to quantify as there is always a degree of asymmetry in any animal whether it is a biped or a quadruped (Back and Clayton 2007). The asymmetric gaits of canter and gallop are not used when assessing gait symmetry. Asymmetry is due in part to laterality or natural 'sidedness' (Fredricson *et al*., 1980; Deuel and Lawrence, 1987; Drevemo *et al*., 1987) in the same way that humans are either left or right handed. An additional influence on laterality in horses is the standard procedure of handling horses from one side in preference of the other. Laterality causes difficulties in defining a threshold to be used to aid discrimination between natural asymmetry and asymmetry associated with lameness.

Gait is often measured in horses in order to assess lameness for which trot is the most commonly used gait (Buchner *et al.*, 1994a). Trot is the most symmetrical gait with emphasized flexion/extension and vertical displacements, where a sound diagonal pair can be used as a control for the lame diagonal pair. Vertical displacements are commonly studied when assessing lameness in horses. However, care must be taken not to misinterpret asymmetry found during trot when determining if a forelimb or a hind limb is lame as forelimb lameness has been shown to cause a 'false' lameness to appear in the hind limbs and *vice versa* due to compensatory load redistribution (Kelmer *et al.*, 2005). Trot can also be controlled in terms of speed with handlers being able to maintain pace with the horse when conducting overground in hand studies. This ease of control enables a good number of repeatable strides to be recorded, likewise trotting on a treadmill or ridden trot maintains consistency of the stride when compared to in hand trotting. Quantitative analysis of lameness commonly involves the calculation of symmetry ratios, which refers to the calculation of a ratio aimed at objectively quantifying describing parameter asymmetry between the left and right phases of a symmetrical trot stride (Walker *et al.*, 2010). Kinematic studies have calculated symmetry ratios to enable asymmetry to be quantified numerically for analysis between horses and conditions (Peham *et al.*, 1996; Pourcelot *et al.*, 1997; Keegan *et al.*, 2001, Keegan *et al.*, 2004; Audigie *et al.*, 2002; Keegan, 2007; Church *et al.*, 2009; Thomsen *et al.*, 2010a; Keegan *et al.*, 2011).

To address the low levels of inter-observer agreement, quantitative analysis of lameness is required when making judgements on symmetry and lameness (Keegan *et al.*, 1998; Arkell *et al.*, 2006; Fuller *et al.*, 2006; Hewetson *et al.*, 2006; Keegan *et al.*, 2010; Thomsen *et al.*, 2010b; Dyson, 2011; Rhodin *et al.*, 2013; Hammarberg *et al.*, 2016; Rhodin *et al.*, 2017). This low rate of agreement requires that objective quantitative assessment of gait can be made not only in laboratory studies but in the field in particular during Veterinary assessments, which may aid in pre-clinical detection of analysis, and early diagnosis and therefore treatment may reduce time out of work and recovery time and costs and increasing longevity of high level performance.

1.2 Gait analysis

Technological advances have enhanced the ways in which gait can be investigated both in lab based studies and in the field. When measuring gait, it is important to understand the differences between kinetics and kinematics. Kinetics is the study of internal and external forces, energy, power and efficiency involved in the movement of a body (Back and Clayton, 2007). Kinetic analysis is concerned with the forces that initiate and alter motion requiring identification of the external forces acting on a system together with their points of application and lines of action (Jones, 1988). Kinematics is the branch of biomechanics that is concerned with the description of movement (Back and Clayton, 2007). Kinematics deals with linear and angular displacements, velocities and accelerations without regard to the forces producing the motion (Jones, 1988).

Many methods of gait analysis utilise some kind of marker affixed to the skin. Skin mounted markers can introduce errors in data due to skin movement artefact in addition to the movement of underlying soft tissue (Clayton and Schamhardt, 2007). Often it is possible to use joint centres for affixing a marker but this relies on the competences of the person affixing the marker to accurately locate and palpate the joint centre, repeatably, and in a variety of subjects (Leach and Dyson, 1988). Markers are best placed on joints or bony landmarks with limited skin movement (Van Weeren *et al.*, 1992a, 1992b). Skin is known to move over bony landmarks, up to as much as 12cm in proximal parts of the limb particularly at the elbow (humeroradial joint) and stifle (tibiofemoral joint) (Clayton and Schamhardt, 2007). However, skin markers still provide a non-invasive and useful assessment of equine locomotion.

1.2.1 Optical Motion Capture

Optical motion capture (mocap) (e.g. Qualisys (QTM) ProReflex, Qualisys Ltd, Gothenburg, Sweden) is long recognised as the gold standard in gait analysis and has been used to demonstrate symmetry of back movement in healthy horses (Audigie *et al.*, 1999), to study three-dimensional kinematics of the equine spine during walk, trot and canter (Faber *et al.*, 2000; Faber *et al.*, 2001a, b), to show that chiropractic manipulation results in more symmetrical pelvic motion (Gómez Álvarez *et al.*, 2008) and that small asymmetries might be related to subtle lameness (Gómez Álvarez *et al.*, 2007). It has also been used for other equine research such as quantifying hoof deformation (Burn and Brockington, 2001) and studying mandibular movements of chewing (Bonin *et al.*, 2007).

Optical Motion Capture works using sophisticated infra-red cameras that reflect light from light-reflective hemispherical markers affixed to usually bony anatomical landmarks of an animal to reconstruct the shape of the animal in three-dimensions. However, the sophisticated technology is very much restricted to use in laboratories due to the large and bulky nature of the equipment and the requirement of having to maintain consistent light qualities. Also, the cost of the equipment restricts it to usually only enough cameras to be stationed around a treadmill so that data from many consistent strides can be collected rather than on an outside track where there may only be enough cameras to collect data of ten strides or so. This produces another potential problem of the changes that are documented to occur to gait when exercising on a treadmill rather than overground (Buchner *et al.*, 1994a). However, optical motion capture has been demonstrated to reliably capture the small ranges of motion in the equine back that are otherwise difficult to assess (Faber *et al.*, 2000; Faber *et al.*, 2001a, 2001b, 2001c).

1.2.2 Videography

Videographic gait analysis would seem the most accessible method, especially when skin fixated markers are used, as there are many widely available pieces of software that enable comprehensive, semi-automated tracking and analysis in both two and three dimensions. However, when dealing with videographic systems there are many more complications to consider that vary between the systems such as: placement of the video camera ensuring it is precisely perpendicular to the plane of interest which restricts how many strides may be visible in the field of view, calibration of the frame, digitization, transformation, smoothing and normalization of the data (Back and Clayton, 2007, chp.3).

High speed video cameras are generally less accessible but are very useful for studies of short duration events or rapid movements as they can record at rates of up to 10 million Hz (Xing *et al.*, 2017), whereas a standard camcorder may record at only 25-30 Hz. However, one study compared sampling rates of 60 and 1000 Hz and determined that with no differences in the measured parameters of the equine stride, stance and swing duration greater than 3.3 metres per second a camera that records at a rate of 60Hz is adequate for kinematic gait analysis in the walk, but that faster gaits require a greater sampling rate for increased accuracy (Linford, 1994). Quantifying movement symmetry with video filming in front or behind the horse also requires complex post processing as the subject is constantly moving towards or away from the camera thus making the calibration and calculation of measurements more difficult and prone to errors.

1.2.3 Force plates

Kinetic analysis of gait refers to the measurements of the forces involved, for which force plates, force-measuring horse shoes and strain gauges can be used. Force plates are restricted to lab-based studies and are limited to providing data for only one or two strides at a time with the added difficulty in making sure the horse steps consistently in the middle of the plate to prevent artefacts. Also, it is difficult to gauge the effects of different surfaces. This has resulted in the development of a horseshoe for measuring three-dimensional ground reaction forces in horses moving overground and across different surfaces (Frederick and Henderson, 1970; Barrey, 1990; Ratzlaff *et al.*, 1990; Roepstorff and Drevemo, 1993; Kai *et al.*, 2000; Roland *et al.*, 2005; Chateau *et al.*, 2009). Early force measuring shoe models were unable to provide a complete description of three-dimensional ground reaction forces and moment vectors, and the 2005 version by Roland *et al.* (2005) used strain gauge technology but was reported to be too heavy at 860g to accurately assess gait without itself causing changes to the gait. The most recent development of a dynamometric device using piezoelectric sensors (Chateau *et al.*, 2009) has been reported to accurately measure three-dimensional ground reaction forces at walk and trot and on different surfaces, and more recently in ridden horses, canter, circling, jumping and when measuring grip on different surfaces (Robin *et al.*, 2010; Camus *et al.*, 2012; Crevier-Denoix *et al.*, 2014).

1.2.4 Inertial Measurement Units

Inertial measurement units have advanced enormously over recent years. Simple hoof mounted accelerometers with a small size and mass have been shown to not adversely affect locomotion (Witte *et al.*, 2004; Pfau *et al.*, 2006) and accelerations of the withers when integrated through velocity have been shown to provide displacement scores comparable to optical motion capture (Pfau *et al.*, 2005). However, the drawback of simple accelerometery devices is the absence of their own recording capabilities requiring the use of at least a simple data logger using MP3 recorders (Parsons and Wilson, 2006) or passing wires up the limbs to a recording device attached to the trunk or distal limb (Witte *et al.*, 2004, Pfau *et al.*, 2006).

Inertial measurement systems have been developed which incorporate tri-axial accelerometers, gyroscopes and magnetometers. Xsens© is one example of such a system (Xsens©, MTx; Xsens, Enschede, The Netherlands). This sophisticated small and lightweight system has been developed to provide accurate measurement of movement in all directions and rotations. Due to the high accuracy and repeatability of the data recorded with little impact of environment, they have been recruited for thorough investigation of locomotion outside of laboratory conditions. Studies have identified that these inertial measurement units allow data to be collected during unrestricted movement giving up to six degree of freedom information (both orientation and displacement) (Keegan *et al.*, 2004; Pfau *et al.*, 2005; Pfau *et al.*, 2006; Pfau *et al.*, 2007). Along with the double integration of accelerations to provide displacement data (Pfau *et al.*, 2005), symmetry related parameters such as Energy Ratios can also be calculated for each stride (Keegan *et al.*, 2004; Pfau *et al.*, 2007). These inertial measurement systems have been utilized to document and quantify vertical head, trunk and pelvic movements along with movement symmetry of sound horses being lunged on hard and soft surfaces where information has previously been sparse (Walker *et al.*, 2010; Starke *et al.*, 2012a; Pfau *et al.*, 2012; Pfau *et al.*, 2016b; Greve *et al.*, 2017).

One study specifically compared data from the inertial measurement units to that of the gold standard of motion capture and determined that accuracy of dorsoventral and mediolateral displacement data calculated from inertial measurement units mounted along the spine of horses trotting over ground lay within ± 4–8 mm (± 2 SD) of the gold standard of motion capture, and Audigié energy ratios varied by ± 0.03 (± 2 SD) between the two methods (Audigié *et al.*, 2002; Warner *et al.*, 2010). More recently, a smartphone (Apple iphone6) was tested against the IMU based dedicated gait analysis system of Xsens© for assessment of pelvic (a)symmetry and limits of agreement were similar enough to suggest that smartphones could be utilized by first opinion practitioners as a convenient alternative to the specialist more expensive devices (Pfau and Weller, 2017). Accuracy as a percentage of the range of movement was found to be in the range of 9–21% (dorsoventral) and 10–24% (mediolateral). Considering that experienced clinicians have been evidenced not to be able to consistently detect asymmetries below a level of 25% (Parkes *et al.*, 2009) therefore suggested that the ease of use of the inertial units along with the accuracy and objectivity of the data coupled with the potential to collect a large amount of simultaneous data renders the inertial unit system a valid technique to collect objective limb and back movement data during overground locomotion (Warner *et al.*, 2010).

Since the initial development and validation of inertial measurement units for gait analysis, other inertial measurement systems have been developed ranging from simple accelerometery devices to the sophisticated Xsens© system described above. Inertial measurement units have been used more and more in the literature for investigating a variety of locomotor related areas, such as demonstrating that inertial sensor-based systems can objectively assess the response to proximal hindlimb flexion tests (Marshall *et al.*, 2012). Inertial measurement units (Xsens©) have been used to accurately quantify hoof on/hoof off timings with the inertial measurement units mounted on the distal metacarpus or distal metatarsus and *sacrum* (Olsen *et al.*, 2012). Another 2012 study reported that a single pelvis-mounted inertial sensor was accurate in quantifying hind limb foot contact timings when compared to data from hoof-mounted accelerometers (Starke *et al.*, 2012b). It has also been determined that an inertial sensor-based system can distinguish between a positive and negative response to diagnostic anaesthesia of the foot and objectively assess the effect of a positive response on the trot (Maliye *et al.*, 2013).

Studies have evolved that compare the data reported from inertial measurement systems to the experience of Veterinary Clinicians. McCracken *et al.* (2012) reported that an inertial sensor system was able to identify lameness in the sole of the hoof at a lower level of lameness than by the consensus of three experienced equine Veterinarians. Keegan *et al.* (2013) also compared data from an inertial measurement system with subjective lameness examinations from three experienced equine Veterinarians and reported that an inertial sensor-based lameness evaluation may enhance but not replace subjective lameness examination of horses even though all inertial sensor measures for forelimb and hind limb lameness were positively and significantly correlated with results of subjective evaluations.

It is pertinent to remember that there is now a wide range of inertial measurement units available on the commercial market but the software may vary tremendously between systems. A 2016 study sought to quantify differences in two leading inertial measurement systems and determined that regression-based correction for systematic differences between the two systems the widths of limits of agreement values for comparison of straight line trials was within, or only marginally outside current proposed thresholds of detecting lameness in horses (6 mm for head movement, 3 mm for pelvic movement) (Pfau *et al.*, 2016a).

1.2.5 Treadmill locomotion

Treadmills have routinely been used in locomotion studies, particularly before the development of inertial measurement systems that can be used in the field. The first records of horses on treadmills for research purposes was in 1967 (Persson, 1967). On a treadmill, it is possible to control speed allowing gait to be evaluated under different conditions, eliminating or controlling for some influencing factors. However, treadmills have been reported to cause changes in kinematic stride variables (Fredricson *et al*., 1983; Leach and Drevemo, 1991; Barrey *et al*., 1993; Buchner *et al.*, 1994a; Buchner *et al.*, 1994b). The ability to control speed on a treadmill may be beneficial, however, it has been reported that speed on a treadmill is achieved with a higher stride frequency and a longer stride length (Barrey *et al*., 1993). There has also been shown to be an increase in stance duration on a treadmill associated with a 9% decrease in speed of the treadmill belt due to the frictional effect of the vertical force applied through the horse's limb, with earlier placement of the forelimbs, greater retraction of both fore and hind limbs, forelimb preceding hindlimb placement by 22ms and a reduction in vertical excursions of the hooves and reduced vertical displacement of the hooves (Buchner *et al.*, 1994a). Clayton and Schamhardt (2007) report that horses use less energy during treadmill locomotion than during comparative speeds and gaits overground, which is likely to be due, in part, to an energy transfer from the treadmill belt to the horse.

It has been documented that although horses adapt very well to treadmill exercise a period of habituation to the treadmill belt is required before horses move consistently, with faster gaits habituating quicker and only three five-minute sessions required to see stabilization of stride kinematics in the trot, whereas the walk kinematics are not fully stabilized even at the tenth session (Buchner *et al.*, 1994b). Treadmills have been used countless times in research to study the movements of the back and limbs, responses to training and in lameness investigations. Treadmills also now exist that have a force plate embedded in them which enables consistent measurement of all four limbs over a number of successive strides (Weishaupt *et al.*, 1996). It is therefore, important to remember that whilst treadmills can offer the opportunity to collect data from a repeated number of successive strides at a constant speed, that horses used in treadmill studies will need a period of acclimatisation to treadmill exercise in each gait.

1.3 Hydrotherapy

Equine hydrotherapy is an area of interest that has been rapidly expanding in recent years. Currently, equine hydrotherapy exists in three main forms: swimming, cold water static spa therapy, and movement through water in either a water walker or water treadmill. The water or aqua-treadmill is a piece of equipment that has rapidly grown in popularity amongst professional and leisure riders and Veterinary clinicians, and increasingly research is being conducted to quantify the positive responses reported anecdotally by nearly all that use them.

1.3.1 Human Hydrotherapy

Hydrotherapy is a branch of physiotherapy that has been long recognised for its benefits which are reported to be relief from pain, swelling and stiffness, joint mobilisation and increased range of motion, cardiovascular fitness and muscle strengthening, maintenance & restoration. Historically, ancient human civilisations are documented to have used hydrotherapy for its physical benefits, notably the use of hot spas and baths in Roman times, and the Greek philosopher Hippocrates is reported to have recommended hydrotherapy for relieving medical conditions (Jackson, 1990). In more recent times, Charles Darwin recognised that he would not have lived long enough to publish his famous work ‘On the Origin of Species’ without the hydrotherapy, water cure and homeopathic treatments that he underwent for his own health, although he was reluctant to admit the benefits of these alternative therapies for fear of his major works not being taken seriously (Ullman, 2009). Hydrotherapy specifically involves therapeutic exercise in the form of swimming or walking (Prankel, 2008). In humans, aquatic exercise has been used extensively in rehabilitation where there is evidence to suggest that exercise in water is preferable to land exercise for patients suffering from orthopaedic disease, and that aquatic therapy may aid ease of movement, swelling reduction and pain relief (Hinman *et al*., 2007), due to the pressure and temperature of the water. There are also effects of the water buoyancy and water resistance (Denning *et al.*, 2012). A recent review article about humans sought to qualify the differences in physiological and biochemical responses that are related to aquatic exercise and how these responses differ relative to land-based exercise so that clinicians can provide more accurate rehabilitation programmes for their patients finding that pain levels were similar between water calisthenics and land exercise but that pain levels tended to decrease after underwater treadmill exercise (Denning *et al.*, 2012).

1.3.2 Animal Hydrotherapy

Specific animal hydrotherapy centres have greatly increased in number due to the sufficient evidence for the effectiveness of hydrotherapy in humans (McGowan, 2008). Canine hydrotherapy centres in the UK have increased rapidly in the last decade from zero centres in 1996 to over eighty centres in 2008 (Waining *et al.*, 2011). The recognition of the importance of rehabilitation after injury has grown which has resulted in Veterinary Surgeons consulting trained therapists in order to put together specific rehabilitation programmes for each animal (McGowan, 2008). They attempt to rehabilitate the animal in a more thorough way so that there is less chance of a repeat injury or an injury occurring elsewhere before the animal is fully fit.

Currently, no specific qualification exists for equine hydrotherapy, but there are regulating bodies for professional practice in animal physiotherapy that strive to promote professional practice in Veterinary physiotherapy to ensure the highest standards of physiotherapy care are delivered to animals. The main one is the Association of Chartered Physiotherapists in Animal Therapy (ACPAT) (ACPAT, 2017) which is recognised by BEVA (BEVA, 2017) as a Musculo-Skeletal Allied Professional. Buchner and Schildboeck stated in 2006 that there were, however, only a small number of reliable studies offering sufficient evidence for clinical efficacy of physiotherapy in equines. A level 3 qualification has been accredited in small animal hydrotherapy, originally pioneered by Hawksmoor in 2002 (Hawksmoor, 2017) but now more widely available throughout the country including qualifications at Level 4 as well (Moulton College, 2014).

1.3.3 Equine Hydrotherapy

Equine hydrotherapy centres have emerged in a similar way to small animal therapy centres, as the future of the equine industry has focused far more on rehabilitation of the sports horse in recent years. Due to the increased focus on the horse as a performance and sporting animal research has grown in the area of retraining and rehabilitation from injury. Unlike the racing industry, in sports such as Eventing, Dressage and Showjumping, the horse's career as a performance animal can be increased and prolonged with the use of specific training and maintenance of the horse's ability to perform. As a result of this, maintaining soundness in performance has led to increases in the use of equine physiotherapy along with other complementary and alternative methods including hydrotherapy. Due to the lack of clinical evidence many Veterinary Surgeons can be reluctant to refer an animal for treatment, especially in the current economic climate when additional therapy would incur further costs to the owner. Although many insurance companies do have policies that cover alternative therapies, the

Veterinary Surgeon can suggest the more traditional recovery approach of trying a period of rest for the horse which would cost next to nothing, hence current efforts by some researchers to document findings within the state-of-the-art therapy centres where they could be invaluable as part of a therapeutic programme and aid a faster and perhaps better recovery, so by returning an animal to training and competition in a shorter amount of time.

Much of what is practiced in equine hydrotherapy centres is based on anecdotal reports and systems of trial and error in conjunction with the Veterinary Surgeon that first referred the horse. Famously the racehorse Red Rum was trained on Southport Beach and his successes and recovery from lameness were accredited to the benefits of bathing in the sea. Currently, there are no laws or legislation that govern equine hydrotherapy and operation of hydrotherapy equipment and practices rely on an experienced horse person to safely and confidently train the horse appropriately in these novel practises.

1.3.4 Modes of equine hydrotherapy

Equine hydrotherapy is limited to three main modes: swimming (whether it be round pool or straight line but completely non-weight bearing), cryotherapy (whether it be cold water spa therapy or ice or cold water boots), and water walking or water treadmill exercise. Specific equine hydrotherapy centres have been proliferating in the UK over the last twenty years with apparently bigger and better centres housing more sophisticated and advanced pieces of equipment. These include the centre at Moulton College in Northamptonshire that uniquely houses a straight-line swimming lane with optional water jets to provide a mild current for horses to swim against, a cold-water saline spa (CET Equine Spas, 2017) and an aqua-treadmill (FMBs, 2017). This hi-tech equipment is both costly and requires ample space for safe and effective operation, which seem the most dominant reasons for opting to have hydrotherapy equipment installed. Centres such as the one at Moulton College operate commercially so the initial financial outlay can be considered an investment. However, due to the enormous amount of positive reports from owners, allied professionals (such as equine physiotherapists and chiropractors) and Veterinary Surgeons, many private training and livery yards are opting to include one or more piece of hydrotherapy equipment for their own use. For example, there are many private equine swimming pools in racehorse training yards, and many professional event riders have installed their own cold water spa or at least own a pair of cold water or ice boots. Aqua-treadmills have increased in popularity enormously over the last few years, with some equestrian centres specifically opting to invest in this piece of equipment to use privately and commercially due to the enormous amount of positive anecdotal evidence and the recent increased surge in scientific evidence supporting the positive changes the aqua-treadmill can make to a horse's way of going (Nankervis *et al.*, 2016; Tranquille *et al.*, 2016; Tabor and Williams, 2017; Nankervis *et al.*, 2017).

1.3.4.1 Swimming horses

Swimming has been a popular mode of exercising horses for training or as a means of maintaining cardiorespiratory fitness during layups since the late 1970s although it is difficult to determine the exercise load. An early study by Thomas *et al.* (1980) had the horses dragging weights behind them in an attempt to standardise the exercise load when swimming, but Hobo *et al.* (1998) determined the exercise load by evaluating swimming speed, heart rates and blood lactate concentrations which all indicated that the swimming load had been aerobic. Bromiley (2000), however, states that swimming exercise is nearly always anaerobic as the horse is unable to breathe efficiently. Hobo *et al.* (1998) noted that arterial haematological parameters degenerated significantly after the swimming started suggesting that there was an effect of water pressure on the horse's body preventing adequate ventilation yet still the exercise was aerobic, which is contradictory, as if it is aerobic then there is by definition sufficient ventilation and O_2 saturation. Hobo *et al.* (1998) also interestingly noted that respiration patterns change from the 'one-pitch-one-breath' method that is seen in field running, suggesting that swimming horses need a greater amount of ventilation to compensate for the restricted respiratory rates due to the water pressure on the chest and abdomen, and that expiratory times were longer than inspiratory times during swimming indicating that longer expiratory times may limit sudden airway collapse caused by the water pressure preventing a radical decrease of air space volume and therefore maintain buoyancy.

The physiological responses of humans exercising in water have been well documented including the energy required for an exercise in water compared to the same exercise on land, the energy expended, maximal oxygen uptake, circulation and thermoregulation (Cureton, 1997). However, in horses, studies are more limited and tend to look at the metabolic and cardiopulmonary responses (Jones and Hiraga, 2006). The Jones and Hiraga (2006) report made comparisons to metabolic and cardiopulmonary responses during swimming and metabolic and cardiopulmonary responses during treadmill galloping and noted some significant conclusions; overall it was found that during swimming horses do not reach their VO_{2max} and consequently maximum heart rates during swimming tend to be lower than at VO_{2max} on a dry high-speed treadmill (Jones and Hiraga, 2006).

In general, swimming is considered to have a similar training effect on cardiorespiratory function but without the load on the legs so is therefore beneficial for horses with locomotorial disease (Misumi *et al.,* 1994b, 1994c). It is difficult to draw comparisons between the existing swimming studies as they have all measured different variables in different ways in both round pools and straight-line pools. Another study determined that evaluation of the exercise fitness from swimming horses was comparable to the exercise fitness of running horses and that poor performance swimming was then a useful indicator to predict poor performance on the track (Misumi *et al.,* 1994a; 1995). An early study by Irwin and Howell (1980) stated that when swimming horses they always had a Veterinary Surgeon present in case of accidents, but there are no legal requirements in existence today.

1.3.4.2 Cryotherapy

Many livery yards and training yards now own a cryotherapy spa as an added benefit for their customers and clients as these pieces of equipment are *relatively* cheap, easy to install and require less space than a pool or aqua-treadmill. When studying the literature, although there may be many papers investigating the efficacy of cryotherapy, particularly in humans, only one paper is directly concerned with cold spa therapy in horses (Hunt, 2001). Hunt (2001) through successful trials with twenty-seven horses with various lower leg injuries stated that hypertonic cold water spa therapy was a valuable addition to therapeutic regimes with horses returning to work sooner and competing successfully without re-injury. A lot of work has been conducted into the principles of cryotherapy in all its different forms and recommendations exist for the practical application of the different forms in different situations particularly with reference to effects of cooling on survival of tendon cells (Petrov *et al.*, 2003; Reesink *et al.*, 2012). It does not appear that cold water spa therapy has been included as a mechanism of cooling by cryotherapy studies and therefore the added benefits of the direct control over the temperature of the water, the depth of the water and the salinity or mineral content of the water have not been studied. Humans are less tolerant of cold water immersion than horses due to the increased blood pressure due to peripheral vasoconstriction and associated risk of hypothermia (Michlovitz, (1990), cited in Hunt, (2001)). However, cold water immersion is used regularly in humans as a popular recovery intervention after exercise although the physiological and biochemical rationale is still unclear (Bleakley and Davison, 2009).

The composition of natural spa waters has been well documented and research has been conducted into the therapeutic effects of these waters on various skin conditions in humans such as psoriasis, and very specific research exists on the effects of spa waters on cutaneous immunological responses (Joly *et al.*, 2000). Recently, more investment has been made in the cosmeceutical benefits of spa waters where anti-inflammatory responses, anti-carcinogenic properties and anti-radical properties have all been noted (Polefka *et al.*, 2012; Seite, 2013). With regard to the mechanical effect of balneotherapy (bathing in thermal or mineral waters) it is reported that immersion allows the mobilisation of joints and the strengthening of muscles with minimal discomfort and reduction in inflammation in diseases such as rheumatoid arthritis (Nasermoaddeli and Kagamimori, 2005). Being immersed in water has been documented in humans to bring about increased diuresis and natriureses, haemodilation, increased cardiac output and reduced plasma levels (O'Hare *et al*., 1985) and mechanical stimulation includes a loss of some body weight thus allowing for easier movement where the body resists the flotation force and the output and the rhythm of the heart increases and breathing becomes deeper (Matz *et al*., 2003). One paper has studied the effect of immersing horses in warm spring water but this looked at the effect on the autonomic nervous system and not any mechanical changes finding that immersion in warm spring water increased parasympathetic nervous activity and may therefore be a means of relaxation for horses (Kato *et al.*, 2003).

The majority of the research conducted into different forms of balneotherapy has been conducted in humans and it may not be possible to draw direct comparisons in horses due to the overt physiological and anatomical differences. However, work that specifically relates to cryotherapy can probably be extrapolated to horses in many instances as it seems currently that it is only promoted to immerse horse lower limbs in cold water where it can be assumed that the make-up of tendon, ligament and bone structures is comparable to that of humans, but with there being far less fat and muscle in the distal limb of horses.

1.3.4.3 Aqua-treadmills and walkers

Equine aqua-treadmill exercise has become increasingly popular as a mode of rehabilitation and training for horses due to the ability to standardise and monitor many more variables than swimming, thereby potentially being able to deduce the exercise load. Aqua-treadmills in a crude form were first described in the literature for horses by Auer in 1989, but then no reference appears again until 1999, over ten years later (Tokuriki *et al.*, 1999). Auer described the idea of the combined use of a treadmill and a whirlpool whereby jets of water were positioned at strategic locations so that the horse could work against the resistance of the water but also then intensifying the massaging action of the water against the limbs and the effect of the water significantly reducing the shock impact to the legs and reducing the risk of injury (Auer, 1989). It was suggested at this time that "the combination of all these features renders such a unit the ideal system for rehabilitation of a previously injured horse" (Auer, 1989).

There is documentation from the United States Patent Office where an invention for a piece of equipment for 'animal exercising, conditioning and therapy' was proposed by an E. J. Scanlon in 1969 stating, "This invention is a novel piece of apparatus wherein the animal is made to run in place on a treadmill, the treadmill being disposed in an enclosure partially filled with liquid" (Scanlon, 1969). Aqua-treadmills now feature as the *pièce de résistance* in equine therapy centres and training yards. As the technology develops and the price becomes more affordable, more equestrian enterprises are having them installed. Aqua-treadmills also exist in a different form of an aqua-walker. These are essentially horse walkers that are submerged in water, enabling several horses to be exercised at the same time, thereby giving obvious advantages in time saving but potentially losing the ability to create individual exercise programmes developed to the specific needs of each horse. There are fewer aqua-walkers in the UK than in USA and no research has been detected on the use of an aqua-walker. A further advantage, however, of an aqua-walker, is that no mechanical belt is needed. It is well documented that equine treadmill locomotion has biomechanical differences to over ground locomotion (Fredricson *et al*., 1983; Leach and Drevemo, 1991; Barrey *et al.*, 1993; Buchner *et al.*, 1994a, 1994b) with a horse apparently first put on a treadmill for research purposes in 1967 (Persson, 1967). Although it seems that the aqua-walker may be more beneficial here for research purposes the most obvious advantage of aqua-treadmills to aqua-walkers is the ability to go in a controlled straight line for several strides and therefore the aqua-treadmill may provide locomotion that is more comparable to overground locomotion. Control of locomotion aided with knowledge of the properties of water such as buoyancy, viscosity, hydrostatic pressure, surface tension, specific gravity and temperature effects, should equate to constructive, specific and controlled therapeutic rehabilitation techniques.

1.4 Current research into aqua-treadmills

As indicated previously, the idea of an aqua-treadmill was first proposed in academic literature in 1989 as potentially the ideal system to rehabilitate an injured horse (Auer, 1989) but a patent exists in the USA dating back to 1969 (Scanlon, 1969). The next time aqua-treadmills appear in the literature is not until 1999 where Tokuriki *et al.* uses an aqua-treadmill as part of a methodology assessing electromyography activity of different muscles during different types of exercise suggesting that, at this point, aqua-treadmills are already a recognised and established exercise and rehabilitation medium for horses. Over the following ten years (1999-2009) academic literature featuring aqua-treadmills was sparse, but with the team led by Dr Kathryn Nankervis at Hartpury College made some significant contributions to introductory aqua-treadmill research. Over the last few years there has been a significant increase in aqua-treadmill research, possibly due to a combination of the increase in aqua-treadmills available in the UK and Europe so improving accessibility. But also due to a significant improvement in technology enabling a wider variety of options for collecting data specifically relating to gait benefits and adaptations. The aqua-treadmill being an enclosed metal box, coupled with the ferocious wash of water over the limbs does not lend itself well to data collection by visual methods or by non-waterproof electronic devices, making objective quantification of locomotion difficult. Current research into aqua-treadmills can be divided into a few key areas.

1.4.1 Heart rates

The effects of aqua-treadmill exercise on heart rates has been investigated more than any other parameter in the literature. A key study from 2006 measured heart rate responses during acclimation to aqua-treadmill exercise and found that acclimation to walking on an aqua-treadmill at a depth of mid-radius takes two fifteen minute sessions with the horses not sedated for heart rates to reach a steady threshold of around 70 beats per minute (Nankervis and Williams, 2006). These results confirmed heart rates reported in an earlier study, and in fact the first study to measure heart rate responses in the literature by Voss *et al.* in 2002, where horses were walked or trotted for twenty minutes at water depths of above carpus, above elbow or on a dry treadmill belt, finding that the most intense exercise load of trotting for twenty minutes at a water depth of above elbow only produced a heart rate of 125 beats per minute, and there was only a small significant difference of 18 beats per minute between the two paces of walk and trot as different workloads, and deducing that aqua-treadmill exercise only constitutes a medium intensity workload (Voss *et al.*, 2002).

A later study investigated the effect of water temperature on heart rates during aqua-treadmill exercise and found that increased water temperature from only 13 to 19 degrees Celsius significantly increases heart rate even when exercise intensity was low (walk) and water depth was considered low at the scapulohumeral joint (Nankervis *et al.*, 2008a). A further study by the team at Hartpury College went on to investigate the effect of water depth on heart rates finding no significant differences in heart rate between each of the wide-ranging water depths from hoof height (mean HR 62.0 ± 10.2 bpm), to the proximal interphalangeal joint (mean HR 61.1 ± 8.3 bpm), to carpus (mean HR 60.6 ± 6.7 bpm) and to ulna (mean HR 64.7 ± 8.0 bpm), but this study looked at walk only (Scott *et al.*, 2010).

A 2003 study reported much higher heart rates of between 120 and 160 beats per minute when trotting in deep water but with no significant differences found between water depths ranging from 10 to 80% of the height of the withers or speeds ranging through walk and trot (Lindner *et al.*, 2003). The higher heart rates reported here than seen in any of the other studies may therefore be due to perhaps in increase in water temperature, lack of acclimatisation to the treadmill, or an increased workload in the trot in deeper water, however, the heart rates are still not high enough to be consistent with highest maximum values of 210-240 beats per minute described previously in literature (Asheim *et al.*, 1970) therefore still only producing a medium sized workload which all these studies appear to agree on, possibly due to the published differences in treadmill versus overground locomotion.

A more recent study of aqua-treadmill exercise and heart rates was in 2011 by the German team of Lindner *et al.* (2011) where they again reported slightly different results with maximum heart rates reaching around 140 beats per minute from an assortment of variations of depth (ranging from 10 to 77 % of the height of the withers) and speed (including trotting up to 5.5 metres per second) but that the fluctuations in heart rate at the different stages of the set exercise tests make it difficult to use heart rate as an accurate evaluation of conditioning in horses exercising on an aqua-treadmill and perhaps heart rate results need to be interpreted differently than from overground studies (Lindner *et al.*, 2011) but it may well be the temperature of the water that affects heart rates most significantly (Nankervis *et al.*, 2008a). More recent studies have found similar results where exercising in an aqua-treadmill does not necessarily produce significant differences in heart rates (Borgia *et al.*, 2010; Firshman *et al.*, 2015). The variations in reported heart rates would therefore not appear to be a useful indicator of workload during equine aqua-treadmill exercise.

1.4.2 Physiological responses

Other physiological responses have often been measured in conjunction with heart rate studies including blood parameters such as lactate and haemoglobin concentration. Voss *et al.*'s (2002) early study measured physiological variables of lactate and haemoglobin concentration as they have previously been reported as reliable markers to indicate exercise induced changes (Persson, 1969) but found none of the exercise tests on the aqua-treadmill (walking and trotting and variable water depths) produced lactacidaemia concluding that horses were able to provide for the higher oxygen demand via respiration presuming an aerobic workload. Voss *et al.* (2002) determined that haemoglobin concentrations increased with increasing workloads which concurs with well-known previously reported data that haemoglobin concentration increases as a result of the mobilization of the erythrocyte reservoir in the spleen during physical exertion (Persson, 1969).

Lindner *et al.*'s 2003 and 2011 studies also studied blood lactate concentrations and found that it was not possible to determine v_4 (speed at which a blood lactate concentration of 4mmol/L is determined under the defined conditions) in horses exercising on aqua-treadmills as the maximum speed of around 5.5 metres per second was not fast enough to induce significant responses in blood lactate even when exercising at the water depth of 80% of withers height did not impose sufficient additional stress to obtain lactate concentrations of ≥4 mmol/L blood coming to the same conclusions reported in the heart rate literature, that it is difficult to evaluate the workload of aqua-treadmill training (Lindner *et al.*, 2003; Lindner *et al.*, 2011).

A more recent study has assessed not only blood lactate but lactate dehydrogenase, creatine kinase, aspartate aminotransferase, glucose and triglyceride levels in horses fed different dietary energy sources and then trained on an aqua-treadmill and made similar overall conclusions in that the measured lactate values suggest that the energy requirement of the aqua-treadmill training (even with twenty minutes trotting in water to 85% of the withers) was provided by an aerobic energy supply as the measured values were below the generally accepted anaerobic threshold of 2-4 mmol/L but there were more significant changes in the creatine kinase and aspartate aminotransferase levels which therefore may be a better indicator of workload intensity (Vincze *et al.*, 2016).

1.4.3 Muscle

An alternative indicator of workload or intensity would be to measure the electromyographic (EMG) activity of some key muscles during aqua-treadmill exercise. This was undertaken in the early 1999 study by Tokuriki *et al.* where eight skeletal muscles (seven in the forelimb and one in the hindlimb) were tested during overground walking, swimming and walking and trotting in deep water on an aqua-treadmill (Tokuriki *et al.*, 1999). Interestingly EMG activity during swimming tended to have continuous activity within a burst of a cycle but walking or trotting in the aqua-treadmill often had intermittent activity within a burst and four horses had more intensified EMG activity at the trot in the aqua-treadmill than the walk but two horses showed the opposite pattern. The muscle that showed the most intense EMG activity was the *extensor digitorum communis* during walking and trotting in the aqua-treadmill suggesting that this muscle probably aided in protracting the distal limb against the resistance of the water (Tokuriki *et al.*, 1999). The water in this study was very deep at 1.2 metres (approximately the depth of the point of shoulder) so this is very plausible. The study concludes that trotting in the aqua-treadmill may provide less intensive training for some forelimb muscles than walking but the depth of the water is important to note here, and frustratingly, the study does not report the results of the EMG activity of the hindlimb muscle tested.

Over ten years later, another study measured the responses of muscles to aqua-treadmill measuring muscle lactate, glycogen, and ATP concentrations from muscle biopsies of the gluteal and superficial digital flexor muscles (SDF) taken before and after four weeks walking training of up to 20 minutes at a water depth to the ventral abdomen but found that this exercise protocol was not strenuous enough to produce any change in the skeletal muscle parameters measured suggesting a more strenuous exercise protocol was required to therefore have a training and hypertrophic effect in muscles (Borgia *et al.*, 2010).

A very similar study by the same team in 2015 included modifying the set exercise test to eight weeks training rising to 40 minutes walking at a depth of the olecranon but again found no evidence of adaptations within the SDF or gluteal muscles (Firshman *et al.*, 2015). In 2016, an interesting study was presented at the 8th International Conference on Canine and Equine Locomotion by a team from Belgium that measured cross sectional area of fifteen skeletal muscles after eight weeks of training on an aqua-treadmill for twenty minutes a day in walk at a water depth of about 40cm. They reported a significant hypertrophy of muscles in the forelimb, back and hindlimb, particularly muscles involved in elevation and forward movement of the forelimb, flexion of the hind limb and muscles used for extension of the spine (Van de Winkel *et al.*, 2016).

1.4.4 Gait and locomotory parameters

Recent studies have focussed more on gait and locomotory parameters suggesting that the development in modern technologies has contributed to this progression. Gait analysis on an aqua-treadmill was first reported in the literature in 2010 where the effect of water depth on stride length and frequency was reported for the first time. The focus was in assessing how many sessions it took for a steady stride length and frequency to occur in horses that had no experience of aqua-treadmill exercise (Scott *et al.*, 2010). An accelerometer (Pegasus) was mounted to the left forelimb on a brushing boot in a waterproof packet and horses were walked on the aqua-treadmill every day for six days for 15-30 minutes with water depths increasing to ulna height by the end of the fourth session. Results showed that horses reached a steady stride frequency and length within the first six sessions of aqua-treadmill exercise and stride length increased with water depth up to carpus height but then decreased again when water was increased to the depth of the ulna and stride frequency decreased at the deeper depths suggesting that at depths between carpus and ulna the horse may find it easier to adopt a rounder flight arc by increasing flexion of the hip, stifle and hock joints (Scott *et al.*, 2010). The Pegasus accelerometer system has previously been validated against 3D motion capture (Nankervis *et al.*, 2008b) but there does not appear to be any evidence in the literature of how accelerometers respond once submerged in water, even if waterproofed, as the forces acting on them are likely to be altered.

A more recent study investigated flexion and extension of joints of the distal limbs when walking on an aqua-treadmill at increasing water depths up to stifle level collecting data using a standard video camera at 60Hz finding that any depth of water above the baseline level of mid hoof significantly increased distal limb joint flexion, extension and range of motion and as water depth increased percentage duration of stance phase decreased and swing phase increased in both fore and hindlimbs (Mendez-Angulo *et al.*, 2013). This supports the earlier work of Scott *et al.* (2010) who reported a decreased stride frequency when the water was raised above carpus level suggesting perhaps that the buoyancy and resistance of the water therefore slows the passage of the limb. Although two-dimensional videography is not perhaps the most current and sophisticated gait analysis tool, the corrections made for the effects of light refraction in water were well considered here and allayed the potential problems of accelerometer use in water.

Another study compared distal limb range of motion in fore and hindlimbs using waterproofed accelerometery devices to measure cannon angles from walking on an aqua-treadmill and walking on a high-speed treadmill finding that walking in deep water (up to stifle depth) results in a lower forelimb range of motion but a higher hindlimb range of motion when compared to walking on a dry treadmill suggesting that there is an effect of drag from the water on the limb (Lefrancois and Nankervis, 2016). Again, it is important to consider here the application of the accelerometry devices that have been calibrated for overground use, but the effects of the system in water has not yet been independently evaluated.

Two recent papers have discussed the effect of aqua-treadmill exercise on the kinematics of the back, one using high speed video cameras to assess axial rotation, lateral bending and pelvic rotation at the walk at increasing depths of water up to shoulder joint level (Mooij *et al.*, 2013) and the most recent using the most sophisticated optical motion capture (Qualisys©) to measure the flexion-extension range of motion of the thoracolumbar spine and pelvic vertical displacement in horses walking on an aqua-treadmill at depths of up to the stifle (Nankervis *et al.*, 2016). Aqua-treadmill training in deep water of elbow and shoulder depth has been found to significantly increase lateral bending, axial rotation, and pelvic flexion at each deeper water depth when compared to the control suggesting at water depths up to the carpus the horse can continue to step over the water which creates greater axial rotation in the horse's back and at water depth to the elbow and shoulder, the horse is forced into a different movement pattern where the water is too deep to step over so the movement pattern changed to increase pelvic flexion and reduce lateral bending (Mooij *et al.*, 2013). Repeated aqua-treadmill training showed no significant changes in movement patterns in any of the aforementioned parameters (Mooij *et al.*, 2013).

Kinematic data from Nankervis *et al.* (2016) found that walking in any depth of water above the control depth of hoof depth was associated with a significantly greater flexion-extension range of motion in all but the most caudal region (L5) of the spine which was expected as a result of the increase in stride length with increasing water depth (Scott *et al.*, 2010). This lack of agreement with the flexion-extension data at L5 in the 2013 study where walking in all depths of water was found to increase pelvic flexion may be due to the different data collection methods used to study this region (Nankervis *et al.*, 2016) with perhaps a bias towards the data from the 2016 study being more accurate as there is potential less error assuming correct placement of the recording cameras. Interestingly, increasing water depth was not found to increase pelvic vertical displacement (Nankervis *et al.*, 2016) suggesting again, that horses adopt different locomotion patterns to move through the deeper waters. Both these studies only investigated the changes seen at the walk. There is clear scope to investigate how movement patterns of the axial skeleton change in the trot using these more sophisticated high frequency, objective data collection technologies.

1.4.5 Other studies

A handful of other studies have utilised aqua-treadmills for research protocols. Aqua-treadmills have been proposed as potential useful tools in treating tendon and ligament injuries mainly due to the beneficial properties of water but no actual evidence of healing was documented (Adair, 2011). A 2013 study reported that aqua-therapy benefits osteoarthritis due to its different mechanisms of action in reducing inflammation and pain and promoting range of motion, but again, no quantitative data was presented (King *et al.*, 2013a). Later in 2013, the same team published some quantitative data on the effects of aqua-treadmill training on postural sway in horses with experimentally induced osteoarthritis, finding that horses that had undergone a period of aqua-treadmill training walking in shoulder deep water had improved postural stability in both base-narrow and blindfolded stance conditions (King *et al.*, 2013b).

Infrared technology was able to non-invasively detect muscle activity and associated changes in blood flow when horses were exercised on an aqua-treadmill (Yarnell *et al.*, 2014) but that opens a further array of discussion points not related to the present study.

1.5 Conclusion

The desire to sustain sport horses to remain competitive for longer has resulted in increased investment in areas of Veterinary Science that promote health, wellbeing and rehabilitation with more sophisticated pieces of technology being developed that can accurately evaluate a horse's ways of going in order to make major or minor improvements where necessary to give that horse either an extra competitive edge, or prolonged competitive career.

From substantial anecdotal evidence and an increasing number of clinical trials, hydrotherapy appears to be a beneficial alternative and/or additional therapy to promote rehabilitation, healing and training in equines. Despite the increase in state-of-the-art therapy centres in the UK, evidence of their beneficial use and application remains predominantly anecdotal but this does not appear to be deterring Veterinary Surgeons from promoting their use. It is well documented that movement in water has significant benefits in rehabilitation and the effect of the temperature of the water has also been studied. The benefits of cryotherapy are well understood as are the effects of treadmill exercise for fittening and controlled exercise comparable to that over-ground. Individually, the components of aqua-treadmill exercise are positive and well understood, but there is little research evidencing the efficacy of them when they are all added together within the aqua-treadmill unit. The aqua-treadmill may be a useful tool in manipulating positive changes to equine locomotion, performance and rehabilitation. There may even be contraindications that become evident through prolonged use. Therefore, more research needs to be conducted to be able to quantify the benefits of the aqua-treadmill so that therapists and Veterinary Surgeons can tailor rehabilitative and training exercise regimes to individuals to faster and better rehabilitate from injury to improve fitness and prevent the likelihood of reoccurrence.

1.6 Opportunity for Research

Anecdotal reports of the aqua-treadmill include claims that the aqua-treadmill is useful at rehabilitating horses at any stage of any injury. Such injuries may include anything from tendon and ligament strains in the lower limbs to overriding dorsal spinous processes. It has been claimed that if a horse can walk over ground, then there is no reason why they should not be walking on an aqua-treadmill and likewise with trot (Baumann, 2015, *personal correspondence*). To aid in aqua-treadmill rehabilitation protocols for specific injuries, further research on the effects of water depth on locomotory parameters is required. It is apparent that there is a clear gap in the literature to add to the clinical evidence researching the effects of the aqua-treadmill on equine locomotion to inform and support the use of the aqua-treadmill in equine rehabilitation and training. In particular, there is an opportunity for investigation into the effect of water depths on specific locomotory parameters that are routinely investigated in overground and treadmill studies.

This thesis sets out to make some quantification of the locomotory biomechanics of the horse, particularly of the axial skeleton during trot as this has not yet been demonstrated in scientific literature. This study seeks to use the trot as the gait to study in order to more easily investigate symmetries that may be comparable to overground studies. And this project sets out to use the most state-of-the-art gait analysis technologies in order to provide very reputable data for quantitative analysis, that have currently only been used in overground studies and not on an aqua-treadmill.

This overall research project can be broken down into four distinct aims that provides the four main chapters of this thesis (Chapters 3, 4, 5 and 6):

- Determine the impact of water depth on vertical pelvis and wither displacement while trotting on an aqua-treadmill (Chapter 3).
- Determine the effect of side reins on vertical displacements of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths (Chapter 4).
- Determine the impact of water depth on mediolateral pelvis and wither displacements when trotting on an aqua-treadmill (Chapter 5).
- Determine the difference a few centimetres of water makes to pelvis and wither displacements when trotting on the aqua-treadmill compared to overground trotting (Chapter 6).

CHAPTER 2: Methods

2.0 Methods

Data collection was separated into two distinct data collection methodologies; that collected by optical motion capture (Qualisys©; ProReflex, Qualisys© Ltd, Gothenburg, Sweden) between 19th and 21st December 2011 and that collected by inertial sensor (Xsens©; Xsens© Ltd, Enschede, The Netherlands) between 30th Oct and 1st November 2013.

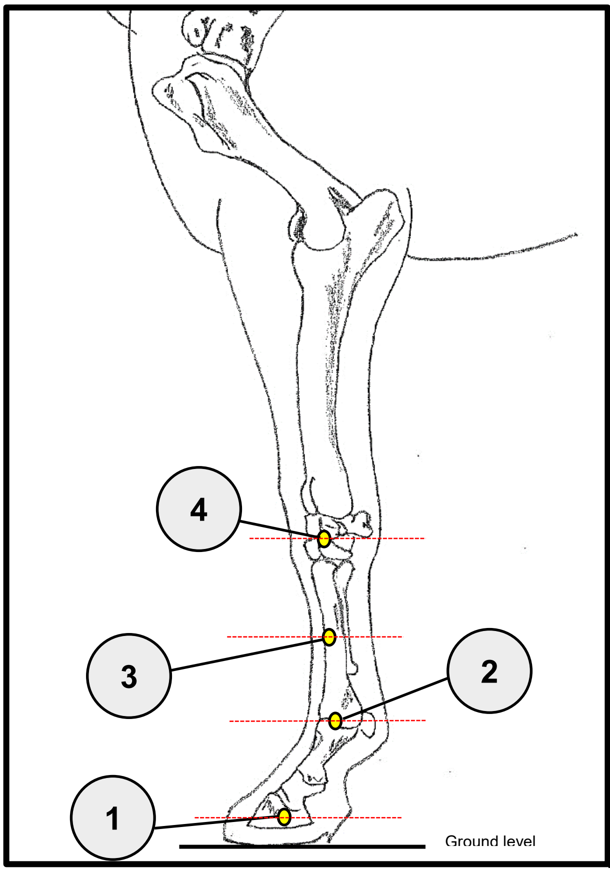
2.0.1 Subjects

Overall, 23 horses took part in the trials. Those with incomplete data sets were removed leaving a total of 18 for analysis to take place (8 for Qualisys© and 10 for Xsens©). The ultimate reason for withdrawing a horse before data analysis was due to an incomplete set of data, and the reasons for this included; horses being withdrawn from the trial due to apparent fatigue, failure of equipment, and a power cut. One horse took part in both trials (Horse 3 in the Qualisys© trial and Horse 1 in the Xsens© trial) in order to perform a validation between the two systems, so his data was then discounted from the Xsens© data set to avoid replication. Horse 11 from the Xsens© trial completed all trials apart from side rein data collection on the aqua-treadmill, so her data is included in most, but not all analyses. All horses were part of Moulton College working environment where the aqua-treadmill was located, therefore they all had regular access to the aqua-treadmill prior to the study and had been on it on multiple occasions but with no real continuity and no specific records kept of the frequency or details of the exercise programmes used. However, all horses had been exercised on the aqua-treadmill frequently in the month prior to data collection and were considered habituated. Nankervis and Williams (2006) identified the necessity of aqua-treadmill habituation and recommended a minimum of at least two fifteen-minute acclimatising runs to ensure a steady heart rate.

Breed, size, type, age or fitness of horse were not qualifying factors for this trial. The majority of horses used were at livery at Moulton College, some belonging to the college, some on full loan to the college. Two horses taking part in this trial were not liveried at Moulton College but regular users of the aqua-treadmill. The horses' fitness levels were all deemed to be good, as they were all in regular riding school type work taking part in flatwork, ground schooling and jump sessions for a maximum three times a day. All horses were considered sound by their owners, had no history of lameness and had consent from the owner and approval of their Veterinary Surgeon to take part in the trial. Horses' heights ranged from 147-180cm (mean ± 1 SD 165.22 ± 6.18) and ages ranged from 8-19 years (mean ± 1 SD 12.83 ± 3.28).

2.0.2 Protocol

Horses were measured perpendicularly from the ground to four specific locations on the distal left forelimb which were subsequently used for the different water depths. These were; the distal phalanx, measured to be half way up the lateral aspect of the hoof wall (P3); mid metacarpophalangeal joint the centre of the joint located from palpation (mid MCP), the mid-point of metacarpal 3, identified by locating the centre of both the MCP joint and the carpus joint from palpation and measuring between them to find the centre (mid MC3); and the mid-point of the carpus joint located by palpation (mid carpus) (Figure 2.1). Water depth was matched to the correct depth for each horse during each stage of the trial by use of a tape measure taped to the outside of the aqua-treadmill window. Water depth was matched as the horses were moving on the aqua-treadmill during the trials, so as to not disrupt the horses' movement patterns and ultimately make the trial last for a lot longer by having to stop and wait for the water to settle and relying on the horse to stand still patiently. Horses went on to complete an exercise protocol on the aqua-treadmill which was a set programme for the Qualisys© trial (Table 2.1) and a different set programme to include side reins for the Xsens© trial (Table 2.2).



4. Mid Carpus – the centre of the carpus joint located by palpation and then measured perpendicularly from the ground.
3. Mid MC3 – the centre of the long cannon bone, meta carpal 3 (MC3) determined from measuring the distance from the centre of the fetlock joint and the carpus joint. This point then measured perpendicularly from the ground.
2. Mid Fetlock – the centre of joint of the fetlock palpated and the distance to the mid-way part of the joint measured perpendicularly from the ground.
1. Mid P3 – the position of the third phalanx within the hoof measured approximately half way up perpendicularly from the ground on the lateral aspect of the hoof wall.

Figure 2.1: An illustrative view of the forelimb of the horse identifying the four positions on the limb that were measured to define the four water depths.

2.1 Use of Qualisys©

2.1.1 Subjects

A total of eight horses were used in this trial. As stated previously, Horse 3 was used as a test horse for both the Qualisys© and Xsens© trials. These eight horses' heights ranged from 157-175cm (mean ± 1 SD 166.25 ± 6.18) and ages ranged from 11-19 years (mean ± SD 13.13 ± 2.59).

2.1.2 Qualisys© Protocol

Six Qualisys© Oqus 300 cameras (ProReflex, Qualisys Ltd, Gothenburg, Sweden) were positioned around the aqua-treadmill and the area inside the aqua-treadmill was calibrated using a standard dynamic wand-based calibration procedure which defines the orientation of the co-ordinate system with a static frame and wand of defined length (QTM, 2015). This system is based on passive infrared markers and infrared cameras. The positive y-axis was orientated in the line of progression, parallel to the treadmill. The positive z-axis was orientated upward and the positive x-axis was orientated perpendicular to the y- and z-axes.

A digital video camera (Panasonic HS60 HD) on a tripod was stationed centrally immediately behind the aqua-treadmill at the height of the *tuber sacrale* for each horse when standing as square as possible inside the aqua-treadmill and video footage was recorded for the whole of each trial.

Reflective hemispherical makers were attached to anatomical bony landmarks on each horse with double sided tape. Markers were attached to the poll or occiput, *tuber sacrale*, left and right *tuber coxae*, left and right ischial tuberosities, withers (highest dorsally protruding thoracic vertebral process (T4 or T5)), and mid thorax (approx. half way between T4 and *tuber sacrale*). Figure 2.2 shows a horse taking part in the exercise trial on the aqua-treadmill demonstrating the location of these markers. Horses were taken through an exercise protocol on the aqua-treadmill (Table 2.1) where data were recorded using the commercially available QTM software at a frequency of 240Hz.

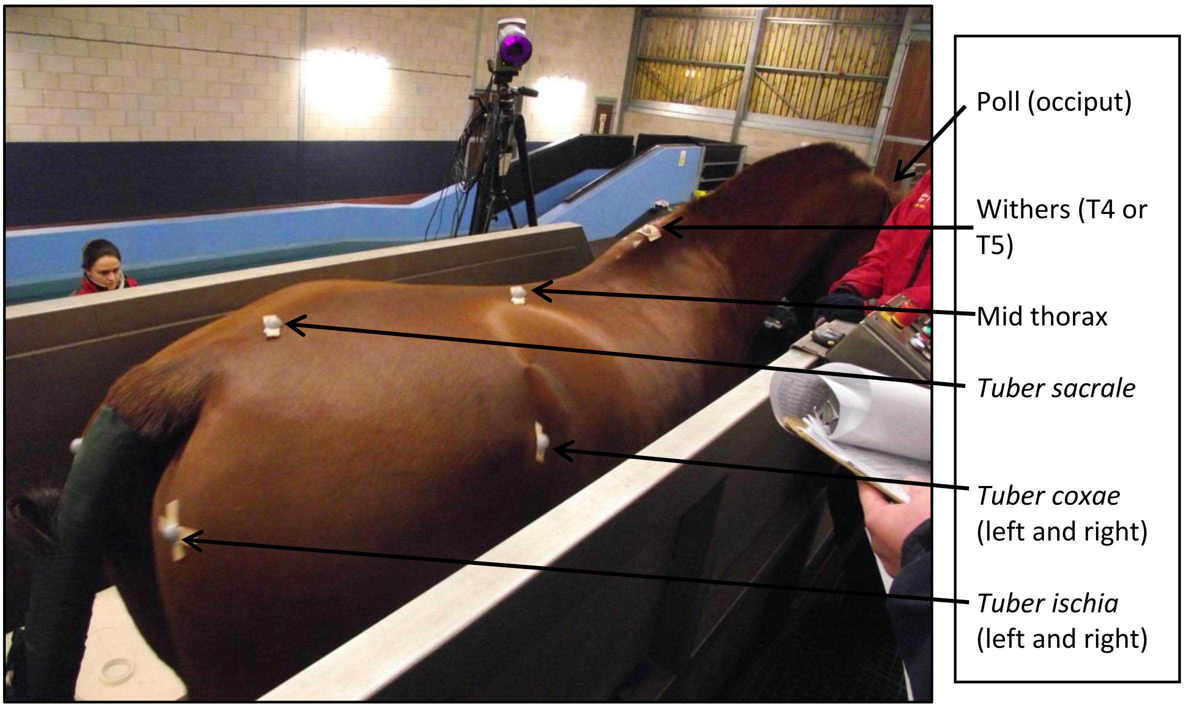


Figure 2.2: A horse on the aqua-treadmill illustrating the location of the light reflective hemi-spherical markers.

Photo credit: Jessica York during data collection, December 2011.

Table 2.1: Aqua-treadmill Qualisys© exercise protocol

WATER DEPTH	SPEED	TIMING (minutes)	DETAILS
Mid P3	Walk	4.0	Warm horse
Mid P3	Trot	1.5	Collect data
Raise water to mid fetlock	Walk	0.5	
Mid fetlock	Walk	1.5	Collect data
Mid fetlock	Trot	1.5	Collect data
Raise water to mid MC3	Walk	0.5	
Mid MC3	Walk	1.5	Collect data
Mid MC3	Trot	1.5	Collect data
Raise water to mid carpus	Walk	0.5	
Mid carpus	Walk	1.5	Collect data
Mid carpus	Trot	1.5	Collect data
Drain water to mid P3	Walk	4.0	Cool horse
	TOTAL TIME	**20**	

2.2 Use of Xsens©

2.2.1 Subjects

Eleven horses were used in this trial (Horse 1 being the test horse used also in the Qualisys© trial so his data was discounted for the Xsens© trial). These eleven horses' heights ranged from 147-180cm (mean ± SD 164.45 ± 10.22) and ages ranged from 8-19 years (mean ± SD 12.73 ± 3.69).

2.2.2 Protocol

Horses were fitted with a roller and a snaffle bridle with Newmarket coupling. Correctly fitted side reins were affixed to the roller to be attached to the bit when required. All horses used in the trial were familiar with wearing and being exercised in these basic items of tack. Side reins are a commonly used training aid for all levels of horses, and all horses in this trial were regularly exercised overground in them.

The Xsens© inertial sensors used in this study were the wired MT9 model. The Xsens© sensor units consist of 3-axis accelerometers, 3-axis gyroscopes, and 3-axis magnetometers that measure 3D linear acceleration, 3D angular velocity, and 3D magnetic field data, measuring 39 x 54 x 28mm, and weighing 35g (Xsens©; Pfau *et al.*, 2005; Findlow *et al.*, 2008; Valentin *et al.,* 2010). The recorded accelerations were subsequently double integrated through velocity to determine displacement of the anatomical bony landmarks in all three axes comparable to that explained in Pfau *et al.* (2007).

This sensor system was fitted to the horses in turn using Animal Polster© (Manufactured by Snogg©) and double-sided tape to hold the sensor onto the Animal Polster©. Large squares of animal polster© were used to securely affix the sensor to the horses' coats. The sensors were connected in series to a wireless transmitter, the XBus (MTx; Xsens, Enschede, The Netherlands), which synchronised data from all sensors and transmitted it via a wireless digital telemetry system (Bluetooth) to a nearby laptop (within 50 metres).

Fitting the Xsens© system to the horses consisted of affixing eight inertial sensor units to the skin overlying bony landmarks commonly used in lameness investigations: poll, withers (highest dorsally protruding thoracic vertebral process (T4 or T5), along the thorax (approximately T13) and lumbar (approximately L4) vertebrae, *tuber sacrale*, left and right *tuber coxae*, and top of tail (1st coccygeal vertebrae). A specially constructed withers mount was used to affix the withers sensor as per recommendation of the equipment and previous studies (Pfau *et al.*, 2005). Figure 2.3 shows the ideal location of the sensors denoted by a red dot, and Figure 2.4 shows the sensors fitted to a horse prior to the start of the trial.

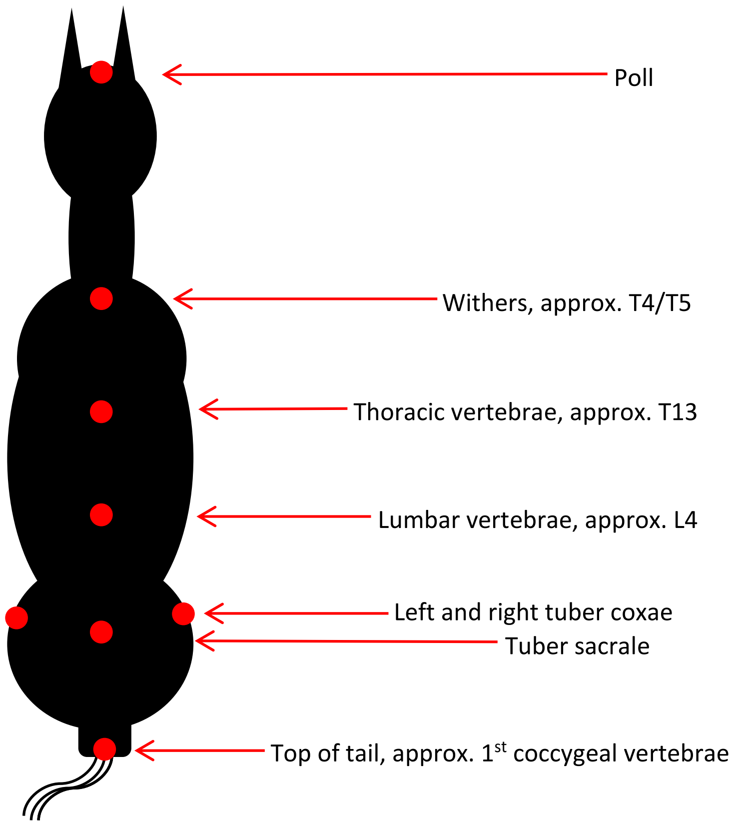


Figure 2.3: Visual representation of the placement of the inertial measurement units of Xsens©.

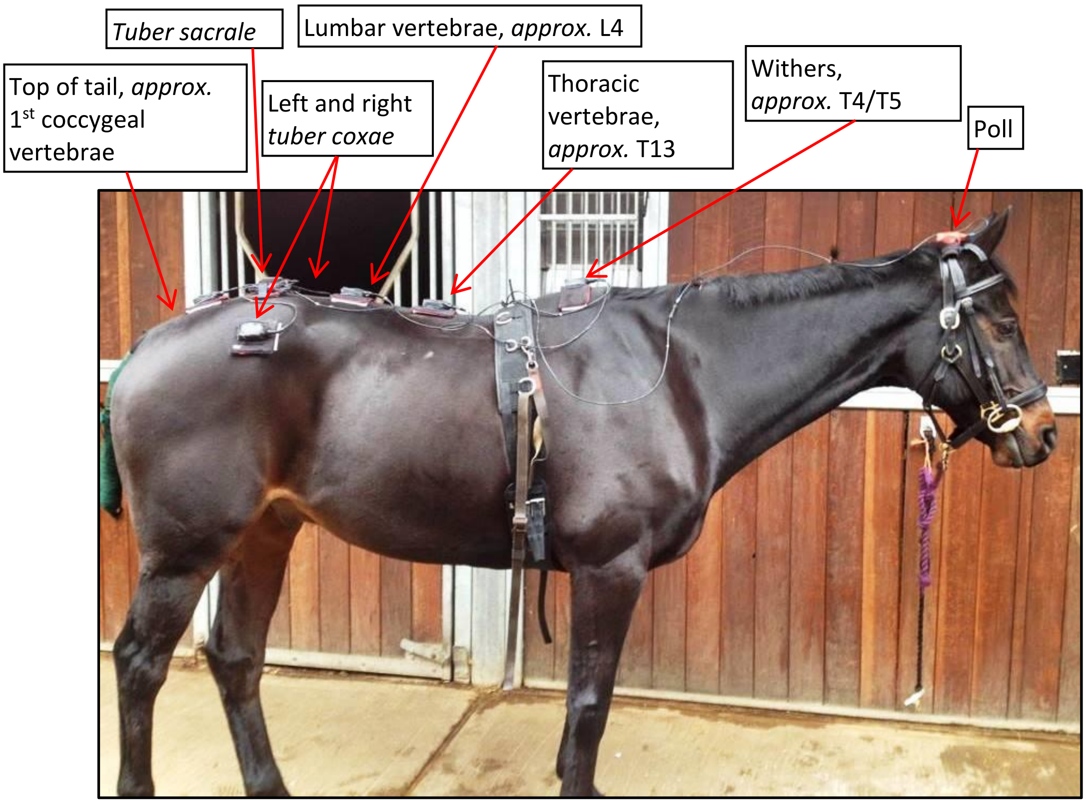


Figure 2.4: A horse fitted with the Xsens© inertial sensors prior to the start of the trial.
Photo credit: Jessica York during data collection, October 2013.

Horses were trotted in hand on a lunge line (to ensure free and non-restricted movement by the handler) overground on a flat tarmac road over approximately 50m. The same handler ran each horse. Horses were engaged and encouraged into a forward-going working trot but not hurried out of their natural rhythm. Horses were trotted three times, a total distance of approximately 150 metres, to obtain adequate stride data and to obtain mean values. The first and last few strides overground were discounted to prevent the effects of acceleration and deceleration. The laptop and operator were stationed mid-point on the tarmac route to ensure optimum recording capacity. The road was fenced with moveable barriers at each end to ensure safety in case the horse became free and to maintain consistency of trotting distances between trial and horses. The side reins were then attached and the horses were trotted up three more times in the same manner. Side reins were fitted according to British Horse Society recommendations (Auty and Linington-Payne, 2013) at a length so as not to inhibit or restrict the horse but to work forward into a steady and straight contact.

Walk and trot speeds of each individual horse were also noted immediately from the GPS and Xsens© software MT Manager, so that the aqua-treadmill speed could be matched to this overground speed. GPS data loggers have been reported as accurate methods for determining speed overground in straight lines (Witte and Wilson, 2004). The Xsens© software collected this information in metres per second. A simple calculation was used to convert this information into kilometres per hour in order to match the method used by the aqua-treadmill.

The horses were then moved to the Therapy Centre to complete the aqua-treadmill exercise protocol straight away (Table 2.2). No rest period was required as the overground trot ups were not deemed to be excessively tiresome. Steps were placed at the near side of the aqua-treadmill – the opposite side to the gallery side to assist in easy attachment and detachment of the side reins. The Xsens© software was used to collect synchronised MTx data via the XBus collecting data at a frequency of 100Hz. Figure 2.5 shows a horse on the aqua-treadmill with the sensors attached completing the Xsens© trial. After the protocol on the aqua-treadmill, horses were cooled down appropriately and had all the equipment removed, and were put back in their stables safely.

Figure 2.5: A horse on the aqua-treadmill with the Xsens© sensors attached undertaking the set exercise protocol.

Photo credit: Anna Walker during data collection, October 2013.

Table 2.2: Aqua-Treadmill Xsens© exercise protocol

The exercise protocol used for the horses on the aqua-treadmill for the collection of Xsens© data. Text in bold includes part of the trial where data was collected. ↑ = increase. ↓ = decrease

WATER DEPTH	SPEED (individualised for each horse)	TIMING	FREE	SIDE REINS	XSens© DATA COLLECT
Raise water	Walk	1 min	Free - raising water		
Mid P3	**Walk**	**1 min**	**Free**		**Yes**
Mid P3	Walk	c.10	Attach side reins		
Mid P3	**Walk**	**1 min**		**Side reins**	**Yes**
Mid P3	Walk	c.20	Detach side reins & increase speed		
Mid P3	**Trot**	**1 min**	**Free**		**Yes**
Mid P3	Walk	c.30	Decrease speed, attach side reins, increase speed		
Mid P3	**Trot**	**1 min**		**Side reins**	**Yes**
↓speed↑water	Walk	c.1 min	Decrease speed to walk, detach side reins, raise		
Mid fetlock	**Walk**	**1 min**	**Free**		**Yes**
Mid fetlock	Walk	c.10	Attach side reins		
Mid fetlock	**Walk**	**1 min**		**Side reins**	**Yes**
Mid fetlock	Walk	c.20	Detach side reins & increase speed		
Mid fetlock	**Trot**	**1 min**	**Free**		**Yes**
Mid fetlock	Walk	c.30	Decrease speed, attach side reins, increase speed		
Mid fetlock	**Trot**	**1 min**		**Side reins**	**Yes**
↓speed	Walk	c.1 min	Decrease speed to walk, detach side reins, raise		
Mid MC3	**Walk**	**1 min**	**Free**		**Yes**
Mid MC3	Walk	c.10	Attach side reins		
Mid MC3	**Walk**	**1 min**		**Side reins**	**Yes**
Mid MC3	Walk	c.20	Detach side reins & increase speed		
Mid MC3	**Trot**	**1 min**	**Free**		**Yes**
Mid MC3	Walk	c.30	Decrease speed, attach side reins, increase speed		
Mid MC3	**Trot**	**1 min**		**Side reins**	**Yes**
↓speed	Walk	c.1 min	Decrease speed to walk, detach side reins, raise		
Mid carpus	**Walk**	**1 min**	**Free**		**Yes**
Mid carpus	Walk	c.10	Attach side reins		
Mid carpus	**Walk**	**1 min**		**Side reins**	**Yes**
Mid carpus	Walk	c.20	Detach side reins & increase speed		
Mid carpus	**Trot**	**1 min**	**Free**		**Yes**
Mid carpus	Walk	c.30	Decrease speed, attach side reins, increase speed		
Mid carpus	**Trot**	**1 min**		**Side reins**	**Yes**
↓speed Drain Water Cool down	Walk	2 min	Decrease speed, detach side reins & empty treadmill. Cool horse down at walk		
	TOTAL WALK	c.16 minutes 40 seconds			
	TOTAL TROT	8 minutes			
	TOTAL SESSION LENGTH	c.24 minutes 40 seconds (25 minutes max.)			

2.3 Data Processing

2.3.1 QTM (Qualisys©)

Each of the eight markers from each trial was manually tracked and labelled using the QTM software. Labelled data were then exported to 'tsv' (tab separated values) files to enable data to be imported into MATLAB (R14, The Mathsworks INC, Natick, MA, US) for further processing and analysis. Firstly, trot data were cut into individual strides using the minimum *sacrum* vertical coordinates associated with mid stance of the left hindlimb. The data at beginning and end of each trial were excluded to discount for acceleration and deceleration, and only data which had an apparent clear sine wave were included to discount data where the horse may have spooked, tripped or lost concentration. This 'good' data once cut into strides, had the minimum and maximum coordinates associated with left and right trot diagonals extracted in all three axes (vertical, cranio-caudal and medio-lateral). Only these good data strides were used for further analyses and any strides that did not correspond with a correct stride pattern were excluded. Every trial had a minimum of twenty strides used for data analysis.

2.3.2 MTX (Xsens©)

Raw inertial data were calibrated and exported to text files using the MTmanager© software. Exported data included both accelerations and the Euler angles, namely roll, pitch and heading. Calibrated acceleration data was processed semi-automatically into strides using custom written scripts in MATLAB where it was filtered (using a Butterworth high pass filter with a cut off frequency of 10Hz) and double integrated through velocity to displacement in all three axes. As with the QTM data, displacement data were cut into strides, in this case by identifying the vertical acceleration of the left *tuber coxae* sensor which was used to identify timings of foot-on of the left hind leg which then allowed automatic detection of the maximum upward acceleration (approximate time of mid-stance) of the left hind limb stance phase from the smoothed withers data and the minimum and maximum vertical values for each stride could be identified and recorded. The frame numbers for each stride were recorded and utilised to extract the corresponding Euler angles for calculation of mean pitch and roll amplitudes and associated comparison between conditions. Every trial had a minimum of twenty strides used for data analysis.

2.4 Reliability

Reliability for each measure was determined for each condition using the standard error of the mean (SEM) for each of the trials (c.20 strides per trial). Reliability of the vertical displacement data is shown in Table 2.3. Reliability of the pitch data is shown in Table 2.4. Reliability of the mediolateral displacement data is shown in Table 2.5. Reliability of the roll data is shown in Table 2.6. Reliability of the absolute position data is shown in Table 2.7. Reliability of the mediolateral flexion data is shown in Table 2.8. As very low SEMs were calculated for all measures (range = 0.68 – 2.37 millimetres for displacement data and range = 0.12 – 1.60 degrees for angle data), these data are indicative that the analyses and specific measures are highly reliable across all conditions. Therefore, differences calculated between conditions >2.40 mm or >1.60 degrees are likely to be a consequence of the condition rather than normal variation (error) in the measure.

Table 2.3 Vertical Displacement Reliability Data

A table of the SEMs for the raw millimetre data of each horse trotting on the aqua-treadmill at a water depth of mid P3 and trotting overground, both with and without side reins, on the left and right parts of the stride for both the pelvis and withers. Considering the low mean SEM for each condition tested here further water depths were not tested.

		TROTTING ON THE AQUA-TREADMILL AT A WATER DEPTH OF MID P3								**TROTTING OVERGROUND**							
		NO SIDE REINS				**YES SIDE REINS**				**NO SIDE REINS**				**YES SIDE REINS**			
		PELVIS		**WITHERS**		**PELVIS**		**WITHERS**		**PELVIS**		**WITHERS**		**PELVIS**		**WITHERS**	
		LEFT	**RIGHT**	**LEFT**	**RIGHT**	**LEFT**	**RIGHT**	**LEFT**	**RIGHT**	**LEFT**	**RIGHT**	**LEFT**	**RIGHT**	**LEFT**	**RIGHT**	**LEFT**	**RIGHT**
QUALISYS	**1**	0.96	1.22	0.83	0.88												
	2	1.73	1.64	0.94	0.93												
	3	1.78	1.42	1.63	2.02												
	4	1.13	1.66	1.03	1.66	NO QUALISYS SIDE REIN DATA				NO QUALISYS OVERGROUND DATA							
	5	1.19	1.17	1.01	1.04												
	6	1.56	1.26	1.43	1.20												
	7	1.37	1.21	1.16	1.28												
XSENS	**8**	1.16	1.70	1.12	1.45	1.31	1.48	0.76	0.95	1.35	1.57	1.58	0.96	1.09	1.52	1.73	0.95
	9	1.81	1.61	1.81	1.74	1.47	1.19	2.05	1.64	1.02	1.10	0.88	0.93	1.26	1.05	2.15	1.49
	10	1.37	1.34	3.33	3.05	1.26	0.93	1.42	1.37	1.56	1.27	2.19	1.61	1.54	1.10	1.40	2.43
	11	1.38	1.20	2.73	2.03	0.95	1.38	2.19	1.20	0.96	0.84	0.64	0.90	0.98	0.71	2.14	1.75
	12	1.10	1.04	2.01	1.76	1.06	0.89	1.43	2.13	0.75	0.84	1.66	1.17	0.63	1.16	1.27	1.96
	13	0.85	0.64	1.64	1.28	0.95	0.71	1.52	1.07	0.80	1.43	1.16	1.76	0.70	1.03	1.52	1.33
	14	1.30	1.15	2.57	1.98	1.44	1.71	1.98	1.23	0.78	0.97	1.40	1.45	0.88	0.74	1.75	1.54
	15	1.04	1.34	1.01	0.79	0.87	0.97	1.61	1.88	1.65	1.28	1.66	2.30	0.95	1.71	1.93	0.91
	16	1.27	2.08	0.99	1.36	1.10	1.20	2.73	0.78	0.72	0.98	1.01	0.97	0.49	0.65	1.20	1.07
	17	1.53	1.93	2.22	2.56	0.65	0.86	3.53	1.21	1.72	1.02	3.32	3.99	1.20	1.15	2.23	1.06
mean SEM		**1.32**	**1.39**	**1.61**	**1.59**	**1.10**	**1.13**	**1.92**	**1.35**	**1.13**	**1.13**	**1.55**	**1.61**	**0.97**	**1.08**	**1.73**	**1.45**

Table 2.4 Pitch Reliability Data

A table of the SEMs of the raw pitch data (in degrees) for each horse trotting on the aqua-treadmill at a water depth of mid P3 and trotting overground, both with and without side reins, on the left and right parts of the stride for both the pelvis and withers. Considering the low mean SEM for each condition tested here further water depths were not tested.

		TROTTING ON THE AQUA-TREADMILL AT A WATER DEPTH OF MID P3								TROTTING OVERGROUND							
		NO SIDE REINS				YES SIDE REINS				NO SIDE REINS				YES SIDE REINS			
		PELVIS		WITHERS		PELVIS		WITHERS		PELVIS		WITHERS		PELVIS		WITHERS	
		LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT
QUALISYS	1 - 7	NO QUALISYS PITCH DATA								NO QUALISYS OVERGROUND DATA							
XSENS	8	0.61	0.63	2.47	2.44	0.59	0.46	2.10	2.02	0.05	0.07	0.10	0.11	0.08	0.10	0.11	0.15
	9	0.97	1.42	0.95	1.06	0.81	1.24	0.90	0.74	0.11	0.16	0.08	0.11	0.14	0.21	0.11	0.12
	10	1.23	1.16	1.52	0.99	0.92	1.04	1.43	1.44	0.24	0.17	0.13	0.15	0.33	0.27	0.12	0.17
	11	1.54	1.19	1.44	1.37	0.79	1.26	1.07	1.04	0.22	0.22	0.09	0.16	0.24	0.25	0.14	0.12
	12	0.89	1.16	1.81	2.61	0.89	1.27	2.56	2.28	0.08	0.11	0.13	0.12	0.12	0.17	0.16	0.16
	13	0.68	0.73	1.39	1.60	0.54	0.47	0.94	0.93	0.09	0.10	0.15	0.15	0.10	0.11	0.13	0.12
	14	1.47	1.26	1.13	1.30	1.54	0.99	1.24	1.10	0.13	0.17	0.12	0.11	0.20	0.16	0.18	0.21
	15	1.67	2.00	2.45	2.67	1.06	1.15	1.78	2.18	0.19	0.36	0.16	0.20	0.13	0.30	0.15	0.20
	16	0.65	1.11	1.11	0.95	0.67	0.87	1.33	1.15	0.07	0.08	0.08	0.08	0.06	0.07	0.09	0.08
	17	1.42	1.18	0.82	1.01	0.56	0.74	0.96	0.88	0.13	0.24	0.15	0.11	0.18	0.35	0.13	0.13
mean SEM		**1.11**	**1.18**	**1.51**	**1.60**	**0.84**	**0.95**	**1.43**	**1.38**	**0.13**	**0.17**	**0.12**	**0.13**	**0.16**	**0.20**	**0.13**	**0.15**

Table 2.5 Mediolateral Reliability Data

A table of the SEMs of the raw mediolateral displacement data (in millimetres) for each horse trotting on the aqua-treadmill at a water depth of mid P3 and trotting overground, both with and without side reins, on the left and right parts of the stride for both the pelvis, withers and poll. Considering the low mean SEM for each condition tested here further water depths were not tested.

		TROTTING ON THE AQUA-TREADMILL AT A WATER DEPTH OF MID P3						**TROTTING OVERGROUND**					
		PELVIS		**WITHERS**		**POLL**		**PELVIS**		**WITHERS**		**POLL**	
		NO SIDE REINS	**YES SIDE REINS**	**NO SIDE REINS**	**YES SIDE REINS**	**NO SIDE REINS**	**YES SIDE REINS**	**NO SIDE REINS**	**YES SIDE REINS**	**NO SIDE REINS**	**YES SIDE REINS**	**NO SIDE REINS**	**YES SIDE REINS**
QUALISYS	**1 - 7**	NO QUALISYS MEDIOLATERAL DATA						NO QUALISYS MEDIOLATERAL DATA					
XSENS	**8**	0.69	0.43	1.28	0.80	1.76	1.09	0.64	0.78	0.89	0.94	1.37	1.73
	9	3.38	0.70	1.71	0.73	3.39	1.80	1.10	1.54	0.75	1.10	1.94	1.88
	10	1.43	0.86	1.91	1.11	2.30	1.08	0.79	1.13	0.94	1.21	2.01	1.50
	11	2.69	1.21	2.00	1.00	3.57	2.46	0.64	1.09	1.01	0.90	0.93	2.50
	12	1.44	1.00	2.83	0.94	4.67	2.46	0.73	0.88	0.61	1.05	1.06	1.30
	13	0.83	0.48	1.15	1.00	1.50	1.41	1.35	1.15	1.24	0.82	1.21	0.97
	14	1.97	1.15	1.10	1.07	1.38	1.51	1.24	0.00	1.10	0.00	1.50	2.36
	15	3.57	1.00	2.19	1.00	2.52	1.51	1.26	1.03	0.96	1.43	2.71	2.72
	16	1.45	0.87	1.70	0.83	1.30	1.24	0.91	0.73	1.01	0.71	1.29	1.48
	17	1.87	1.29	1.40	0.88	1.30	1.62	1.41	1.45	1.38	1.30	1.50	1.71
mean SEM		**1.93**	**0.90**	**1.73**	**0.94**	**2.37**	**1.62**	**1.01**	**0.98**	**0.99**	**0.95**	**1.55**	**1.81**

Table 2.6 Roll Reliability Data

A table of the SEMs of the raw roll displacement data (in degrees) for each horse trotting on the aqua-treadmill at a water depth of mid P3 and trotting overground, both with and without side reins, on the left and right parts of the stride for both the pelvis, withers and poll. Considering the low mean SEM for each condition tested here further water depths were not tested.

		TROTTING ON THE AQUA-TREADMILL AT A WATER DEPTH OF MID P3						TROTTING OVERGROUND					
		PELVIS		WITHERS		POLL		PELVIS		WITHERS		POLL	
		NO SIDE REINS	YES SIDE REINS	NO SIDE REINS	YES SIDE REINS	NO SIDE REINS	YES SIDE REINS	NO SIDE REINS	YES SIDE REINS	NO SIDE REINS	YES SIDE REINS	NO SIDE REINS	YES SIDE REINS
QUALISYS	1 - 7	NO QUALISYS ROLL DATA						NO QUALISYS ROLL DATA					
XSENS	8	0.24	0.42	0.29	0.43	0.33	0.17	0.20	0.21	0.28	0.30	0.27	0.33
	9	0.92	0.18	0.51	0.26	0.92	0.20	0.27	0.29	0.57	0.42	0.33	0.27
	10	0.35	0.24	0.60	0.28	0.26	0.18	0.23	0.25	0.24	0.26	0.74	0.42
	11	0.43	0.20	0.76	0.39	0.43	0.30	0.26	0.27	0.20	0.58	0.29	0.56
	12	0.56	0.24	0.59	0.71	0.94	0.30	0.25	0.28	0.18	0.23	0.17	0.32
	13	0.20	0.20	0.30	0.22	0.19	0.21	0.30	0.18	0.39	0.31	0.40	0.17
	14	0.22	0.25	0.39	0.36	0.22	0.16	0.26	0.20	0.34	0.22	0.22	0.40
	15	0.32	0.19	0.89	0.56	0.26	0.16	0.25	0.19	0.43	0.36	0.56	0.36
	16	0.39	0.25	0.49	0.27	0.46	0.32	0.28	0.22	0.37	0.28	0.14	0.23
	17	0.35	0.24	0.38	0.35	0.46	0.26	0.23	0.22	0.43	0.26	0.43	0.51
mean SEM		**0.40**	**0.24**	**0.52**	**0.38**	**0.44**	**0.23**	**0.25**	**0.23**	**0.34**	**0.32**	**0.36**	**0.36**

Table 2.7 Absolute Position Reliability Data

A table of the SEMs of the raw absolute positions of displacement data (in millimetres) for each horse trotting on the aqua-treadmill at increasing water depths for the most minimum and most maximum parts of the stride for both the left and right parts of the stride for both pelvis and withers. Low mean SEM for each condition were established.

			TROT MID P3				TROT MID FETLOCK				TROT MID MC3				TROT MID CARPUS			
			min1	min2	max1	max2	min1	min2	max1	max2	min1	min2	max1	max2	min1	min2	max1	max2
QUALISYS	1	PELVIS	0.66	0.96	0.85	0.91	0.70	0.66	0.70	0.66	1.24	1.16	1.03	0.99	1.15	1.22	1.14	1.02
	2		1.71	1.36	1.02	0.73	1.05	1.15	0.99	0.98	1.49	1.38	1.04	1.10	1.46	1.24	1.30	1.47
	3		1.12	0.94	0.96	0.87	2.18	1.98	1.63	1.34	2.76	1.99	1.41	1.19	1.98	1.10	1.09	1.14
	4		0.85	0.92	1.44	0.90	1.77	1.59	2.29	2.51	4.40	1.71	2.03	2.32	2.19	1.49	2.68	3.88
	5		0.97	1.24	0.75	0.85	0.98	1.77	1.03	1.21	1.66	1.56	1.31	1.15	3.73	1.20	1.40	2.11
	6		1.00	0.80	0.78	0.82	1.86	1.20	1.06	1.22	1.96	0.76	1.02	0.91	0.83	0.70	0.88	0.81
	7		0.97	1.49	0.94	0.97	2.36	2.15	1.75	1.53	1.50	2.35	1.17	1.55	2.82	2.74	1.88	2.30
	8		1.08	0.82	0.90	0.87	1.08	1.14	1.08	1.14	1.54	1.75	1.21	1.39	1.94	1.40	1.14	1.12
	mean SEM		**1.05**	**1.07**	**0.95**	**0.86**	**1.50**	**1.45**	**1.32**	**1.32**	**2.07**	**1.58**	**1.28**	**1.32**	**2.01**	**1.39**	**1.44**	**1.73**
QUALISYS	1	WITHERS	0.45	0.41	0.63	0.81	0.47	0.38	0.60	0.61	0.53	0.50	0.72	0.73	0.35	0.49	0.54	0.56
	2		0.60	0.52	0.66	0.68	0.52	0.48	0.55	0.51	0.75	0.90	0.68	0.63	0.53	0.93	0.80	0.66
	3		0.78	0.86	0.68	0.79	0.81	0.71	0.93	0.94	0.72	0.62	0.78	0.91	0.56	0.52	0.73	0.60
	4		0.83	1.19	1.04	1.55	1.28	1.30	2.17	1.74	1.63	1.35	2.17	3.34	1.39	1.85	4.35	3.59
	5		0.57	0.89	0.85	1.20	1.22	1.47	1.38	1.44	1.09	1.54	1.51	1.71	0.65	0.69	1.19	1.01
	6		0.60	0.59	0.73	0.68	0.72	0.65	1.15	0.81	0.73	0.65	0.79	0.71	0.49	0.59	0.95	0.91
	7		0.69	0.59	1.09	1.02	0.62	0.73	0.84	1.01	0.71	0.60	0.84	0.97	0.75	0.86	1.49	1.32
	8		0.91	0.88	0.96	1.03	0.99	1.07	0.99	0.64	1.44	1.27	1.19	1.26	1.27	1.11	1.19	1.12
	mean SEM		**0.68**	**0.74**	**0.83**	**0.97**	**0.83**	**0.85**	**1.08**	**0.96**	**0.95**	**0.93**	**1.09**	**1.28**	**0.75**	**0.88**	**1.41**	**1.22**

Table 2.8 Mediolateral Flexions Reliability Data

A table of the SEMs of the raw mediolateral flexion data (in degrees) for each horse trotting on the aqua-treadmill at increasing water depths. Low mean SEM for each condition were established.

		Mid P3	Mid Fetlock	Mid MC3	Mid Carpus
QUALISYS	**3**	0.19	0.22	0.21	0.21
	4	0.47	0.45	0.54	0.70
	5	0.34	0.30	0.28	0.24
	6	0.31	0.27	0.19	0.21
	7	0.17	0.21	1.93	0.24
	8	0.24	0.36	0.48	0.48
mean SEM		0.29	0.30	0.61	0.35

2.5 Validating Xsens© against Qualisys©

Optical Motion Capture has long been recognised as the gold standard to assess 3-dimensional movement and orientation with a high degree of accuracy, of which Qualisys© is one available system. Inertial measurement systems have been developed as a more practical but objective method to assess movement of which the Xsens© system is now considered as the gold standard in inertial measurement technologies (Cutty *et al.*, 2008; Chung *et al.*, 2011; Mourcou *et al.*, 2015). Xsens© has also been validated against electromagnetic 3D motion tracking technologies (Saber-Sheikh *et al.*, 2010) and against Optotrack motion capture technologies (Robert-Lachaine *et al.*, 2017).

Specifically, in horses, Xsens© has been validated against Qualisys© in a study assessing kinematics of the equine spine where the reflective hemispherical markers of the Qualisys© system were directly secured to the six inertial measurement units of the Xsens© system that were located at T6, T10, T13, L1, S3 (identified by palpation of the respective dorsal spinous processes) plus the left *tuber coxae*, and horses were trotted along an 18-metre track surrounded by ten Qualisys© cameras (Warner *et al.*, 2010). Using this technique, differences between the two systems were reported to be 8–16 mm (± 2 s.d.) for dorsoventral movement and 7–11 mm (± 2 s.d.) for mediolateral movement which were both similar to values reported during treadmill exercise (Pfau *et al.*, 2005). These values expressed as a percentage were reported as 9–21% accurate for dorsoventral movement and 10–24% accurate for mediolateral movement, and the higher mediolateral values were explained by the lower range of movement in this plane (Warner *et al.*, 2010). Considering that it has previously been reported that even experienced clinicians are not able to reliably detect movement asymmetries below a level of 25% (Parkes *et al*., 2009) it was concluded that inertial sensors provided consistent data between trials and between strides that was sufficiently comparable to that recorded by motion capture, although due to the relatively small range of motion in the mediolateral direction, subtle mediolateral asymmetries might potentially go undetected.

A previous study compared Xsens© to Qualisys© on a treadmill in a similar fashion focussing on movement at the withers (Pfau *et al.*, 2005) A wand with three orthogonal arms each bearing a retro-reflective spherical motion capture marker (the positive x-axis pointing towards the front of the treadmill, the positive y-axis pointing towards the left side of the treadmill and the positive z-axis pointing upwards) was fixed to an inertial measurement unit (IMU) located at the withers of a horse on a treadmill thus rendering a virtual marker at the site of the IMU. Mean x, y and z displacement and roll, pitch and heading traces obtained using the inertial sensor were virtually indistinguishable from those obtained using optical motion capture in walk, trot and canter (Pfau *et al.*, 2005). During trot, compared with optical motion capture, 50% of the values for the x, y and z displacement obtained from the inertial sensor were found within (–2.8, +1.4) mm, (–0.9, +0.9) mm, and (–4.3, +4.9) mm, which compared with the true values derived from the optical motion capture correspond to a relative error of (–6.5, +3.2)%, (–2.6, +2.6)%, (–5.6, +6.4)% concluding that the inertial sensors captured cyclical movements with comparable accuracy to optical motion capture systems (Pfau *et al.*, 2005).

A more recent study investigated dorsoventral flexion-extension of a single horse's back when trotting and cantering on a treadmill with synchronised motion capture and inertial measurement systems (Martin *et al.*, 2014). The difference (mean ± SD) between the IMU and motion capture during trot were, respectively for the thoracic and thoracolumbar angles, 0.57 ± 0.44 and 0.65 ± 0.47 degrees concluding that angle values calculated with IMU data showed acceptable accuracy consistency for quantification of flexion-extension movement in a horse's back (Martin *et al.*, 2014).

Despite current evidence in the literature, it was deemed pertinent to conduct a validation of the equipment specific to the unique environment of this project and as such, one horse was used in both the Qualisys© and Xsens© data collection trials to ensure validity between both operating systems. Qualisys© has been previously validated in a treadmill environment (Pfau *et al.*, 2005) and Xsens© has also been validated against Qualisys© in a treadmill environment (Pfau *et al.*, 2005), but there are currently no reports in the literature that validate either system in the environment of an aqua-treadmill where the increased mass of metal may have an effect on the operation of the systems. It is more likely that the magnetometers of the Xsens© system may be affected by any increased magnetism of the environment. Ideally, the validation would have occurred utilising both systems simultaneously, but unfortunately the equipment was only available for this project separately, two years apart. Also, ideally, both systems would have been validated simultaneously overground, before moving on to the aqua-treadmill, but again, the gap of two years in availability of the equipment did not make this possible and the Qualisys© system was not used at all in overground trials due to the limited time-frame the equipment was available to us.

Due to the cumbersome nature of the Qualisys© equipment, it is best suited to lab based studies thus expecting that Qualisys© would work well for the aqua-treadmill in this situation as the aqua-treadmill was enclosed in a barn where conditions were consistent and the cameras were less likely to be affected by light anomalies and weather conditions. One horse and one water depth were selected for a validation trial.

A statistical validation was conducted on one horse that was used in both the Qualisys© trial and then the Xsens© trial. As discussed, no validation could be completed for the overground data as the Qualisys© system was not utilised in this project overground, but both experimental protocols involved trotting on the aqua-treadmill at a water depth of mid P3. The mean (±SEM) vertical displacements of the pelvis and withers (in millimetres) of the test horse when trotting on the aqua-treadmill at a water depth of mid P3 for both Qualisys© and Xsens© are shown in Table 2.9 and plotted in Figure 2.6.

Table 2.9 Reliability and validation of Xsens© against Qualisys© using one test horse
The mean and SEM of the vertical displacements of the pelvis and withers (in millimetres) of the test horse when trotting on the aqua-treadmill at a water depth of mid P3 for both Qualisys© and Xsens©.

		QUALISYS		**XSENS**	
		mean	SEM	mean	SEM
PELVIS	Left	105.89	1.28	105.59	1.16
	Right	98.97	1.24	100.48	1.70
WITHERS	Left	61.88	0.98	69.90	1.12
	Right	65.58	0.99	67.72	1.45

Results of a two-way repeated measures ANOVA of the main effect of data collection system showed no significant difference in the actual values of vertical displacement amplitude (mm) between Qualisys© and Xsens© ($F_{(1,19)}$ = 4.15, *p*

>0.05) (SEM 1.31). In the pelvis there is a similar displacement difference between left and right in both Qualisys© and Xsens©. Mean (±SEM) displacement values for the pelvis on the left using Qualisys© were 105.89 (±1.28) mm compared to 105.59 (±1.16) mm using Xsens©, a difference of 0.30 mm. Mean (±SEM) displacement values for the pelvis on the right using Qualisys© were 98.97 (±1.24) mm compared to 100.48 (±1.70) mm using Xsens©, a difference of 1.51 mm. These differences between the two systems (0.3 mm left and 1.51 mm right) are smaller than previously reported differences by Warner *et al.* (2010) that reported differences in dorsoventral movement of 8–16 mm between the two systems along the back.

In the withers there is also a similar displacement difference between left and right in both Qualisys© and Xsens©. Mean (±SEM) displacement values for the withers on the left using Qualisys© were 61.88 (±0.98) mm compared to 69.90 (±1.12) using Xsens©, a difference of 8.02 mm. Mean (±SEM) displacement values for the withers on the right using Qualisys© were 65.58 (±0.99) mm compared to 67.72 (±1.45) mm using Xsens©, a difference of 2.14 mm. These differences between the two systems (8.02 mm left and 2.14 mm right) compare to the previously reported values of 8-16 mm of difference in dorsoventral movement of the horse's back (Warner *et al.*, 2010) although the withers in the current study are slightly more cranial than the first marker used of T6 in the Warner *et al.* (2010) study. The Pfau *et al.* (2005) study directly recorded vertical displacements of the withers, but the study did not report mean raw data values, but reported 50% of the values of dorsoventral displacement of the inertial sensor compared to the optical motion capture were found within (–4.3, +4.9) mm for trot which suggests a range of 9.2 mm, which again concurs with the differences reported in the present study.

The difference between left and right changes round in the withers from Qualisys© to Xsens© and the difference between them becomes smaller. This is perhaps accounted for by the two years' difference in data collection and that the horse may have become more symmetrical in front or perhaps fitter for the second trial. Two years difference in data collection is a long time, so it is more likely that there is a difference in the placement of the sensor to the marker used for Qualisys©. Nevertheless, the overall result shows no significant difference between Qualisys© and Xsens©. This validation facilitated confidence in the recording systems and considering the satisfactory levels of agreement reported between the two systems in this validation trial, it was considered acceptable to amalgamate the data for the analysis of vertical displacements (seen in Chapter 3).

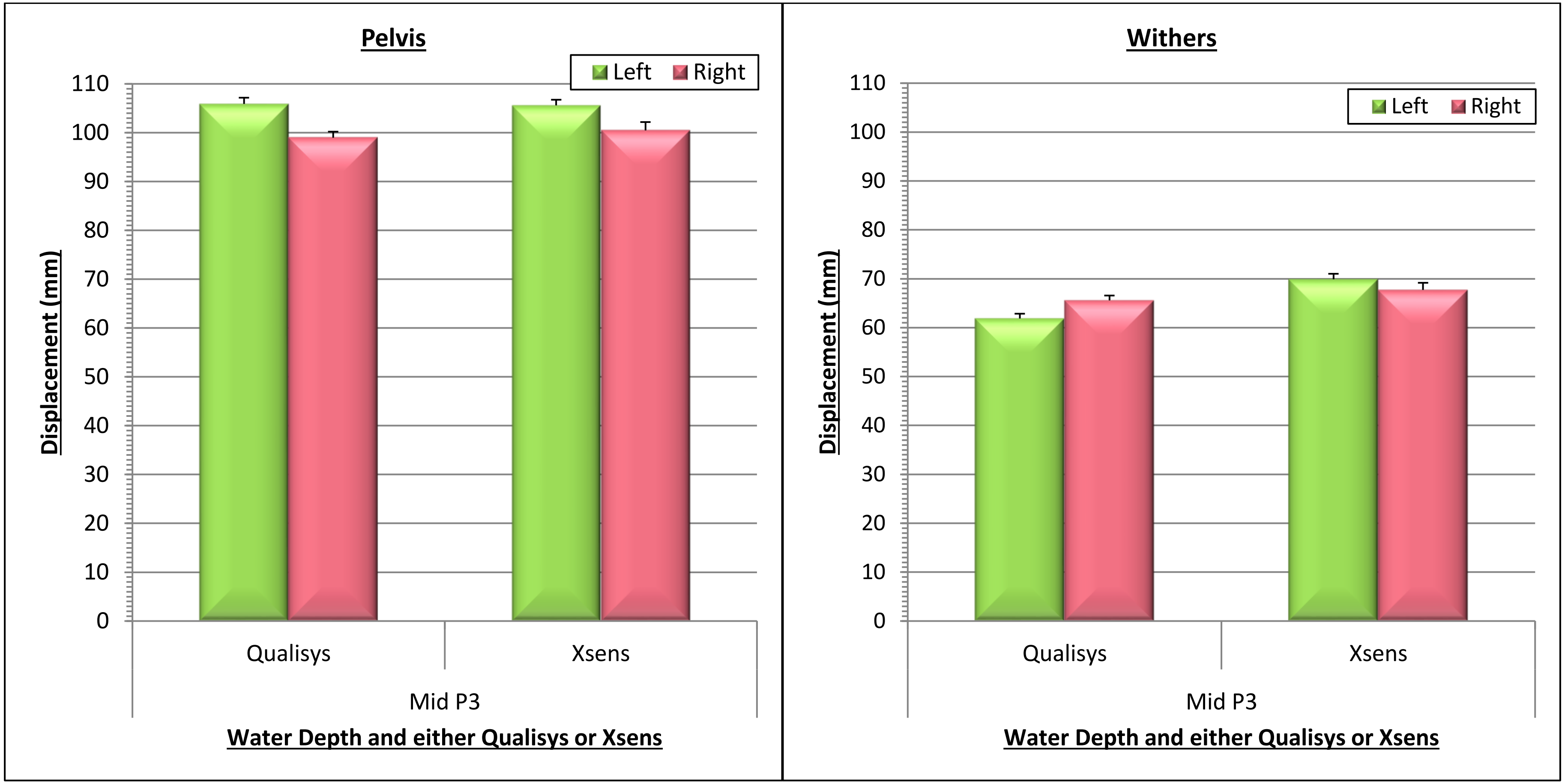


Figure 2.6: Mean (±SEM) vertical displacement amplitude (mm) of the pelvis (left) and withers (right) for the test horse in both the Qualisys© and Xsens© trials at the water depth of mid P3 for both left diagonal pair (green) and right diagonal pair (pink). No significant differences were seen between the two systems.

2.5 Statistical analyses

A variety of statistical analyses were used throughout the projects and are described in more detail in each individual chapter. Data analyses were conducted using IBM SPSS Statistics Version 22 software (IBM Corp. Released 2013). All data were tested for normality throughout all stages of analyses using Shapiro-Wilk tests and data were normally distributed unless otherwise stated ($p > 0.05$) (Shapiro and Wilk, 1965). Ultimately, all trials were tested for significance using repeated measures analysis of variance (ANOVA) tests. Mauchly's test for sphericity was investigated and interpreted (Mauchly, 1940) in accordance with the assumptions of the ANOVA, and where Mauchly's test of sphericity was violated, the Greenhouse-Geisser correction was applied when interpreting the results (Laerd Statistics, 2015).

2.6 Health and Safety and Ethics

All horses were fit, well and sound as determined by a Veterinary Surgeon prior to their involvement in either trial. They also had full owner consent to partake.

Extensive risk assessments were carried out for both data collection trials. For the Qualisys© trial, risk assessments were carried out to ensure all cables were as safely covered as possible to enable horses and handlers to negotiate them in a safe manner with no risk of tripping, damaging the equipment or spooking the horses. For Xsens© it was necessary to ensure that all wires and cables over the horses were as safely and securely attached to the horses as possible to ensure that they could not be caught up in anything or hang too low and be tripped over by the horses or to spook the horses. The laptop receiving the data was positioned never more than 50 metres away from the horse when data collection was taking place, and when collecting the data from the aqua-treadmill the laptop was positioned on a table next to the aqua-treadmill.

CHAPTER 3: Vertical displacements of the equine pelvis and withers when trotting on the aqua-treadmill at increasing water depths

3.0 Introduction

Equine aqua-treadmills have increased in popularity as a mode of exercise and rehabilitation. As discussed in Chapter 1.4 a limited number of scientific studies have focussed on quantifying the biomechanical movement of the horse during exercise on the aqua-treadmill. As a result, current practice is based on anecdotal evidence as little objective, quantitative evidence exists. The study of aqua-treadmill exercise has been hampered by the ability to collect useful objective, quantitative data from within the confines of the aqua-treadmill apparatus. Being an enclosed metal box, access to the limbs is difficult, but the axial skeleton is exposed leaving scope to collect valid and useful movement data of the horses' back. The static nature of the treadmill enables a greater quantity of consecutive stride data to be collected for analysis compared with overground studies. The aqua-treadmill therefore, should render itself the ideal environment to observe a horse's way of going in walk and trot. Utilising sophisticated technologies enables the consistent observation and analysis of minute changes in movement which would otherwise be unobservable by the human eye. Comparisons can then be made to overground movement and movement on a non-water treadmill.

A notable area of interest in movement on an aqua-treadmill is the apparent vertical lift that is achieved when horses trot through water. Visibly, horses appear to have to raise their body to aid an increase in movement of the limbs up and over the water but currently there is no evidence to suggest how water depth may affect this vertical biomechanical movement. This study seeks to determine the effect of water depth of the vertical displacement amplitude on both the fore quarters (the withers) and hind quarters (the pelvis) of the horse.

The trot is a symmetrical gait (Hildebrand, 1965) with each of the diagonal limb pairs (left hind/right fore or right hind/left fore) being dynamically coupled which results in a symmetrical movement pattern during the left and right diagonal phases of the stride. Vertical displacement, joint angles and protraction and retraction within each phase of the trot stride (during left and right diagonal stance and swing) are symmetrical in a sound horse (Robilliard *et al.,* 2007). Each stride contains two vertical pelvic displacement minima corresponding to left hind mid-stance and then right hind mid-stance. The *tuber coxae* being displaced laterally from the midline exhibit vertical displacements that differ in amplitude during each trot diagonal which allows clear identification of left hind (prior to the largest displacement amplitude) and right hind (prior to the smallest amplitude) stance phases (May and Wyn-Jones, 1987). Figure 3.1 graphically explains the patterns seen.

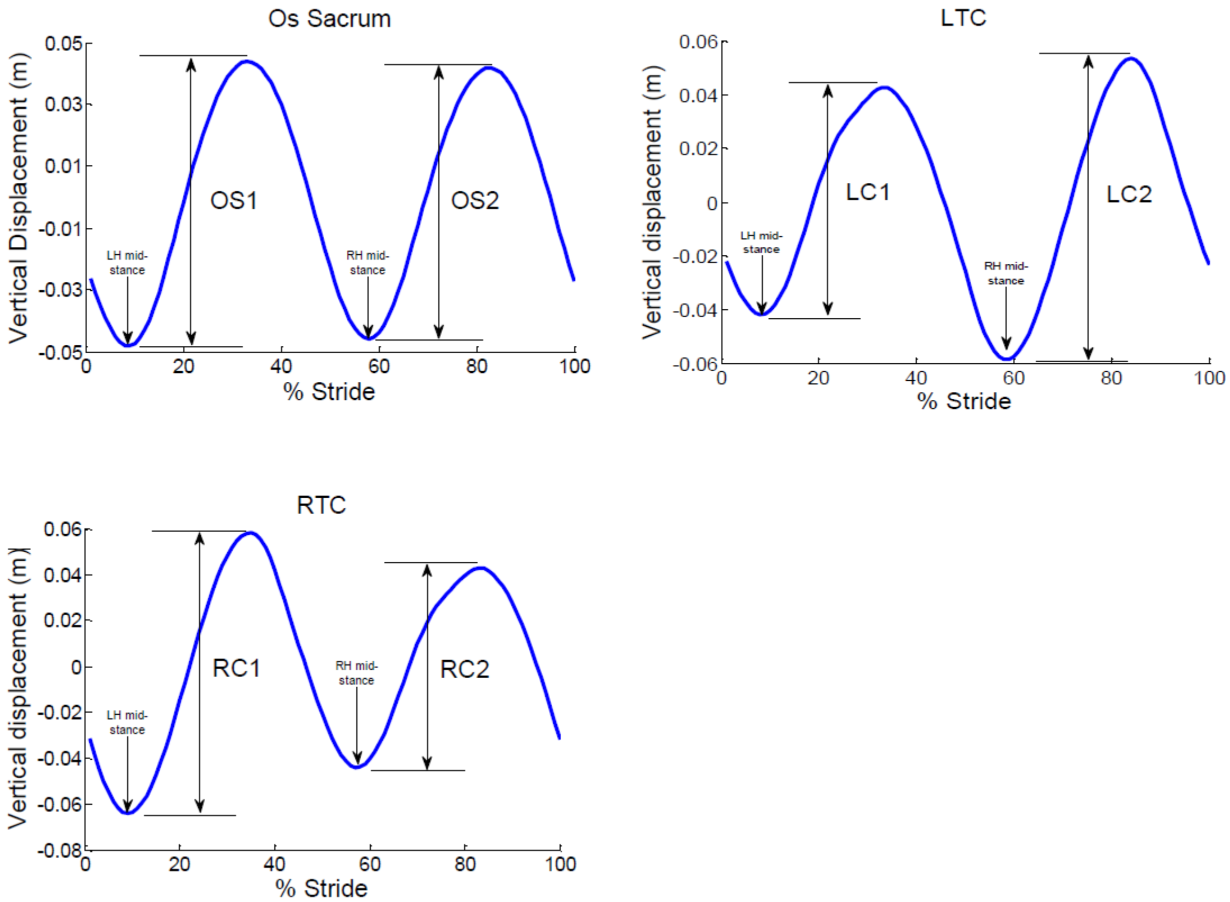


Figure 3.1: Graphical representation of the vertical displacements achieved by the os sacrum and the left and right tuber coxae (LTC or RTC) during a single stride from a sound symmetrical horse cut from left hind stance. The vertical arrows indicate amplitude. OS1 and OS2 correspond to the 2 amplitudes per stride of the os sacrum. LC1 and LC2 identify the 2 amplitudes of the left coxae and RC1 and RC2 identify the two amplitudes of the right coxae.

Unilateral lameness of horses is characterised by asymmetry in the vertical displacements between the left and right phases of a stride. The presence of ‘hip hike’ is often used as a parameter to evaluate lameness in the hind limbs, which is documented as a rapid upwards movement before foot contact of the lame limb (May and Wyn-Jones, 1987; Buchner *et al.*, 1993; Pfau *et al.*, 2015). Displacement asymmetry in horses has reported a symmetry of less than 95% in the *os sacrum* to be associated with a lameness score of 1/4 or higher (Peham *et al.*, 2001, Audigie *et al.*, 2002) with symmetry of less than 85% in the *os sacrum* being associated with a lameness score of 2/10 (Church *et al.*, 2009). Even though these studies use different lameness grading scales, both values are below the reported 25% threshold which has been reported as the limit for human visual discrimination performance and the threshold for clinicians to consistently agree (Parkes *et al.*, 2009).

The vertical displacements of the *tuber sacrale* are studied in conjunction with the vertical displacements of the *tuber coxae* in order to study and assess lameness. Whilst movement of the sacrum can be used independently to investigate movement symmetry, the tuber coxae, to date, does not appear to have been studied in isolation with respect to movement symmetry. One study investigated mean vertical displacement amplitudes of both parts of the stride in trotters in both the fore and hindlimbs using an accelerometer on the withers and *tuber sacrale*, investigating the influence of speed plus a different track surface (Pauchard *et al.*, 2014). The consideration of a different type of track surface in trotters may draw some parallel observations to exercising horses in trot through water. Pauchard *et al.* (2014) found that the mean vertical displacement amplitude of the overall stride (the left and right parts of the stride were not reported independently, only the overall mean) as measured at either the withers or croup in both the fore and hind limbs, decreased with speed (25-40km/h) regardless of the track type used (either soft or hard surface); for every 10km/h between 25 and 40km/h the overall vertical displacement amplitude of the stride decreased approximately 9mm in the withers and 6mm in the croup. The influence of the hard track was more significant in the croup than the withers, however, these horses were trotting at speed. This perhaps suggests that water may provide a cushioning effect equivalent to a softer track surface that then exhibits vertical displacements not as high as a harder surface, but a test of speed would also need to be conducted in order to draw true parallels.

Trotting on an aqua-treadmill does not reach speeds comparable to those recorded in trotting horses by Pauchard *et al.* (2014). The aqua-treadmill used for this study is only calibrated to reach speeds of c.18km/h. Ridden trot speeds range from 11.5km/h in collected trot, 13km/h in working trot, 16.1km/h in medium trot and 17.8km/h in extended trot (Clayton, 1994a) while trotting in hand has been reported to be approximately 14.25km/h (Galisteo *et al.*, 1998). It is previously reported that the speed at which horses move without being forced is that which requires the minimum expenditure of energy (Hoyt and Taylor, 1981) which is arguably the opposite of the requirement of trotting horses where extreme trotting speeds are reached. Pauchard *et al.* (2014) concluded that vertical displacements decrease with speed and the decrease is larger at the withers than the pelvis, also that a soft track increased the vertical amplitudes of the pelvis compared to a hard track but not that of the withers. At the highest speeds, track surface no longer had a significant effect on vertical displacements.

To the author's knowledge, no other studies appear to have investigated the vertical displacement amplitudes of the pelvis and withers when trotting through water. This study aims to determine the effect of water depth on the vertical displacement amplitudes of both the fore quarters (the withers) and hind quarters (the pelvis) of the horse.

3.1 Aims

The aim of this chapter is to investigate and quantify the effect of water depth on objective vertical displacements of the horse when exercising in trot on an aqua-treadmill. Specifically:

1. Analyse the vertical displacement amplitudes of the pelvis and withers when trotting on an aqua-treadmill at different water depths

2. Investigate the percentage change in vertical displacement amplitudes at the pelvis and withers when trotting on an aqua-treadmill at different water depths

3. Determine vertical displacement amplitude symmetry at the pelvis and withers when trotting on an aqua-treadmill at different water depths

4. Investigate the changes in vertical position of the pelvis and withers throughout a stride cycle when trotting on an aqua-treadmill at different water depths

5. Investigate the percentage change in vertical position of the pelvis and withers throughout a stride cycle when trotting on an aqua-treadmill at different water depths

6. Evaluate the pitch amplitudes of the pelvis and withers throughout a stride cycle when trotting on an aqua-treadmill at increasing water depths.

3.2 Methods and Statistical Analysis

Data were collected and the raw data were processed and analysed as explained in Chapter 2. Data were processed using custom written scripts in Matlab© and accelerations were used to calculate displacements before the data were cut into strides. Only good stride data cut from left hind stance were used for further analysis. Every trial had a minimum of twenty trot strides used for data analysis. To compute vertical displacements (namely at the pelvis (*tuber sacrale*) and withers (T4-5), a double numerical integration of the accelerations was completed in conjunction with a high-pass 4^{th} order Butterworth high pass filter applied with a cut off of 0.5Hz. Vertical displacement as a function of time presents as a double sinusoidal pattern per stride corresponding to the left and right diagonals (Audigie *et al*., 1999; Buchner *et al*., 1994a; Faber *et al*., 2001a, 2001b, 2001c; Warner *et al.*, 2010). All data were cut from the first minima representing mid stance of the left hind leg determined by the corresponding movement patterns of the markers or sensors on the *sacrum* and *tuber coxae*. Each stride has two minima (min1 and min2) and two maxima (max1 and max2) indicating the descent and rise for each diagonal half cycle during trot. Amplitudes were calculated (Equation 1).

Equation 1:
$Ampl_1 = Max_1 - Min_1$ and $Ampl_2 = Max_2 - Min_2$

Mean displacement amplitudes along with their standard deviations and standard errors were calculated for each trial for each horse.

The inertial measurement units (IMUs) (Xsens©) with their tri-axial accelerometers, gyroscopes and magnetometers, are able to record synchronised accelerations, roll, pitch and heading of each sensor on each bony anatomical landmark. As such, it was possible to cut the data from all sensors in all axes using the frame numbers recorded from the cutting of the vertical displacement into cycles. Once cut into strides it is possible to extract the minimum and maximum values of the pitch angle for each half stride and at increasing water depths. Pitch amplitudes were then compared between pelvis and withers and then compared against the displacement amplitudes in order to provide a detailed analysis of the movement to enable identification of changes in locomotion associated with different depths of water.

3.2.1 Statistical Analysis

Repeated measures ANOVAS were used for all statistical analyses and these are described in detail for each individual analysis. Shapiro-Wilk tests for Normality were used throughout and data were normally distributed unless otherwise stated ($p > 0.05$) (Shapiro and Wilk, 1965), and Mauchly's test for sphericity was investigated and interpreted (Mauchly, 1940) in accordance with the assumptions of the ANOVA, and where Mauchly's test of sphericity was violated, the Greenhouse-Geisser correction was applied when interpreting the results (Laerd Statistics, 2015).

3.3 Results

Data from both the Qualisys© and Xsens© trials were used for this study. Where data from both trials could be amalgamated (as per Chapter 2, Section 2.5), 17 complete sets of data were available and horses' heights ranged from 147-180cm (mean ± SD 165.24 ± 9.11) and ages ranged from 8-19 years (mean ± SD 12.88 ± 3.37). Where analyses utilised only the data from the Qualisys© trial there were 8 complete sets of data available for analysis where horses' heights ranged from 162-175cm (mean ± SD 165.25 ± 6.18) and ages ranged from 11-19 years (mean ± SD 13.13 ± 2.59). Where analyses utilised only the data from the Xsens© trials there were 10 complete sets of data available for analysis where horses' heights ranged from 147-180cm (mean ± SD 163.20 ± 9.84) and ages ranged from 8-19 years (mean ± SD 12.88 ± 3.84).

3.3.1 Vertical Displacement of the Pelvis and Withers

This analysis utilised the combined data of both the Qualisys© and Xsens© trials. The vertical displacement values were plotted for the pelvis and withers at each water depth for both the left and right parts of the stride (Figure 3.2). A repeated measures ANOVA (2x4x2) was conducted to determine the effects of pelvis or withers, water depth, and the left or right parts of the stride on mean vertical displacement amplitudes of 17 horses trotting on the aqua-treadmill at increasing water depths.

There were no interactions between the variables but two significant main effects. Firstly, there was a large significant effect of the anatomical location ($F_{(1,16)}$ = 42.053, p < 0.001); with *post hoc* analysis showing that overall, the pelvis had significantly larger mean vertical displacement amplitudes than the withers with a mean (±SEM) of 115.28 (±2.52) mm for the pelvis and a mean (±SEM) of 91.53 (±2.36) mm for the withers, a mean (±SEM) significant difference of 23.74 (±2.68) mm (Table 3.1).

There was also a significant main effect of water depth ($F_{(3,48)}$ = 144.269, p < 0.001), with both the pelvis and withers showing an increase in vertical displacement amplitudes with each increasing water depth. Table 3.2 shows the results of the *post hoc* analysis where there was a significant difference at every depth.

Table 3.1: Post hoc analysis of the significant main effect of pelvis versus withers on vertical displacements of the pelvis and withers when trotting on the aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction. Mean difference shown in mm.

				95% Confidence Interval for Difference[a]	
	Mean	Standard error	Sig.[a]	Lower Bound	Upper Bound
Pelvis mean estimate	115.28	2.52		110.14	120.41
Withers mean estimate	91.53	2.36		86.74	96.33
Pelvis – Withers mean difference	23.743*	2.678	<0.001	18.294	29.192

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.01 level

Table 3.2: Post hoc analysis of the significant main effect of water depth on vertical displacements of the pelvis and withers when trotting on the aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in millimetres with * denoting significance at the 0.05 level. In the pelvis, means were 96.19, 112.49, 122.29, and 130.14 mm, and in the withers, 74.69, 87.26, 96.81, and 107.37 mm at each increasing depth.

					95% Confidence Interval for Difference[a]	
		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Mid P3	Mid Fetlock	-14.43*	1.01	<0.001	-17.26	-11.60
	Mid MC3	-24.11*	1.60	<0.001	-28.60	-19.63
	Mid Carpus	-33.32*	1.57	<0.001	-37.72	-28.91
Mid Fetlock	Mid P3	14.43*	1.01	<0.001	11.60	17.26
	Mid MC3	-9.68*	1.08	<0.001	-12.72	-6.64
	Mid Carpus	-18.88*	1.15	<0.001	-22.11	-15.66
Mid MC3	Mid P3	24.11*	1.60	<0.001	19.63	28.60
	Mid Fetlock	9.68*	1.08	<0.001	6.64	12.72
	Mid Carpus	-9.20*	1.01	<0.001	-12.03	-6.38
Mid Carpus	Mid P3	33.32*	1.57	<0.001	28.91	37.72
	Mid Fetlock	18.88*	1.15	<0.001	15.66	22.11
	Mid MC3	9.20*	1.01	<0.001	6.38	12.03

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.01 level

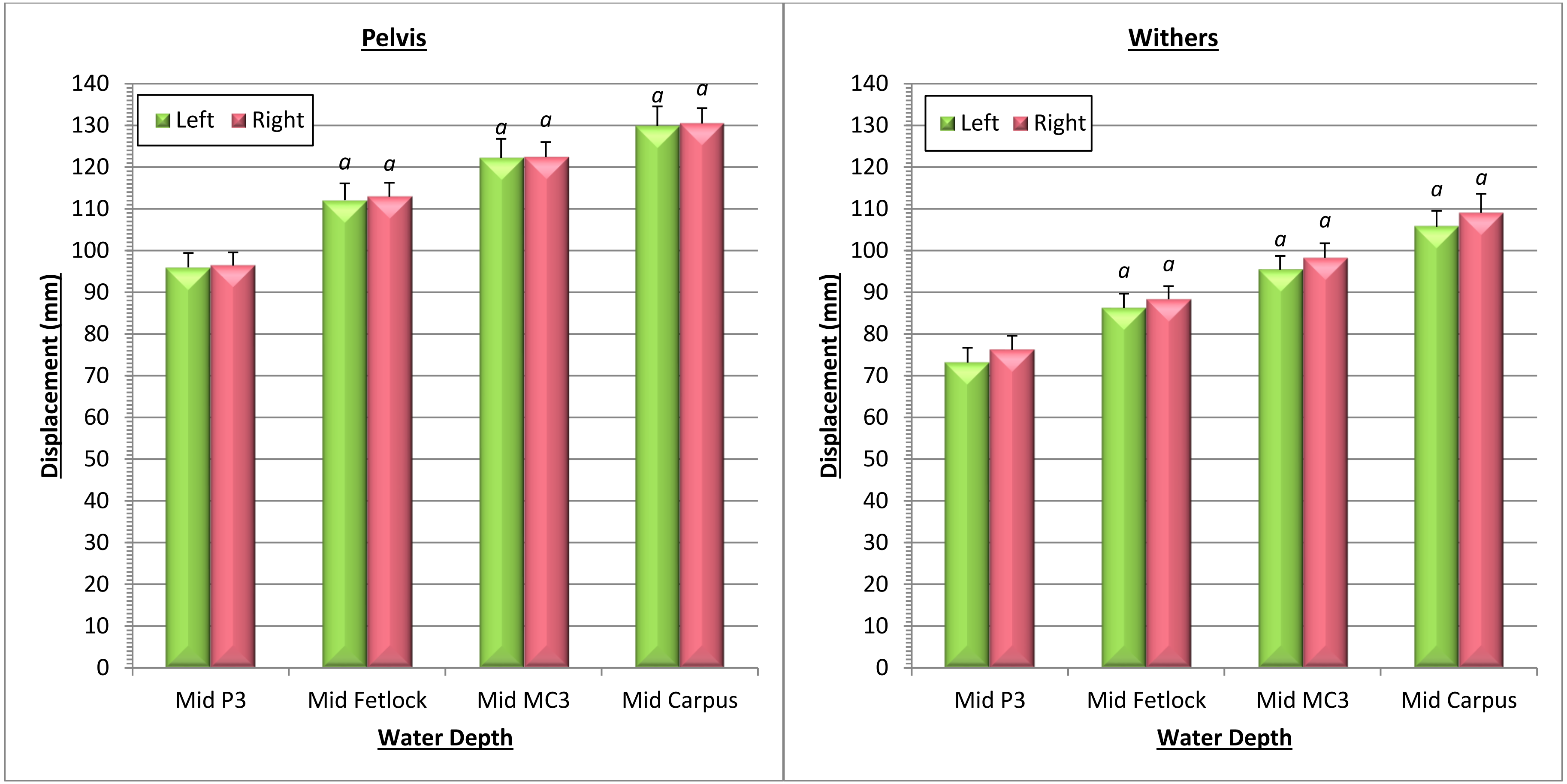


Figure 3.2: Mean (±SEM) vertical displacement (in millimetres) of the equine pelvis (left) and withers (right) at increasing water depths when trotting on an aqua-treadmill at increasing water depths for both left diagonal pair (green) and right diagonal pair (pink) (n=17). Overall the pelvis has significantly larger vertical displacements than the withers ($p < 0.01$). Vertical displacements increase at each increasing water depth ($p < 0.01$), [a] significantly different from all lower water depths.

3.3.2 Percentage Change in Displacement of the Pelvis and Withers

It was considered necessary to take the height of the horse into consideration for analysis as it could be expected that the taller the horse the larger the vertical displacement amplitude within a stride as all movements are relative. To address this, a repeated measure ANOVA was conducted using horse height as a co-variate. The covariate (horse height) met most requirements (correlating with the dependent variable, not correlating with the independent variable, and having a linear relationship with the dependent variable), but the analysis failed on homogeneity of regression with the slope of the regression line for the overall pelvis scoring -0.745, but for the overall withers data scoring -0.500 where the slope should have been the same for the different conditions. Therefore, it was deemed not valid to use horse height as a covariate in the analysis. An alternative solution was proposed using the proportional increase or percentage change in vertical displacement amplitudes.

Percentage Change in Displacement

Percentage change in displacement was calculated by using the first water depth (water at mid P3) as the baseline depth from which to calculate percentage change for the three increasing water depths. Percentage change in displacement was calculated for each horse using the mean values that were obtained from the mid P3 water depth and the means were plotted (Figure 3.3).

To ascertain if this was a suitable method of adjusting for height of horse, correlations were performed of the percentage data against the millimetre data and the correlations were analysed. The correlation and confidence intervals were found to be notably smaller in the percentage change data set (mean correlation coefficient -0.37 for the mm data set versus -0.21 for the percentage data set, a decrease in the percentage data set of -0.16) suggesting that this adjustment was a valid procedure to improve the data by removing variability associated with the height of the horse.

A repeated measures ANOVA (2x3x2) was conducted to determine the effects of pelvis or withers, water depth, and the left or right parts of the stride on mean percentage increases in vertical displacement amplitudes of 17 horses trotting on the aqua-treadmill at increasing water depths.

Results showed a highly significant main effect of water depth ($F_{(2,32)} = 79.562$, $p < 0.001$) but also one significant two-way interaction between pelvis or withers and water depth ($F_{(2,32)} = 4.901$, $p = 0.031$). Displacements increased as water depth increased and *post hoc* analyses determined a simple main effect of pelvis versus withers with percentage displacement value scores being mean (±SEM) 10.74 (±4.43) % higher in the withers than the pelvis at the deepest water depth of mid carpus on the left diagonal pair ($p = 0.028$) (Table 3.3). Pairwise comparisons of the simple main effect of water depth showed significant differences at every combination ($p < 0.001$), showing significant increases in percentage displacement at each depth in both the pelvis and withers and on both the left and right diagonal pairs depth (Table 3.4).

Table 3.3: Post hoc analysis of the significant simple main effect of pelvis versus withers on percentage change in vertical displacements of the pelvis and withers when trotting on the aqua-treadmill at increasing water depths. Pairwise comparisons with a Bonferroni correction for multiple comparisons shown. Mean percentage differences shown.

Pelvis to Withers		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Mid Fetlock	Left	-1.82	2.33	0.45	-6.76	3.13
	Right	0.91	2.20	0.68	-3.75	5.58
Mid MC3	Left	-4.70	2.90	0.12	-10.84	1.44
	Right	-2.29	2.61	0.39	-7.81	3.24
Mid Carpus	Left	-10.74*	4.44	0.03	-20.14	-1.33
	Right	-7.62	4.72	0.13	-17.62	2.37

Based on estimated marginal means

[a] Bonferroni adjustment for multiple comparisons

* The mean difference is significant at the 0.05 level

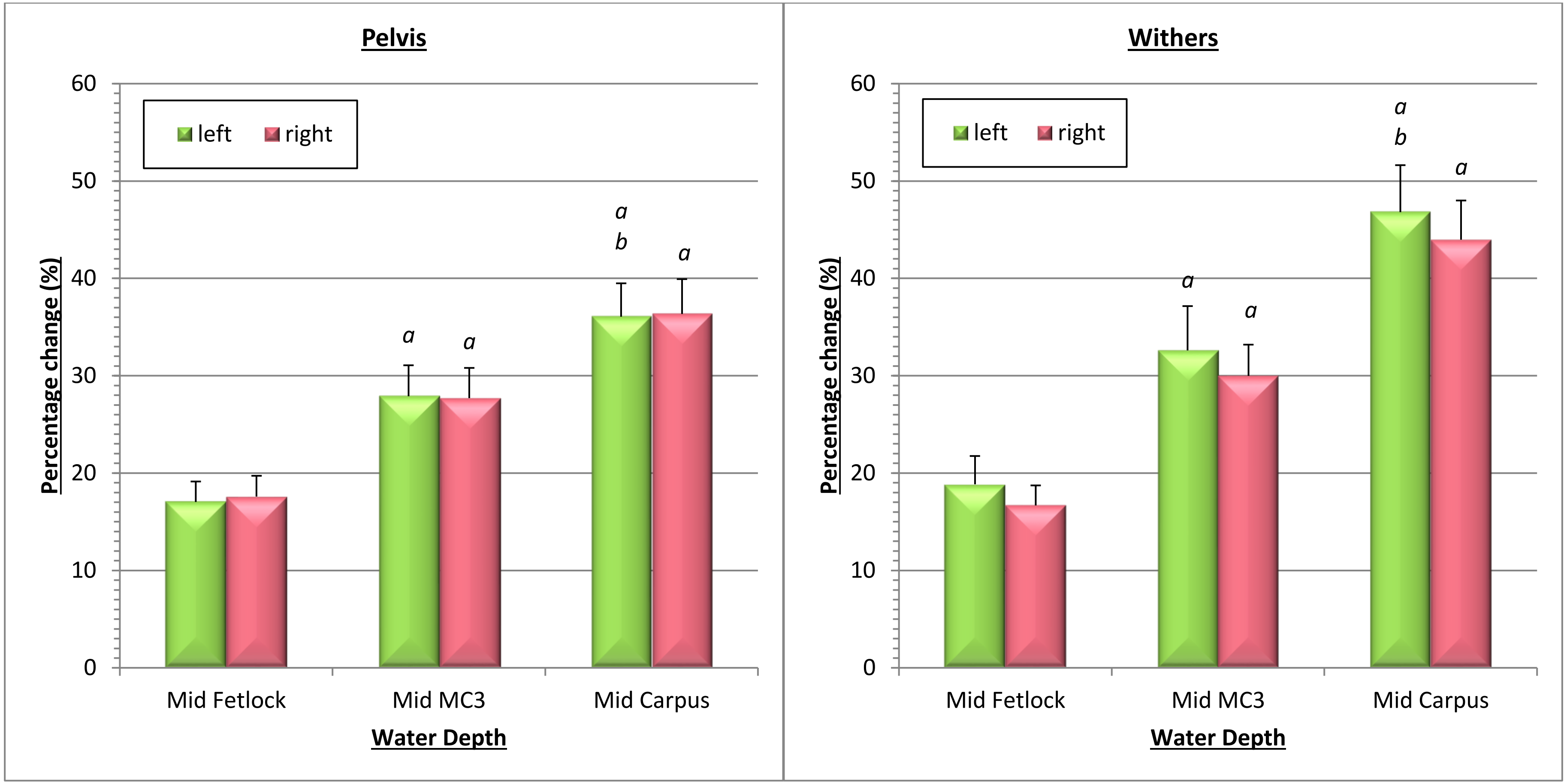


Figure 3.3: Mean (±SEM) percentage increase in vertical displacements of the equine pelvis (left) and withers (right) when trotting on an aqua-treadmill at increasing water depths for both left diagonal pair (green) and right diagonal pair (pink) (n=17). Significant increases in percentage displacement at each depth in both pelvis and withers on both left and right diagonal pair, [a] significantly different from all lower depths to $p < 0.001$. [b] significant difference between pelvis and withers on the left diagonal pair at depth of mid carpus ($p = 0.028$).

Table 3.4: Post hoc analysis of the significant simple main effect of water depth on percentage change in vertical displacements of the pelvis and withers when trotting on the aqua-treadmill at increasing water depths. Pairwise comparisons with a Bonferroni correction for multiple comparisons shown. Mean percentage differences shown. A significant effect of depth was found in both the pelvis and withers at both the left and right parts of the stride for each combination.

				Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
PELVIS	LEFT	Mid Fetlock	Mid MC3	-10.87*	1.56	<0.001	-15.03	-6.70
			Mid Carpus	-19.03*	2.21	<0.001	-24.93	-13.14
		Mid MC3	Mid Fetlock	10.87*	1.56	<0.001	6.70	15.03
			Mid Carpus	-8.17*	1.69	0.001	-12.69	-3.64
		Mid Carpus	Mid Fetlock	19.03*	2.21	<0.001	13.14	24.93
			Mid MC3	8.17*	1.69	0.001	3.64	12.69
	RIGHT	Mid Fetlock	Mid MC3	-10.11*	1.95	<0.001	-15.31	-4.91
			Mid Carpus	-18.76*	1.93	<0.001	-23.91	-13.61
		Mid MC3	Mid Fetlock	10.11*	1.95	<0.001	4.91	15.31
			Mid Carpus	-8.65*	1.71	<0.001	-13.23	-4.07
		Mid Carpus	Mid Fetlock	18.76*	1.93	<0.001	13.61	23.91
			Mid MC3	9.65*	1.71	<0.001	4.69	13.23
WITHERS	LEFT	Mid Fetlock	Mid MC3	-13.75*	2.86	0.001	-21.38	-6.12
			Mid Carpus	-27.95*	3.82	<0.001	-38.16	-17.74
		Mid MC3	Mid Fetlock	13.75*	2.86	0.001	6.12	21.38
			Mid Carpus	-14.20*	3.18	0.001	-22.70	-5.70
		Mid Carpus	Mid Fetlock	27.95*	3.82	<0.001	17.74	38.16
			Mid MC3	14.20*	3.18	0.001	5.70	22.70
	RIGHT	Mid Fetlock	Mid MC3	-13.31*	2.61	<0.001	-20.29	-6.33
			Mid Carpus	-27.29*	2.99	<0.001	-35.30	-19.29
		Mid MC3	Mid Fetlock	13.31*	2.61	<0.001	6.33	20.29
			Mid Carpus	-13.98*	2.64	<0.001	-21.05	-6.92
		Mid Carpus	Mid Fetlock	27.29*	2.99	<0.001	19.29	35.30
			Mid MC3	13.98*	2.66	<0.001	6.92	21.05

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.01 level

3.3.3 Symmetry

Analysis of both left and right parts of the trot stride independently allowed investigation into the symmetry of the horses at each water depth and to investigate if water depth influences the symmetry of the stride. Symmetry indices were determined by calculating the difference between the left and right parts of the stride for each depth (Equation 1). Both the millimetre and percentage score data were investigated in this study and analysed separately, and the means were plotted (Figure 3.4).

In the millimetre data, a two-way repeated measures ANOVA (2x4) was conducted to determine the effect of water depth on the symmetry of the stride in both the pelvis and withers of 17 horses trotting on the aqua-treadmill at increasing water depths. Difference value scores were not normally distributed as assessed by Shapiro-Wilk's test of normality ($p > 0.05$), however, the ANOVA was run without a transformation of the data as transforming it did not result in a statistically different result. There was no significant interaction between water depth and pelvis or withers ($F_{(3,48)} = 0.266$, $p = 0.850$). The main effect of water depth on symmetry was not significant ($F_{(3,48)} = 0.335$, $p = 0.800$), and the main effect of pelvis or withers on symmetry was not significant ($F_{(1,16)} = 0.101$, $p = 0.755$).

In the percentage change data, a two-way repeated measures ANOVA (2x3) was conducted to determine the effect of water depth on the symmetry of the stride in both the pelvis and withers of 17 horses trotting on the aqua-treadmill at increasing water depths. Difference value scores were not normally distributed as assessed by Shapiro-Wilk's test of normality ($p > 0.05$), however, the ANOVA was run without a transformation of the data as transforming it did not result in a statistically different result. There was no statistically significant interaction between water depth and pelvis or withers ($F_{(2,32)}$ = 0.801, p = 0.458). The main effect of water depth on symmetry was not statistically significant ($F_{(2,32)}$ = 3.114, p = 0.084). The main effect of pelvis or withers on symmetry was statistically significant ($F_{(1,16)}$ = 4.957, p = 0.041) and *post hoc* pairwise comparisons showed that overall, the withers are 3.73 % less symmetrical than the pelvis (Table 3.5).

Table 3.5: Post hoc analysis of the significant main effect of pelvis versus withers on symmetry of vertical displacements of the pelvis and withers when trotting on the aqua-treadmill at increasing water depths. Pairwise comparisons with a Bonferroni correction for multiple comparisons shown. Mean percentage differences shown with the withers to be overall 3.73% less symmetrical than the pelvis.

					95% Confidence Interval for Difference[a]	
		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Pelvis	Withers	-3.73*	1.68	0.041	-7.29	-0.18

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

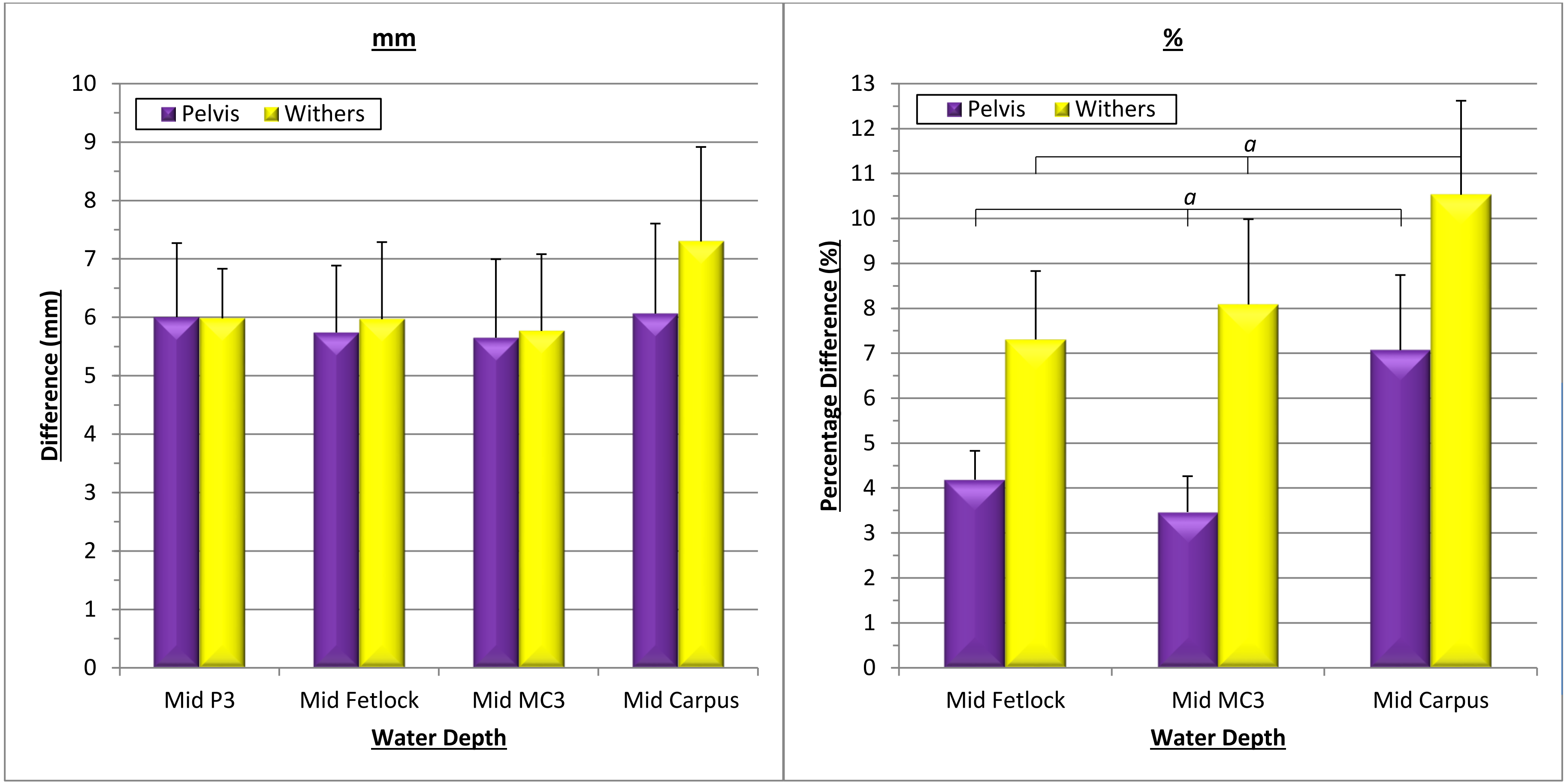


Figure 3.4: Mean (±SEM) difference between the mean vertical displacements for left and right diagonal for the equine pelvis (purple) and withers (yellow) when trotting on an aqua-treadmill at increasing water depths with raw measurement of mm (left) and percentage change in displacement (right) (n = 17). In the percentage change data, overall the withers are significantly less symmetrical than the pelvis, [a] (p < 0.05).

3.3.4 Changes in Position

The Qualisys© data can provide information for the actual movement patterns in terms of looking at the markers in space in time, to show not only the vertical displacement amplitudes, but the minimum and maximum vertical points reached through the stride cycle for each marker (both pelvis and withers in this study) which can give a good visual descriptor of the actual movement patterns within a stride cycle (n = 8).

Figure 3.5 shows the minimum and maximum vertical positions reached throughout a stride cycle of both the pelvis and withers. For this dataset, separate analyses were conducted on the minimum values and then the maximum values.

<u>Minimum positions</u>

A repeated measures ANOVA (2x4x2) was conducted to determine the effects of pelvis or withers, water depth, and the left or right parts of the stride on the minimum vertical position reached in the stride cycle of 8 horses trotting on an aqua-treadmill at increasing water depths. There was no significant three-way interaction between 'pelvis or withers', 'water depth' or 'left or right' ($F_{(3,21)}$ = 0.260, p = 0.853). There were no significant two-way interactions. There was, however, a significant main effect of pelvis versus withers ($F_{(1,7)}$ = 42.038, p < 0.001). *Post hoc* analyses of pairwise comparisons show that the pelvis overall has a 43.89 ± 6.77 mm lower minimum position than the withers at the lowest point of the stride (Table 3.6).

There was also a significant main effect of water depth ($F_{(3,21)}$ = 72.122, p < 0.001) where at each increasing water depth the minimum part of the stride was significantly lower than the minimum of the preceding depth (means (±SEM) of 20.434 (±20.41), 14.31 (±20.90), 10.96 (±20.01), 5.97 (±21.30) mm) (Table 3.7).

Table 3.6: Post hoc analysis of the statistically significant main effect of pelvis versus withers when analysing the mean minimum part of the stride of 8 horses trotting on an aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in mm. (means Pelvis -9.028 versus Withers 34.866mm with a mean difference of 43.894 ± 6.770 mm).

					95% Confidence Interval for Difference[a]	
		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Pelvis	Withers	-43.89*	6.77	<0.001	-59.90	-27.89

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.01 level

Table 3.7: Post hoc analysis of the statistically significant main effect of water depth when analysing the mean minimum part of the stride of 8 horses trotting on an aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in mm.

					95% Confidence Interval for Difference[a]	
		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Mid P3	Mid Fetlock	6.13*	1.00	0.003	2.49	9.76
	Mid MC3	9.48*	1.31	0.001	4.71	14.24
	Mid Carpus	14.46*	1.15	<0.001	10.28	18.64
Mid Fetlock	Mid P3	-6.13*	1.00	0.003	-9.76	-2.49
	Mid MC3	3.35*	0.80	0.024	0.45	6.26
	Mid Carpus	8.34*	0.83	<0.001	5.31	11.37
Mid MC3	Mid P3	-9.48*	1.31	0.001	-14.24	-4.71
	Mid Fetlock	-3.35*	0.98	0.024	-6.23	-0.45
	Mid Carpus	4.98*	0.87	0.004	1.82	8.15
Mid Carpus	Mid P3	-14.46*	1.15	<0.001	-18.64	-10.28
	Mid Fetlock	-8.34*	0.83	<0.001	-11.37	-5.31
	Mid MC3	-4.98*	0.87	0.004	-8.15	-1.82

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

Maximum positions

A repeated measures ANOVA (2x4x2) was conducted to determine the effects of pelvis or withers, water depth, and the left or right parts of the stride on the most maximum vertical position reached in the stride cycle of 8 horses trotting on an aqua-treadmill at increasing water depths (Figure 3.5).

There was no significant three-way interaction between 'pelvis or withers', 'water depth' or 'left or right' ($F_{(3,21)}$ = 2.529, p = 0.085). There were no significant two-way interactions. There was one significant main effect of water depth ($F_{(3,21)}$ = 28.581, p < 0.001) where *post hoc* analyses showed significant increases from mid P3 to mid fetlock (p = 0.002), from mid P3 to mid MC3 (p = 0.003), and from mid P3 to mid carpus (p = 0.003) (Table 3.8).

Table 3.8: Post hoc analysis of the statistically significant main effect of water depth when analysing the mean maximum part of the stride of 8 horses trotting on an aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in mm.

					95% Confidence Interval for Difference[a]	
		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Mid P3	Mid Fetlock	-9.17*	1.35	0.002	-14.09	-4.25
	Mid MC3	-14.07*	2.37	0.003	-22.68	-5.45
	Mid Carpus	-18.79*	3.09	0.003	-30.04	-7.47
Mid Fetlock	Mid P3	9.17*	1.35	0.002	4.25	14.09
	Mid MC3	-4.90	1.62	0.114	-10.77	0.98
	Mid Carpus	-9.63*	2.13	0.016	-17.37	-1.88
Mid MC3	Mid P3	14.066*	2.37	0.003	5.45	22.68
	Mid Fetlock	4.90	1.62	0.114	-0.98	10.77
	Mid Carpus	-4.73	1.71	0.167	-10.94	1.49
Mid Carpus	Mid P3	18.79*	3.09	0.003	7.55	30.04
	Mid Fetlock	9.63*	2.13	0.016	1.88	17.37
	Mid MC3	4.73	1.71	0.167	-1.49	10.94

Based on estimated marginal means

[a] Bonferroni adjustment for multiple comparisons

* The mean difference is significant at the 0.05 level

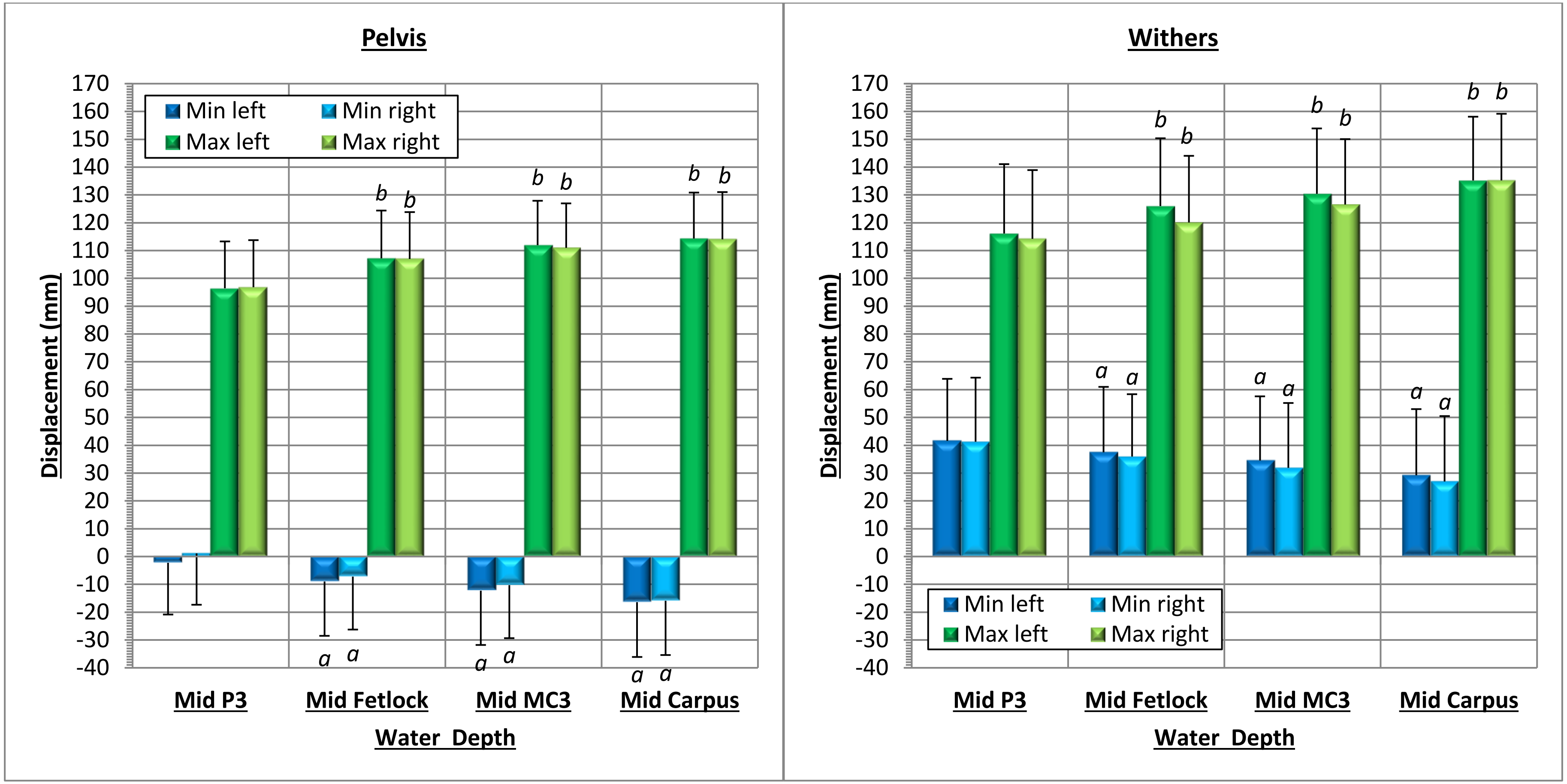


Figure 3.5: Mean (±SEM) position (mm) in the absolute displacement of the equine pelvis (left) and withers (right) from the minimum starting point (at left hind stance) and maximum vertical point of the stride cycle when trotting on an aqua-treadmill at increasing water depths (n = 8). Left minimum vertical position (min left) (blue). Right minimum vertical position (min right) (light blue). Maximum left vertical position (max left) (green). Maximum right vertical position (max right) (light green). Overall, the pelvis has significantly lower minimum positions than the withers (43.89 ± 6.77 mm, p < 0.001). [a] At increasing water depths each minimum position was significantly lower than the last (p < 0.001). [b] Maximum positions all significantly different from water depth of mid P3 (p < 0.01).

3.3.5 Percentage Change in Position

Again, considering the influencing factor of horse height on the vertical displacement amplitudes and therefore also positions, the percentage change in position was also investigated and analysed using the information obtained from the Qualisys© data set. The percentage change data is plotted in Figure 3.6. For this dataset, separate analyses were conducted on the minimum values and then the maximum values.

<u>Minimum positions</u>

A repeated measures ANOVA (2x3x2) was conducted to determine the effects of pelvis or withers, water depth, and the left or right parts of the stride on the percentage change in minimum position from the baseline water depth of mid P3 of 8 horses trotting on an aqua-treadmill at increasing water depths. There was no significant three-way interaction between 'pelvis or withers', 'water depth' or 'left or right' ($F_{(2,14)} = 0.105$, $p = 0.901$), and there were no significant two-way interactions. There was one significant main effect of water depth ($F_{(2,14)} = 28.677$, $p < 0.001$) where *post hoc* analyses showed a significant decrease in percentage change in position from mid fetlock to mid MC3 ($p = 0.012$) and from mid MC3 to mid carpus ($p = 0.020$) (Table 3.9).

Table 3.9: Post hoc analysis of the significant main effect of water depth when analysing the mean percentage change in the minimum part of the stride of 8 horses trotting on an aqua-treadmill. Pairwise comparison with Bonferroni correction shown. Mean difference shown in %.

					95% Confidence Interval for Difference[a]	
		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Mid Fetlock	Mid MC3	3.35*	0.80	0.012	0.86	5.85
	Mid Carpus	8.29*	1.15	0.001	4.70	11.87
Mid MC3	Mid Fetlock	-3.35*	0.80	0.012	-5.85	-0.86
	Mid Carpus	4.93*	1.30	0.020	0.87	8.99
Mid Carpus	Mid Fetlock	-8.29*	1.15	0.001	-11.87	-4.70
	Mid MC3	-4.93*	1.30	0.020	-8.99	-0.87

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

Maximum positions

A repeated measures ANOVA (2x3x2) was conducted to determine the effects of pelvis or withers, water depth, and the left or right parts of the stride on the percentage change in maximum vertical position from the baseline water depth of mid P3 of 8 horses trotting on an aqua-treadmill at increasing water depths. There was no statistically significant three-way interaction between ‘pelvis or withers’, ‘water depth’ or ‘left or right’ ($F_{(2,14)}$ = 2.748, p = 0.098). There was one statistically significant two-way interaction between pelvis or withers and water depth ($F_{(2,14)}$ = 3.789, p = 0.048).

Post hoc analyses determined that in fact there was no simple main effect of pelvis or withers but the simple main effect of water depth was significant (Table 3.10) at the withers on the left diagonal pair where the significant difference lay between the depths of mid Fetlock and mid Carpus with a mean (±SEM) increase of 9.19 (±2.36) % (*p* = 0.018), and on the right diagonal pair where the significant differences lay between the depths of mid Fetlock and mid Carpus with a mean increase of 15.15 (±2.34) % (*p* = 0.002), and between mid MC3 and Mid Carpus with a mean increase of 8.67 (±1.88) % (*p* = 0.007).

Table 3.10: Post hoc analysis of the significant simple main effect of water depth when analysing the mean percentage change in the maximum part of the stride of 8 horses trotting on an aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in %.

							95% Confidence Interval for Difference[a]	
				Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
PELVIS	Left	Mid Fetlock	Mid MC3	-4.73	2.14	0.187	-11.41	1.95
			Mid Carpus	-7.10	3.13	0.172	-16.89	2.68
		Mid MC3	Mid Fetlock	4.73	2.14	0.187	-0.95	11.41
			Mid Carpus	-2.37	1.83	0.705	-8.08	3.34
		Mid Carpus	Mid Fetlock	7.10	3.13	0.172	-2.68	16.89
			Mid MC3	2.37	1.83	0.705	-3.34	8.08
	Right	Mid Fetlock	Mid MC3	-3.99	2.11	0.300	-10.58	2.60
			Mid Carpus	-7.07	2.72	0.106	-15.56	1.42
		Mid MC3	Mid Fetlock	3.99	2.11	0.300	-2.60	10.58
			Mid Carpus	-3.08	2.14	0.581	-9.78	3.62
		Mid Carpus	Mid Fetlock	7.07	2.72	0.106	-1.42	15.56
			Mid MC3	3.08	2.14	0.581	-3.62	9.78
WITHERS	Left	Mid Fetlock	Mid MC3	-4.39	1.58	0.082	-9.33	0.55
			Mid Carpus	-9.19*	2.36	0.018	-16.55	-1.82
		Mid MC3	Mid Fetlock	4.39	1.58	0.082	-0.55	9.33
			Mid Carpus	-4.79	2.27	0.217	-11.89	2.30
		Mid Carpus	Mid Fetlock	9.19*	2.36	0.018	1.82	16.55
			Mid MC3	4.79	2.27	0.217	-2.30	11.89
	Right	Mid Fetlock	Mid MC3	-6.48	2.11	0.054	-13.08	0.12
			Mid Carpus	-15.15*	2.64	0.002	-23.41	-6.88
		Mid MC3	Mid Fetlock	6.48	2.11	0.054	-0.12	13.08
			Mid Carpus	-8.67*	1.88	0.007	-14.55	-2.78
		Mid Carpus	Mid Fetlock	15.15*	2.64	0.002	6.88	23.41
			Mid MC3	8.67*	1.88	0.007	2.78	14.55

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

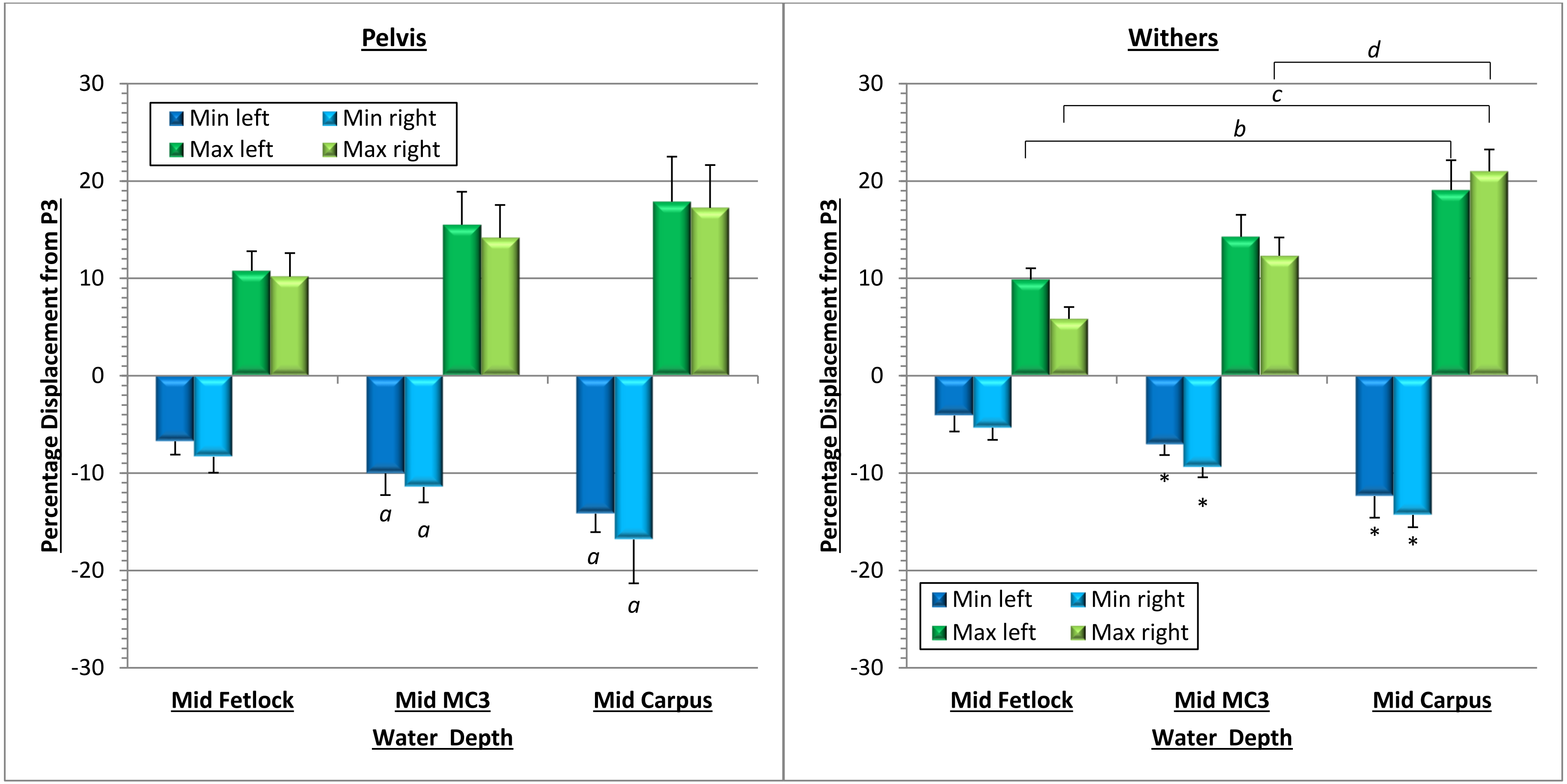


Figure 3.6: Mean (±SEM) percentage change in position (%) from the baseline water level of mid P3 of the equine pelvis (left) and withers (right) from the minimum vertical starting point (at left hind stance) and maximum vertical point of the stride cycle when trotting on an aqua-treadmill at increasing water depths (n = 8). Left minimum vertical position (min left) (blue). Right minimum vertical position (min right) (light blue). Maximum left vertical position (max left) (green). Maximum right vertical position (max right) (light green). Percentage change in minimum displacements decreased at each increasing water depth, [a] significantly different from all lower water depths ($p < 0.01$). Maximum values showed significance in the withers only, [b] significant difference between mid fetlock and mid carpus on the left diagonal pair ($p < 0.05$), [c] and on the right ($p < 0.01$). [d] significant difference between mid MC3 and mid carpus on the right ($p < 0.01$).

3.3.6 Pitch

The inertial measurement units of the Xsens© system could record the pitch of the sensor throughout the trials. This information was extracted for analysis using methods previously described and the means were plotted (Figure 3.7).

A repeated measures ANOVA (2x4x2) was conducted to determine the effects of pelvis or withers, water depth, and the left or right parts of the stride on mean pitch amplitude throughout a stride cycle of 10 horses trotting on the aqua-treadmill at increasing water depths. There was no significant three-way interaction between 'pelvis or withers', 'water depth' or 'left or right' ($F_{(3,27)}$ = 0.686, p = 0.568). There were no statistically significant two-way interactions. There were no statistically significant main effects. Water depth had no effect on pitch amplitudes throughout a stride cycle, neither was there an effect of pelvis or withers, or the left or right part of the stride.

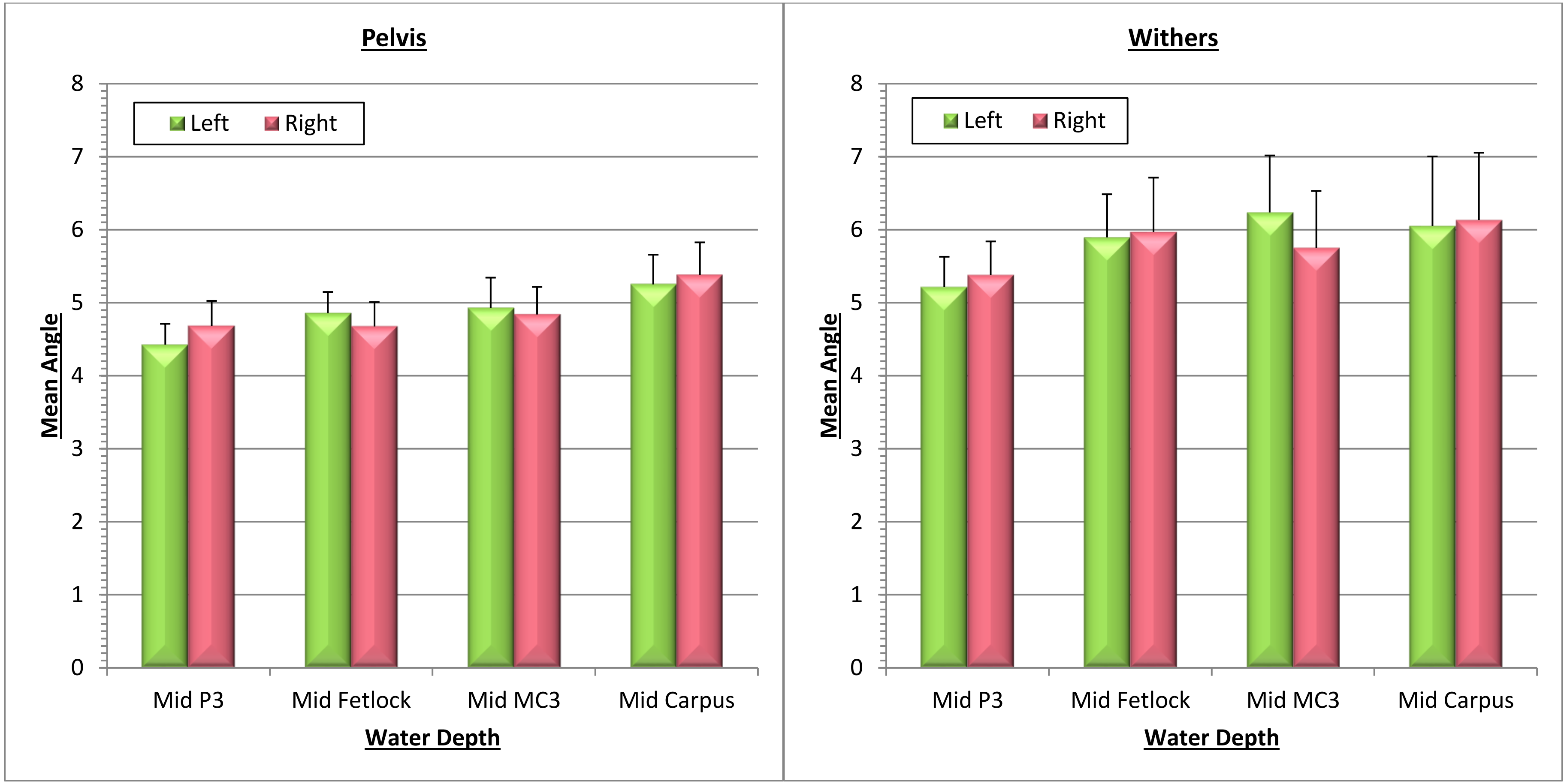


Figure 3.7: Mean (±SEM) pitch of the pelvis (left) and withers (right) throughout a stride cycle when trotting on an aqua-treadmill at increasing water depths for both left diagonal pair (green) and right diagonal pair (pink) (n = 10). No significant differences were found in pitch angle.

3.4 Discussion

The aim of this study was to determine if there was an effect of water depth on the vertical displacement amplitudes of the fore and hind quarters of the horse (pelvis and withers) when trotting on an aqua-treadmill. Water depth was found to have a significant effect on vertical displacement amplitudes of the pelvis and withers, as water depth increased so did the vertical displacement amplitudes. This was found to be the case both in terms of the actual values in millimetres and when looking at the proportionate data in terms of percentage change in displacement at increasing water depths. It was necessary to look at the proportionate data as the height of the horse was found to have a positive correlation with the amount of displacement; as you would expect, taller horses had larger displacements than shorter horses. It was found not valid to use height as a covariate within statistical analyses; therefore, using the proportionate change in displacements was proposed and was found to be effective in reducing the effect of height of horse.

Increase in vertical displacement as water depth increases demonstrates that the horse is perhaps working harder to push themselves up higher out and over the top of the water, which may link to the Pauchard *et al*. (2014) study where a softer surface increased vertical displacement amplitudes in the pelvis. It may be that the buoyancy of the water provides a cushioning effect akin to a softer surface even though the treadmill belt itself would not be categorized as a soft surface. Studies investigating the effect of water depth on heart rate of horses exercising on an aqua-treadmill determined that water height had no effect on heart rate (Voss *et al*., 2002; Nankervis and Williams, 2006; Nankervis *et al*., 2008a; Scott *et al*., 2010), which would suggest actually no greater energy expenditure or effort.

However, all but one of these studies investigated only the walk and not trotting through water. Voss *et al*. (2002) determined that there were only small significant differences of 18 beats per minutes of the mean values of the medians between walking and trotting and that the small difference plus the relatively low levels of heart rates and blood lactate concentrations near resting values meant that trotting on a water treadmill at water depths of carpus or elbow was only a medium-sized workload and an aerobic activity.

An earlier study that investigated electomyographic (EMG) activity of seven skeletal muscles in the forequarters and one in the hindquarters of horses both walking and trotting on an aqua-treadmill showed that the intensity of EMG activity within the *extensor digitorum communis* (a forelimb muscle) was higher during walking on an aqua-treadmill than during trot on an aqua-treadmill (Tokuriki *et al*., 1999). Unfortunately, the results of the one muscle investigated in the hindlimb, the *vartus lateralis* of the *quadriceps femoris* were not reported (Tokuriki *et al*., 1999) so no comparison can be made between the fore and hind quarters of the horse. It is known, however, that horses exhibit increased elastic storage at faster speeds (Biewener, 1998) which may account for the lack of notable EMG activity reported in the hindlimb (Tokuriki *et al*., 1999) and the non-significant increase in workload (heart rates or blood lactate concentrations) at either increasing water depth (Voss *et al*., 2002; Nankervis and Williams, 2006; Nankervis *et al*., 2008; Scott *et al*., 2010) or increasing speed from walk to trot (Tokuriki *et al*., 1999; Voss *et al*., 2002). With regards to the results reported in the current study, it is likely that the increased vertical displacements recorded with increasing water depths are due to elastic energy storage, the buoyancy of the water and an increased workload, and it is therefore probable that trotting in water provides a greater stimulus to develop more muscle. Heart rates were not measured in this study so it is impossible to conclude that increased water depth whilst trotting did or did not have an effect on heart rate or workload.

The pelvis was found to have vertical displacements significantly greater than those found at the withers. This suggests that the hind quarters of the horse are in some way working harder than the forequarters. No other study has made a comparison between the fore and hindquarters in this way. It is likely that the hindquarters are more engaged than the forequarters due at large to the anatomy of the hind limb and the reciprocal apparatus where during the stance phase the extension of the fetlock joint and stance flexion of the stifle, tarsal and coffin joints illustrate the shock absorption of the hind limb and in the swing phase the reciprocal apparatus, which forms the coupling mechanism between stifle and tarsal joint, also influences the fetlock joint because synchronous flexion and extension between these three joints have been demonstrated (Wyn-Jones, 1988; Back *et al*., 1995b). It is also probable that the hindquarters have an increased engagement by perhaps tucking under to work harder to create the larger displacements, however, investigation of the pitch showed no corroboration with this theory – if the pelvis was significantly ‘tucking under’ then it would be expressed by the pitch amplitude of the IMU on the *tuber sacrale*. Increasing water depth was shown to have no effect on pitch amplitudes throughout the stride cycle and there was no difference in pitch amplitudes between the pelvis and withers, in fact if anything, the withers appear to exhibit overall larger pitch amplitudes than the pelvis. It is very likely that the smaller vertical displacements seen in the withers are due to the forequarters of the horse being able to compensate the depth of the water by flexing at the carpus. The slightly larger (but actually non-significant) pitch amplitudes seen in the withers are likely due to the anatomy of the withers and the difficulty in affixing the IMU to this area (Pfau *et al*., 2005). One further study that has extensively investigated trotting on a treadmill, albeit it not an aqua-treadmill, found that vertical displacement of the trunk of the horse was reduced (Buchner *et al*., 1994a) when compared to overground trotting. Perhaps the small amount of water seen in this study induces the effect of significantly larger vertical displacements.

When investigating the symmetry of the horse at increasing water depths there was found to be no statistical significance between increasing water depth and asymmetry. Although it appeared that there may be an increase in symmetry at a water depth of mid MC3 in the pelvis, this trend was found to be non-significant. At the greatest water depth of mid carpus, the horse seemed to become more asymmetrical, particularly at the withers, but again this trend was not found to be significant. This suggests that this cohort of horses was perhaps struggling with this deep water exercise and having to adapt their gait to 'jump' over the water in order to cope with the exertion rather than being able to continue to compensate the water depth by flexing at the carpus.

The overall mean values obtained for the level of asymmetry between left and right hind stance were found to be quite high, for example in the pelvis with water at mid P3, there was approximately 6 millimetres difference between left and right hind stance. Although the horses used in this study were assessed by a Veterinary Surgeon prior to the study and were deemed to be sound, this degree of asymmetry agrees with findings in the literature that state that around 40% of competition and leisure horses in normal work are found to be lame (Greve and

Dyson, 2014) and that the traditional visual assessment of lameness according to specific grading systems (Dyson, 2011) can often have low inter-observer agreement (Keegan *et al*., 1998; Keegan *et al*., 2010), with within-observer agreement on lameness levels being higher in more senior clinicians than interns and residents and concluding that increased experience leads to increased agreement of lameness identification (Keegan *et al*., 1998). As horses used in the current study were only observed by the hydrotherapist and owner and had no history of lameness, it is possible if observed by more experienced Veterinary Clinicians some may have been identified as clinically asymmetric i.e. lame. It has also been demonstrated that there is an observer bias when the observer knows that a limb or area has received a nerve block showing a difference therefore in the lameness grade given (Arkell *et al.*, 2006). It is also worth noting that it has been demonstrated that the human eye struggles to perceive asymmetries of less than 10-20%, with a difference in hip hike or *tuber coxae* asymmetry needing to be 25% to be visible making subtle lamenesses difficult to detect (Parkes *et al.*, 2009) which is perhaps more pertinent to this study as all horses that took part were riding school type horses in regular work that were potentially less well scrutinized than a competition type horse. In this study, it is unclear if an average asymmetry of 6mm between the *tuber coxae* would be an asymmetry obvious enough to detect by eye.

When looking at the percentage change data rather than the actual values, a different trend exhibited in the withers data where there was an increase in percentage change of asymmetry at increasing water depths suggesting, that the deeper the water the more asymmetrical the stride becomes in front. This potentially indicates that horses whilst engaging the hind quarters and compressing the forelimb to create larger vertical lift may compensate on balance losing the symmetry of the lift in front. As there are no previous studies investigating symmetry on an aqua-treadmill there is currently no data available for comparison. Symmetry could be investigated further with a larger number of horses of perhaps a more precisely equivalent level of fitness (for example all 3-year-old race horses) to identify if specific or heavier workloads through deeper water depths induced changes in symmetry; likewise if shallower water depths have a more positive effect on symmetry. A study to investigate horses' symmetry patterns overground that then subsequent completed a period of exercising in an aqua-treadmill to analyse changes in patterns of symmetry would be particularly interesting.

Horses started the stride significantly lower in the pelvis than they did in the withers. This suggests that the hindquarters compress more to produce the larger vertical displacements seen, which agrees with previous discussion on the reciprocal apparatus of the hind limb (Wyn-Jones, 1988; Back *et al*., 1995b). The withers perhaps, are more stable, and again, able to compensate the depth of the water by flexing at the carpus. The starting (most minimum) point of the stride was also significantly lower in both the pelvis and withers as the water got deeper, suggesting an increase in both hindlimb and forelimb compression to increase push off to compensate for the increasing depth of the water. It could be speculated that this increase in limb compression may be linked to an increase in stance time or increased peak forces as peak vertical displacement does not alter, and the same (if not more) force needs to be produced for push off which either requires a longer time to do it with a longer stance time or results in increased peak forces which could be detrimental for the musculoskeletal system of the horse and unlikely due to the buoyancy of the water working to reduce concussion. This, however, conflicts with recent evidence where stance times were found to decrease as water depth increased but the horses in the previous study were walking not trotting (Mendez-Angulo *et al.*, 2013), however, studies of treadmill trotting (without water) have reported an increased stance time as the hoof remains in contact with the treadmill belt for longer in the retraction phase but the hoof is placed cranially comparatively with overground trot (Buchner *et al.*, 1994a). It is suggested that perhaps the addition of water also increases the cranial placement of the hoof as vertical displacements are increased suggesting an increased range of motion throughout the limb to create the vertical lift which may therefore correspond to a subsequent increased cranial placement.

In another study, (Scott *et al.,* 2010) horses walking in water at the level of the carpus and ulna joints (deep to very deep water) had a lower stride frequency and a higher stride length of forelimbs compared with those walking at shallower water depths. This makes sense in that the buoyancy of the water increases and slows the aerial phase of the walk stride. In the present study in the trot stride there is likely to be both an increase in the stance time and aerial phase as the horse compresses the limb to increase push off to create the greater vertical displacement, which is then large enough to overall slow the stride frequency meaning that the horses are potentially producing fewer strides in the same amount of time (as they would overground or with no water) but actually working harder in those fewer strides which would create a larger workload. Workload or heart rates were not measured in this study.

An increase in limb compression with subsequent greater vertical displacement may also correspond with a greater range of motion (ROM) being produced throughout the hindlimb (Mendez-Angulo *et al.*, 2013), although ROM was not directly measured here, it is biologically logical, as the only other way to increase vertical displacement would be to drastically increase the work and power output of the muscles which would be very costly and probably not desirable in the horses utilised in this study.

The maximum vertical point of the stride was shown to significantly increase with increasing water depth but there was no significant difference in the magnitude between the pelvis and the withers, concluding that both the fore and hindlimbs of the horse reached a similar maximum point of the stride. This makes sense; otherwise the horse would appear to be very lopsided cranio-caudally when trotting (a range of 96.43–114.30mm in the pelvis, and 114.24–135.23mm in the withers).

3.5 Conclusion

Vertical displacement of the equine pelvis and withers increases with increasing water depth when trotting on an aqua-treadmill and there is a greater displacement in the pelvis than the withers. Minimum and maximum positions of the pelvis and withers were found to alter with increasing water depth, with minimum values decreasing significantly indicating an increase in limb compression during stance. Maximum vertical positions also increased significantly indicating greater maximum lift out of the water as a result of the increased compression. No significance was found in displacement symmetry for either the pelvis or withers. And no significance was found with regards to the change in the pitch of the IMUs throughout a stride cycle with increasing water depths.

There is plenty of scope for further research here to practically apply the information found. Further studies to make comparisons to overground trotting and the vertical displacement values here would be relevant, as would a longitudinal study to compare starting values before an extended period of aqua-treadmill exercise to perhaps see if repeated use of an aqua-treadmill has an overall or long lasting effect on vertical displacements. For example, there is clearly some muscle training involved with producing the greater vertical displacements, so how long lived is this training and is there an increasing effect with increasing aqua-treadmill use? For example, do horses reach a threshold of increased vertical displacement with repeated aqua-treadmill training?

Understanding how a horse moves on an aqua-treadmill is vital for tailoring specific therapy treatments and exercise programmes in order to most successfully rehabilitate the horse from injury and prepare the horse for competition. Investigation to quantify the effects of increasing water depth on asymmetric horses should be carried out to further inform and support its application as a tool for rehabilitation. An investigation into the specific structures involved and the effects of increased compression should be carried out, including looking further along the axial skeleton and investigating pelvic and thoracic rotations, perhaps investigating changes in forces through the limbs, stance and stride times, distal limb joint kinematics, and changes in ranges of motion in both walk and trot.

CHAPTER 4: The Effect of Side Reins on the Vertical Displacements of the Pelvis and Withers when Trotting on an Aqua-Treadmill at Increasing Water Depths

4.0 Introduction

Chapter 3 of this thesis reported a change in the vertical displacement amplitudes of the pelvis and withers in horses trotting on an aqua-treadmill at increasing water depths. When exercising horses overground, some form of training aid is normally always used on the horse in an attempt to positively alter a horse's way of going. There are currently no reports in the literature of training aids being used whilst horses are exercising on an aqua-treadmill, therefore there is an opportunity for further research to investigate the effect of a commonly used training aid when exercising horses on an aqua-treadmill. Therefore, the effect of side reins on the parameters measured in Chapter 3 could be investigated and compared.

Training aids have been recognised and reported to be beneficial for over 300 years yet there is limited evidence to support their application in the scientific literature (Cottriall *et al.*, 2009). Side reins are a commonly used British Horse Society (BHS) approved training aid that are routinely used when working horses from the ground (i.e. non-ridden work) and are first introduced into the BHS's syllabus at Stage 2 when exam candidates are expected to be able to competently lunge a horse using side reins (BHS, 2017). The aqua-treadmill is a therapeutic exercise medium for horses so it seems a logical progression to include a training aid whilst the horses are exercising.

Historically, there are few studies that report biomechanical changes when using training aids, but due to technological advances in measuring biomechanical parameters there is plenty of opportunity for research in this area. With specific regards to side reins in the literature, only one study could be found that directly investigated the use of side reins to measure a musculoskeletal parameter, where it was hypothesized that using side reins when lungeing horses would increase electromyographic (EMG) activity of the *longissimus dorsi* muscle (Cottriall *et al*., 2009). In fact, it was found that horses walking and trotting with no side reins on the lunge had a significantly increased EMG activity of the *longissimus dorsi* when compared to walking and trotting with side reins, but that always, the part of the *longissimus dorsi* on the inside of the circle had a significantly increased EMG activity when compared to the outside portion of the muscle (Cottriall *et al*., 2009). It would be interesting for some biomechanical parameters other than that of the left forefoot contact with the ground to have been measured in order to quantify if any biomechanical changes were occurring at the same time, but of course, this was not the focus of this particular study. Aqua-treadmills work horses in straight lines so a comparison between muscle activity on a circle and in straight lines would also be useful.

Biau *et al.* (2002) used a gait analysis system to determine the effects of three different types of reins on kinematic variables. All three types of reins; an elastic band, a Chambon, and a 'Back Lift' showed increased forelimb propulsion at the trot, with Chambons increasing the dorsoventral activity of the hindlimbs at the trot and hindlimb propulsion at the walk; the Back Lift increased forelimb dorsoventral activity both at the walk and trot; and the Chambon increased the activity of the hindlimbs. It was concluded that all three rein types have effects on the kinetic variables of both forelimbs and hindlimbs but with few significant effects on the hindlimbs, which is possibly more important in training, but with all three reins increasing forelimb activity, in spite of the different head-neck angles they produced, concluding that these training aids are probably better at training the neck muscles (Biau *et al.*, 2002).

A recent comprehensive study utilised sophisticated technologies to assess rein tensions in horses trotting in-hand wearing three different types of side rein made from three materials representing different degrees of elasticity: stiff and inelastic, stiff elastic, and compliant elastic, and each type of rein was tested at three different lengths; neutral, short (-10cm) and long (+10cm) (Clayton *et al*., 2011). The ultimate recommendation of the study was that side reins with an elasticized component should be used as they reduce maximal tension and loading rates and horses seemed more willing to seek a contact with the most elastic side rein as shown by the higher minimal tensions (Clayton *et al*., 2011). However, this study measured nothing other than the rein tensions, rate of loading and impulse on the reins, and the speeds of the horses when trotting, but did not assess the effect of these different side reins on any musculoskeletal or biomechanical parameter.

Other studies have focussed on rein tensions in the trot in ridden horses (Singleton, 2001; Clayton *et al*., 2003; Clayton *et al*., 2005; Heleski *et al.*, 2009) where rein tensions were found to have a consistent and repeatable tension pattern of two peaks in the tension in one trot stride but in the range of 19 – 80N dependent on type of horse, bit used, and whether on a straight line or circle, but again, did not discuss the effect of side reins on the locomotory patterns of the horse.

The 'Pessoa' is a modern training aid that was invented by the famous showjumper and horse trainer Nelson Pessoa (GFS, 2017) and although marketed to be beneficial for training and developing horses, again there is a lack of evidence in the scientific literature to support these claims. One study sought to document temporal, linear and angular variables of the working trot when a Pessoa training aid was used with a comprehensive data collection strategy using both high speed video to track skin markers and inertial measurement units and a Global Positioning System (GPS) to measure stride duration, stride length and speed (Walker *et al.*, 2013). Ultimately it was determined that the Pessoa may indeed have benefits for general training and rehabilitation as it appeared to improve gait scores and encouraged the horse to maintain posture and lumbosacral flexion without apparent concurrent increase in loading of the fore and hind limbs. The Pessoa Training Aid incorporates a system of ropes with pulleys and clips that run around the hocks, down the sides of the horse, clip to the bit and then from the bit back either in-between the horse's front legs to a D-ring on the roller, or back to the roller on the outside of the horse. Due to the low hanging ropes on the Pessoa, it was deemed unsafe by this team of researchers and equine hydrotherapists to use a Pessoa training aid in the aqua-treadmill as the ropes may potentially present as a hazard to the horse within the aqua-treadmill which overground would not present so much of a problem. Due to the small space inside the aqua-treadmill and the impossibility of a quick way to evacuate the horse from the Pessoa or the aqua-treadmill, the Pessoa could potentially pose a risk with the horse becoming entangled in the ropes if the horse performed any irregular or exaggerated gait or any startling behaviour. The use of side reins in an aqua-treadmill was not considered unsafe as they could be quickly clipped on and off the bit and clipped back to the roller out of the way with no potential risk of the horse becoming entangled in them.

To date, there appear to be no reports in the literature that document biomechanical changes when exercising horses in side reins. And no evidence to document the use of any training aid when exercising horses in an aqua-treadmill. For this study, it was hypothesized that the use of side reins would potentially have an impact on the biomechanical parameters measured previously with changes in the vertical displacement amplitudes achieved at the pelvis and withers.

4.1 Aims

The aim of this chapter was to investigate and quantify the effect of side reins on the vertical displacements of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths. Specifically:

1. Analyse the effect of side reins on the vertical displacement amplitudes of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths.

2. Analyse the effect of side reins on the percentage change in vertical displacement amplitudes of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths.

3. Analyse the effect of side reins on the symmetry of the vertical displacement amplitudes of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths.

4. Analyse the effect of side reins on the pitch amplitudes of the pelvis and withers throughout a stride cycle when trotting on an aqua-treadmill at increasing water depths.

4.2 Methods and Statistical Analysis

Data were collected as detailed in Chapter 2.2 and the raw data were processed and analysed as explained in Chapter 2.3. However, it was only the data collected during the Xsens© trial that was used for this study as it was during the initial Qualisys© data collection trial where it was observed that the use of side reins may be an interesting and valid addition to the investigation. As previously, data were processed using custom written scripts in Matlab© and only good stride data cut from left hind stance were used for further analysis. Every trial had a minimum of twenty strides used for data analysis. To compute vertical displacements (namely at the pelvis (*tuber sacrale*) and withers (T4-5), a double numerical integration of the accelerations was completed with a high-pass 4th order Butterworth filter applied with a cut off of 0.5Hz. Methodology procedures have been reported in Chapter 3.2 so will not be repeated here.

4.2.1 Statistical Analysis

Repeated measures ANOVAS were used for all statistical analyses and these are described in detail for each individual analysis. Shapiro-Wilk tests for Normality were used throughout and data were normally distributed unless otherwise stated ($p > 0.05$) (Shapiro and Wilk, 1965), and Mauchly's test for sphericity was investigated and interpreted (Mauchly, 1940) in accordance with the assumptions of the ANOVA, and where Mauchly's test of sphericity was violated, the Greenhouse-Geisser correction was applied when interpreting the results (Laerd Statistics, 2015).

4.3 Results

The vertical displacement amplitudes of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths without side reins are reported in Chapter 3.3.1. However, for this analysis, only the horses used in the Xsens© data collection trial were included as it was only these horses that had the use of side reins incorporated into their exercise protocol. As such there were a total of 10 good and complete sets of data from the Xsens© data trial to complete this analysis. Horses heights ranged from 147-180cm (mean ± SD 163.2 ± 9.84) and ages ranged from 8-19 years (mean ± SD 12.90 ± 3.84).

Considering that only the 10 Xsens© horses were used for this analysis, the results of vertical displacement of the pelvis and withers may have been slightly different from the values reported in Chapter 3 which includes the amalgamated results of both the Qualisys© and Xsens© trials. Table 4.1 shows the comparison of mean values obtained from the Qualisys© and Xsens© data collection trials together (Q+X, *n*=17) and then the Xsens© trial on its own (*n*=10). Results of a repeated measures ANOVA showed no significant difference (p = 0.456) between the values with the results from Qualisys© plus Xsens© and the values from just the Xsens© trial with all results being within 5% for each corresponding value (Table 4.1). Therefore, it can be deemed valid to use only the results of the Xsens© trials in further analyses where necessary. A further validation of Xsens© against Qualisys© was completed prior to data analysis and can be found in Chapter 2.

Table 4.1: A comparison of the mean vertical displacement values (in mm) for the pelvis and withers obtained from the amalgamation of both Qualisys© (Q) and Xsens© (X) data collection trials (Q+X, n=17) and then just the values obtained from the Xsens© data collection trial (X, n=10). Percentage difference between the two means is shown (%diff).

		Mid P3			Mid Fetlock			Mid MC3			Mid Carpus		
		Q+X	**X**	*% diff*	**Q+X**	**X**	*% diff*	**Q+X**	**X**	*% diff*	**Q+X**	**X**	*% diff*
PELVIS	**Left**	**95.97**	**95.77**	0.21	**112.06**	**109.31**	2.46	**122.22**	**116.99**	4.28	**129.85**	**125.46**	3.38
	Right	**96.40**	**97.40**	1.04	**112.91**	**111.65**	1.12	**122.37**	**119.26**	2.54	**130.44**	**126.15**	3.29
WITHERS	**Left**	**73.16**	**71.60**	2.13	**86.19**	**84.73**	1.70	**95.38**	**93.53**	1.94	**105.70**	**106.16**	0.43
	Right	**76.23**	**76.19**	0.06	**88.32**	**88.71**	0.44	**98.24**	**97.33**	0.93	**109.04**	**108.96**	0.08

4.3.1 The effect of side reins on vertical displacements of the pelvis and withers

A four-way repeated measures ANOVA (2x4x2x2) was conducted to determine the effect of side reins on the vertical displacement of the pelvis and withers of ten horses trotting on an aqua-treadmill at increasing water depths. Mean results were plotted (Figure 4.1).

The four-way interaction between pelvis or withers, depth, use of side reins and left or right was not significant ($F_{(3,27)} = 0.154$, $p = 0.927$). There were no significant three-way or no significant two-way interactions. The main effect of side reins was not found to be significant ($F_{(1,9)} = 3.839$, $p = 0.082$). Otherwise the same trends, significances and effects that were seen in Chapter 3.3.1 were also seen here; there was a significant effect of pelvis versus withers ($F_{(1,9)} = 47.712$, $p < 0.001$) with the pelvis showing overall greater displacement amplitudes than the withers, and a significant effect of water depth ($F_{(3,27)} = 137.239$, $p < 0.001$), where increasing water depth showed an increase in vertical displacement amplitudes.

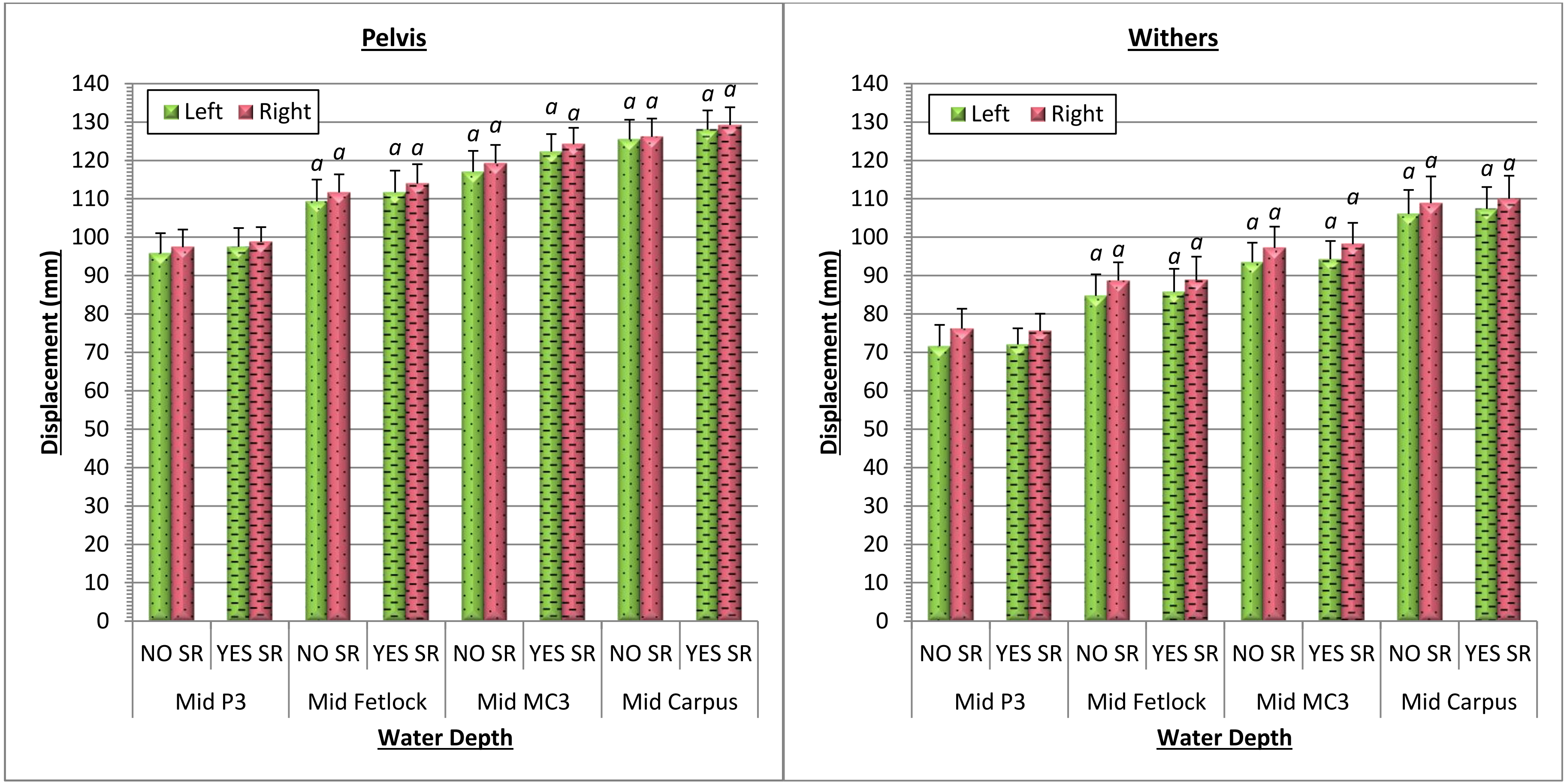


Figure 4.1: Mean (±SEM) vertical displacement (in millimetres) of the equine pelvis (left) and withers (right) at when trotting on an aqua-treadmill at increasing water depths both without and with side reins for both left (green) and right (pink) diagonal pair (n=17). No side reins indicated by dots. Side reins indicated by dashes. There was no significant effect of side reins. Overall the pelvis has significantly larger vertical displacements than the withers ($p < 0.01$). Vertical displacements increase at each increasing water depth ($p < 0.01$), [a] significantly different from all lower water depths

4.3.2 Percentage change in vertical displacements of the pelvis and withers with and without side reins

As explained previously in Chapter 3.3.2, the height of the horse is positively correlated with vertical displacement amplitudes, so once again, it is worth considering the proportionate data in terms of percentage changes from the baseline level of water at mid P3 to investigate any impact of side reins. Figure 4.2 shows the data from the percentage change in displacement from the baseline level of water at mid P3 to the increasing water depths at both the pelvis and withers.

A four-way repeated measures ANOVA (2x3x2x2) was conducted. In concordance with the results Chapter 4.3.1, there was no effect of side reins on the change in vertical displacement amplitudes of the pelvis and withers at increasing water depths when trotting on an aqua-treadmill from a baseline level of water at mid P3 ($F_{(1,9)}$ = 0.218, p = 0.652).

In concordance with the results reported in Chapter 3.3.2 there is a highly significant effect of increasing water depth increasing the changes in displacement amplitudes ($F_{(2,18)}$ = 60.982, p < 0.001) and also a significant difference overall between pelvis and withers ($F_{(1,9)}$ = 10.962, p = 0.009).

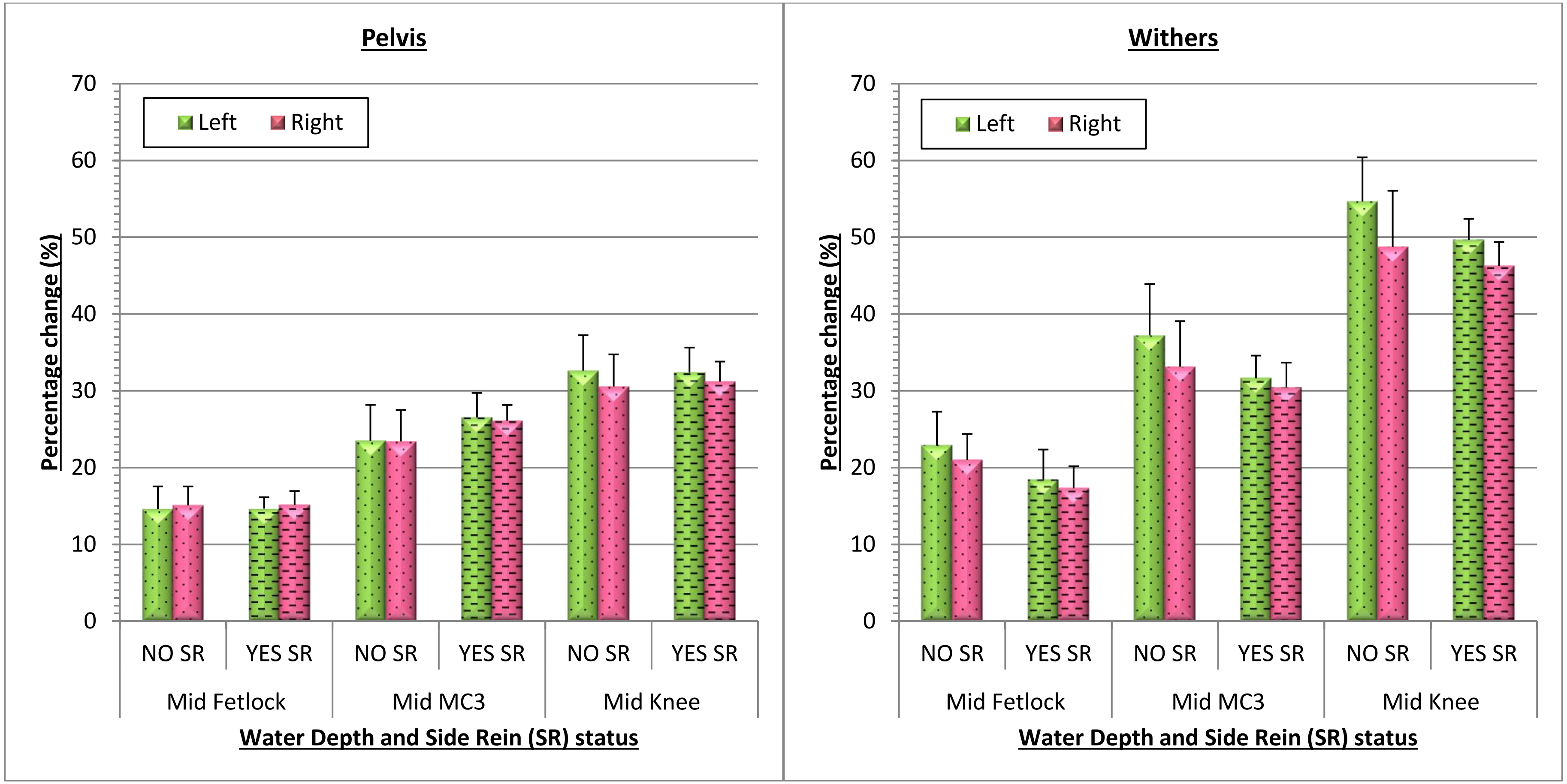


Figure 4.2:* *Mean (±SEM) percentage change in vertical displacements of the equine pelvis (left) and withers (right) when trotting on an aqua-treadmill at increasing water depths without and with side reins for both left (green) and right (pink) diagonal pair (n=10). No side reins indicated by dots. Side reins indicated by dashes. There was no significant effect of side reins on the percentage change in vertical displacement amplitudes.

4.3.3 The effect of side reins on symmetry of vertical displacement amplitudes

Chapter 3 (3.3.3) established that there was no effect of water depth on the symmetry of the horse. This section seeks to quantify the effect of side reins on symmetry. Both the millimetre and percentage score data were investigated in this study and analysed separately, and the means were plotted (Figure 4.3).

In the millimetre data, a repeated measures ANOVA (2x4x2) was conducted to determine the effect of side reins on the symmetry of the stride in both the pelvis and withers of 10 horses trotting on the aqua-treadmill at increasing water depths. There was no significant three-way interaction between pelvis or withers, water depth and side reins ($F_{(3,27)}$ = 1.623, p = 0.229). There were no significant two-way interactions, and the main effect of side reins on symmetry was not significant ($F_{(1,9)}$ = 0.285, p = 0.606). As reported in Chapter 3.3.3, the main effect of pelvis or withers on symmetry was not significant ($F_{(1,9)}$ = 0.648, p = 0.442), and the main effect of water depth was also not significant ($F_{(3,27)}$ = 1.072, p = 0.377).

In the percentage change data, a repeated measures ANOVA (2x3x2) was conducted to determine the effect of side reins on the symmetry of the stride in both the pelvis and withers of 10 horses trotting on the aqua-treadmill at increasing water depths in terms of the percentage change in displacement. There was no statistically significant three-way interaction between pelvis or withers, water depth and side reins ($F_{(2,18)}$ = 2.843, p = 0.085), and there were no statistically significant two-way interactions.

The main effect of side reins on symmetry on the percentage change in displacement was not statistically significant ($F_{(1,9)}$ = 1.444, p = 0.260). As reported in Chapter 3 (3.3.3) and again seen here, the main effect of pelvis or withers on percentage change in displacement symmetry was statistically significant ($F_{(1,9)}$ = 9.366, p = 0.014), but there was no main effect of water depth on percentage change symmetry ($F_{(2,18)}$ = 1.758, p = 0.201).

Side reins have no impact on displacement symmetry of horses trotting on an aqua-treadmill at increasing water depths.

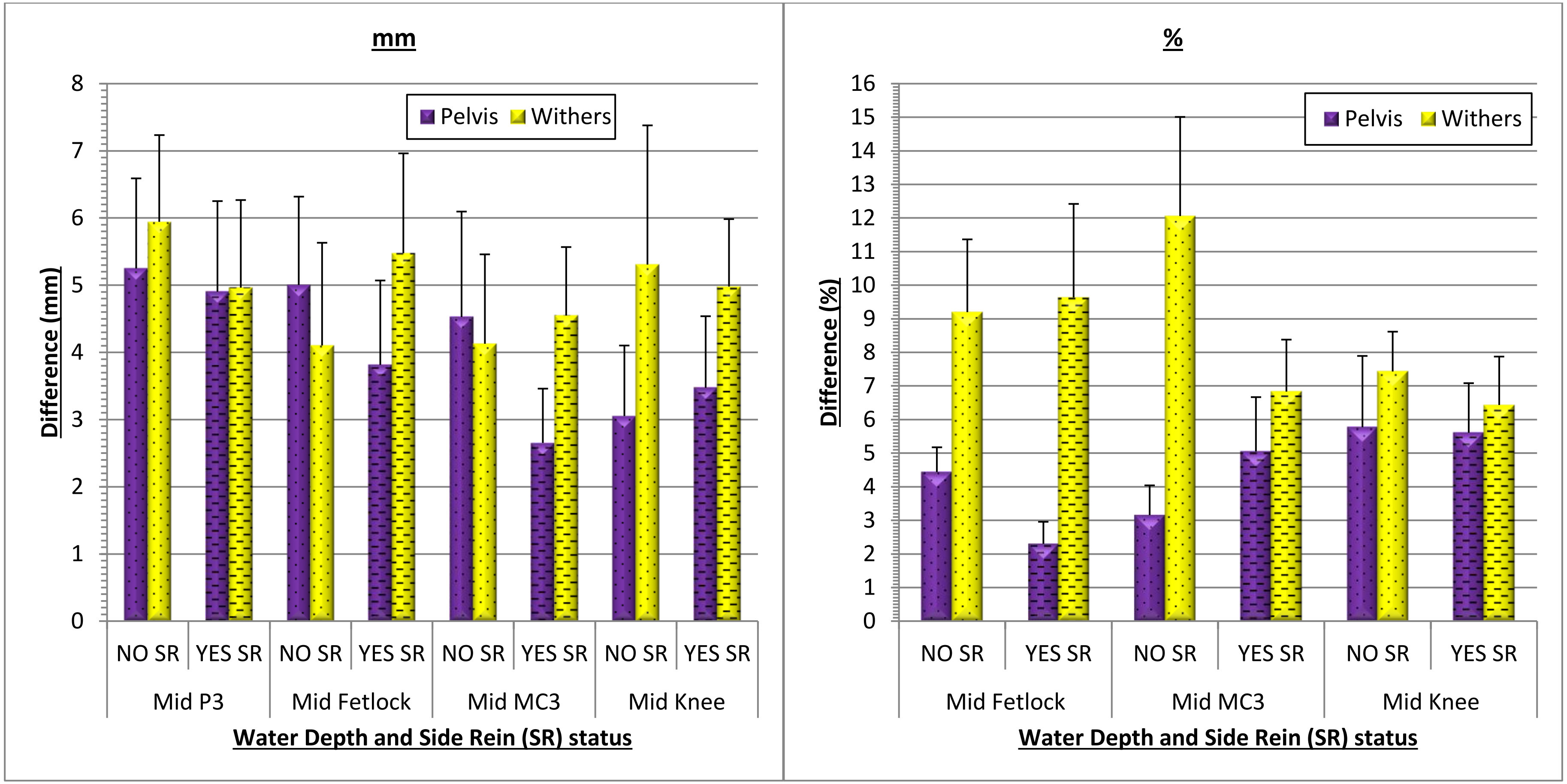


Figure 4.3: Mean (±SEM) difference between the mean vertical displacements following left and right hind stance for the equine pelvis (purple) and withers (yellow) when trotting on an aqua-treadmill at increasing water depths with raw measurement of mm (left) or considering the percentage change in displacement (right) both without AND with side reins (n=10). No side reins indicated by dots. Side reins indicated by dashes. There was no effect of side reins on displacement symmetries either in the raw mm data or in the percentage change data.

4.3.4 Impact of side reins on pitch

The effect of side reins on the pitch of the pelvis and withers throughout a stride cycle when trotting on an aqua-treadmill at increasing water depths was investigated and the means plotted (Figure 4.4).

Results of a four-way repeated measures ANOVA (2x4x2x2) showed no significant four-way interaction ($F_{(3,27)} = 1.187$, $p = 0.333$), no significant three-way interactions and no significant two-way interactions. There was no main effect of side reins on changes in pitch ($F_{(1,9)} = 1.083$, $p = 0.325$).

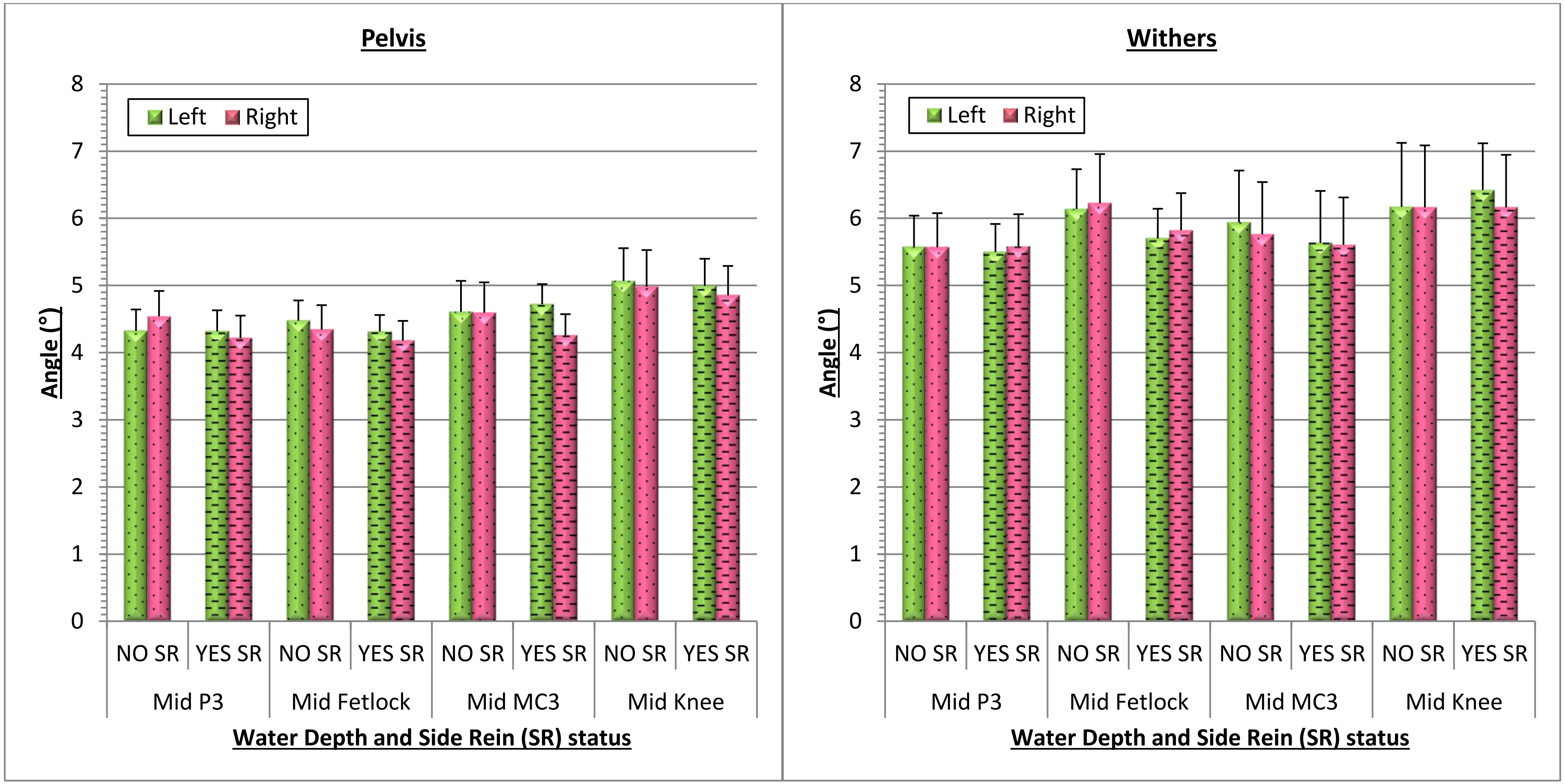


Figure 4.4: Mean (±SEM) pitch of the pelvis (left) and withers (right) throughout a stride cycle when trotting on an aqua-treadmill at increasing water depths both without and with side reins for both left (green) and right (pink) diagonal pair (n = 10). No side reins indicated by dots. Side reins indicated by dashes. There was no effect of side reins on changes in pitch amplitude in the pelvis or withers.

4.4 Discussion

The aim of this study was to determine if side reins have an effect on vertical displacement amplitudes of the pelvis and withers of horses trotting on an aqua-treadmill at increasing water depths. Prior to this study, it was observed that side reins or some form of training aid are routinely used when working horses overground so it was considered pertinent to also use some form of training aid when working horses in an aqua-treadmill. It was also noted through experience and observation of working with horses on an aqua-treadmill that often, the horse would lose concentration in their work, raising their heads and altering their body position instead of maintaining a relaxed and balanced head and neck position. It was considered that the use of a training aid would be beneficial in helping to maintain a more correct head and neck posture and side reins were deemed to be the most straightforward, easy to use, and safe option within the aqua-treadmill environment. All horses taking part in the study were regularly lunged in side reins overground so the concept of side reins was not a new phenomenon to them. However, it was the first time that any of the horses had been exercised on an aqua-treadmill in side reins. The regular use of side reins in overground training informed the decision to include side reins in the exercise protocol within the aqua-treadmill in order to make some quantifiable recording and measurement of the impact of side reins on movement patterns.

Ultimately it was determined in this study that the use of side reins had no significant effect on the vertical displacement amplitudes achieved by either the pelvis or the withers. No larger or no smaller vertical displacements were recorded with the use of side reins. However, the same trends as seen previously and reported in Chapter 3 were still observed – increasing water depth increased vertical displacement amplitudes. This implies, as previously, that a greater depth of water encourages the horse to work harder to raise themselves up over the water therefore possibly building greater strength through muscular development. It also implies that the use of side reins does not alter these vertical displacement amplitudes and suggests that side reins have no effect in how hard the horse has to work to achieve the greater amplitudes at greater depths. It could be suggested however, that although no difference in vertical displacements were observed, other muscles were perhaps engaged in order for comparative vertical displacement amplitudes to be achieved both with and without the side reins. It has previously been claimed in the literature that when working horses, lowering the neck and working the horse downwards and forwards increases the movement of the back and strengthens the back muscles (Denoix *et al.*, 2001) and that the hindlimbs become more engaged when the horse has a low head position where the poll is at the same level or lower than the withers and with a wider head/neck angle (Roepstorff *et al.*, 2002). The results of this study perhaps concur with these reports and in fact, by lowering the horses head and neck, greater engagement of the back and hindlimb muscles are generated, along with the greater compression of the limbs previously reported in Chapter 3 therefore working the horse harder to create the vertical displacement required to work up and over the water. A further study to investigate the effect of side reins on vertical displacement amplitudes overground would be very beneficial.

As defined by the Federation Equestre Internationale (FEI) Rulebook for Dressage 2017, dressage desires lightness in the forehand and the engagement of the hindquarters with a lively impulsion (Anon, 2017). Strasser (1913) cited in Slijper

(1946) and in Jeffcott (1979c) proposed the bow and string mechanism that is now widely accepted which suggests that both the fore and hind limbs as well as the abdominal and axial muscles are all involved in controlling trunk movement, and it is well thought that a low head position and greater engagement of the hind quarters is the best way to improve and develop equine trunk muscle (Cottriall *et al.*, 2009). In order for the hindlimbs to be engaged and the head and neck to stretch downwards and forwards, the dorsal and ventral muscles must act to stabilize the back against excessive flexion and extension as well as stabilising lateral flexion and axial rotation (Cottriall *et al.*, 2009). It has been suggested that side reins, and other training aids, may aid in the activation and development of these muscles to perform these stabilisations, however, when surface electromyography (EMG) in the *longissimus dorsi* muscle was measured in horses at trot on the lunge there was no significant effect of side reins on EMG activity but there was a significantly greater EMG activity of the *longissimus dorsi* on the inside of the circle (Cottriall *et al.*, 2009). There is currently no information on surface EMG activity of the *longissimus dorsi* when exercising horses in an aqua-treadmill but as horses are maintained in a straight line in an aqua-treadmill it would be very interesting to assess surface EMG in this way both with and without side reins, and also, in both walk and trot as Cottriall *et al.* (2009) found surface EMG the highest when walking on the lunge with no training aid (and again the inside *longissimus dorsi* showed higher levels of activity than the outside) and highest in the trot when only the hindquarter strap of a Pessoa training aid was used (again inside muscle showed higher activity levels than the outside muscle). Although not demonstrated in the EMG work by Cottriall *et al*. (2009) it is recognised that side reins maintain a low position of the head and neck which should ensure maximal hind limb engagement and development of the horse's topline muscles.

A later study specifically investigating the effect of the Pessoa Training Aid (Walker *et al.*, 2013) found that the Pessoa resulted in decreased stride length and a decreased head angle and lowered head and neck position which agreed with the findings of the Cottriall *et al.* (2009) study with both the Pessoa and side reins where a shorter stride length and shorter neck length was noted. The shortened neck and shortened stride length is most likely due to an influence of pressure on the bit. In the present study, it is suggested that the element of water in the aqua-treadmill goes somewhere to counteract the shorter stride lengths seen in previous studies when training aids are applied as previous studies investigating the aqua-treadmill have identified longer stride lengths and increased range of motions in limb joints when exercising through water albeit these studies investigated walk, not trot (Scott *et al.*, 2010; Mendez-Angulo *et al.*, 2013). A further consideration to the counteraction of a potential shortened stride length with the addition of side reins is that of the weight of the water and the tactile stimulation of the water where studies have shown that the addition of weight to the pasterns along with tactile stimulation of the pasterns resulted in a significant increase in the flexion of the fetlock, tarsal and stifle joints when trotting (Clayton *et al.*, 2010; Clayton *et al.*, 2011). Water could be considered as both a weight and a tactile stimulator thereby increasing range of motion in the limb joints. Even small changes in digital mass may have significant effects on kinematics due to the rapid accelerations experienced during the gait cycle (Back *et al.,* 1995a).

It is likely that the ventral muscles such as the abdominals and pectorals and deeper muscles of the back become more engaged when exercising within an aqua-treadmill when the horse must control their balance and rhythm on the treadmill belt and work to lift themselves up over the water. Working over the water likely requires a greater amount of muscle engagement as demonstrated by the greater vertical amplitudes achieved in deeper water. Plus, the resistance of the water creates a weight that also should be counteracted by a greater muscle engagement. Further studies must be conducted to assess and determine if exercising in side reins in the aqua-treadmill effects the overall workload of the exercise session and where these changes in muscle engagements occur. Indeed, a study that determines the workload of individual muscles when exercising through different depths of water would also be highly relevant.

Previous studies have also identified that head and neck position have an effect on stride length and the kinematics of the back (Faber *et al.*, 2002; Johnston *et al.*, 2002; Rhodin *et al.*, 2005; Gomez Alvarez *et al.*, 2006; Rhodin *et al.*, 2009). Interestingly, in the Rhodin *et al*. (2005) study, stride length was shortest with the head restricted in the highest position in the walk, but in trot, stride length was independent of head and neck position, but it has been previously determined that movement of the back is related to stride length where horses with longer strides extend and flex their backs in the caudal saddle region to a greater extent at the walk (Johnston *et al*., 2002) and an increasing stride length was correlated with an increasing flexion/extension range of movement for most of the vertebrae at both walk and trot (Faber *et al.*, 2002; Rhodin *et al*., 2005). Elevating the head and neck has been found to lead to extension in the cranial part of the spine and flexion in the caudal part and lowering the head and neck has the opposite effect in the unridden horse when trotting on a treadmill (Gomez Alvarez *et al.*, 2006). Unfortunately, no further parameters of the back were investigated in this present study, but a consideration to the flexion/extension of the back when using side reins in the aqua-treadmill would give scope for further investigation.

Anecdotally, riders and trainers are impressed with muscle developments in horses that are regular uses of an aqua-treadmill which are displayed by an apparent better core strength, stability and balance. This study only investigated a single parameter of two bony anatomical landmarks moving in one plane to assess the effect of water depth and side reins on vertical displacement amplitudes, but there are of course, many integral essential body systems that contribute to understand these findings. Side reins may change the shapes a horse makes with regards to the positioning of the head and neck but have no apparent effect on the workload in terms of vertical displacements achieved in the pelvis and withers even at increasing water depths, so it may have been expected that there were some changes in the results of the pitch data. There was no effect of side reins on the pitch amplitudes throughout a stride cycle in both the pelvis and withers.

Side reins may not be the only training aid to act in this manner in the aqua-treadmill, although as previously discussed, the training aid used needs to have further safety considerations than would be considered overground due to the nature of the confined environment within the aqua-treadmill – it is not possible to get to the horse quickly to free them from a line trapped around a leg for example. For this reason, it was considered that training aids such as a Pessoa are not suitably safe to be used within an aqua-treadmill. However, there are a number of other training aids that may be utilised on the aqua-treadmill that produce similar or maybe more significant results than side reins owing to their different actions that may be more suitable to different horses. Other potential possible training aids include; an elastic bungie (Old Mill Saddlery, 2017), a Kavalkade HO Lungeing Aid (Sydney Free Saddlery, 2017), a Chambon (Equestrian and Horse, 2017a), or a de Gogue (Equestrian and Horse, 2017b). Further consideration must also be given to how side reins effect the vertical displacement amplitudes of the pelvis and withers when trotting overground.

4.5 Conclusion

Ultimately, side reins appear to have no effect on vertical displacement amplitudes of the pelvis or withers when trotting on an aqua-treadmill at increasing water depths. It could be recommended or suggested from this study that it is advantageous to work horses in side reins or another safely attachable training aid (that does not directly interfere with the limb movement of the horse). It can be concluded that the use of side reins do not appear to affect how hard a horse is working including at different water depths as the vertical displacement amplitudes achieved were similar to those achieved when working without side reins. Therefore, the horse is perhaps not exerting any more energy or working any harder, but it could be argued that the side reins are maintaining a more correct head and neck position so that the horse may be working more constructively over the back and aiding the development of the topline muscles along with engaging the abdominal muscles for correct posture and balance plus engaging the hindquarters. Repeated use of the aqua-treadmill with or without side reins would perhaps be beneficial in promoting the engagement of the hindquarters, the lightness of the forehand and the strengthening and development of the abdominal and back muscles.

CHAPTER 5: Mediolateral displacements of the equine pelvis and withers when trotting on the aqua-treadmill

5.0 Introduction

The equine vertebral column is well documented in anatomical texts (Jeffcott 1979a; Jeffcott 1979b; Goody and Goody, 2000). Biomechanical analysis is traditionally carried out during treadmill locomotion and often in relation to investigation of back pain, lameness or poor performance (Butler *et al.*, 2000; Faber *et al.*, 2000; Faber *et al.*, 2001a, 2001b, 2001c; Johnston *et al.*, 2002; Faber *et al.*, 2002; Erichson, 2003; Wennerstrand *et al.*, 2004; Johnston *et al.*, 2004; Erichson *et al.*, 2004; Barrett *et al.*, 2006; Van Weeren *et al.*, 2010; Allen *et al.*, 2010; Findley and Singer, 2015; Burns *et al.*, 2016). Recent advances in technology have enabled more detailed investigation into the movement of anatomical structures during locomotion overground (Audigié *et al.*, 1999) which can provide a greater insight into abnormalities that may be altered or difficult to detect with treadmill locomotion. Buchner *et al.* (1994a) has documented differences in treadmill versus overground locomotion such as an increased stance phase of the forelimbs and an increase in caudal movement during the retraction phase of both fore and hind limbs on the treadmill compared to overground. These studies are important in aiding understanding of thoracolumbar biomechanical changes during exercise and may provide a baseline for comparison when investigating biomechanical changes during aqua-treadmill exercise.

Aqua-treadmill exercise has been shown to alter the movement pattern of the limbs during walk (Scott *et al*., 2010; Mendez-Angulo *et al*., 2013; Lefrancois and Nankervis, 2016). As limb and back movement are dynamically linked (van Weeren, 2009; van Weeren *et al*., 2010) there is an expectation that changes in water depth will impact back kinematics where limbs are influenced. Three major movements of equine intervertebral joints have been described (Jeffcott, 1980; Townsend *et al*., 1983; Clayton & Townsend, 1989; Denoix, 1999; van Weeren, 2009):

- flexion and extension movements occurring in the median plane around a transverse axis,
- lateral flexion to the left and right sides in the horizontal plane around a dorsoventral axis,
- left or right rotation occurring around a longitudinal axis.

It is only in the last five years that changes in the vertebral column have been reported when walking through water (Mooij *et al*., 2013; Nankervis *et al*., 2016). To date it seems that only walk has been investigated during aqua-treadmill locomotion despite the treadmills being capable of safely imitating speeds of up to 18kmh which is more than adequate for most horses to achieve a ‘working trot’. Ridden trot speeds have been documented as ranging from 11.5km/h in collected trot, 13km/h in working trot, 16.1km/h in medium trot and 17.8km/h in extended trot (Clayton, 1994a) while trotting in hand has been reported to be approximately 14.25km/h (Galisteo *et al.*, 1998). Mooij *et al*. (2013) used videography to assess pelvic rotation, axial rotation (roll) and lateral bend in horses walking at increasing water depths and determined that pelvic flexion increased with water depth which subsequently leads to increased stride length (Scott *et al*., 2010). Lateral bend decreased with increasing water depth (Mooij *et al.,* 2013) indicating that the increased resistance of water leads to a compensatory pattern of movement. Axial rotation increased when the water was at carpus level, but as the water deepened to elbow and shoulder depths, axial rotation decreased. This is thought to be as the horses were no longer able to step over the water forcing them to adapt their movement as a result of the resistance of the water which lead to increased pelvic flexion and reduced lateral bending (Mooij *et al.,* 2013).

Nankervis *et al*. (2016) utilised a sophisticated three-dimensional gait analysis system (Qualisys©) to investigate flexion and extension of the vertebral column whilst horses were walking on an aqua-treadmill at increasing water depths. They determined that deeper water increases the flexion-extension range of motion when compared to walking at very low water levels; however, very deep water (elbow height) resulted in increased mid thoracic extension. Whilst no effect of water depth on pelvic displacement was found which contradicts the earlier study where significant changes in axial rotation and pelvic displacement were seen (Mooij *et al*., 2010). It is suggested in the most recently published study by Nankervis *et al.* (2016), that the absence of a trend in pelvic displacement and subsequent axial rotation with increasing water depths is indicative of the horses adopting different hindlimb movement strategies. This change in movement is possibly due to differences in muscle strength and activation levels required suggesting possible contraindications if an inappropriate water depth is utilised for a specific horse.

Using the main methodologies already detailed in Chapter 2, the aim of this chapter was to quantify mediolateral displacement of the withers and pelvis to identify an increase or decrease in lateral flexion with increasing water depths. It is understood that this is not perhaps lateral flexion in the true sense as the horses are moving in a straight line on the treadmill belt, but assessing the total deviation from midline through a stride cycle and the subsequent amplitude of movement in the horizontal plane representing range of motion. Further, this study aimed to investigate the change in mediolateral angles and roll around the midline in order to give an overall impression of changes in equine vertebral joints in the horizontal plane and longitudinal axis when exercising through increasing water depths from mid P3 to mid carpus.

5.1 Aims

The aims of this chapter were to investigate the effect of water depth and side reins on mediolateral displacement of the pelvis and withers; lateral flexion of the spine and roll of the pelvis and withers. Specifically:

1. Analyse the mediolateral displacement amplitudes of the pelvis and withers when trotting on the aqua-treadmill at increasing water depths.
    a. Determine the effect of side reins on these displacements, including any effect on symmetry

2. Analyse the roll of the pelvis and withers when trotting on the aqua-treadmill at different water depths.
    a. investigate the effect of side reins on the roll

3. Analyse the change in mediolateral flexions from the withers to T13 to the pelvis at increasing water depths.

5.2 Methods and Statistical Analysis

5.2.1 Mediolateral displacement amplitudes

The methods and protocol used to collect the data were the same as previously described in Chapter 2. A custom written script was generated for Matlab© to this time extract the displacement data along the x-axis to calculate mediolateral displacement amplitudes rather than vertical displacement amplitudes along the z-axis. Figure 5.1 shows the orientation of the axes in the inertial measurement units.

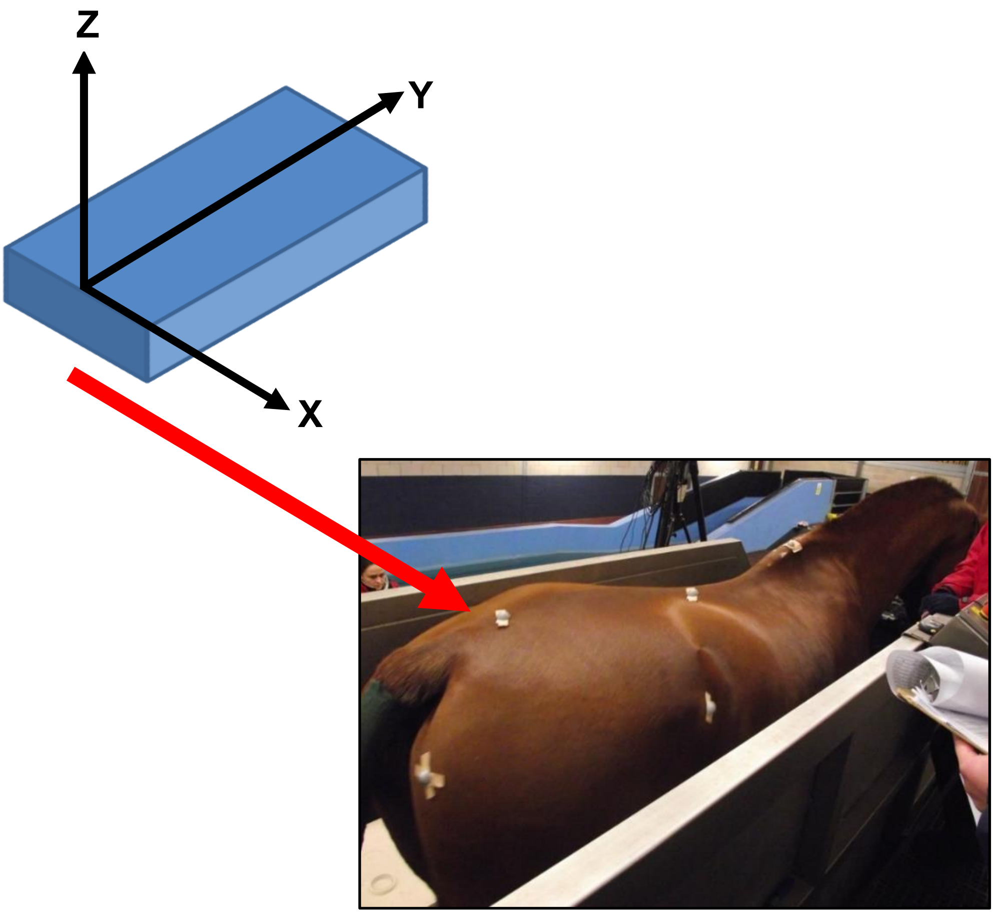


Figure 5.1: Orientation of the axes in a triaxial inertial measurement unit. Showing also the correct orientation of the unit on a horse (although the horse in the image has hemispherical light reflective markers attached for the optical motion capture methodology). The z-axis has been previously utilised in this project when researching vertical displacement amplitudes. And the x-axis is now being used in this chapter to investigate mediolateral displacement amplitudes. In all cases the y-axis denotes forward movement on the aqua-treadmill.

Mediolateral displacements were calculated in the same initial way as for vertical displacements with regards to cutting the raw data into individual strides according to left hind stance patterns with a double numerical integration from acceleration to calculate displacements. Strides were cut using the minima of vertical displacement at left hind stance. Only complete whole strides were utilised in the analysis. Mediolateral displacements are presented as an approximate single sinusoidal pattern for each stride with deviation away from the midline (either positive or negative) calculated by establishing the distance deviated from a central point which was considered as the sensor at T13 according to previous literature stating that T13 most closely corresponds to the body centre of mass movement (Buchner *et al.*, 2000; Warner *et al.*, 2010). The position of the sensor at T13 was then subtracted from the position of the sensors either at the pelvis or withers to determine movement to the left (negative) and movement to the right (positive) thereby giving the relative positions of both the pelvis and withers. Total mediolateral displacement amplitudes were then able to be calculated by subtracting the relative position of T13 for the withers and pelvis.

5.2.2 Roll amplitudes

To investigate the roll of each of the inertial measurement units on the pelvis and withers, a custom written Matlab© script was used to extract the data from the gyroscopes.

5.2.3 Mediolateral flexions

Using the sophisticated QTM Track Manager© software it was possible to investigate the angle data between the labelled points of the withers, mid back and pelvis. There were six horses that had a mid-back (T13) light reflective marker attached for investigation. The change in angle between these points was calculated at the increasing water depths and a repeated measures one-way ANOVA was used to test for significance. Figure 5.2 illustrates the mediolateral flexions of the spine with T13 as the central point.

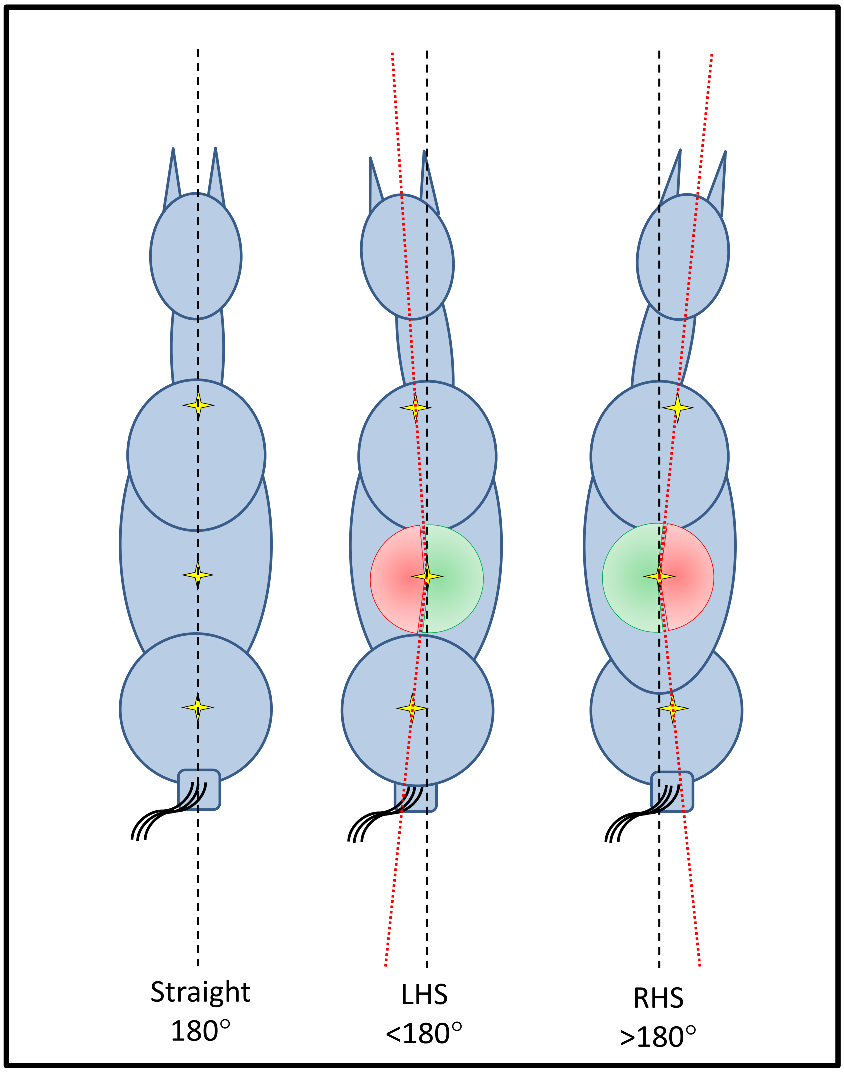


***Figure 5.2:** An illustration of how the anatomical landmarks of the withers (T4/5), mid back (T13) and pelvis (tuber sacrale) form a straight line and at left hind stance (LHS) there is flexion to the left which changes to a flexion to the right as the horse changes to a right hind stance (RHS).*

5.2.4 Statistical Analyses

Repeated measures ANOVAS were used for all statistical analyses and these are described in detail for each individual analysis. Shapiro-Wilk tests for Normality were used throughout and data were normally distributed unless otherwise stated ($p > 0.05$) (Shapiro and Wilk, 1965), and Mauchly's test for sphericity was investigated and interpreted (Mauchly, 1940) in accordance with the assumptions of the ANOVA, and where Mauchly's test of sphericity was violated, the Greenhouse-Geisser correction was applied when interpreting the results (Laerd Statistics, 2015).

5.3 Results

There were a total of ten complete sets of data for the analyses on mediolateral displacements and roll amplitudes (Chapters 5.3.1 and 5.3.2). Horses heights ranged from 147-180cm (mean ± SD 163.20 ± 9.84) and ages ranged from 8-19 years (mean ± SD 12.88 ± 3.84). For the mediolateral flexions analysis (Chapter 5.3.3) there were six sets of data with heights ranging from 157-172cm (mean ± SD 163.8 ± 4.956) and ages ranging from 11-13 years (mean ± SD 12.00 ± 0.894).

5.3.1 Mediolateral Displacement Amplitudes

A repeated measures ANOVA (2x4x2) was conducted to determine the effects of pelvis or withers, side reins and water depth on the mean mediolateral displacement amplitudes of ten horses trotting on the aqua-treadmill at increasing water depths; the means were plotted (Figure 5.3).

There was no significant three-way interaction between ‘pelvis or withers’, ‘water depth’ or ‘side reins’ ($F_{(3,27)} = 0.208$, $p = 0.326$). There was a statistically significant two-way interaction between ‘pelvis or withers’ and ‘water depth’ ($F_{(3,27)} = 8.819$, $p < 0.001$) which required further analysis.

Post hoc analyses of the simple main effect of pelvis versus withers (Table 5.1) determined that the withers had significantly greater mediolateral displacements than the pelvis without side reins at a water depth of mid P3 ($F_{(1,9)} = 9.036$, $p = 0.015$), a mean (±SEM) increase in the withers of 15.49 (±5.15) mm; and with side reins at a water depth of mid P3 ($F_{(1,9)} = 29.917$, $p < 0.001$), a mean (±SEM) increase in the withers of 21.38 (±3.91) mm. At a water depth of mid fetlock, the withers had greater mediolateral displacement amplitudes than the pelvis without side reins ($F_{(1,9)} = 7.177$, $p = 0.025$), a mean (±SEM) increase in the withers of 16.81 (±6.28) mm.

Post hoc analyses of the simple main effect of water depth (Table 5.2) determined that in the pelvis, there was a significant increase in mediolateral displacement amplitudes with the use of side reins between the depths of mid P3 and mid carpus ($F_{(3,27)}$ = 5.192, p = 0.006) with a mean (±SEM) increase of 12.73 (±2.64) mm.

Post hoc analyses of the simple main effect of water depth determined that in the withers, (Table 5.2), there was a trend of decreasing mediolateral displacement amplitudes with increasing water depth both without ($F_{(3,27)}$ = 7.806, p = 0.001) and with side reins ($F_{(3,27)}$ = 6.673, p = 0.009), with the significant differences laying between the depths of mid P3 and mid carpus without side reins (p = 0.046), with a mean (±SEM) decrease of 10.51 (±3.07) mm, and between mid fetlock and mid carpus without side reins (p = 0.003), with a mean (±SEM) decrease of 9.89 (±1.90) mm. With the use of side reins in the withers, the significant differences lay between mid P3 and mid carpus (p = 0.011), a mean (±SEM) decrease of 8.66 (±1.99) mm, and between mid fetlock and mid carpus (p = 0.012), with a mean (±SEM) decrease of 8.60 (±2.01) mm.

Table 5.1: Post hoc analysis of the significant simple main effect of pelvis versus withers on mediolateral displacement amplitudes of 10 horses trotting on the aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in mm.

				Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
NO SIDE REINS	Mid P3	Pelvis	Withers	-15.49*	5.15	0.015	-27.15	-3.83
	Mid Fetlock	Pelvis	Withers	-16.81*	6.28	0.025	-30.99	-2.62
	Mid MC3	Pelvis	Withers	-10.99	5.93	0.097	-24.41	2.43
	Mid Carpus	Pelvis	Withers	-0.94	6.37	0.885	-15.36	13.47
YES SIDE REINS	Mid P3	Pelvis	Withers	-21.38*	3.91	<0.001	-30.22	-12.54
	Mid Fetlock	Pelvis	Withers	-14.95	7.50	0.077	-31.92	2.02
	Mid MC3	Pelvis	Withers	-6.16	8.17	0.470	-24.64	12.31
	Mid Carpus	Pelvis	Withers	0.02	5.19	0.997	-11.72	11.75

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

Table 5.2: Post hoc analysis of the significant simple main effect of water depth on mediolateral displacement amplitudes of the pelvis and withers of 10 horses trotting on the aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in mm.

				Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
PELVIS	NO SIDE REINS	Mid P3	Mid Fetlock	1.94	5.89	1.000	-17.88	21.75
			Mid MC3	0.68	3.87	1.000	-12.36	13.70
			Mid Carpus	-4.04	3.77	1.000	-16.73	8.46
		Mid Fetlock	Mid P3	-1.94	5.89	1.000	-21.75	17.88
			Mid MC3	-1.26	4.33	1.000	-15.82	13.30
			Mid Carpus	-5.98	5.21	1.000	-23.50	11.55
		Mid MC3	Mid P3	-0.68	3.87	1.000	-13.71	12.36
			Mid Fetlock	1.26	4.33	1.000	-13.30	15.82
			Mid Carpus	-4.72	1.54	0.082	-9.91	0.48
		Mid Carpus	Mid P3	4.04	3.77	1.000	-8.64	16.73
			Mid Fetlock	5.98	5.21	1.000	-11.55	23.50
			Mid MC3	4.72	1.54	0.082	-0.48	9.91
	YES SIDE REINS	Mid P3	Mid Fetlock	-6.36	4.07	0.912	-20.04	7.31
			Mid MC3	-11.44	3.83	0.091	-24.31	1.43
			Mid Carpus	-12.73*	2.64	0.006	-21.63	-3.84
		Mid Fetlock	Mid P3	6.36	4.07	0.912	-7.31	20.04
			Mid MC3	-5.08	4.55	1.000	-20.37	10.21
			Mid Carpus	-6.37	2.27	0.122	-13.99	1.25
		Mid MC3	Mid P3	11.44	3.83	0.091	-1.43	24.31
			Mid Fetlock	5.08	4.55	0.000	-10.21	20.37
			Mid Carpus	-1.29	3.66	0.000	-13.60	11.03
		Mid Carpus	Mid P3	12.73*	2.64	0.006	3.84	21.63
			Mid Fetlock	6.37	2.27	0.122	-1.25	13.99
			Mid MC3	1.29	3.66	1.000	-11.03	13.60
WITHERS	NO SIDE REINS	Mid P3	Mid Fetlock	0.62	2.08	1.000	-6.36	7.60
			Mid MC3	5.18	2.25	0.283	-3.40	12.76
			Mid Carpus	10.51*	3.07	0.046	0.17	20.84
		Mid Fetlock	Mid P3	-0.62	2.08	1.000	-7.60	6.36
			Mid MC3	4.56	2.32	0.484	-3.23	12.35
			Mid Carpus	9.89*	1.90	0.003	3.48	16.29
		Mid MC3	Mid P3	-5.18	2.25	0.283	-12.76	2.40
			Mid Fetlock	-4.56	2.32	0.484	-12.35	3.23
			Mid Carpus	5.33	2.94	0.619	-4.56	15.21
		Mid Carpus	Mid P3	-10.51*	3.07	0.046	-20.84	-0.17
			Mid Fetlock	-9.89*	1.90	0.003	-16.29	-3.48
			Mid MC3	-5.33	2.93	0.619	-15.21	4.56
	YES SIDE REINS	Mid P3	Mid Fetlock	0.06	1.87	1.000	-6.22	6.34
			Mid MC3	3.77	3.08	1.000	-6.61	14.15
			Mid Carpus	8.66*	1.99	0.011	1.98	15.35
		Mid Fetlock	Mid P3	-0.06	1.87	1.000	-6.34	6.22
			Mid MC3	3.71	1.53	0.231	-1.45	8.86
			Mid Carpus	8.60*	2.01	0.012	1.83	15.37
		Mid MC3	Mid P3	-3.77	3.08	1.000	-14.15	6.61
			Mid Fetlock	-3.71	1.53	0.231	-8.86	1.45
			Mid Carpus	4.89	2.60	0.556	-3.86	13.65
		Mid Carpus	Mid P3	-8.66*	1.99	0.011	-15.35	-1.98
			Mid Fetlock	-8.60*	2.01	0.012	-15.37	-1.83
			Mid MC3	-4.89	2.60	0.556	-13.65	3.86

Based on estimated marginal means

[a] Bonferroni adjustment for multiple comparisons

* The mean difference is significant at the 0.05 level

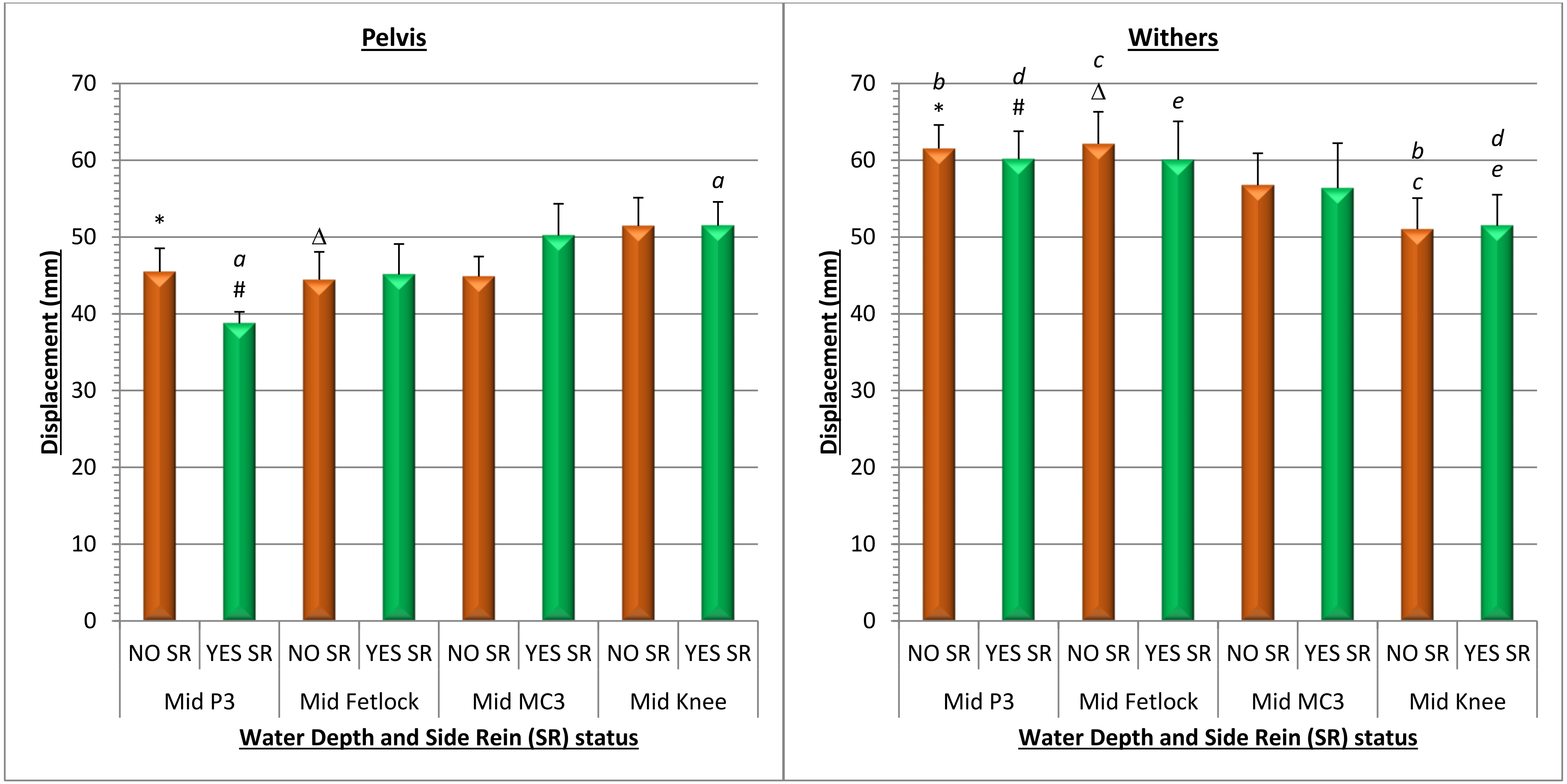


***Figure 5.3:** Mean (±SEM) mediolateral displacement amplitudes (in millimetres) of the equine pelvis (left) and withers (right) when trotting on an aqua-treadmill at increasing water depths both without side reins (orange) and with side reins (green) (n=10). The withers had significantly larger mediolateral displacements than the pelvis at mid P3 without side reins * ($p < 0.05$), and with side reins # ($p < 0.01$). The withers had significantly larger mediolateral displacements than the pelvis at mid fetlock without side reins Δ ($p < 0.05$). In the pelvis with side reins there was a significant difference in mediolateral displacements between mid P3 and mid carpus [a] ($p < 0.01$). In the withers there was a trend of decreasing mediolateral displacements with increasing water depth, with a significant difference between mid P3 and mid carpus without side reins [b] ($p < 0.05$), and between mid fetlock and mid carpus without side reins [c] ($p < 0.01$); with side reins there was a significant difference between mid P3 and mid carpus [d] ($p < 0.05$), and between mid fetlock and mid carpus [e] ($p < 0.05$).*

5.3.2 Roll Amplitudes

The second element of this chapter investigated the roll of the inertial measurement units of the pelvis and withers when trotting on the aqua-treadmill at increasing water depths and the means were plotted (Figure 5.4).

A repeated measures ANOVA (2x4x2) was conducted to determine the effects of pelvis or withers, side reins and water depth on the mean roll amplitudes of ten horses trotting on the aqua-treadmill at increasing water depths. There was no statistically significant three-way interaction between 'pelvis or withers', 'water depth' or 'side reins' ($F_{(3,27)}$ = 0.695, p = 0.458). There was a statistically significant two-way interaction between 'pelvis or withers' and 'side reins' ($F_{(1,9)}$ = 8.535, p = 0.017) which required further analysis. *Post hoc* analyses determined that when the water depth was at mid P3, the withers had significantly greater mean (±SEM) roll amplitudes than the pelvis both without side reins at 6.47 (±1.64)° ($F_{(1,9)}$ = 15.618, p = 0.003), and with side reins at 7.34 (±3.01)° ($F_{(1,9)}$ = 5.949, p = 0.037) (Table 5.3). Also, when the water depth was at mid fetlock, the withers had significantly greater roll amplitudes than the pelvis both without side reins at 9.21 (±3.38)° ($F_{(1,9)}$ = 7.410, p = 0.024), and with side reins at 6.30 (±2.54)° ($F_{(1,9)}$ = 6.162, p = 0.035) (Table 5.3).

*Post hoc a*nalysis on the simple main effect of side reins determined that there was a significant decrease in mean (±SEM) roll amplitude of 3.34 (±1.34)° in the withers at a water depth of mid fetlock when the side reins were attached ($F_{(1,9)}$ = 6.230, p = 0.034) (Table 5.4). There was no effect of water depth on roll amplitudes.

Table 5.3: Post hoc analysis of the significant simple main effect of pelvis versus withers on roll amplitudes of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in degrees.

Pelvis to Withers		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
NO Side Reins	Mid P3	-6.47*	1.64	0.003	-10.18	-2.77
	Mid Fetlock	-9.21*	3.38	0.024	-16.86	-1.56
	Mid MC3	-6.69	3.18	0.065	-13.88	0.50
	Mid Carpus	-5.34	3.26	0.136	-12.71	2.03
YES Side Reins	Mid P3	-7.34*	3.01	0.037	-14.14	-0.53
	Mid Fetlock	-6.30*	2.54	0.035	-12.03	-0.56
	Mid MC3	-6.01	2.82	0.061	-12.39	0.36
	Mid Carpus	-4.36	2.82	0.156	-10.73	2.01

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

Table 5.4: Post hoc analysis of the significant simple main effect of side reins on roll amplitudes of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths. Pairwise comparison with Bonferroni correction shown. Mean difference shown in degrees.

No Side Reins to Yes Side Reins		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
PELVIS	Mid P3	-0.42	0.42	0.344	-1.38	0.54
	Mid Fetlock	0.43	0.62	0.509	-0.97	1.82
	Mid MC3	-0.25	0.58	0.675	-1.56	1.06
	Mid Carpus	-0.47	0.88	0.605	-2.48	1.53
WITHERS	Mid P3	-1.29	2.83	0.660	-7.68	5.11
	Mid Fetlock	3.34*	1.34	0.034	0.31	6.36
	Mid MC3	0.42	1.23	0.738	-2.35	3.20
	Mid Carpus	0.51	1.45	0.735	-2.78	3.79

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

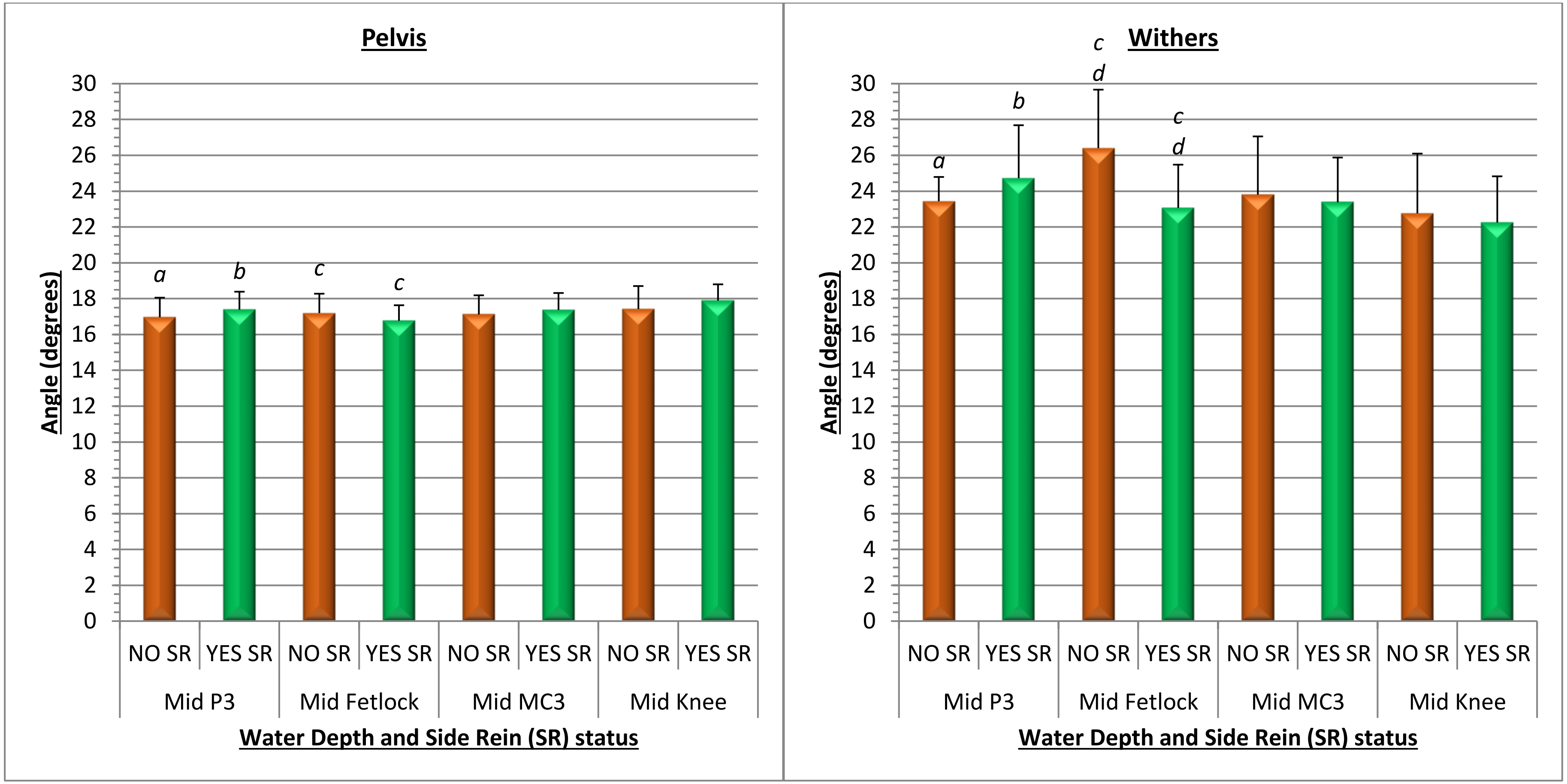


Figure 5.4: Mean (±SEM) roll amplitudes (in degrees) throughout a stride cycle of the inertial measurement units stationed at the pelvis (left) and withers (right) when trotting on an aqua-treadmill at increasing water depths both without side reins (orange) and with side reins (green) (n=10). The withers had significantly greater roll amplitudes than the pelvis at a depth of mid P3 without side reins [a] ($p < 0.01$) and with side reins [b] ($p < 0.05$). The withers had significantly greater roll amplitudes than the pelvis at a depth of mid fetlock both without and with side reins [c] ($p < 0.05$). In the withers at the depth of mid fetlock there was a significant decrease in roll amplitudes when side reins were applied [d] ($p < 0.05$). There was no effect of water depth on roll amplitudes.

5.3.3 Mediolateral Flexions

The third element of this chapter, involved the investigation of the mediolateral flexions from the withers through T13 to the pelvis. Mean flexions were plotted (Figure 5.5).

A one-way repeated measures ANOVA was conducted to determine the effects of water depth on the change in mediolateral angle through withers-T13-pelvis of six horses trotting on the aqua-treadmill at increasing water depths. Changes in the water depth did not elicit statistically significant changes in the change in mediolateral spine angle ($F_{(3,15)} = 0.426$, $p = 0.737$, partial $\eta^2 = 0.079$).

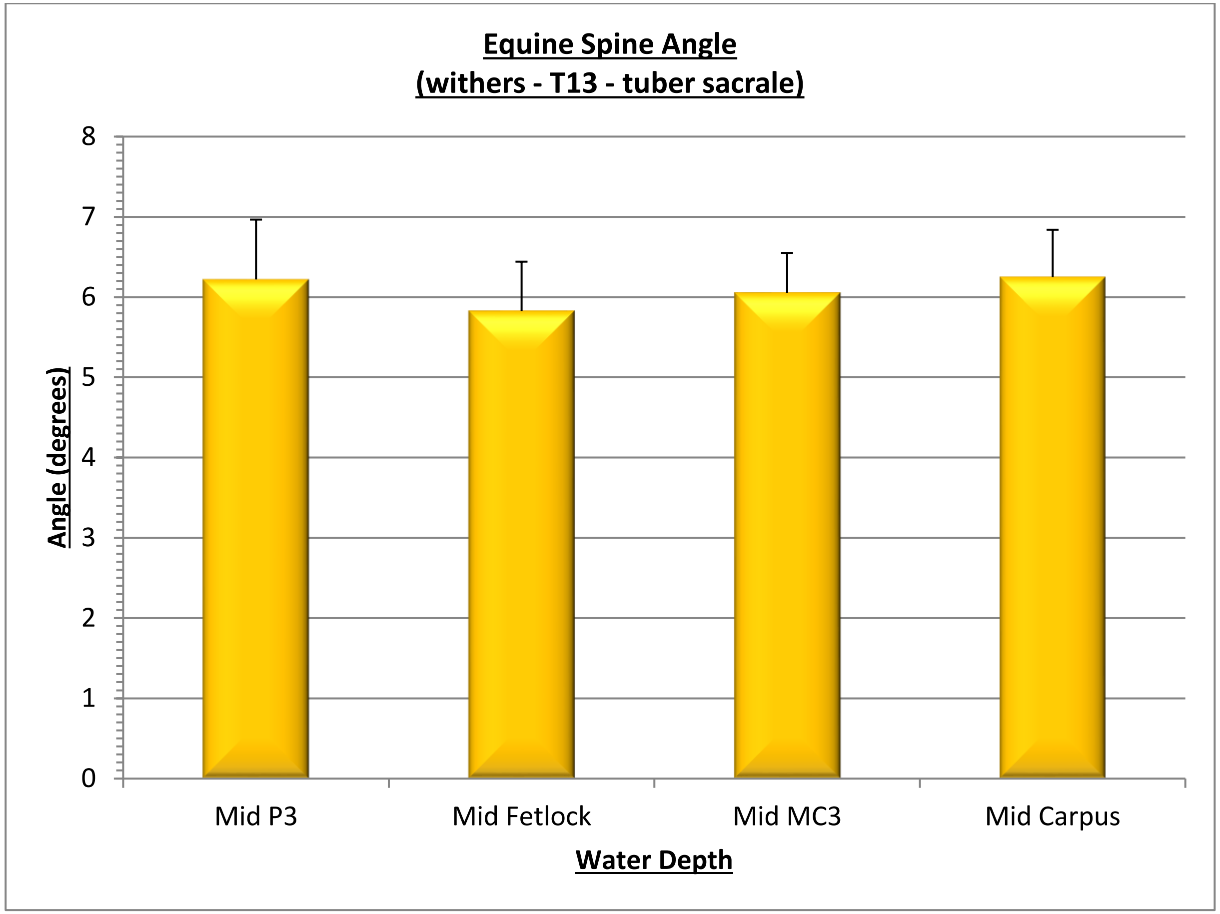


Figure 5.5: Mean (±SEM) change in mediolateral angle of the equine spine (Angle XY, Withers - T13 - tuber sacrale) when trotting on the aqua-treadmill at increasing water depths (n=6). There was no significant effect of water depth on mediolateral spine flexions.

5.4 Discussion

The aims of this study were to determine if water depth has a significant effect on how the axial skeleton moves mediolaterally in a horizontal plane around a dorsoventral axis, including investigating any effect of side reins, investigating the roll of the inertial measurement units and investigating the change in mediolateral angle throughout a stride cycle. No studies have appeared to investigate these parameters previously with a horse exercising on an aqua-treadmill at trot.

5.4.1 Mediolateral displacement amplitudes

Mediolateral displacement amplitudes throughout the stride were found not to change with increasing water depths. This is similar to a previous study where lateral bending range of motion was found not to change significantly when the horses were walking at a water depth of the fetlock or carpus but decreased significantly when the water depth was increased to the levels of the elbow and shoulder joints (Mooij *et al.*, 2013). The present study did not take the water above the level of the carpus and only included trot not walk, so no direct comparison can be made; however, it seems likely that the mechanisms for trotting and walking over a low level of water may be similar whereby the horse is required to step up over the water. The Mooij *et al*. (2013) study suggests that at the deepest water levels of elbow and shoulder depth, that the water provides some stability as lateral bending was significantly decreased but pelvic flexion was significantly increased so suggesting a change in the engagement of different muscles to produce a different walking pattern. Pelvic flexion was not measured in the current study so again, no direct comparison can be made.

It appeared from Figure 5.1 that overall, the withers had a greater overall mediolateral displacement than the pelvis but this was only found to be significant at the lower water depths of mid P3 without side reins ($p = 0.015$) and with side reins ($p < 0.001$), and mid fetlock ($p = 0.025$). It is interesting that the withers exhibited a greater mediolateral displacement than the pelvis at a lower water depth suggesting that the front end of the horse is perhaps rocking from side to side to jump over the water when the water is first introduced. This perhaps is comparable to the evidence that the withers overall appeared to have lower overall vertical displacement amplitudes than the pelvis suggesting that the front end of the horse compensated the water by bending at the carpus, and so here suggesting that the bending action of the carpus one leg at a time creates a rocking action that actually sways the horse from side to side thereby creating these greater mediolateral displacement amplitudes. At the deeper water depths of mid MC3 and mid carpus, the mediolateral displacement amplitudes of the pelvis and withers were almost the same. This perhaps suggests that the front end of the horse is no longer rocking by simply bending at the carpus, but actually having to create a jump up over the deeper water. This would also correspond with the evidence from Chapter 3 where vertical displacement amplitudes in the withers were found to increase with deeper water, so the horse is now jumping up over the water creating greater vertical lift and less mediolateral sway. Overall, the withers exhibited a trend where increasing water depth reduced mediolateral displacement amplitudes. This again would suggest that there is an increased compression of the joints in the forehand to create this lift, which again correlates with the findings of Chapter 3.

There was no apparent overall effect of side reins on mediolateral displacement amplitudes. It is suggested that side reins may have had a stabilizing effect on the side to side sway seen in the withers at lower water depths, and although a reduction in mediolateral displacement amplitudes was seen with the addition of side reins at the lower water depths (Figure 5.1), this trend was not found to be significant. In the pelvis, there was no effect of side reins at all on mediolateral displacement amplitudes. The application of side reins did not have the effect of reducing mediolateral displacement amplitudes and therefore perhaps creating any kind of stabilizing effect. Likewise, the application of side reins did not increase mediolateral displacement amplitudes which would have been unexpected.

5.4.2 Roll amplitudes

There was one clear trend in the roll data, the withers overall showed greater changes in roll than the pelvis (p = 0.039). As discussed in the Methods chapter (Chapter 2), a specially constructed withers mount was used to affix the inertial measurement unit to the withers due to previous studies stating the difficulty of affixing inertial measurement units to this anatomical area (Pfau *et al.*, 2005) due to the movement of the skin over bony protuberances which is well documented (Van Weeren and Barneveld, 1986; Van den Bogert *et al*., 1990; Van Weeren *et al.*, 1990a; Van Weeren *et al.*, 1990b; Van Weeren *et al.*, 1992a, 1992b). It is therefore suspected that these greater roll amplitudes may well be due to the movement of the skin over the withers.

There was no effect of water depth on the roll amplitudes. Roll amplitudes in the pelvis remained fairly constant (between 16 – 18 degrees) with the increasing water depths evidencing that neither water depth nor side reins had an impact on these displacements. In the withers, roll amplitudes appeared more variable but again, there was no effect of water depth or side reins. It could have been suggested perhaps, that the addition of side reins may have decreased the variability in the roll due to perhaps having an effect on stabilising the front end of the horse but this was not seen. Roll amplitudes in the withers decreased slightly as the water depth increased which tallies with the decrease also seen in mediolateral displacements but this effect was not found to be significant. It is suggested that the lack of change in roll seen in the pelvis may be due to the location of placement of the inertial measurement unit on the *tuber sacrale*. It is perhaps not expected that a large variation would be seen here due to the anatomical location being fairly level, so perhaps not subject to large changes in roll. However, a previous study investigating axial rotation in the walk at increasing water depths found that axial rotation increased significantly at each successive water depth from the hoof control level to a level of the shoulder joint (Mooij *et al.*, 2013). It is possible that the significant increases seen in the walk and not the trot are due to the fundamental difference in the kinematics of the gaits and not due to any effect of water (Haussler *et al.*, 2001; Johnston *et al.*, 2002; Johnston *et al.*, 2004). However, it must also be noted that it is documented that horses do modify their gait mechanics to compensate for injury and pain (Cadiot and Almy, 1924; Buchner *et al.*, 1995; Buchner *et al.*, 1996a, 1996b; Uhlir *et al.*, 1997; Denoix and Audigie, 2001; Weishaupt *et al*., 2004; Weishaupt *et al*., 2006; Gomez Álvarez *et al*., 2007; Gomez Álvarez *et al*., 2008). Although all horses were deemed non-lame by a Vet prior to taking part in the study, minute changes in gait that may not have been detectable by eye overground may have been exacerbated or accentuated in the aqua-treadmill and this could potentially have resulted in changes in range of motion in the thoracolumbar spine without producing detectable changes in the kinematics of the limbs, for example a slightly increased range of motion of the thoracolumbar back, a slightly decreased range of motion of the lumbosacral segment and rotational motion changes of the pelvis which have been noted when inducing a slight hindlimb lameness overground in walk and trot (Gomez Álvarez *et al*., 2008). This potentially may have had individual effects on each horse in the present study thereby reducing the likelihood of finding a trend in this roll data.

5.4.3 Mediolateral flexions

Mediolateral flexions were investigated along with the mediolateral displacement amplitudes and roll data. There was no effect of water depth on the change in mediolateral spinal angle. It is suggested that perhaps as water depth increased there may have also been an increase in the change in that mediolateral angle but there were no changes seen. This actually suggests that even with greater depths of water the spine actually remains very stable and is not perhaps ‘over’ or ‘hyper’ flexed by the horse trotting in greater depths of water. This suggests that you can continue to work the horse hard benefitting from the benefits of the deeper water but without any detrimental effects to over lateral flexing of the spine. It does not appear that these mediolateral angles have been investigated in any previous study.

5.5 Conclusion

This chapter has identified the relationships between the mediolateral movements and roll of the equine spine when trotting on an aqua-treadmill at increasing depths of water. Water depth had no effect on mediolateral displacements either in the pelvis or withers of horses trotting on an aqua-treadmill. At lower water depths, the withers exhibited greater mediolateral displacements than the pelvis suggesting a side to side sway motion was initiated. At deeper water depths, the mediolateral displacements in the withers had reduced to a level comparable with those of the pelvis suggesting that deeper water controls front end mediolateral movement. Side reins had no effect on mediolateral displacement amplitudes or on roll amplitudes of horses trotting on an aqua-treadmill at increasing water depths.

Roll amplitudes were significantly greater in the withers than the pelvis. This is likely due to the movement of the skin over the bony protuberance of the withers. The lack of significance in the roll data suggests that roll is therefore not a parameter that has any bearing on studies investigating biomechanical parameters of the axial skeleton. Mediolateral flexions of the spine were not affected by water depth or side reins. This suggests that the horse can be worked harder at greater water depths without over stressing the mediolateral capabilities of the spine.

CHAPTER 6: A comparison of overground and aqua-treadmill locomotion

6.0 Introduction

It is understood that horses were first put on a treadmill for research into locomotion by Persson in 1967 and the treadmill has facilitated major advances in biomechanical research by allowing the integration of biomechanical, physiological and biochemical data to be collected under controlled conditions (Clayton, 1989). A state-of-the-art treadmill for use with cinematography was described by Fredricson *et al*. in 1983 where he reported that reproducibility between strides was good but that there was a reduction in stride length and stride frequency. Since then, treadmills have been commonly used for research purposes due to the ability to move the horse in a straight line for a repeated number of reproducible successive strides. Also, biomechanical differences between treadmills and overground locomotion have been well reported and discussed earlier in this thesis (Fredricson *et al*., 1983; Leach and Drevemo, 1991; Barrey *et al*., 1993; Buchner *et al*., 1994a, 1994b).

The concept of aqua-treadmills was first introduced into scientific literature in 1989, (Auer, 1989) but the effect of water on any biomechanical parameters was not studied until 2010 when stride length and frequency were investigated with changes in water depth (Scott *et al.*, 2010). As yet, there does not appear to be any reports making a direct comparison between overground locomotion and locomotion through water on an aqua-treadmill. This study sought to investigate the effects of a very low water depth of just a few centimetres on the locomotion parameters previously investigated in this project.

6.1 Aims

This chapter aimed to make a comparison of the locomotion of the pelvis and withers overground and on the aqua-treadmill with just a small amount of water, to determine the effect a water depth of mid P3 has on the locomotory parameters previously measured in this project. Specifically, to:

1. Analyse the vertical displacement amplitudes achieved by the pelvis and withers when trotting overground
    a. investigate the effect of side reins on these displacements, including any effects on symmetry

2. Compare and analyse the vertical displacement amplitudes of the overground work to the aqua-treadmill work (water at mid P3) and investigate the effect of side reins

3. Analyse the pitch of the inertial measurement units at the pelvis and withers when trotting overground.

4. Compare and analyse the pitch of the inertial measurement units at the pelvis and withers when trotting overground to trotting on the aqua-treadmill (water at mid P3).

5. Analyse the mediolateral displacement amplitudes at the pelvis, withers and poll when trotting overground.
    a. investigate the effect of side reins on these displacements

6. Compare and analyse the mediolateral displacement amplitudes of the overground work to the aqua-treadmill work (water at mid P3) in the pelvis, withers and poll.
    a. investigate the effect of side reins

7. Analyse the roll of the pelvis, withers and poll when trotting overground.
    a. investigate the effect of side reins on the roll

8. Compare and analyse the roll from the overground work to the aqua-treadmill work (water at mid P3)
    a. investigate the effect of side reins on these comparisons

6.2 Methods and Statistical Analysis

For this investigation, only the data from the Xsens© data collection was utilised as this included the overground work and the side reins work. Data were collected and handled in the manner as described previously in Chapter 2 for the Xsens© data.

6.2.1 Statistical Analysis

Repeated measures ANOVAS were used for all statistical analyses and these are described in detail for each individual analysis. Shapiro-Wilk tests for Normality were used throughout and data were normally distributed unless otherwise stated (p > 0.05) (Shapiro and Wilk, 1965), and Mauchly's test for sphericity was investigated and interpreted (Mauchly, 1940) in accordance with the assumptions of the ANOVA, and where Mauchly's test of sphericity was violated, the Greenhouse-Geisser correction was applied when interpreting the results (Laerd Statistics, 2015).

6.3 Results

The effects of water on vertical and mediolateral displacement amplitudes of the pelvis and withers when trotting on an aqua-treadmill have previously been reported in Chapters 3, 4 and 5, but now a comparison can be made with values obtained from trotting overground. The same ten horses that were exercised on the aqua-treadmill using the Xsens© technology were also trotted overground on tarmac in a straight line both without and with side reins. There was a total of 10 sets of Xsens© data to complete this analysis. Horses' heights ranged from 147-180cm (mean ± SD 163.20 ± 9.84) and ages ranged from 8-19 years (mean ± SD 12.88 ± 3.84).

6.3.1 Overground Vertical Displacements

Vertical displacements of the pelvis and withers in horses trotting overground were measured and the means plotted (Figure 6.1). A repeated measures ANOVA (2x2x2) was conducted to determine the effects of pelvis or withers, side reins and left or right diagonal pair stride on the mean vertical displacement amplitudes of ten horses trotting overground.

There was no significant three-way interaction between 'pelvis or withers', 'side reins' or 'left or right diagonal pair' ($F_{(1,9)}$ = 0.023, p = 0.956). There was a significant two-way interaction between 'pelvis or withers' and 'side reins' ($F_{(1,9)}$ = 7.844, p = 0.021) and a significant two-way interaction between 'pelvis or withers' and 'left or right' ($F_{(1,9)}$ = 6.837, p = 0.028) which required further analysis.

Post hoc analyses of the simple main effects of pelvis versus withers (Table 6.1), showed that with the addition of side reins on the left diagonal pair, vertical displacement amplitudes were significantly lower in the withers than the pelvis ($F_{(1,9)}$ = 9.719, p = 0.012), a mean (±SEM) difference of 22.90 (±7.35) mm.

Post hoc analyses of the simple main effects of Side Reins (Table 6.2) showed that in the withers, there was a significant decrease in vertical displacement amplitudes with the addition of side reins in both the left ($F_{(1,9)}$ = 10.555, p = 0.010) and right ($F_{(1,9)}$ = 11.281, p = 0.008) diagonal pairs; a mean (±SEM) difference on the left of 23.18 (±7.13) mm, and on the right of 22.99 (±6.85) mm. Principally, the mean displacement in the withers was less when side reins were added. Side reins had the effect of reducing displacement in the withers but not in the pelvis.

Table 6.1: Post hoc analysis of the significant simple main effect of pelvis versus withers on vertical displacements in horses trotting overground (n=10). Pairwise comparison with Bonferroni correction shown. Mean difference shown in millimetres.

Pelvis to Withers		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
NO Side Reins	Left	1.81	2.52	0.491	-3.90	7.52
	Right	-3.90	3.57	0.304	-11.98	4.19
YES Side Reins	Left	22.90*	7.35	0.012	6.28	38.52
	Right	17.06	9.47	0.105	-4.36	38.47

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

Table 6.2: Post hoc analysis of the significant simple main effect of side reins on vertical displacements of the pelvis and withers in horses trotting overground (n=10). Pairwise comparison with Bonferroni correction shown. Mean difference shown in millimetres.

No Side Reins to Yes Side Reins		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
PELVIS	Left	2.09	1.17	0.107	-0.55	4.73
	Right	2.04	1.95	0.323	-2.37	6.44
WITHERS	Left	23.18*	7.13	0.010	7.04	39.32
	Right	22.99*	6.85	0.008	7.51	38.47

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

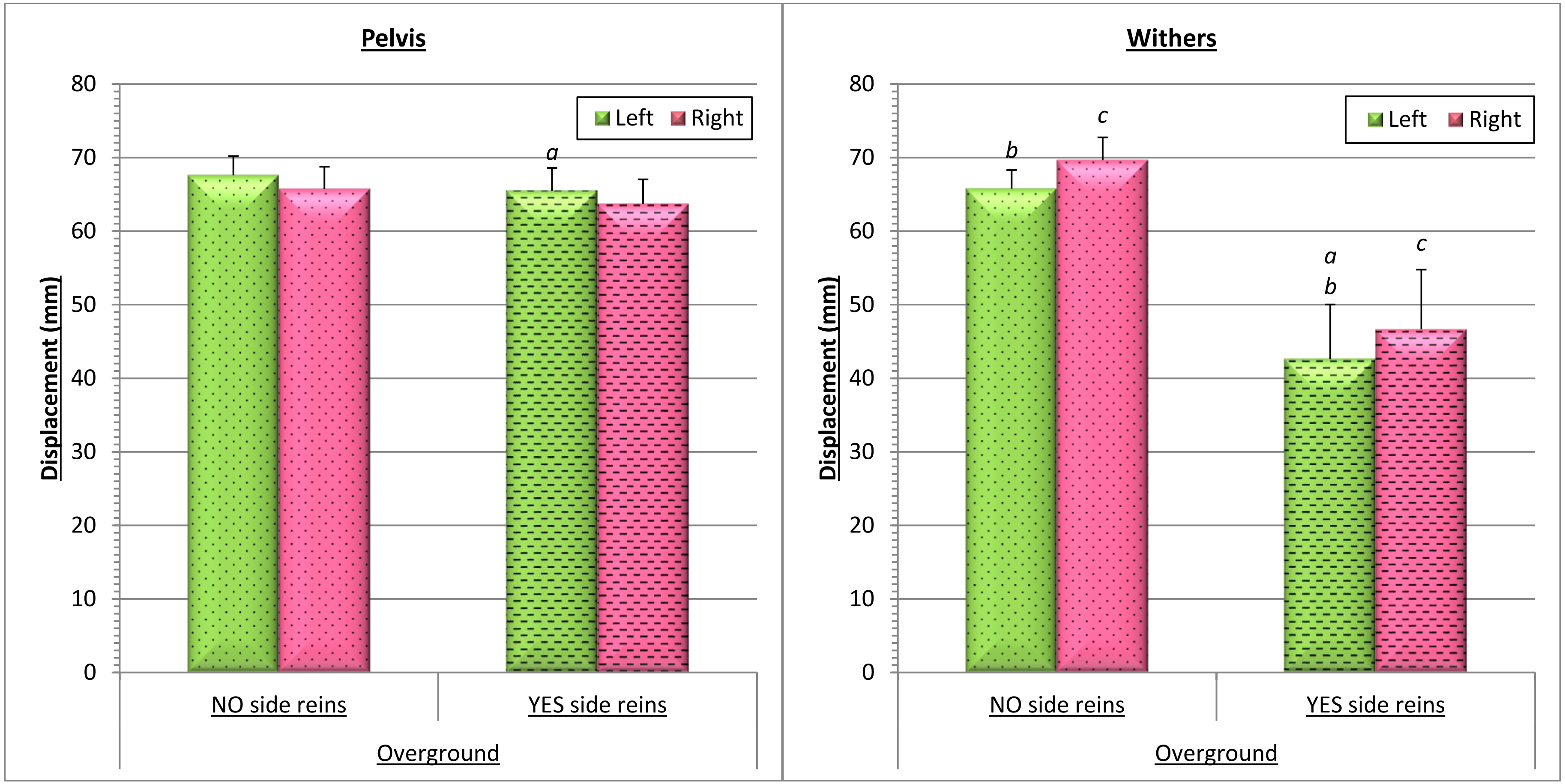


Figure 6.1: Mean (±SEM) vertical displacement amplitudes (in mm) of the equine pelvis (left) and withers (right) when trotting in a straight line overground as informed by the Xsens© data (n=10), both with and without side reins on left (green) and right diagonal (pink) stride pair. No side reins indicated by dots. Side reins indicated by dashes. Vertical displacement amplitudes were significantly lower in the withers than the pelvis on the left diagonal pair with side reins [a] ($p < 0.05$). In the withers vertical displacement amplitudes were significantly less when side reins were added on both the left [b] ($p < 0.05$), and right diagonal pair [c] ($p < 0.01$).

6.3.1.a Overground Vertical Displacements – Symmetry

Considering the apparent effect of side reins creating asymmetry in the withers in the previous analysis (6.3.1), it was worth studying the effects of the side reins on the symmetry of the vertical displacements when the horse is trotting overground in more detail. Figure 6.2 is created from the raw data and outlines the difference in displacement of the left and right diagonal pair in the pelvis and withers both with and without side reins, appearing to show that symmetry is increased in the withers when side reins are applied.

A two-way repeated measures ANOVA showed no statistically significant two-way interaction between pelvis or withers and side reins ($F_{(1,9)}$ = 2.726, p = 0.133). There was no significant difference in the main effect of pelvis or withers ($F_{(1,9)}$ = 0.545, p = 0.479) and there was no significant difference in the main effect of side reins ($F_{(1,9)}$ = 1.884, p = 0.203). Adding side reins made no significant difference to the symmetry of the horses trotting overground.

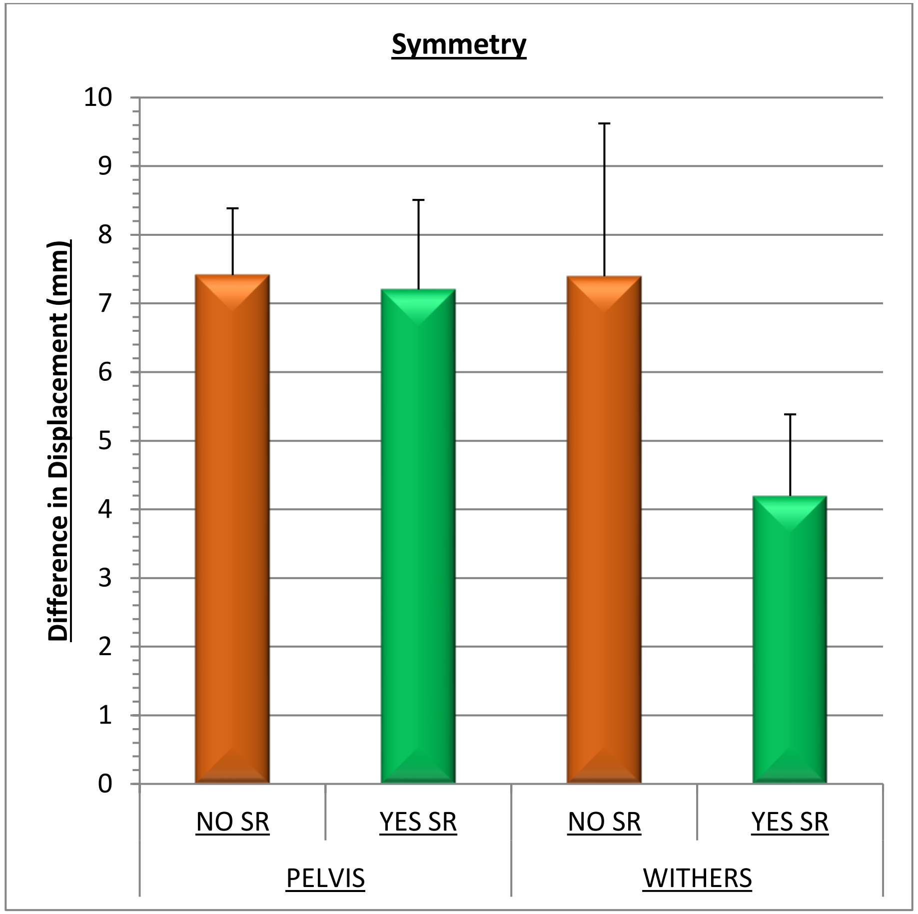


***Figure 6.2:** Mean (±SEM) difference (mm) in vertical displacement amplitudes between left and right diagonal pair in the pelvis and withers without side reins (orange) and with side reins (green) (n=10). There was no effect of side reins on the vertical displacement symmetries of horses trotting overground.*

6.3.2 Comparison between overground and ATMP3 vertical displacements

A comparison could be made between the vertical displacements of the pelvis and withers trotting overground to the vertical displacements when the same ten horses trotted on the aqua-treadmill at a low water depth (depth of mid P3) (reported in Chapters 3 and 4) to determine what effect, if any, a very low depth of water has on vertical displacement amplitudes. The means were plotted in Figure 6.3.

A repeated measures ANOVA (2x2x2x2) was conducted. Results of a four-way analysis of variance are vast and take some deciphering but ultimately, the significant elements of the analysis are reported as follows.

There was no statistically significant four-way interaction between the variables ($F_{(1,9)} = 0.067$, $p = 0.801$). But there was one statistically significant three-way interaction between pelvis or withers, overground or ATMP3, and side reins ($F_{(1,9)} = 5.923$, $p = 0.038$).

Post hoc analysis of simple two-way interactions, simple simple main effects and pairwise comparisons (Table 6.3) showed that trotting on the aqua-treadmill at a water depth of mid P3 showed significantly greater vertical displacements in the pelvis both without and with side reins than trotting overground both without and with side reins ($p < 0.001$). In the pelvis, mean (±SEM) vertical displacements were 28.18 (±4.51) mm higher on the aqua-treadmill than overground, without side reins, and 32.01 (±3.66) mm higher with side reins. In the withers, trotting on the aqua-treadmill at a water depth of mid P3 with side reins had significantly greater vertical displacements than trotting overground with side reins ($p = 0.003$) a mean difference of 29.46 (±7.46) mm, but there was no significant effect without side reins.

Trotting on the aqua-treadmill at a water depth of mid P3 both without and with side reins showed significantly lower vertical displacements in the withers than the pelvis ($p < 0.001$) (*as reported in Chapter 3 – the pelvis displaces higher than the withers*). When trotting overground with side reins, the pelvis exhibited significantly greater vertical displacements than the withers ($p = 0.012$) and the application of side reins had the significant effect of reducing vertical displacements in the withers ($p = 0.010$) *(also reported in chapter 6.3.1).*

Table 6.3: Post hoc analysis of the significant simple two-way interactions and simple simple main effects for the comparison in vertical displacements of the pelvis and withers of horses trotting overground and on an aqua-treadmill at water depth of mid P3. Pairwise comparisons with Bonferroni correction shown. Mean difference shown in millimetres.

					95% Confidence Interval for Difference[a]	
Pelvis to Withers		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
OG	NO SR	1.81	2.52	0.491	-3.90	7.52
	YES SR	22.90*	7.35	0.012	6.28	39.52
ATMP3	NO SR	24.16*	3.89	<0.001	15.37	32.95
	YES SR	25.45*	3.54	<0.001	17.45	33.46
OG to ATMP3		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Pelvis	NO SR	-28.178*	4.51	<0.001	-38.37	-17.98
	YES SR	-32.01*	3.66	<0.001	-40.29	-23.73
Withers	NO SR	-5.83	4.21	0.200	-15.35	3.71
	YES SR	-29.46*	7.46	0.003	-46.32	-12.59
NO SR to YES SR		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Pelvis	OG	2.09	1.17	0.107	-0.55	4.73
	ATMP3	-1.75	1.48	0.267	-5.08	1.59
Withers	OG	23.18*	7.13	0.010	7.04	39.32
	ATMP3	-0.45	2.51	0.861	-6.14	5.23

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

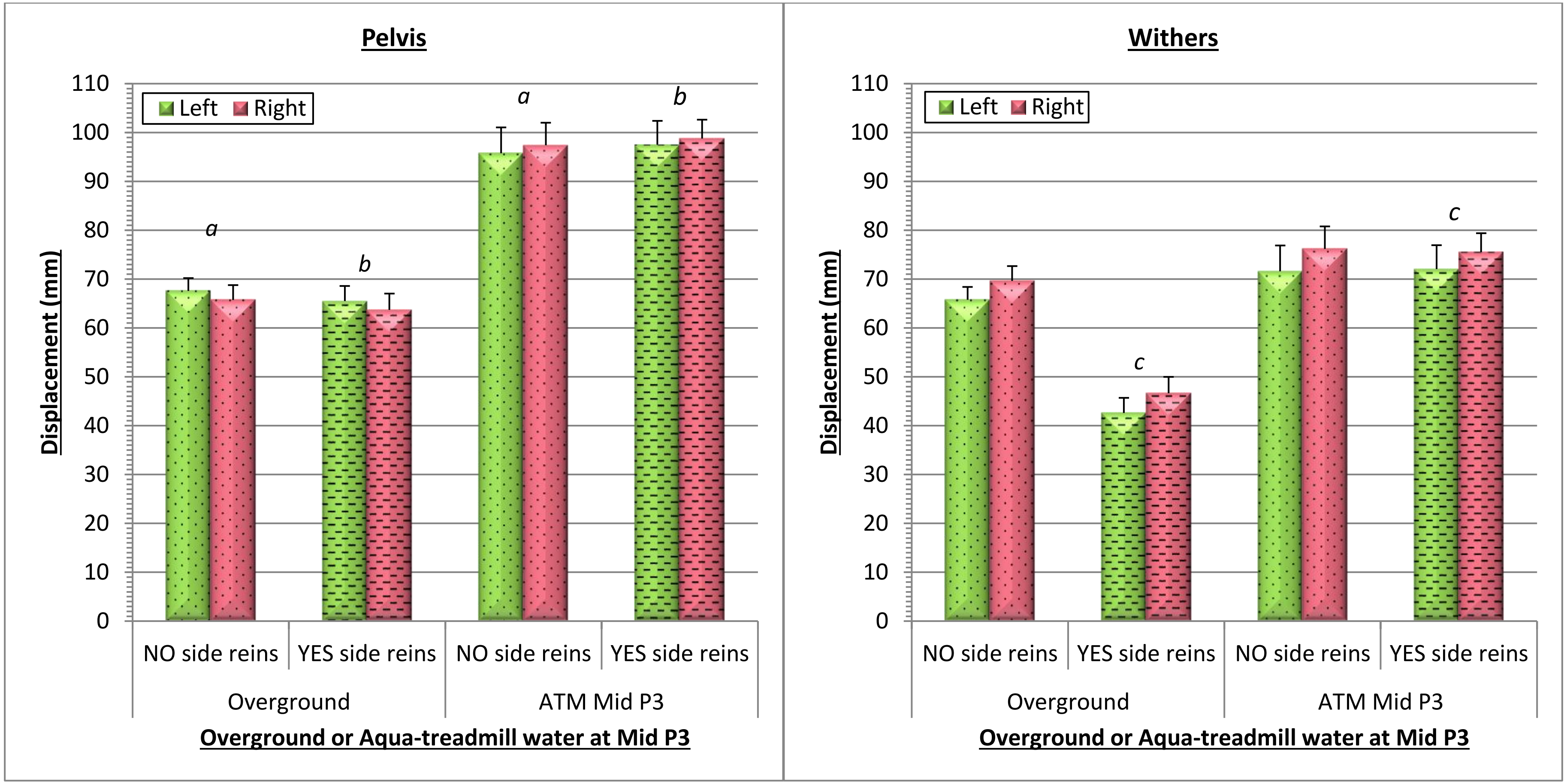


***Figure 6.3:** Mean (±SEM) vertical displacement amplitudes (in mm) of the equine pelvis (left) and withers (right) when trotting in a straight line overground and when trotting at a water depth of mid P3 on the aqua-treadmill as informed by the Xsens© data both with and without side reins on the left (green) and right (pink) diagonal stride pair (n=10). No side reins indicated by dots. Side reins indicated by dashes. In the pelvis, vertical displacement amplitudes were significantly larger on the aqua-treadmill than overground both without [a] ($p < 0.01$) and with [b] ($p < 0.01$) side reins. In the withers, vertical displacement amplitudes were significantly larger on the aqua-treadmill with side reins [c] ($p < 0.01$).*

6.3.3 Pitch Angles Trotting Overground

The pitch of the inertial measurement units at the pelvis and withers when trotting overground was quantified and the means plotted (Figure 6.4).

A repeated measures ANOVA (2x2x2) was conducted to determine the effects of pelvis or withers, side reins and left or right diagonal pair stride on the mean pitch angle amplitudes throughout a stride cycle of ten horses trotting overground. There was no statistically significant three-way interaction between 'pelvis or withers', 'side reins' or 'left or right diagonal pair' ($F_{(1,9)} = 0.867$, $p = 0.376$). There was one statistically significant two-way interaction between 'pelvis or withers' and 'left or right' ($F_{(1,9)} = 18.459$, $p = 0.002$) requiring further analysis.

Post hoc analysis of simple main effect and pairwise comparisons showed that in the pelvis there was an apparent asymmetry in pitch amplitudes between the left and right diagonal pair with the right diagonal pair reaching significantly greater pitch amplitudes both without side reins ($p = 0.001$), a mean (±SEM) difference of 1.82 (±0.37) degrees, and with side reins ($p < 0.001$), a mean (±SEM) difference of 1.52 (±0.21) degrees (Table 6.4).

Overall, the withers had lower pitch amplitudes throughout a stride cycle than the pelvis ($p = 0.039$), and specifically these differences were on the right diagonal pair both without side reins ($p = 0.013$), a mean (±SEM) difference of 2.69 (±0.87) degrees and with side reins ($p = 0.006$), a mean (±SEM) difference of 2.43 (±0.68) degrees (Table 6.5).

Table 6.4: Post hoc analysis of the significant simple main effect of left versus right on the pitch of the pelvis or withers in horses trotting overground (n=10). Pairwise comparison with Bonferroni correction shown. Mean difference shown in degrees.

Left to Right		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	95% Confidence Interval for Difference[a] Upper Bound
Pelvis	NO SR	-1.82*	0.37	0.001	-2.65	-0.99
	YES SR	-1.52*	0.21	<0.001	-2.00	-1.03
Withers	NO SR	0.07	0.35	0.851	-0.72	0.86
	YES SR	-0.00	0.27	0.993	-0.62	0.61

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

Table 6.5: Post hoc analysis of the significant simple main effect of pelvis versus withers on the pitch of the pelvis or withers in horses trotting overground (n=10). Pairwise comparison with Bonferroni correction shown. Mean difference shown in degrees.

Pelvis to Withers		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	95% Confidence Interval for Difference[a] Upper Bound
NO Side Reins	Left	0.81	0.85	0.369	-1.12	2.73
	Right	2.69*	0.87	0.013	0.73	4.65
YES Side Reins	Left	0.91	0.65	0.194	-0.56	2.38
	Right	-2.43*	0.68	0.006	0.89	3.96

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

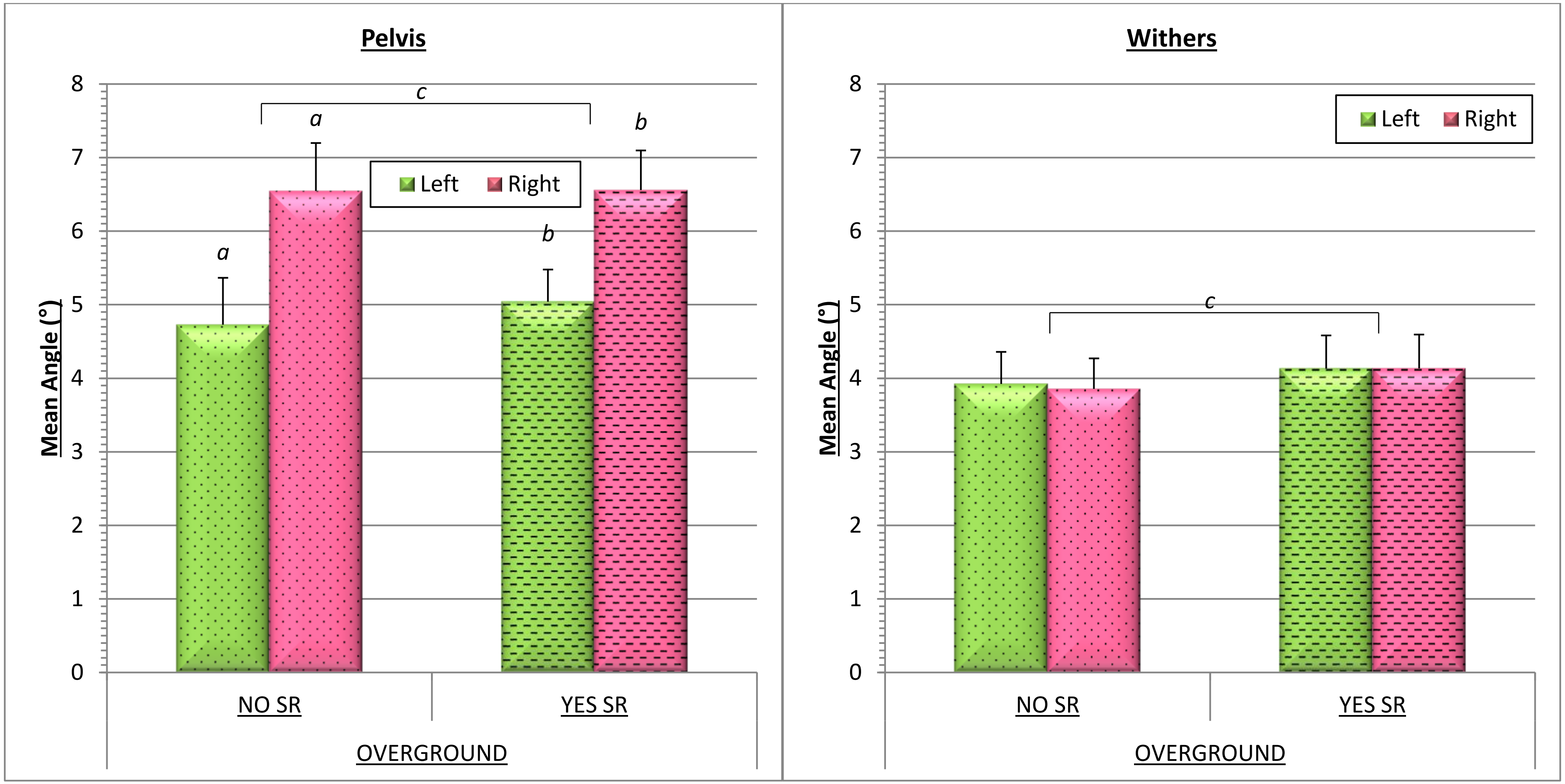


Figure 6.4: Mean (±SEM) pitch (in degrees) of the equine pelvis (left) and withers (right) when trotting in a straight line overground as informed by the Xsens© data both with and without side reins on the left (green) and right (pink) diagonal stride pair(n=10). No side reins indicated by dots. Side reins indicated by dashes. In the pelvis, pitch amplitudes were significantly greater on the right diagonal pair both without [a] ($p < 0.01$) and with side reins [b] ($p < 0.01$). The withers had significantly lower pitch amplitudes than the pelvis [c] ($p < 0.05$).

6.3.4 Comparison between overground and ATMP3 Pitch Angles

A comparison between the overground pitch amplitudes and the amplitudes achieved when trotting on the aqua-treadmill with just a very low water depth (mid P3) was conducted to identify what effect, if any, a small amount of water has on pitch amplitudes throughout a stride cycle (Figures 6.5).

A repeated measures ANOVA (2x2x2x2) was conducted. The four-way interaction between 'OG or ATMP3', 'P or W', 'side reins' and 'L or R' was not statistically significant ($F_{(1,9)} = 0.004$, $p = 0.949$). There was one statistically significant three-way interaction; 'OG or ATMP3', 'P or W', 'L or R' ($F_{(1,9)} = 12.711$, $p = 0.006$) which required further analysis.

Post hoc analyses of simple two-way interactions and simple simple main effects with a Bonferroni correction showed that there was no significant effect of the use of side reins on the pitch amplitudes, as side reins did not feature in the only significant three-way interaction. The important comparison between overground pitch amplitudes and ATMP3 pitch amplitudes showed that pitch amplitudes were significantly higher in the pelvis when trotting overground than when trotting on the aqua-treadmill with water at mid P3 (on the right diagonal pair only) ($p = 0.002$), a mean (±SEM) difference of 2.01 (±0.45) degrees (Table 6.6). In the withers, pitch amplitudes were significantly higher when trotting on the aqua-treadmill at a water depth of mid P3 than they were when trotting overground on both the left diagonal pair ($p = 0.001$), a mean (±SEM) difference of 1.66 (±0.36) degrees and right diagonal pair ($p < 0.001$), a mean (±SEM) difference of 1.71 (±0.31) degrees (Table 6.6).

Overground, pitch amplitudes were higher on the right diagonal pair than the left diagonal pair (*p* = 0.001), and higher in the pelvis than withers on the right diagonal pair (*p* = 0.013) as reported in Chapter 6.3.3 (Table 6.6).

Table 6.6: Post hoc analysis of the significant simple two-way interactions and simple simple main effects for the comparison in pitch amplitudes of the pelvis and withers of horses trotting overground and on an aqua-treadmill at water depth of mid P3. Pairwise comparisons with Bonferroni correction shown. Mean difference shown in degrees.

					95% Confidence Interval for Difference[a]	
Pelvis to Withers		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
OG	Left	0.81	0.85	0.369	-1.12	2.73
	Right	2.69*	0.87	0.013	0.73	4.65
ATMP3	Left	-1.26	0.57	0.056	-2.55	0.04
	Right	-2.01*	0.45	0.002	0.99	3.02
OG to ATMP3		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Pelvis	Left	0.41	0.67	0.559	-1.11	1.92
	Right	2.01*	0.45	0.002	0.99	3.02
Withers	Left	-1.66*	0.36	0.001	-2.47	-0.84
	Right	-1.71*	0.31	<0.001	-2.42	-1.00
Left to Right		Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
Pelvis	OG	-1.82*	0.37	0.001	-2.65	-0.99
	ATMP3	-0.22	0.25	0.407	-0.78	0.35
Withers	OG	0.07	0.35	0.851	-0.72	0.86
	ATMP3	0.01	0.23	0.967	-0.52	0.54

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

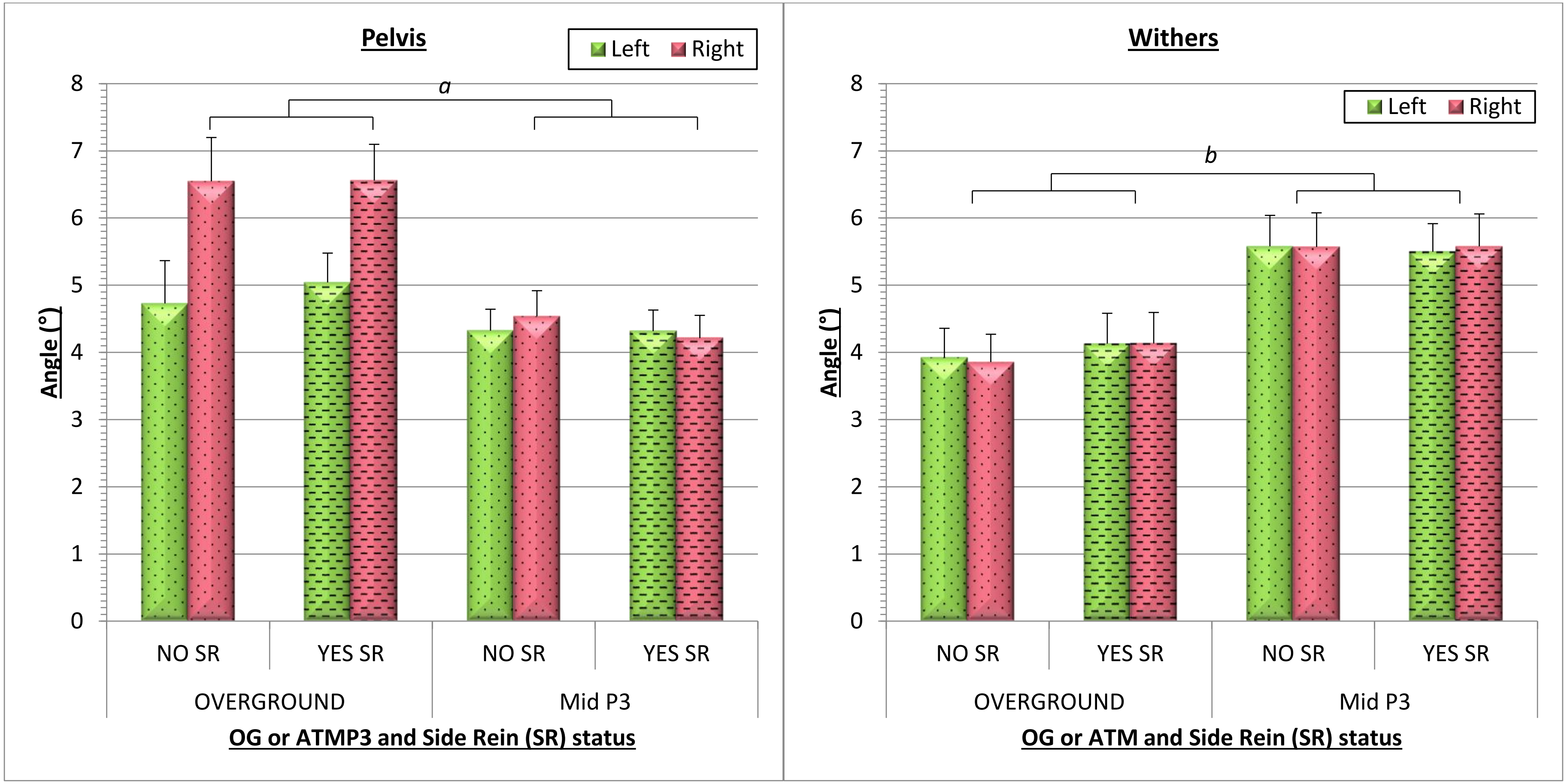


***Figure 6.5:** Mean (±SEM) pitch amplitudes throughout a stride cycle (in degrees) of the equine pelvis (left) and withers (right) when trotting in a straight line overground and when trotting at a water depth of mid P3 on the aqua-treadmill as informed by the Xsens© data, both with and without side reins on the left (green) and right (pink) diagonal stride pair (n=10). No side reins indicated by dots. Side reins indicated by dashes. In the pelvis on the right diagonal pair, pitch amplitudes were significantly higher overground than on the aqua-treadmill [a] ($p < 0.01$). In the withers, pitch amplitudes were significantly higher on the aqua-treadmill than overground [b] ($p < 0.01$).*

6.3.5 Mediolateral Displacements at the pelvis, withers and poll and when trotting overground

Mediolateral displacement amplitudes when trotting on an aqua-treadmill at increasing water depths were discussed in Chapter 5. Here, mediolateral displacements at the pelvis and withers were investigated when the horse is trotting in a straight line overground, and for the first time, the poll is also included in the analysis to determine any effect of side reins on the mediolateral movements of the head. Means were plotted and can be seen in Figure 6.6.

A two-way repeated measures ANOVA (3x2) was conducted. There was no significant two-way interaction between anatomical area and the use of side reins ($F_{(2,18)}$ = 0.525, p = 0.494). With no significant interaction, the main effects could be interpreted and analysed.

There was no significant main effect of anatomical location (pelvis, withers, poll) on mediolateral displacement amplitudes ($F_{(2,18)}$ = 0.678, p = 0.520). No one area appeared to have smaller or larger mediolateral displacements than another.

The main effect of side reins on mediolateral displacements proved not to be significant ($F_{(1,9)}$ = 1.319, p = 0.280). It was anticipated that the use of side reins would perhaps have had the effect of reducing mediolateral displacement amplitudes particularly at the withers and poll but while a trend was seen (Figure 6.6) this trend was not found to be significant.

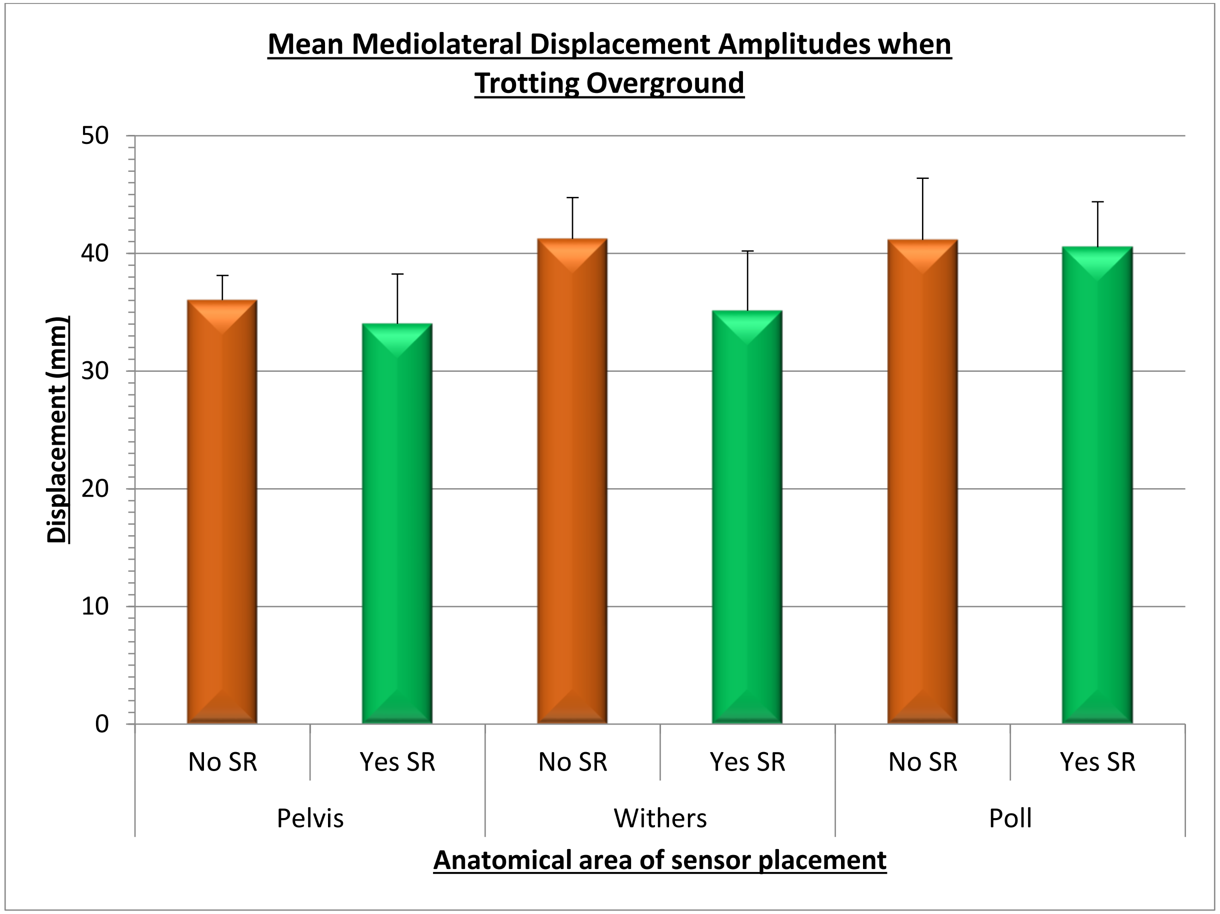


Figure 6.6: Mean (±SEM) mediolateral displacement amplitudes (in mm) of the equine pelvis, withers and poll when trotting in a straight line overground as informed by the Xsens© data both without side reins (orange) and with side reins (green) (n=10). There was no significant effect of side reins on mediolateral displacements of the pelvis, withers or poll when trotting overground.

6.3.6 Comparison between overground and ATMP3 mediolateral displacements (pelvis, withers and poll)

As previously stated, mediolateral displacement amplitudes on the aqua-treadmill are reported in Chapter 5. A comparison was made between mediolateral displacements achieved when trotting overground to trotting on the aqua-treadmill at a low water depth of mid P3 at the pelvis, withers and poll and the means were plotted (Figure 6.7).

A repeated measures ANOVA (2x3x2) was conducted to determine any effects of overground or ATMP3, pelvis, withers or poll, or the use of side reins on the mediolateral displacement amplitudes of ten horses trotting. There was no statistically significant interaction between OG or ATMP3, pelvis withers or poll, and the use of side reins ($F_{(2,18)} = 2.894$, $p = 0.081$). There was a statistically significant two-way interaction between OG or ATMP3 and pelvis, withers or poll ($F_{(2,18)} = 7.698$, $p = 0.004$) so further analysis was required. *Post hoc* analyses of simple main effects and pairwise comparisons were performed, and six significant effects were found.

When comparing overground trotting to trotting on the aqua-treadmill at a water depth of mid P3 without side reins, the pelvis showed significantly greater mediolateral displacements on the aqua-treadmill ($p = 0.041$), a mean (±SEM) difference of 9.97 (±4.18) mm. This was not significant when side reins were applied ($p = 0.731$). The withers showed significantly greater mediolateral displacements on the aqua-treadmill both without and with side reins ($p < 0.001$), a mean (±SEM) difference of 20.26 (±2.62) mm without side reins, and a mean

(±SEM) difference of 21.08 (±2.46) mm with side reins. (Table 6.8). There was no significant difference in mediolateral displacement amplitudes in the poll from trotting overground to trotting on the aqua-treadmill with a small amount of water (Table 6.7).

When studying the changes in mediolateral displacements between the pelvis, withers and poll, no significant differences were found in the overground data but when trotting on the aqua-treadmill at a water depth of mid P3, the withers have a significant greater mean (±SEM) mediolateral displacement than the pelvis of 15.49 (±5.15) mm when there are no side reins (*p* = 0.044) and when the side reins are applied the mediolateral displacement is even greater at 21.38 (±3.91) mm (*p* = 0.001) (Table 6.9). Also, with side reins, the withers have a significantly greater mediolateral displacement than the poll of 19.47 (±5.61) mm (*p* = 0.021) (Table 6.8).

Table 6.7: Post hoc analysis of the significant simple main effect of OG versus ATMP3 on mediolateral displacements of the pelvis, withers and poll of horses trotting overground and on an aqua-treadmill at water depth of mid P3. Pairwise comparisons with Bonferroni correction shown. Mean difference shown in millimetres.

OG to ATMP3		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Pelvis	NO side reins	-9.97*	4.18	0.041	-19.42	-0.52
	YES side reins	-0.86	2.43	0.731	-6.35	4.63
Withers	NO side reins	-20.26*	2.62	<0.001	-26.18	-14.35
	YES side reins	-21.08*	2.46	<0.001	-26.63	-15.52
Poll	NO side reins	-3.67	5.51	0.523	-16.13	8.80
	YES side reins	-0.14	3.13	0.967	-7.21	6.94

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

Table 6.8: Post hoc analysis of the significant simple main effect of anatomical location on mediolateral displacements of the pelvis, withers and poll of horses trotting overground and on an aqua-treadmill at water depth of mid P3. Pairwise comparisons with Bonferroni correction shown. Mean difference shown in millimetres.

							95% Confidence Interval for Difference[a]	
				Mean difference	Standard error	Sig.[a]	Lower Bound	Upper Bound
OVERGROUND	NO SR	Pelvis	Withers	-5.20	3.74	0.592	-16.16	5.76
			Poll	-5.12	6.29	1.000	-23.55	13.32
		Withers	Pelvis	5.20	3.74	0.592	-5.76	16.16
			Poll	0.09	6.67	1.000	-19.47	19.64
		Poll	Pelvis	5.12	6.29	1.000	-13.32	23.55
			Withers	-0.09	6.67	1.000	-19.64	19.47
	YES SR	Pelvis	Withers	-1.16	3.29	1.000	-10.80	8.48
			Poll	-2.63	5.58	1.000	-19.00	13.74
		Withers	Pelvis	1.16	3.29	1.000	-8.48	10.80
			Poll	-1.47	4.96	1.000	-16.01	13.07
		Poll	Pelvis	2.63	5.58	1.000	-13.74	19.00
			Withers	1.47	4.96	1.000	-13.07	16.01
ATMP3	NO SR	Pelvis	Withers	-15.49*	5.15	0.044	-30.61	-0.37
			Poll	1.19	2.84	1.000	-7.13	9.52
		Withers	Pelvis	15.49*	5.15	0.044	0.37	30.61
			Poll	16.69	5.69	0.050	-0.00	33.74
		Poll	Pelvis	-1.19	2.84	1.000	-9.52	7.13
			Withers	-16.69	5.69	0.050	-33.37	0.00
	YES SR	Pelvis	Withers	-21.38*	3.91	0.001	-32.84	-9.91
			Poll	-1.91	3.65	1.000	-12.60	8.79
		Withers	Pelvis	21.38*	3.91	0.001	9.91	32.84
			Poll	19.47*	5.61	0.021	3.01	35.93
		Poll	Pelvis	1.91	3.65	1.000	-8.79	12.60
			Withers	-19.47*	5.61	0.021	-35.93	-3.01

Based on estimated marginal means

[a] Bonferroni adjustment for multiple comparisons

* The mean difference is significant at the 0.05 level

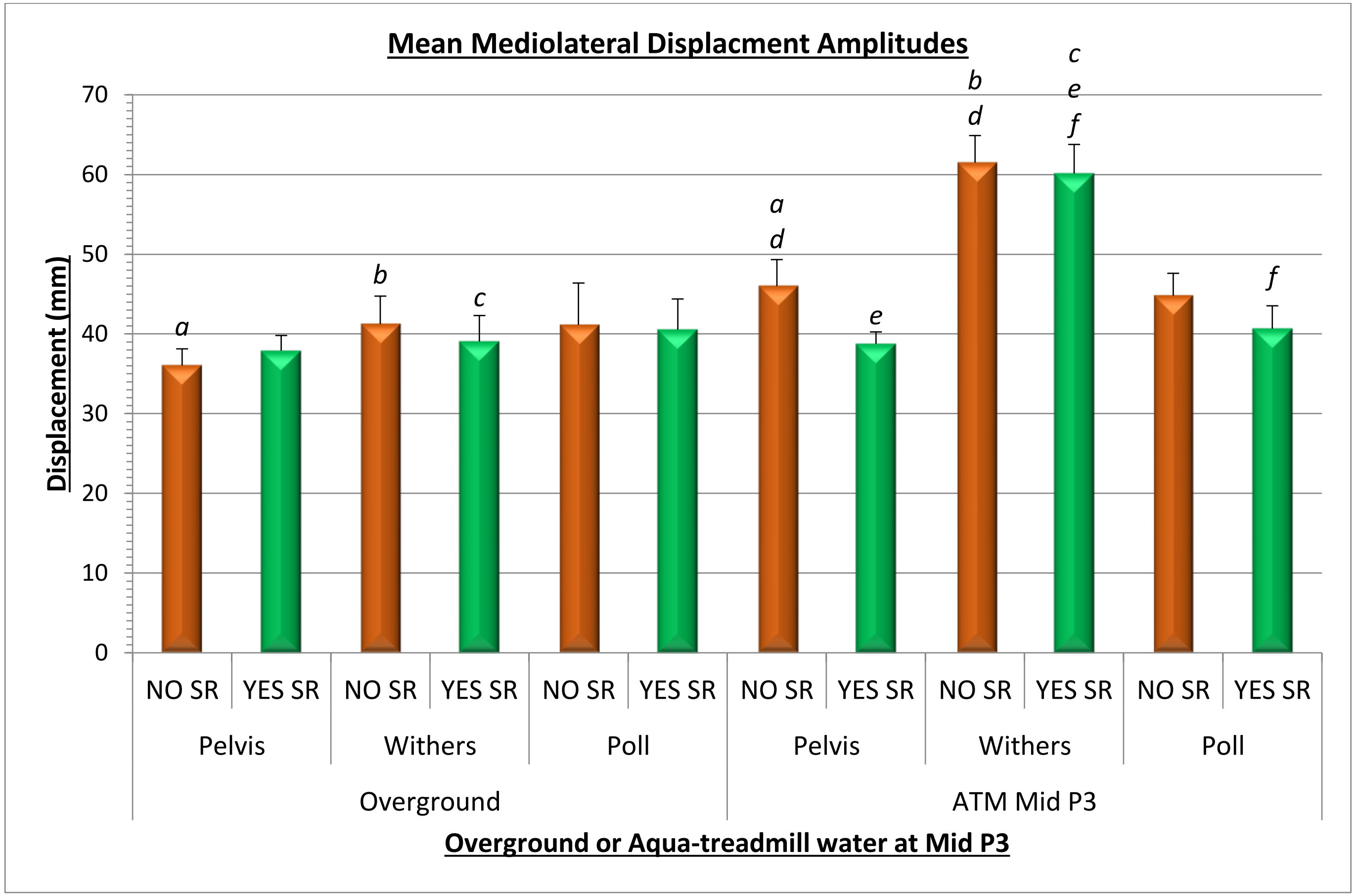


Figure 6.7: Mean (±SEM) mediolateral displacement amplitudes of the equine pelvis, withers and poll (in mm) in trot both overground and on the aqua-treadmill at a water depth of mid P3 both without side reins (orange) and with side reins (green) as informed by the Xsens© data (n=10). Mediolateral displacements were significantly larger on the aqua-treadmill than overground in the pelvis without side reins [a] ($p < 0.05$). Mediolateral displacements were significantly larger on the aqua-treadmill than overground in the withers both without [b] ($p < 0.01$) and with side reins [c] ($p < 0.01$). On the aqua-treadmill, the withers have a greater mediolateral displacement than the pelvis without side reins [d] ($p < 0.05$) and with side reins [e] ($p < 0.01$); the withers have a greater mediolateral displacement than the poll with side reins [f] ($p < 0.05$).

6.3.7 Roll of the pelvis, withers and poll when trotting overground

The roll amplitudes of the inertial measurement units when trotting on an aqua-treadmill at increasing water depths were discussed in Chapter 5. The roll at the pelvis and withers were also investigated when the horse was trotting in a straight line overground, and for the first time, the poll was also included in the analysis to determine any effect of side reins on the mediolateral movements of the head. Means were plotted and san be seen in Figure 6.8.

A repeated measures ANOVA (3x2) was conducted. There was no statistically significant two-way interaction between pelvis, withers or poll and the use of side reins on roll amplitudes ($F_{(2,18)}$ = 0.565, p = 0.578). With no significant interaction, the main effects were interpreted and analysed.

There was no main effect of side reins on the roll amplitudes, but there was a significant main effect of anatomical location on roll amplitudes ($F_{(2,18)}$ = 69.372, p < 0.001) where pairwise comparisons showed that the poll had a significantly lower mean (±SEM) roll amplitude than both the pelvis of 10.26 (±0.98) degrees (p < 0.001) and the withers of 11.56 (±1.27) degrees (p < 0.001) (Table 6.9).

Table 6.9: Post hoc analysis of the significant main effect of anatomical location on the roll of the pelvis withers and poll of horses trotting overground (n=10). Pairwise comparison with Bonferroni correction shown. Mean difference shown in degrees.

Anatomical Location		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
Pelvis	Withers	-1.30	0.94	0.63	-4.06	1.46
	Poll	10.26*	0.98	<0.001	7.39	13.13
Withers	Pelvis	1.30	0.94	0.603	-1.46	4.06
	Poll	11.56*	1.27	<0.001	7.82	15.29
Poll	Pelvis	-10.26*	0.98	<0.001	-13.13	-7.39
	Withers	-11.56*	1.27	<0.001	-15.29	-7.82

Based on estimated marginal means

[a] Bonferroni adjustment for multiple comparisons

* The mean difference is significant at the 0.05 level

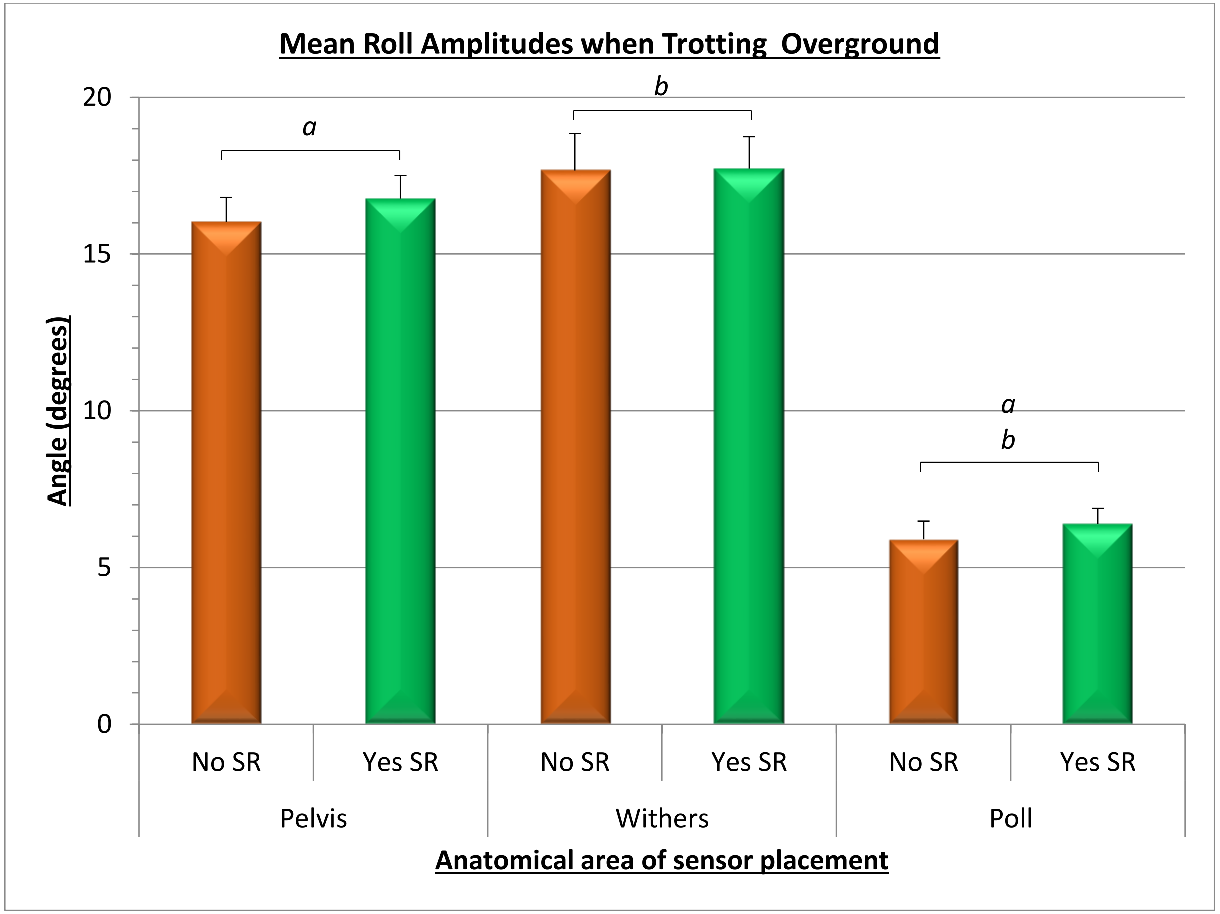


Figure 6.8: Mean (±SEM) mediolateral roll amplitudes (in degrees) of the equine pelvis, withers and poll when trotting in a straight line overground both without side reins (orange) and with side reins (green) as informed by the Xsens© data (n=10). There was no effect of side reins on roll amplitudes. The poll had significantly lower roll amplitudes than the pelvis [a] ($p < 0.01$) and withers [b] ($p < 0.01$).

6.3.8 Comparison between overground and ATMP3 roll amplitudes

A significant effect on roll amplitudes at the poll was reported in Chapter 6.3.7 (both with and without side reins) so it was pertinent to again include the poll in the analysis of comparing the roll amplitudes from trotting overground to the roll amplitudes when trotting on an aqua-treadmill at a water depth of mid P3. The means were plotted and can be seen in Figure 6.9.

A repeated measures ANOVA (2x3x2) was conducted determine any effects of OG or ATMP3, pelvis, withers or poll, or the use of side reins on the roll amplitudes of ten horses trotting. There was no significant three-way interaction between 'OG or ATMP3', 'pelvis, withers or poll', and 'side reins' ($F_{(2,18)} = 1.431$, $p = 0.265$). With no significant three-way interaction, the two-way interactions were investigated and there was one significant two-way interaction between 'OG and P3' and 'anatomical area' ($F_{(2,18)} = 5.263$, $p = 0.016$). *Post hoc* analyses of simple main effects and pairwise comparisons were then run, and several significant effects were found.

The comparison between overground trotting and trotting on the aqua-treadmill showed only a significant difference in the withers. The withers showed a statistically significant increase in roll amplitudes on the aqua-treadmill at a water depth of mid P3 compared with overground, both without side reins ($p < 0.001$), a mean (±SEM) difference of 5.77 (±1.07) degrees, and when side reins were added ($p = 0.038$), a mean (±SEM) difference of 6.99 (±2.87) degrees. There were no other significant differences in roll amplitudes between trotting overground and trotting on the aqua-treadmill at a water depth of mid P3 (Table 6.10).

When trotting overground, the poll had significantly lower roll amplitudes than the pelvis and withers both with and without side reins ($p < 0.001$) (as reported in chapter 6.3.7) but there was no significant difference in the roll amplitudes achieved between the pelvis or withers (Table 6.11).

When trotting on the aqua-treadmill at a water depth of mid P3 without side reins, the withers had significantly greater roll amplitudes than the pelvis ($p = 0.010$), a mean (±SEM) difference of 6.47 (±1.64) degrees, and the poll ($p < 0.001$), a mean (±SEM) difference of 14.80 (±2.19) degrees, and the poll had significantly lower roll amplitudes than the pelvis ($p = 0.001$), a mean (±SEM) difference of 8.33 (±1.57) degrees. When side reins were added, the withers still had greater roll amplitudes than the poll ($p < 0.001$), a mean (±SEM) difference of 19.05 (±2.80) degrees, but not the pelvis ($p = 0.112$). The poll still had significantly lower roll amplitudes than the pelvis ($p < 0.001$), a mean (±SEM) difference of 11.71 (±1.12) degrees (Table 6.11).

Table 6.10: Post hoc analysis of the significant simple main effect of overground versus ATMP3 on roll amplitudes of the pelvis, withers and poll of horses trotting overground and on an aqua-treadmill at water depth of mid P3. Pairwise comparison with Bonferroni correction shown. Mean difference shown in degrees.

OG to ATMP3		Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
Pelvis	NO side reins	-0.94	0.90	0.323	-2.97	1.09
	YES side reins	-0.62	0.58	0.317	-1.93	0.70
Withers	NO side reins	-5.77*	1.07	<0.001	-8.18	-3.36
	YES side reins	-6.99*	2.87	0.038	-13.49	-0.49
Poll	NO side reins	-2.73	2.27	0.260	-7.87	2.41
	YES side reins	0.71	0.50	0.191	-0.43	1.85

Based on estimated marginal means
[a] Bonferroni adjustment for multiple comparisons
* The mean difference is significant at the 0.05 level

Table 6.11: Post hoc analysis of the significant simple main effect of anatomical location on roll amplitudes of the pelvis, withers and poll of horses trotting overground and on an aqua-treadmill at water depth of mid P3. Pairwise comparison with Bonferroni correction shown. Mean difference shown in degrees.

				Mean difference	Standard error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
OVERGROUND	NO SR	Pelvis	Withers	-1.64	1.14	0.553	-5.00	1.71
			Poll	10.12*	1.01	<0.001	7.16	13.08
		Withers	Pelvis	1.64	1.14	0.553	-1.71	5.00
			Poll	11.77*	1.24	<0.001	8.12	15.41
		Poll	Pelvis	-10.12*	1.01	<0.001	-13.08	-7.16
			Withers	-11.77	1.24	<0.001	-15.41	-8.12
	YES SR	Pelvis	Withers	-0.96	0.80	0.778	-3.29	1.38
			Poll	10.39*	1.10	<0.001	7.17	13.60
		Withers	Pelvis	0.96	0.80	0.778	-1.38	3.29
			Poll	11.35*	1.36	<0.001	7.35	15.35
		Poll	Pelvis	-10.39*	1.10	<0.001	-13.60	-7.17
			Withers	-11.35*	1.36	<0.001	-15.35	-7.35
ATMP3	NO SR	Pelvis	Withers	-6.47*	1.64	0.010	-11.28	-1.67
			Poll	8.33*	1.57	0.001	3.72	12.95
		Withers	Pelvis	6.47*	1.64	0.010	1.67	11.28
			Poll	14.80*	2.19	<0.001	8.38	21.22
		Poll	Pelvis	-8.33*	1.57	0.001	-12.95	-3.72
			Withers	-14.80	2.19	<0.001	-21.22	-8.38
	YES SR	Pelvis	Withers	-7.34	3.01	0.112	-16.58	1.49
			Poll	11.71*	1.12	<0.001	8.43	15.00
		Withers	Pelvis	7.34	3.01	0.112	-1.49	16.16
			Poll	19.05*	2.80	<0.001	10.83	27.27
		Poll	Pelvis	-11.71*	1.12	<0.001	-15.00	-8.43
			Withers	-19.05*	2.80	<0.001	-27.27	-10.83

Based on estimated marginal means

[a] Bonferroni adjustment for multiple comparisons

* The mean difference is significant at the 0.05 level

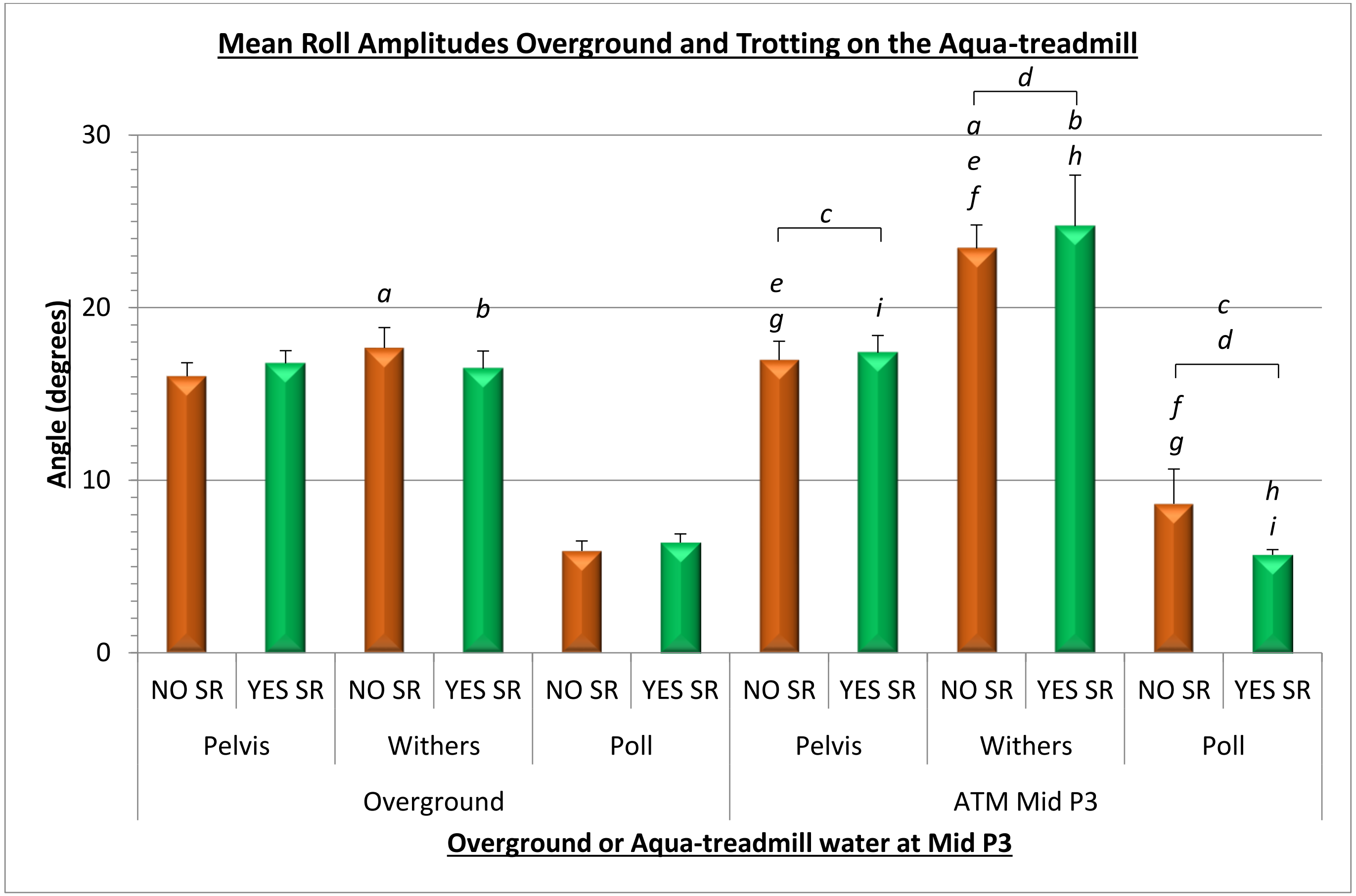


Figure 6.9: Mean (±SEM) roll amplitudes of the equine pelvis, withers and poll (in degrees) in trot both overground and on the aqua-treadmill at a water depth of mid P3 both without side reins (orange) and with side reins (green) as informed by the Xsens© data (n=10). Roll amplitudes were significantly larger on the aqua-treadmill than overground in the withers both without [a] ($p < 0.01$) and with [b] ($p < 0.05$) side reins. On the aqua-treadmill, the poll had significantly lower roll amplitudes than the pelvis [c] ($p < 0.01$) and withers [d] ($p < 0.01$). On the aqua-treadmill without side reins, the withers had larger roll amplitudes than the pelvis [e] ($p < 0.01$) and the poll [f] ($p < 0.01$), and the poll had significantly lower roll amplitudes than the pelvis [g] ($p < 0.01$). On the aqua-treadmill with side reins, the withers had larger roll amplitudes than the poll [h] ($p < 0.01$), and the poll had significantly lower roll amplitudes than the pelvis [i] ($p < 0.01$)

6.4 Discussion

6.4.1 Overground vertical displacements

There were many elements to this study, but the key areas for discussion begin with the information gleaned from trotting horses overground.

This study reported overground vertical pelvic displacement amplitudes in the trot of 66.66 ± 8.76 mm (mean ± 1 SD) with a range of 49.86 to 83.15 mm which concurs with previous studies of amplitudes of around 60mm in non-lame horses using the Xsens© system for data collection (Church *et al.*, 2009). Overground withers vertical displacements were quantified as 67.71 ± 8.94 (mean ± 1 SD) with a range of 51.97 to 86.84 mm, which agrees with an earlier study where horses were trotted overground and withers vertical displacements were reported as a mean of 66.0 ± 12.0 mm (mean ± 1 SD) (Buchner *et al.*, 1994a). This agreement in vertical displacement amplitudes gives confidence in the data collection and handling techniques employed in this study and so gives confidence that any effect of side reins is notable.

This study reported a significant effect of the use of side reins decreasing vertical displacement amplitudes in the withers. This effect was not seen in the pelvis. This suggests perhaps, that side reins have a stabilising effect on the front end of the horse, perhaps controlling erratic head movements by keeping the head and neck straighter and lowering the poll, thereby stabilising the movement of the withers. It is possible that more energy is exerted into forward thrust rather than vertical displacements and stride length may therefore become longer with the use of side reins. However, stride length was not measured in this study, but a simultaneous study investigating vertical displacements and stride parameters would be interesting to further quantify locomotory changes overground with the use of side reins. There appear to be no reports in the literature discussing the effect of side reins or training aids on stride parameters, only the effect of different length side reins on the resulting rein tensions (Clayton *et al.*, 2011) and the effect of different training aids on EMG activity in the *longissimus dorsi* (Cottriall *et al.*, 2009).

6.4.2 Overground mediolateral displacements

Literature has previously reported mediolateral displacements at T6 of around 34 mm but with a range of 16 to 37 mm throughout different locations on the back decreasing from cranial to caudal and then increasing in the sacral region with the 1st lumbar vertebrae recording mediolateral displacements of around 17mm (Warner *et al.*, 2010). The current study reports a larger mean mediolateral displacement in the pelvis of 36.03 ± 6.63 mm (mean ± 1 SD) with a range of 24.86 to 48.28 mm, and in the withers; 41.24 ± 11.11 mm (mean ± 1 SD) with a range of 26.01 to 55.85 mm but does exhibit the same trend as the previous study (Warner *et al.*, 2010) with greater mediolateral displacements shown at the withers than at the pelvis. This pattern of lateral displacement amplitudes corresponds to the body centre of mass which is most similar to the movement at T13 (Buchner *et al.*, 2000) and the further away from this, the greater the lateral movement (Buchner *et al.*, 2000; Warner *et al.*, 2010).

Mediolateral displacements appear to be less studied in the wider scientific literature possibly due to vertical displacements most often being studied with regards to lameness, and symmetry indices determined from the asymmetries in vertical displacements between the *tuber coxae* and *sacrum*. With regard to the mediolateral displacement amplitudes when trotting overground, side reins appeared to reduce mediolateral displacements in the withers, and again, perhaps stabilise the front end of the horse. Although this trend was not found to be significant, a supplementary investigation utilising a greater cohort of horses may develop this trend further. It is suggested, however, that mediolateral displacements in the withers are also subject to perhaps a greater variation in skin rolling over the withers as skin is known to move over bony landmarks, and up to as much as 12cm in proximal parts of the limb (Clayton and Schamhardt, 2007). Plus, there can be difficulty in the fixation of the inertial measurement unit over the withers hence a specific withers mount is required (Pfau *et al.*, 2005).

6.4.3 Overground roll

The atlantooccipital joint has been documented as able to rotate through a mean of 27 degrees in cadaver studies (Clayton and Townsend, 1989) whereas the current study reports a mean roll at the poll in trotting horses of 5.90 ± 1.84 degrees (mean ± 1 SD) with a range of 3.53 to 9.50 degrees. Roll amplitudes overground in the present study were reported in the pelvis as 16.02 ± 2.45 degrees (mean ± 1 SD) with a range of 11.55 to 19.41 degrees, and in the withers as 17.67 ± 3.72 degrees (mean ± 1 SD) with a range of 12.56 to 25.53 degrees.

Haussler *et al.* (2001) reported axial rotation in segmental vertebrae in the trot as a mean of 4.4 ± 0.8 through the region of L6 to S1 which was as close anatomically as could be found in current literature that could be compared to the roll of the *tuber sacrale* which was measured in the current study. Haussler *et al.* (2001) reported figures a lot less than the mean 16.02 ± 2.45 degrees reported in the pelvis in the current study but this could be due to the fact that the horses were trotting on a dry treadmill not overground, and actually the 2001 study only reported the mean of three horses. On a high-speed treadmill, pelvic roll was reported to be 6.9 ± 2.1 degrees at trot with a relaxed head and neck position (Gomez Alvarez *et al.*, 2006) which is still not as high as the values seen overground in the present study. There may simply be a significant change in pelvic roll in treadmill locomotion compared to overground which has not yet been quantified in current literature and so is a potential area for further research. However, Pfau *et al.* (2005) also reports lesser roll amplitudes at the withers of around 10 degrees recorded from inertial measurement units, compared to 17.67 ± 3.72 degrees (mean ± 1 SD) in the present study. Again, this may simply be due to differences in dry treadmill and overground locomotion which warrants further investigation.

In the present study, the roll of the inertial measurement units in horses trotting overground, showed the poll had significantly less roll than both the pelvis and withers. This is to be expected due to the anatomical structure of the atlantooccipital joint where there is only limited rotation permissible at this joint. Side reins had no effect of increasing or decreasing this roll, and had no effect on the roll in the pelvis or withers either. The lack of difference in roll amplitudes between the pelvis and withers suggests perhaps that the horses in this study were actually well balanced with no apparent lameness or asymmetries which corroborates with the Vet that deemed them non-lame and suitable to take part in the study.

6.4.4 Effect of water on vertical displacements

Investigating the effect of a small amount of water on vertical and mediolateral displacements had interesting findings. Firstly, it seems that a small amount of water does indeed induce a significant effect in vertical displacements particularly at the pelvis and without the use of side reins. However, this result could simply be due to the differences in treadmill locomotion compared to overground, rather than specifically due to the water but a dry treadmill was not included in the current study. Previous literature has shown that vertical displacements of the withers were comparable from overground trotting on asphalt to trotting on a dry treadmill (Buchner *et al.*, 1994a). The speed of the aqua-treadmill was matched to the speed the horses trotted in hand overground which was an important factor, as kinematic stride variables have been reported to be velocity dependent (Leach and Drevemo, 1991; Buchner *et al.*, 1994a). The pelvis exhibited significantly greater vertical displacements on the aqua-treadmill with water at a depth of mid P3 than it did when trotting overground. This effect was not seen in the withers which suggests that the withers can compensate the water depth by being able to bend at the carpus, whereas the stay and reciprocal apparatus in the hindlimb creates significant flexion throughout the whole limb increasing compression and therefore increasing vertical lift. It is suggested therefore, that a very low depth of water induces a large response in hindlimb flexions which may in turn create a greater workload for the horse. This potentially corresponds to the effects of tactile stimulation of the pastern and coronary band where significant increases in peak flexion of the stifle, tarsal, MTP and DIP joints throughout the swing phase in trot when a lightweight tactile stimulator (55 g) consisting of a 1 cm wide braided nylon strap with 7 double strands of lightweight brass chain, 6 cm in length, were attached loosely around the hind pasterns (Clayton *et al.*, 2008; 2010). It is suggested that the effect of tactile stimulation would not diminish with increasing water depth but the extra stimulus effects of the resistance and weight of the water would perhaps encourage larger muscle development while maintaining this increased range of motion.

6.4.5 Effect of side reins

It is interesting that when trotting overground, side reins had a significant effect of reducing vertical displacements in the withers, but when trotting on the aqua-treadmill at a water depth of mid P3 this effect was not seen. This could indicate that there is a stabilising effect of water keeping both the hind and front end of the horse more stable. It could be in fact, that overground, the side reins have the effect of controlling the head, but perhaps also making the horse more ‘downhill’. The water could serve to counteract this effect by controlling and straightening the head and neck but without creating the downhill effect that was seen in the significantly lower vertical displacements overground.

6.4.6 Pitch

There was an interesting anomaly seen with regards to the pitch amplitudes achieved by the inertial measurement units when trotting overground; the right diagonal pair had significant greater pitch amplitudes throughout a stride cycle than the left diagonal pair. Considering this effect was not seen when trotting on the aqua-treadmill, it is possible that there may have been an effect of handler, or more likely, an effect of the incline of the tarmac road used to trot the horses. A significant difference in pitch range between level and incline galloping on a treadmill has been reported (Parsons *et al.*, 2008). Side reins had no significant effect of increasing or decreasing pitch amplitudes. The pelvis displayed greater pitch amplitudes when trotting overground, but the reason for the statistical significance here could be due to the right diagonal pair anomaly (which is likely due to the presence of a handler). Looking at the left diagonal pair, there was no greater pitch amplitudes achieved overground in the pelvis.

The most interesting result was the significant greater pitch amplitudes seen in the withers when trotting on the ATMP3 compared to when trotting overground. Even with no effect of side reins, this perhaps suggests that the horse has to ‘pitch up’ and therefore perhaps ‘jump’ over the water at the front end. It has been suggested in this thesis that the front end of the horse may compensate the water depth by being able to bend at the carpus, but the increase in pitch seen here also suggests perhaps that there is hyperextension of the neck from the withers to create a jump at the front end.

6.4.7 Effect of water on mediolateral displacements

No significant effects were seen in the mediolateral displacements overground, but when trotting in a small amount of water on the aqua-treadmill without side reins, both the pelvis and withers had significant increases in mediolateral displacements. This perhaps suggests that the increased effort that results in the increased vertical displacements from an increased compression of the limb, has the effect of tipping the horse side to side thus increasing mediolateral displacements. The results of the investigation into the roll would perhaps confirm this in the withers. The roll of the withers was significantly increased in the aqua-treadmill compared to overground, but again, the potential for skin movement over the withers may be the contributing factor here. Side reins also had the effect of reducing mediolateral displacements on the ATM at mid P3; indicating that perhaps side reins have the effect of stabilising the pelvis when working in water.

It is appreciated that a direct comparison between a dry treadmill belt and a treadmill belt with a small amount of water would perhaps be preferred, but as aqua-treadmill belts do not like to run ‘dry’ then this was not possible on this occasion. Also, it is suggested that the parameters measured in this study may not be directly affected by locomotion on a belt. Vertical and mediolateral displacements are perhaps unlikely to change whether moving on a motorised belt or overground as previously discussed. Also, this study sought to investigate the effect of just a small amount of water on the measured parameters which it was successful in doing. Indeed, it appears that only a small amount of water is required to induce a significant effect in both vertical and mediolateral displacements.

6.5 Conclusion

This chapter has identified that there is a clear effect of a very low depth of water on the biomechanical parameters of vertical and mediolateral displacements, pitch and roll, measured at the pelvis, withers and poll. Side reins decrease vertical displacement amplitudes in the withers overground by controlling erratic movements of the head and neck but perhaps make the horse more downhill. Trotting on an aqua-treadmill with only a small amount of water counteracts this downhill effect. Side reins also supported the stability of the hindquarters when working through water preventing excessive mediolateral movements.

Trotting through a very low water depth generated significantly greater vertical displacements in the pelvis than when trotting overground and significantly greater vertical displacements than the withers suggesting that the withers can compensate the water depth by being able to flex at the carpus, but also having an increased pitch at the withers suggests more extension to create a jump up over the water. A very low water depth is therefore suggestive of a significantly greater workload for the horse in both the front end and hindquarters than overground trotting. The significantly increased vertical displacements in both the pelvis and withers when trotting through a small amount of water compared to overground is also indicative of greater limb compression and a greater workload. It is therefore suggested that the aqua-treadmill is beneficial at increasing the workload for the horse that will have a corresponding effect of increasing muscle mass, strength and condition, but without detrimental effects to cranial-caudal or mediolateral symmetry patterns.

CHAPTER 7 Discussion and Conclusions

7.0 Introduction

Equine aqua-treadmills have become increasingly popular in practice and scientific literature is growing. There is however, still relatively little research documenting how horses move during this method of locomotion and what deviations exist from overground locomotion. With more emphasis and investment in recent years in the competition horse to harness any opportunity to prevent injury, give horses an extra edge in their training, to rehabilitate them from injury more quickly and ultimately promote and prolong their competitive career, riders, trainers and Veterinary Surgeons are anecdotally reporting numerous benefits of aqua-treadmill exercise. The overarching aims of this study were to quantify equine kinematics during aqua-treadmill exercise, with specific focus on the axial skeleton during trot.

There were four main objectives for this study; 1) Determine the impact of water depth on vertical pelvis and wither displacement while trotting on an aqua-treadmill (Chapter 3); 2) Determine the effect of side reins on vertical displacements of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths (Chapter 4); 3) Determine the impact of water depth on mediolateral pelvis and wither displacements when trotting on an aqua-treadmill (Chapter 5); and 4) Determine the difference a small amount of water makes to pelvis and wither displacements when trotting on the aqua-treadmill compared to overground trotting (Chapter 6). In order to address these objectives, novel data were successfully collected, processed and analysed. This data provides an insight, developing a better understanding of equine locomotion in each specific scenario, proposing likely adaptation strategies used by the horses. These data and observations can be used to inform current practices and better tailor training and rehabilitation programmes to the individual needs of the horse from the leisure horse to the elite competition horse.

This chapter provides a summary of the findings presented in previous chapters, tying all the relevant information together to start and build one big picture. This combined information can then be applied to the industry to demonstrate the significance and relationship to current scientific knowledge and the scope of future developments. Furthermore, it will highlight the limitations of current knowledge in conjunction with areas that have raised further questions requiring further investigation.

7.1 Effects of water depth

Water depth was found to have a significant impact on equine kinematics indicating changes in locomotor technique when trotting through water of varied depth. Increasing water depth up to the carpus resulted in increased vertical displacement amplitudes at both the withers and pelvis. These increases in vertical displacement suggest the horse had to alter their locomotor pattern working differently to lift themselves up and out of the water. A previous study on heart rates by Lindner *et al.* (2003) found no significant effect of water depth on heart rates in either walk or trot which would disagree with the suggestion that the horse is having to work harder in increased water depths, although a later study by

Nankervis *et al.* (2008a) concluded that heart rate is not a reliable indicator of work done during partial submersion with a range of different depths, as water temperature has a greater effect on heart rates than depths. The greater vertical displacements achieved at deeper depths may also be due in part to the buoyancy of the water, and of course the speed of the trot where optimum energy preserving system of the spring-mass model is affected by velocity (Blickhan, 1989; Farley *et al.*, 1993). The pelvis had significantly larger vertical displacements than the withers at all water depths. This was an interesting and unexpected finding and was not seen in the overground study indicating that water depth has a larger impact on the hindlimbs than the forelimbs. This difference between withers and pelvis is likely due directly to the anatomy of the horse. The stay and reciprocal apparatus of the hindlimb may be affected by the water depth to a greater extent than the forelimbs, with the forelimbs perhaps being able to compensate for the increasing water depths by flexing at the carpus providing an ease of movement up and over the water. This would provide optimal conditions from a training perspective, where horses are encouraged to work from behind more and lighten the forehand as defined by the Federation Equestre Internationale (FEI) Rulebook for Dressage 2017 (Anon, 2017). It is unknown from the current study which muscles are recruited to create these locomotory changes but it could be expected that undertaking desirable movements such as these would likely activate and develop the same muscles as would be required to provide such movements overground under saddle thus improving performance. To further consolidate this theory and demonstrate this optimal movement and expected weight distribution, establishing that the muscles of the hindlimb are working harder than the forelimb, a further study investigating muscle activity perhaps by electromyography would be required despite the clear changes in the mechanics of the limb. EMG activity of the back muscles and hind limbs of horses has successfully been reported during trotting (Robert *et al.*, 2000; Robert *et al.*, 2001a, 2001b; Robert *et al.*, 2002; Licka *et al.*, 2004) with the most recent study establishing that during take-off of a hind limb, EMG activity of the *longissimus dorsi* muscle ipsilateral to that limb is higher than EMG activity of the contralateral *longissimus dorsi* muscle, which was attributed to the propulsive force generated by the hind limb, and maximum EMG activity was found to precede the maximum vertical excursion, which could be interpreted as pre-emptive tension (Licka, 2004). Increasing speed has also been found to effect EMG activity of muscles of the hindlimb during trot (Robert *et al.*, 2002) where changes in speed could be related to the mechanical function of the muscles involved and greater trotting speeds are associated with greater vertical ground reaction forces (Barr *et al.*, 1995) and require increased muscle activity. A more recent study investigated work done by both fore and hindlimbs using ground reaction forces and found that from inverse dynamic analysis, the forelimb did not appear to do any work (i.e. it neither absorbing or generating energy), while the hindlimb did positive work at all speeds (Dutto *et al.*, 2006). This potentially concurs with the findings of the present study with the hind limb apparently working harder than the forelimb to create the greater vertical displacements seen when trotting on the aqua-treadmill. It would be interesting to also measure vertical ground reaction forces when trotting on an aqua-treadmill to establish if the buoyancy of the water perhaps reduces ground reaction forces, yet increases vertical displacements.

In the forelimb, it is the muscles of the neck that are often studied as dynamic aspects of asymmetric head and neck movements are associated with lameness compensation. Electromyography activity of the splenius muscle at walk and trot has been reported using surface EMG (Robert *et al.*, 2001a, b; Robert *et al.*, 2002; Zsoldos *et al.*, 2010) and needle EMG (Tokuriki *et al.*, 1999), and it has been reported that the splenius muscle reaches maximum activity at the beginning of the forelimb stance phases in trot, indicating functional stabilisation against flexion of the head and neck (Zsoldos *et al.*, 2010). The one study that has investigated EMG activity of the forelimb muscles during aqua-treadmill exercise established that the *extensor digitorum communis* showed the most intense EMG activity at both walk and trot in the aqua-treadmill indicating that this muscle probably aided in protracting the distal limb against the resistance of the water, as the water was deep at a depth of 1.2 metres (Tokuriki *et al.*, 1999). This study concluded that trotting in the aqua-treadmill may provide less intensive training for some forelimb muscles than walking but the depth of the water is important to note here, and frustratingly, the study did not report any results of the EMG activity of hindlimb muscles.

It would also be interesting to further investigate these kinematic changes by examining flexion and extension throughout the limbs, in particular, changes in joint angles at each discrete joint as well as the limb as a whole. Furthermore, studying the changes at water depths deeper than that of mid carpus would allow investigation in to how much more vertical displacement can physically be achieved and therefore perhaps the maximum capacities of flexion and extension in the limbs. Linking these studies with further studies into ground reaction forces, EMG activity and also perhaps heart rates when trotting on an aqua-treadmill, would further establish the potential work load associated with increasing water depths.

When investigating the absolute positions of the pelvis and withers, the effect of water depth was again significant with both pelvis and withers exhibiting lower starting points of the stride during stance, suggesting an increase in both hindlimb and forelimb compression. Increased limb compression could be determined through close analysis of individual joint angles and may have been employed to increase the ease of push off to compensate for the increasing depth of the water through elastic recoil of the tendons (Dimery *et al.*, 1986; Blickhan, 1989; Biewener, 1998; Farley *et al.*, 1993). The pelvis started the stride significantly lower than the withers suggesting that the hindquarters have an increased engagement by perhaps compressing further to work harder to create the larger displacements and therefore increasing range of motion through a greater number of effective joints compressing under load. Forelimbs can flex at the shoulder, elbow and fetlock during stance, the hindlimbs can flex at the hip, stifle, hock and fetlock meaning there is effectively an increased ability to compress the hindlimbs underload. However, investigation of the pitch showed no corroboration with this theory – if the pelvis was significantly 'tucking under' then it would be expected that the pitch amplitude of the IMU on the *tuber sacrale* would highlight this, however this was not found to be true during the current study. In contrast to the minimum absolute vertical position and associate increased hindlimb compression, maximum vertical position during the stride was found to significantly increase with water depth but no significant difference was shown between the pelvis and the withers further supporting the theory of increased hindlimb compression to allow increased push off, aided by elastic recoil to reduce the energy cost of locomotion. In essence the hindlimbs drop slightly lower but then realign with the forelimbs during the aerial phase. Further investigation into limb flexions and extensions and associated EMG activity would provide further insight into the mechanisms utilised, further validating the use of the aqua-treadmill as an optimal training tool for improving muscular strength and musculoskeletal condition in horses.

Water depth was not found to impact on the movement symmetry of the horse in the pelvis. This further supports the application of aqua-treadmill exercise during training and rehabilitation as it suggests that horses can continue to work harder (as demonstrated by increased vertical displacement amplitude and increased hindlimb compression) in the deeper water without detrimental effects to their way of going. In contrast the withers started to show an asymmetry during trot at mid carpus. Perhaps this highlights the physical limitations of the forelimb adaptations, as the ability to bend at the carpus to compensate for water depth reaches its limit and the horses then have to either jump up over the water creating some sway or drag the distal limb through the water. Dragging the distal limb through the water may in turn highlight muscle weakness or asymmetry as there are no energy saving mechanisms that can be recruited. The magnitude of this asymmetric sway may be linked to each horse's individual strength and balance. Stronger more balanced horses may produce less asymmetry at the withers as the water gets deeper. This is an area for further longitudinal investigation, to determine whether training horses on the aqua-treadmill is capable of subsequent improvement in balance and movement symmetry.

These differences in movement symmetry between the withers and pelvis at increased water depth are further supported by investigations into mediolateral displacements. No significant effect of water depth has been shown, however at the lower depths of mid P3 and mid Fetlock, the withers had greater mediolateral displacements than the pelvis. This could be linked to and supported by a published study investigating walk, where mediolateral displacements did not decrease until water depth reached the level of the elbow and shoulder (Mooij *et al.*, 2013), however, it is difficult to directly compare the locomotion patterns of walk and trot although fundamental principles associated with increasing water depth could be expected to have a universal effect. It appeared that mediolateral displacements had a trend of increasing at increasing water depths in the pelvis and decreasing at increasing water depths in the withers, to the point that at the deepest depth studied of mid carpus, the mediolateral displacement amplitudes were comparable. Likewise, there was no effect of water depth found on mediolateral flexions. This suggests that there is stability in the vertebral column during trot throughout all depths of water. A further investigation into the flexion-extension of the individual vertebrae in trot (not just the three points used in this study; withers-T13-pelvis) would be useful to add to this study and make comparisons with previously published data in the walk (Nankervis *et al.*, 2016). From a training perspective, the trends found in this investigation suggest there are not any specific advantages or disadvantages of increased water depth on mediolateral capabilities

Water depth had no effect on roll amplitudes but the withers did show overall greater roll amplitudes than the pelvis which is most likely due to the superficial sensor attachment and associated movement of skin over this area (Clayton and Schamhardt, 2007). Overground, however, there was no significant difference between the pelvis and withers which suggests less effect of skin movement, but a greater effect of water. The deeper water may be creating a change in locomotory pattern in the front end of the horse, causing the horse to really rock from side to side. This rocking may be linked temporally with each stance phase where the limb is compressing more to create the greater vertical lift induced by the presence of water. It is expected that roll amplitudes are not likely to show large variation in the trot, but they have been shown to increase with increasing water depth in the walk as the increased flexion in the hindlimb creates a larger axial rotation (Mooij *et al.*, 2013).

7.1.1 Overground comparisons

The overground work in this study reported pelvic vertical displacements of 66.66 ± 8.76 mm (mean ± 1 SD) which concurs with previous studies of amplitudes of around 60 mm in non-lame horses (Church *et al.*, 2009). Overground withers vertical displacements were reported as 67.71 ± 8.94 (mean ± 1 SD) which agrees with an earlier study that reported withers vertical displacements as a mean of 66.0 ± 12.0 mm (mean ± 1 SD) (Buchner *et al.*, 1994a). This enabled confident comparisons to be made to the work completed in the aqua-treadmill. A small amount of water (water depth at mid P3) had a significant effect on the vertical displacement amplitudes in the pelvis which further supports the theory of tactile stimulation in the hind limbs (Clayton *et al.*, 2008; Clayton *et al.*, 2010) where the water in the present study acts as a very effective tactile stimulator without being of sufficient depth to inhibit the free movement during swing phase of the stride. During trot, horses reduce the amount of work required by the muscles through an exchange of potential and kinetic energy throughout the trajectory of their body's centre of mass. Energy exchange is achieved through the storage and recovery of elastic strain energy in the long tendons and ligaments of the limb (Dimery *et al.*, 1986; Blickhan, 1989; Biewener, 1998; Farley *et al.*, 1993). In the present study, only a small amount of water was required to induce significantly greater vertical displacements in the pelvis, and deeper water depths produced significantly larger vertical displacements at each increasing depth, which were maintained throughout each trial. It is interesting to note here, the differing information from previous studies on the effect of water depth on heart rates that have evidenced that water depth does not have a significant effect on heart rates in walk or trot (Lindner *et al.*, 2003; Scott *et al.*, 2010) indicating that the increased vertical displacement shown here is likely a result of potential and kinetic energy exchange thus limited extra energy requirement. Previous studies on heart rates during walking and trotting in different depths of water have been inconclusive in terms of determining workload for the horse but in deeper water there is the element of the limb being 'pulled' or 'dragged' through the water which is likely to increase the energy requirement and work carried out by the muscles. The additional vertical displacement at low water levels is likely therefore induced by the tactile stimulation effect of the water and achieved through energy exchange in the tendons. The significantly larger vertical displacements were maintained throughout each trial, with no evidence of fatigue, which suggests that also greater elastic storage was maintained throughout each of the trot trials on the aqua-treadmill but that this greater elastic storage was initiated by the presence of water and then amplified with the increasing depth. This does suggest that, along with the tactile stimulation effect of the water and the increased weight of the deeper water, it would reason that there was an increased activation of muscular structures also, therefore deducing that simply water, and then deeper water provides an increased work load to the musculoskeletal system and would therefore have a conditioning and fittening effect on these systems. This is a considerable area for further research which should include work to be carried out on respiratory gasses along with studies on EMG activity to identify muscle activation and potentially also to measure strain in the tendons which could potentially be achieved by implanting a strain gauge. This work would be more invasive but more informative providing a whole picture of achieved workload of different water depths.

Mediolateral displacements in the pelvis and withers were significantly larger when trotting on the aqua-treadmill in a small amount of water than when trotting overground, but increases in water depth made no further significant changes. It is likely that the exertion resulting in increased vertical displacement and associated limb compression on the aqua-treadmill is responsible for the greater mediolateral displacements seen. This theory is further supported by the increased roll amplitude of the withers during aqua-treadmill exercise compared to overground. Despite previous studies concluding that three, five-minute sessions are sufficient to stabilise stride kinematics on a treadmill (Buchner *et al.*, 1994b) an alternative explanation would be that the increased mediolateral displacements shown here were indicative of changes to the balance of the horse on the treadmill belt compared to overground. The horses used in the present study were all habituated to treadmill exercise, but it would be interesting to carry out a longitudinal investigation to determine whether repeated aqua-treadmill use improved balance and reduced mediolateral displacements.

7.2 Effects of side reins

Despite their common application within equine training, when exercising on the aqua-treadmill, side reins had no significant effect on the vertical displacement amplitudes achieved by either the pelvis or withers and side reins had no significant effect on pitch amplitudes or displacement symmetry, leading to the deduction that side reins may be a useful tool in an aqua-treadmill to maintain a straight and correct head and neck position and perhaps to help sharper horses concentrate in their work, but without any detrimental effects to movement patterns and symmetry. It could be expected that alternative muscles were engaged for comparative vertical displacement amplitudes to be achieved both with and without the side reins. Previous studies have concluded that when working horses, lowering the neck and working the horse downwards and forwards increases the movement of the back and strengthens the back muscles (Denoix *et al.*, 2001) and that the hindlimbs become more engaged when the horse has a low head position where the poll is at the same level or lower than the withers and with a wider head/neck angle (Roepstorff *et al.*, 2002). It is also likely that in this long and low position the ventral muscles such as the abdominals and pectorals along with the deeper muscles of the back become more engaged when exercising within an aqua-treadmill as the horse must control their balance and rhythm on the treadmill belt and work to lift themselves up over the water. This promotes development of the desired muscles required for impulsion, engagement and ultimately performance. Other studies have also identified that head and neck position have an effect on stride length and the kinematics of the back (Faber *et al.*, 2002; Johnston *et al.*, 2002; Rhodin *et al.*, 2005; Gomez Alvarez *et al.*, 2006; Rhodin *et al.*, 2009) but stride kinematic variables were not measured in the current study so it is impossible to know if side reins had the same effects as previously seen when exercising through water. Side reins may also be modified to affect or target specific muscle groups in the neck and back but further studies are required, probably using EMG, to determine which muscles are recruited with the use of side reins, and how changes in length and positioning of the side reins, along with changes in water depth affect muscle engagements.

It was suggested that side reins may have had a stabilizing effect on the side to side sway seen in the withers at lower water depths, and although a reduction in mediolateral displacement amplitudes was seen with the addition of side reins at the lower water depths this trend was not found to be significant. This would suggest that again, side reins have no detrimental effect to exercising horses in water but may in fact provide beneficial, stabilising proprioceptive feedback in the form of a contact, encouraging horses to work into the contact as they would under saddle. Interestingly, side reins had the effect of reducing vertical displacements in the withers when trotting overground, to the point that they were significantly lower than that of the pelvis, but without side reins, the pelvis and withers had comparable vertical displacements. This suggests that the side reins had the effect of keeping the head and neck more still and potentially rendering the horse 'downhill'. The effect of water on the aqua-treadmill could therefore serve to counteract this 'downhill' effect in the withers by controlling and straightening the head and neck but without creating the downhill effect that was seen in the significantly lower vertical displacements overground. However, here it is pertinent to acknowledge the importance of speed, where on the aqua-treadmill, speeds were able to be kept constant, but overground, the horses may have been accelerating or decelerating or losing impulsion. Although as far as possible, the overground speeds were matched to those on the aqua-treadmill, but further investigation into the impact of side reins on trot speed would also be useful. The results of this study essentially suggest it may be more beneficial to use side reins in an aqua-treadmill than overground. However, without quantification of the rest of the back movement and the muscles involved, this would be difficult to determine and should therefore be approached with caution. This provides an opportunity for further research, especially since the effects of side reins and other training aids in the literature is under studied.

7.3 Conclusions

This study has succeeded in its overall aim of making a quantification of the locomotory biomechanics of the axial skeleton of the horse when trotting on an aqua-treadmill, using the most state-of-the-art gait analysis technologies. The four chapters of this thesis answered the four main aims which were to; determine the impact of water depth on vertical pelvis and wither displacement while trotting on an aqua-treadmill (Chapter 3), determine the effect of side reins on vertical displacements of the pelvis and withers when trotting on an aqua-treadmill at increasing water depths (Chapter 4), determine the impact of water depth on mediolateral pelvis and wither displacements when trotting on an aqua-treadmill (Chapter 5), and to determine the difference a few centimetres of water makes to pelvis and wither displacements when trotting on the aqua-treadmill compared to overground trotting (Chapter 6). This project has provided useful, quantifiable information which was previously lacking in scientific literature and non-existent with regards to aqua-treadmill studies.

Water was found to have a significant effect on several locomotory parameters. In particular, vertical displacements of the pelvis and withers increased as water depth increased and vertical displacements of the pelvis were significantly greater than those of the withers. A mean (±SEM) of 115.25 (±2.52) mm for the pelvis, compared to a mean (±SEM) of 91.53 (±2.36) mm for the withers. Also, the pelvis exhibited greater minimum and maximum vertical parts of the stride than the withers. These increases in displacement are indicative of greater compression of the limbs and likely therefore, indicative of greater workloads at deeper water depths. Trotting in a small amount of water on an aqua-treadmill induced a mean (±SEM) increase in vertical displacements of the pelvis 28.18 (±4.51) mm compared to trotting overground, believed to be due to tactile stimulation of the water. Side reins had no direct effect on the vertical or mediolateral displacements during aqua-treadmill exercise (with a maximum water depth of mid carpus) so it can be deduced that side reins have no direct impact on the work load and associated changes in parameters measured here, although it is likely that other muscles are engaged and is therefore an area requiring further investigation. The aqua-treadmill may be beneficial in increasing the work load for the horse as it is suggested that changing mechanics are likely to influence loading that could influence strength, endurance and muscle mass but without detrimental effects to cranial-caudal or mediolateral symmetry patterns.

Practical experience of training and exercising horses in conjunction with knowledge of this primary scientific data will enable aqua-treadmill operators to better assist and benefit the horses they are working with whilst also standardising exercise protocols at the same time as personalising the exercise for the individual subject.

7.4 Future research and limitations

It is important to recognise the limitations of any study where appropriate and where suggestions can be made for improvement. Overall, this study has produced some informative primary data but as always, there are areas for improvement. The number of final complete sets of data from the trials was a little disappointing. Initially, for the Qualisys© data collection trial, ten horses were put forward and began the trials, and for the Xsens© data collection period, fifteen horses began the trials, which would have given an overall 25 horses of riding school type all habituated to aqua-treadmill exercise. Unfortunately, due to various reasons, most often equipment malfunction, there were only a final seven sets of complete data from the Qualisys© trials and ten from the Xsens© trials. One horse was withdrawn during the later stages of the exercise protocol due to fatigue, and with one horse in the second last stage of the exercise protocol on the aqua-treadmill, there was a power-cut, meaning that the trial could not be completed. It was deemed important to use only the horses with full and complete sets of data for accuracy in statistical analyses, with each horse being able to act as its own control in each trial. Of course, it would have also been ideal to have been able to select a cohort of horses of all the same breed, type, age and perhaps more specifically, height, due to the variations seen in vertical displacements from different height horses. A further interesting study would be to investigate more fully if height of horse affects the vertical displacement amplitudes they can achieve and therefore the impact of water depth. Although a more comprehensive study on the conformation of the whole horse may be more appropriate as the vertical height a horse can reach during a trot stride is more likely due to its physical make-up, breeding and training.

One paper recognised a specific potential technical issue with the Xsens© system where it is considered that connecting several sensors in series to a single XBus Master in a daisy chain style causes a bias error in the magnetometers, which results in up to a 15° error in heading but only if nine extra IMUs are added as this creates a local magnetic field, but the 'daisy chain' effect can be mitigated by correctly calibrating the sensors (Brodie *et al.*, 2008). However, the issue of magnetic field is possibly very apt in this current study, as data were collected within the confines of the aqua-treadmill environment which is a galvanized steel metal box. It is possible that the metal from the aqua-treadmill itself had an effect on the magnetometers in the inertial measurement units, and possibly the accelerometers and gyroscopes too although to a lesser extent. This may account for the larger roll amplitudes reported in this study compared to overground studies. Roll amplitudes reported overground in this study were larger than in previous literature, but that may be accounted for by the physical transferring of the IMUs from overground to the aqua-treadmill for each horse, and actually the IMUs may need re-calibration after each exposure to the stainless-steel environment of the aqua-treadmill. A validation of Xsens© against Qualisys© was performed on the vertical displacement data (Chapter 2.5), although there do not currently appear to be any reports in the literature validating either piece of gait analysis equipment in the novel environment of an aqua-treadmill. This therefore requires further specific investigation before any fundamental procedural changes to aqua-treadmill exercise should be made based on the output from this study.

A significant area for improvement in this study would be to make a direct comparison between treadmill locomotion on a dry belt and aqua-treadmill locomotion with just a small amount of water. Unfortunately, at the time of data collection, we were misinformed about the operation of the aqua-treadmill and were instructed to ensure that there was always at least a few centimetres of water over the treadmill belt, hence our baseline water depth of mid P3. However, aqua-treadmills can in fact be run with no apparent water on the belt but as the belt is submerged in its own water bath, as long as the belt comes through wet, that is perfectly safe and acceptable operation (Beckmann, 2016, *personal correspondence*). A further, more accurate investigation could then be made into the effects of water on locomotion. It would also be useful to perhaps further validate treadmills in their own right, as there are now several manufacturers of treadmills, including high-speed and aqua-treadmills. There needs to be some standardisation of stated speeds, including calibrations to determine accuracy in maintaining speed, plus some quantification and calibration of the added effect of the weight of the water plus the combined weight of the water and the horse on the speeds of the aqua-treadmill, especially as it has been reported that on a dry high-speed treadmill, the speed of the belt is reduced as much as 9% during stance (Buchner *et al.*, 1994a), so it would be expected that the heavier the weight on the belt the greater the reduction in speed during stance, perhaps dependent upon the power output of the drive motor. Validation and standardisation of treadmill equipment would therefore seem a necessity before further accurate locomotory studies can take place.

During the Xsens© trial, horses were trotted in-hand overground first to collect overground data and to establish an apparent chosen speed of each horse overground. These speeds were matched on the aqua-treadmill for each horse, as previous research has established that the optimum energy preserving system of the spring-mass model is affected by velocity (Blickhan, 1989; Farley *et al.*, 1993). In the Qualisys© trial it was not possible to match the horse's speeds on the aqua-treadmill to their speeds overground as no overground experimental protocol was included due to time constraints of using the equipment. Ideally, this would have taken place, however, the two systems were successfully validated against each other.

Another useful area for investigation, and mentioned previously in this thesis, is the effectiveness and calibration of hoof or distal limb mounted accelerometers. Some previous studies in the literature have employed this methodology, but it would be pertinent to investigate accelerometers in water as there may be some doubt over the efficacy of the data produced as there are likely different forces acting on the accelerometers due to the wash of the water. If hoof or distal limb mounted accelerometers can be validated, then a complementary study with appendicular mounted IMUs as well would be very useful.

This study only employed an exercise protocol that included a water depth of up to mid carpus. It was deemed that the riding school type horses that were used in the study would not be able to manage exercising in deeper water, which is frustrating as many of the previous studies in the literature feature both walking and trotting at very deep water depths such as elbow, point of shoulder and stifle. However, from both personal observation, and now reports in the literature, horses do have a change in locomotion patterns when water becomes so deep that the horse can no longer lift and step over the water but start to have to wade (Mooij *et al.*, 2013; Nankervis *et al.*, 2016). However, these recent studies have so far only investigated walk with the more sophisticated gait analysis technologies leaving scope for further investigations in this area.

Arguably the largest and most understudied area therefore requiring investigation is the long-term effects of aqua-treadmill exercise at different water depths. Longitudinal studies are therefore of imperative importance and should be seen as a priority. As yet, there are no reports in the literature of contra-indications of using an aqua-treadmill, which of course, is positive, but further research must identify specific cases for rehabilitation in an attempt to propose ideal aqua-treadmill protocols to treat specific conditions. This thesis, therefore, is a mere starting point for this type of work having quantified the effect of water depth on certain locomotory parameters and having quantified the potentially beneficial effects of using side reins. It must now be put forward how to best use the knowledge of these locomotory parameters in specific instances.

7.5 Implications and applicability to the equine industry

The implications of this study for the equine industry are useful and important. Quantification of locomotory parameters when exercising through water at the trot are currently unique and add to existing scientific literature that has investigated locomotory parameters at the walk. Significantly, it seems that anyone (with enough funds) can purchase and operate an aqua-treadmill with potentially little or no knowledge of equine locomotion, biomechanics and positive and potential negative effects of different types of exercise in different situations. It has been previously reported in the literature that not all experienced Veterinary Clinicians can agree on lamenesses so it is more likely that a layperson might miss something small, but potentially significant in a horse's way of going on an aqua-treadmill. Thereby, instead of working the aqua-treadmill to alleviate the problem, there is the potential to make a condition worse. Currently, there are no known contra-indications reported in the literature of aqua-treadmill exercise but with the increasing number of aqua-treadmills in the public domain, it is important that all users are educated not only in the best possible practices, to ensure safety of handlers, operators and of course, the horses, but to be educated in how water acts to change a horse's way of going.

Aqua-treadmills are not often purchased and operated by Veterinary Practices and most often not by Veterinary allied professionals either. Producing significant research such as this study, and getting it into peer-reviewed academic literature is a necessity, so that it can be readily available to Veterinary Surgeons and allied scientific professionals so that they can relay this useful information on gait, locomotion and biomechanical features to their clients and colleagues, who can all make educated judgements on how different water depths will most benefit the condition they are trying to treat. It is therefore paramount to produce most of the work of this thesis for publication in peer-reviewed journals now as a matter of priority. There is also an apparent lack in the industry of an appropriate governing body for equine hydrotherapy, where for dogs, the Canine Hydrotherapy Association (CHA, 2018) exists that aids standardisation in procedures and protocols, so the development of an equine equivalent would be highly relevant.

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