Department of Animal Medicine and Surgery.

Equine Sport Medicine Center.

Faculty of Veterinary Medicine, University of Córdoba, Spain

EVALUATION OF THE BIOMECHANICS OF LOCOMOTION IN THE ATHLETIC HORSE BY ACCELEROMETRY

ARITZ SAITUA PENAS

Supervisors:

Dr. Ana Muñoz Juzado

Dr. David Argüelles Capilla

Córdoba, 2022

TITULO: *Evaluation of the biomechanics of locomotion in the athletic horse by accelerometry*

AUTOR: *Aritz Saitua Penas*

© Edita: UCOPress. 2023
Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

https://www.uco.es/ucopress/index.php/es/
ucopress@uco.es

Instituto de Estudios de Postgrado

TÍTULO DE LA TESIS:

EVALUATION OF THE BIOMECHANICS OF LOCOMOTION IN THE ATHLETIC HORSE BY ACCELEROMETRY

(Evaluación de la biomecánica de la locomoción en el caballo atleta mediante acelerometría)

DOCTORANDO/A: ARITZ SAITUA PENAS

INFORME RAZONADO DE LOS DIRECTOR/ES DE LA TESIS

La presente tesis doctoral ha sido realizada bajo la tutela de dos directores pertenecientes al Departamento de Medicina y Cirugía Animal, de la Universidad de Córdoba. La tesis se presenta como un compendio de tres publicaciones, todas ellas publicadas en revistas indexadas, situadas en el primer tercil, según su índice de impacto y en inglés, al optar a mención internacional.

Durante la realización de esta tesis doctoral, el doctorando ha mostrado un gran interés y dedicación al trabajo y ha sido capaz de desarrollar una labor de investigación con rigor científico. Hemos encontrado una importante progresión en cuanto a su capacidad investigadora, además de mejorar su formación clínica, como denota que en la actualidad es residente del Colegio Europeo de Medicina Deportiva Veterinaria y Rehabilitación.

La presente tesis ha generado hasta este momento la siguiente producción científica:

Artículos publicados

Argüelles D, **Saitua A**, Sánchez de Medina A, Muñoz JA, Muñoz A. 2019. Clinical efficacy of clodronic acid in horses diagnosed with navicular syndrome: a field study using objective and subjective lameness evaluation. Res. Vet. Sci. 125: 298-304.

Saitua A, Becero M, Argüelles D, Castejón-Riber C, Sánchez de Medina A, Satué K, Muñoz A. 2020. Combined effects of water depth and velocity on the accelerometric parameters measured in horses exercised on a water treadmill. Animals (Basel.) 10(2):236.

Saitua A, Castejón-Riber C, Requena F, Argüelles D, Calle-González N, Sánchez de Medina A, Muñoz A. 2022. Previous exercise on a water treadmill at different depths affects the accelerometric pattern recorded on a track in horses. Animals 2022, 12, 3086.

Comunicaciones presentadas en congresos

Herrera R, **Saitua A**, Becero M, Castejón-Riber C, Muñoz A. 2019. Longitud de tranco y desplazamiento dorsoventral del centro de gravedad en caballos ejercitados sobre diferentes superficiales, treadmill terrestre y acuático. Congreso de la Asociación de Veterinarios de Équidos AVEE, Alicante, España

Herrera R, **Saitua A**, Becero M, Castejón-Riber C, Riber C, Muñoz A. 2018. Influencia de la superficie en las características acelerométricas del caballo al paso. II Congreso de Veterinaria y Ciencia y Tecnología de los Alimentos, Córdoba, España

Saitua A, Castejón-Riber C, Becero M, Reguera M, Argüelles D, Muñoz A. 2019. Cambios acelerómetros en caballos durante un ejercicio al paso en cinta rodante acuática a diferentes profundidades de agua. II Congreso de Veterinaria y Ciencia y Tecnología de los Alimentos, Córdoba, España

Saitua A, Becero M, Castejón-Riber C, Riber C, Argüelles D, Sánchez de Medina AL, Muñoz A. 2019. Parámetros acelerométricos determinados en miembros torácicos y pelvianos en caballos durante una sesión de ejercicio en treadmill acuático a diferentes profundidades de agua. XXIV Congreso Internacional de la Sociedad Española de Cirugía Veterinaria, Córdoba, España.

Sánchez de Medina AL, **Saitua A,** Argüelles D, Castejón-Riber C, Riber C, Becero M, Muñoz A. 2019. Frecuencia, longitud, simetría y regularidad del tranco cuantificados en miembros torácicos y pelvianos en caballos con síndrome de navicular y caballos sin cojera. XXIV Congreso Internacional de la Sociedad Española de Cirugía Veterinaria, Córdoba, España.

Saitua A, Becero M, Castejón-Riber C, Argüelles D, Sánchez de Medina A, Muñoz A. 2019. Total power measured with accelerometry in horses exercised on a water treadmill at different water depth and velocities. Meeting of the European Society of Veterinary Sport Medicine and Rehabilitation ECVSMR, Ghent, Bélgica.

Saitua A, Becero M, Trueba A, Argüelles D, Sánchez de Medina AL, Castejón-Riber C, Muñoz A., 2019. El entrenamiento en una cinta rodante acuática en caballos aumenta la fuerza muscular durante la locomoción terrestre. IV Congreso Internacional de Medicina Deportiva Equina, Madrid, España

Además, el doctorando ha colaborado en otros trabajos de investigación del grupo AGR-111 (Medicina Deportiva Equina), al cual pertenece. De este modo, ha participado en las siguientes publicaciones:

Muñoz A, **Saitua A**, Becero M, Riber C, Satué K, De Medina AS, Argüelles D, Castejón-Riber C. 2019. The use of the water treadmill for the rehabilitation of musculoskeletal injuries in the sport horses. J. Vet. Res. 63(3): 439-445.

Becero M, **Saitua A**, Argüelles D, Sánchez de Medina A, Castejón-Riber C, Riber C, Muñoz A. 2020. Capacitive resistive electric transfer modifies gait pattern in horses exercised on a treadmill. BMC Vet. Res. 16(1), 236.

Argüelles D, Becero M, Muñoz A, **Saitua A**, Ramón T, Gascón E, Sánchez de Medina A, Prades M. 2020. Accelerometric changes before and after capacitive resistive electric transfer therapy in horses with thoracolumbar pain compared to a sham procedure. Animals (Basel.), 10(12), 2305.

Ha participado en las siguientes comunicaciones a congresos:

Becero M, **Saitua A**, Reguera M, Castejón-Riber C, Herrera R, Muñoz A. 2018. Ejercicio en treadmill acuático en la rehabilitación de un caballo con tendinopatía del flexor digital superficial. II Congreso de Veterinaria y Ciencia y Tecnología de los Alimentos, Córdoba, España.

Reguera M, Becero M, **Saitua A**, Argüelles D, Sánchez de Medina A, Muñoz A. 2018. Cambios en la longitud de tranco en caballos durante un ejercicio en treadmill al paso tras la aplicación de una terapia de transferencia eléctrica capacitiva resistiva. II Congreso de Veterinaria y Ciencia y Tecnología de los Alimentos, Córdoba, España.

Becero M, **Saitua A**, Argüelles D, Sánchez de Medina A, Castejón-Riber C, Muñoz A. 2018. Efectos de la transferencia eléctrica capacitiva resistiva en la locomoción del caballo. Congreso Internacional de Medicina y Cirugía Equina. Sevilla, España. Premio al mejor póster en investigación experimental del congreso.

Vida T, Castejón-Riber C, Riber C, **Saitua A**, Becero M, Méndez-Angulo JL, Roldán J, Muñoz A. 2018. Ejercicio en cinta rodante acuática en la rehabilitación de un caballo con osteoartritis múltiple crónica. Congreso Internacional de Medicina y Cirugía Equina. Sevilla, España.

Sánchez de Medina AL, **Saitua A**, Argüelles D, Castejón-Riber C, Riber C, Becero M, Muñoz A. 2019. Frecuencia, longitud, simetría y regularidad del tranco cuantificados en miembros torácicos y pelvianos en caballos con síndrome de navicular y caballos sin cojera. XXIV Congreso Internacional de la Sociedad Española de Cirugía Veterinaria. Córdoba, España.

Becero M, **Saitua A**, Castejón-Riber C, Argüelles D, Sánchez de Medina A, Muñoz A. 2019. Frecuencia, longitud, simetría y regularidad del tranco cuantificados en miembros torácicos y pelvianos en caballos con síndrome de navicular y caballos sin cojera. XXIV Congreso Internacional de la Sociedad Española de Cirugía Veterinaria. Córdoba, España.

Becero M, **Saitua A**, Castejón-Riber C, Argüelles D, Sánchez de Medina A, Muñoz A. 2019. Changes in the kinematic parameters of the stride in horses after two sessions of capacitive resistive electric transfer applied two consecutive days. Meeting of the European Society of Veterinary Sport Medicine and Rehabilitation ECVSMR, Ghent, Bélgica.

Sánchez de Medina A, Muñoz A, **Saitua A,** Becero M, Serrano-Rodríguez JM. 2019. El benaceprilo oral modifica el equilibrio simpático-parasimpático durante las primeras 8 horas post-administración en caballos normotensos. IV Congreso Internacional de Medicina Deportiva Equina. Madrid, España.

Becero M, **Saitua A**, Argüelles D, Sánchez de Medina AL, Muñoz A. 2019. Los parámetros espaciotemporales del tranco se modifican tras una sesión de transferencia eléctrica capacitiva resistiva en caballos ejercitados en pista y en treadmill. IV Congreso Internacional de Medicina Deportiva Equina. Madrid, España.

Becero M, **Saitua A**, Argüelles D, Luna-Correa P, Muñoz A. 2021. Total power and velocity before and after radiofrequency at 448 kHz in Spanishbred dressage horses performing collected, working, medium and extended trot. Meeting of the European Society of Veterinary Sport Medicine and Rehabilitation ECVSMR, online.

Cobo M, Luna-Correa P, Becero M, **Saitua A**, Argüelles D, Sánchez de Medina A, Muñoz A. 2021. Modificaciones en la temperatura superficial de la región metacarpiana en caballos tras una sesión de crioterapia. III Congreso de Veterinaria y Ciencia y Tecnología de los Alimentos, Córdoba, España.

Muñoz-García C, Miró F, Becero M, Argüelles D, **Saitua A**, Luna-Correa P, Muñoz A. 2021. Cambios termográficos en la región toracolumbar equina tras la aplicación de diversos protocolos de transferencia eléctrica capacitiva resistiva. Congreso Internacional de Medicina y Cirugía Equina. Sevilla, España.

Becero M, Luna-Correa P, Argüelles D, **Saitua A**, Muñoz A. 2021. Mejora de las características locomotoras del trote reunido, passage y piaffé en caballos PRE de doma clásica antes y después de la aplicación de una terapia de radiofrecuencia. Congreso Internacional de Medicina y Cirugía Equina. Sevilla, España.

Saitua A, Argüelles D, Calle N, Nocera I, Vitale V, Sgorbini M, Díez-Carrera JC, Muñoz A. 2022. Application of a capacitive resistive electric transfer therapy 24 hours before exercise

increases velocity and accelerometric activity in Standardbred trotters. Meeting of the European Society of Veterinary Sport Medicine and Rehabilitation ECVSMR, Pula, Croacia.

Calle-González N, Miró F, Argüelles D, **Saitua A**, Valladares L, Muñoz A. 2022. Skin termographic changes in the thoracolumbar region of a horse, induced by the application of a capacitive resistive electric transfer therapy. Meeting of the European Society of Veterinary Sport Medicine and Rehabilitation ECVSMR, Pula, Croacia.

Además, el doctorando ha tenido una implicación muy activa en la dirección de trabajos fin de grado y de master, cooperando con sus directores. Ha codirigido los siguientes trabajos:

Trabajo de fin de grado

Actualización sobre osteocondritis disecante en el caballo de deporte. 2020. Autor/a: Lourdes Pérez Peláez. Directores: Cristina Riber Pérez y Aritz Saitua Penas.

Comparación entre estimuladores sensitivos, inerciales y cavaletis en caballos. 2020. Autor/a: Miguel Ramos Iglesias. Directores: Ana Muñoz Juzado y Aritz Saitua Penas.

Rehabilitación en caballos de deporte con terapia de ondas de choque. 2021. Autor/a: Alejandro Pérez Pérez. Directores: David Argüelles Capilla y Aritz Saitua Penas.

Guía diagnóstica de lesiones tendoligamentosas en el caballo de deporte. 2021. Autor/a: Santiago Rodríguez Ortiz. Directores: Cristina Riber Pérez y Aritz Saitua Penas.

Problemas de dorso en el caballo de deporte. 2021. Autor/a: Irene Martínez Rubio. Directores: David Argüelles Capilla y Aritz Saitua Penas.

Update in the latest treatment options in acute laminitis. 2022. Autor/a: Claudia Valero Esteve. Directores: David Argüelles Capilla y Aritz Saitua Penas.

Evaluación locomotora derivada del entrenamiento de la musculatura “core” a corto y largo plazo en el caballo. 2022. Autor/a: Joaquín Pérez Umbría. Directores: Ana Muñoz Juzado y Aritz Saitua Penas.

Desviaciones en las extremidades en potros. Diagnóstico y tratamiento. 2022. Autor/a: Natalia González Tornel. Directores: David Argüelles Capilla y Aritz Saitua Penas.

Trabajo de fin de máster

Hallazgos patológicos en vías aéreas superiores mediante endoscopia dinámica en el caballo PRE de doma y su relación con la posición de la cabeza y el cuello. 2021. Autor/a: Azahara Camacho Martín. Directores: David Argüelles Capilla y Aritz Saitua Penas.

Efecto locomotor de la movilización dinámica en caballos. 2022. Autor/a: Karelhia Cristina García Álamo. Directores: Ana Muñoz Juzado y Aritz Saitua Penas.

Debido al rigor científico, calidad de la tesis, formación científica y labor realizada por el doctorando, altamente satisfactoria, tanto de investigación, como de formación docente en los trabajos fin de grado y de máster y clínica, se autoriza la presentación de la presente tesis doctoral.

Córdoba, diciembre 2022

Fdo.: Ana Muñoz Juzado

Ana María Muñoz Juzado

Firmado digitalmente por Ana María Muñoz Juzado
Fecha: 2022.12.08 12:13:27 +01'00'

Fdo: David Argüelles Capilla

Firmado por ARGUELLES CAPILLA DAVID - 44178480B el día 08/12/2022 con un certificado emitido por AC FNMT Usuarios

"Slippery-smooth rhytmic motion, absolute single-minded purpose, motion for the pleasure of motion itself. It was terrible in its beauty, the flight of the horse"

Laurence van Cott Niven

A mis padres, Ana y Joseba.
A mis hermanos, Ziortza y Unai.
Y mis sobrinos, Malen y Zuhaitz.
Gracias.

AGRADECIMIENTOS:

Este trabajo solo ha sido posible gracias al trabajo en equipo y al trabajo guiado por un buen líder.

Gracias Ana por toda tu ayuda en todo momento y por todo tu trabajo para que esto haya sido posible. Por hacerme disfrutar de otra manera de los caballos y por enseñarme tanto durante este tiempo. Es un placer poder contar contigo como mentora y aprender de ti. Gracias por confiar en mí.

Gracias David por todo tu ayuda y por tu apoyo cada día, por enseñarme, por guiarme y hacerme crecer profesionalmente. Gracias por creer en mí.

A mis padres, Ana y Joseba, y a mi hermana, Ziortza, que siempre están pendientes de mí y me dan fuerzas para seguir adelante. A Javi, que también me ha apoyado desde muy pequeño en esta locura de ser veterinario. A mi tío Jon, por hacer crecer en mí la pasión por los caballos. También a mi prima Oihane, que ha compartido conmigo el camino de la ciencia. A Julio, por ser mi apoyo cada día, quererme y cuidarme. Y por hacer que me embarcara en esto de ser doctor. A Mireya, por toda su ayuda y el trabajo con los caballos durante el desarrollo experimental. Por ser como mi hermana pequeña y un pilar fundamental en mi vida. A Pablo, por hacer que todo sea fácil y sacarme una sonrisa hasta en mis peores días. A Raquel, por aguantarme y por estar ahí para todo lo que necesito, en lo profesional y en lo personal. Aunque seamos como el perro y el gato, no sabemos vivir el uno sin el otro. A Toñi, que desde que empecé mi andadura como veterinario me ha enseñado y me ha guiado en este camino. A Elisa y Antonio, Sete, Esther, Ana, Pablo, Fernando, Inma, Toñi y el resto de mis compañeros del HCV. A mis amigos Migue y Sabina, que son muy importantes para mí en Córdoba.

A Cristina y a Francisco, por todo lo que me han enseñado y los momentos vividos, en especial, los buenos ratos que hemos pasado en los raids. A Manu, María, Marta, Joaquín, Clara e Irene, por acogerme en el CEMEDE cuando llegué a Córdoba, por todos los momentos que hemos vivido juntos. A Rafi, Alejandra Tamayo, Ana Trueba, Teresa Vida y otros muchos alumnos del CEMEDE que han participado activamente y me han ayudado en el desarrollo de los estudios.

Gracias a toda esa gente que ha hecho que esto sea posible, porque las cosas se consiguen con esfuerzo y sacrificio, pero para ello es muy importante tener a gente que te arrope y confíe en ti.

ETHICS AND WELFARE ANIMAL STATEMENTS

This research has been performed in accordance with the rules of the Bioethics and Biosafety Committee for Animal Experimentation of the University of Córdoba.

The studies I and III included privately-owned horses, submitted for diagnosis and treatment of lameness to the Veterinary Clinical Hospital of the University of Córdoba (study I) and for training and fitness improvement in the Equine Sport Medicine Center (CEMEDE) of the same University (study III). The owners were informed about the procedures, the benefits and risks and they formally signed the inclusion of their horses in the study. Study II included horses used for researching and teaching equine exercise physiology and sport medicine at the CEMEDE.

The studies II and III were also approved by the Ethics Committee for Animal Welfare of the Veterinary Clinical Hospital of the University of Córdoba (Ref: 41/2019).

INDEX:

1. GENERAL INTRODUCTION TO THE STUDY………………………………………………………..1

2. OBJECTIVES AND HYPOTHESYS……………………………………………………………………..6

3. LITERATURE REVIEW…………………………………………………………………………………….8

3.1 INTRODUCTION TO THE LOCOMOTION IN THE HORSE…………………………………8

3.1.1. Terminology and definitions………………………………………………………………..8

3.1.2. Equine gait description……………………………………………………………………….9

3.2. KINEMATIC ANALYSIS……………………………………………………………………………11

3.2.1. Techniques for kinematic analysis………………………………………………………11

3.3.2. Kinematic parameters in the evaluation of equine lameness…………………..13

3.3. KINETIC ANALYSIS…………………………………………………………………………………16

3.3.1. Techniques for kinetic analysis…………………………………………………………..16

3.3.2. Kinematic parameters in the evaluation of equine lameness…………………..19

3.4. ACCELEROMETRY…………………………………………………………………………………20

3.4.1. Use of accelerometry to evaluate locomotion in performance horses…….20

3.4.1.1. Use of accelerometry in racehorses (thoroughbreds and trotters)……….24

3.4.1.2. Use of accelerometry in show jumpers…………………………………………28

3.4.1.3. Use of accelerometry in dressage horses……………………………………..32

3.4.1.4. Use of accelerometry in endurance horses…………………………………..34

3.4.1.5. Use of accelerometry to evaluate lameness…………………………………37

3.4.1.6. Use of accelerometry to evaluate ataxia………………………………………39

4. INDIVIDUAL STUDIES……………………………………………………………………………….41

5. STUDY I. Clinical efficacy of clodronic acid in horses diagnosed with navicular syndrome: a field study using objective and subjective lameness evaluation……43

5.1. INTRODUCTION……………………………………………………………………………………44

5.1.1. Navicular syndrome or disease…………………………………………………………..44

5.1.1.1. Anatomy of the navicular bone and associated structures…………………44

5.1.1.2. Pathophysiological theories of the navicular syndrome……………………..45

5.1.1.3.Clinical characterization of the navicular syndrome……………………………47

5.1.1.4. Diagnosis of the navicular syndrome……………………………………………49

5.1.1.5. Management and treatment of the navicular syndrome……………………..52
5.1.2. Bisphosphonates in the horse…………………………………………………………….55
5.1.2.1. History and development of bisphosphonates……………………………………55
5.1.2.2. Structure and biochemical features of bisphosphonates……………………..57
5.1.2.3. Pharmacodynamics…………………………………………………………………………..58
5.1.2.4. Pharmacokinetics……………………………………………………………………………..60
5.1.2.5. Biphosphonates in equine medicine…………………………………………………62
5.1.2.6. Side effects and concerns of the use of bisphosphonates in equine medicine……………………………………………………………………………………………………66
5.2. OBJECTIVES AND HYPOTHESIS…………………………………………………………….67
5.3. MATERIAL AND METHODS……………………………………………………………………67
5.3.1. Horses' selection: inclusion and exclusion criteria. Diagnostic procedure…………………………………………………………………………………………………67
5.3.2. Accelerometric evaluation and accelerometric parameters……………………69
5.3.3. Statistical analysis…………………………………………………………………………………69
5.4. RESULTS…………………………………………………………………………………………………70
5.4.1. Clinical examination………………………………………………………………………………70
5.4.2. Radiographic results………………………………………………………………………………71
5.4.3. Magnetic resonance imaging results………………………………………………………71
5.4.4. Accelerometric results……………………………………………………………………………71
5.5. MAIN CONCLUSIONS……………………………………………………………………………75
6. STUDY II. Combined effects of water depth and velocity on the accelerometric parameters measured in horses exercised on a water treadmill………………………76
6.1. INTRODUCTION………………………………………………………………………………………77
6.1.1. A brief overview of the exercise on the water in the sport horse……………77
6.1.2. Proposed mechanisms of action of the exercise on water: physical properties of the water…………………………………………………………………………………79
6.1.3. Physiological adaptations to exercise and training on water in the horse………83
6.1.3.1. Respiratory adaptations to exercise and training on water………………….83
6.1.3.2. Cardiovascular adaptations to exercise and training on water…………….85
6.1.3.3. Metabolic adaptations to exercise and training on water……………………86

6.1.3.4. Locomotor adaptations to exercise and training on water……………………88
6.1.3.5. Changes in back kinematic associated with exercise on water……………..90
6.1.4. Use of the water treadmill to rehabilitate injured horses………………………..91
6.2. OBJECTIVES AND HYPOTHESES…………………………………………………………………..95
6.3. MATERIAL AND METHODS…………………………………………………………………………95
6.3.1. Horses……………………………………………………………………………………………………..96
6.3.2. Exercise on the water treadmill…………………………………………………………………96
6.3.3. Accelerometry……………………………………………………………………………………………96
6.3.4. Statistics…………………………………………………………………………………………………..98
6.4. RESULTS……………………………………………………………………………………………………..98
6.4.1. Summary of the results of trial A…………………………………………………………..106
6.4.2. Summary of the results of trial B…………………………………………………………..108
6.5. MAIN CONCLUSIONS………………………………………………………………………………..109
7. STUDY III. Previous exercise on a water treadmill at different depths affects the accelerometric pattern recorded on a track in horses……………………………………111
7.1. INTRODUCTION………………………………………………………………………………………..112
7.2. OBJECTIVES AND HYPOTHESES…………………………………………………………………113
7.3. MATERIAL AND METHODS……………………………………………………………………….114
7.3.1. Horses……………………………………………………………………………………………………114
7.3.2. Exercise on the water treadmill………………………………………………………………114
7.3.3. Accelerometric device, evaluation and parameters………………………………115
7.3.4. Statistics………………………………………………………………………………………………..115
7.4. RESULTS……………………………………………………………………………………………………116
7.4.1. Accelerometric parameters……………………………………………………………………116
7.4.2. Stride coordination parameters……………………………………………………………..123
7.4.3. Spatiotemporal parameters of the stride…………………………………………………125
7.5. MAIN CONCLUSIONS………………………………………………………………………………..129
8. GENERAL DISCUSSION………………………………………………………………………………130
8.1. The use of the accelerometer Equimetrix to monitor changes in lameness in horses diagnosed of navicular before and after clodronic acid administration…………………………………………………………………………………………………132

8.1.1. Clinical improvement after clodronic acid administration in horses diagnosed with navicular syndrome……………………………………………………………..133
8.1.2. Changes in the parameters measured with the accelerometer Equimetrix in horses diagnosed with navicular syndrome after clodronic acid administration…………………………………………………………………………………………….134
8.1.3. Limitations of the study……………………………………………………………………….136
8.2. The use of the accelerometer Equimetrix to assess the locomotor pattern in horses exercised on a water treadmill at different water depth………………………137
8.2.1. Changes in total accelerometric activity during an exercise on a water treadmill at different water depths……………………………………………………………….137
8.2.2. Changes in the accelerometric activity in the three body axes during an exercise on a water treadmill at different water depths…………………………………139
8.2.3. Changes in the coordination parameters of the stride during an exercise on a water treadmill at different water depths………………………………………………….141
8.2.4. Changes in the spatiotemporal parameters of the stride during an exercise on a water treadmill at different depths……………………………………………………….142
8.2.5. Limitations of the study……………………………………………………………………….142
8.3. The use of the accelerometer Equimetrix to assess how exercise on a water treadmill at different water depths influences locomotion on a track in horses………..143
8.3.1. Changes in total accelerometric activity on a track after water treadmill exercise at different water depths……………………………………………………………..143
8.3.2. Changes in the accelerometric activity in the three body axes on a track after water treadmill at different water depths……………………………………………144
8.3.3. Changes in the coordination parameters of the stride on a track after water treadmill exercise at different water depths…………………………………………145
8.3.4. Changes in the spatiotemporal parameters of the stride on a track after water treadmill exercise at different water depths…………………………………………146
8.3.5. Limitations of the study……………………………………………………………………….147
9. CONCLUSIONS………………………………………………………………………………………….148
10. RESUMEN……………………………………………………………………………………………….150
11. SUMMARY………………………………………………………………………………………………153

12. REFERENCES……………………………………………………………………………………………156

TABLE INDEX:

Table 1. Definitions of different terms used in equine locomotion (modified from Brownwyn, 2014)………………………………………………………………………………………..9

Table 2. Definitions of different accelerometric measurements performed by the accelerometer Equimetrix® (modified from Frigerio et al., 2019)………………………22

Table 3. Median and quartiles of lameness scoring according to the criteria established by AAEP in 11 horses diagnosed with navicular syndrome……………..70

Table 4. Median and quartiles of the accelerometric parameters measured at walk in the 6 horses diagnosed of navicular syndrome that had clinical improvement , before treatment (baseline), and after 7 (control-1), 30 (control-2) and 90 (control-3) days after intramuscular administration of a single dose of clodronic acid (different letters indicate significant differences at p<0.05 between baseline and controls performed at defined time after treatment)………………………………………72

Table 5. Brief summary of the results found at walk in six horses that had a clinical improvement during the study……………………………………………………………………73

Table 6. Median and quartiles of the accelerometric parameters measured at trot in the 6 horses diagnosed of navicular syndrome that had clinical improvement , before treatment (baseline), and after 7 (control-1), 30 (control-2) and 90 (control-3) days after intramuscular administration of a single dose of clodronic acid (different letters indicate significant differences at p<0.05 between baseline and controls performed at defined time after treatment)……………………………………..73

Table 7. Brief summary of the results found at trot in six horses that had a clinical improvement during the study……………………………………………………………………74

Table 8. Means and standard deviations of the dorsoventral, longitudinal y mediolateral accelerometric activities expressed as percent of the total

accelerometric activity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05……………………………………………………………………………………………………….103

Table 9. Means and standard deviations of the stride symmetry and regularity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05……………………………………………….103

Table 10. Summary of the results found in trial A with the accelerometer in PECT position (p<0.05)……………………………………………………………………………………….106

Table 11. Summary of the results found in trial A with the accelerometer in SML position (p<0.05)……………………………………………………………………………………….107

Table 12. Summary of the results found in trial B with the accelerometer in SML position (p<0.05)……………………………………………………………………………………….108

Table 13. Median and quartiles (in brackets) of the percent of change from baseline of the dorsoventral displacement measured on a track after WT exercise at four different water depths, at walk and at trot and the accelerometer in two different positions (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05…………………………….121

Table 14. Summary of the main results of the accelerometric parameters measured on a track, comparing before and at 30 min after exercise sessions on a WT at different water levels (WW, without water; FET: water at the level of the fetlock; CAR: water at the level of the carpus; STF: water at the level of the stifle), with the accelerometer in pectoral (PECT) and sacrum midline (SML) position……………………………………………………………………………………………………..122

Table 15. Median and quartiles (in brackets) of the percent of change from baseline of the stride regularity measured on a track after WT exercise at four different water depths, at walk and at trot and the accelerometer in two different positions (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05……………………………………………………….123

Table 16. Median and quartiles (in brackets) of the percent of change from baseline of the stride symmetry measured on a track after WT exercise at four different water depths, at walk and at trot and the accelerometer in two different positions (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05……………………………………………………….124

Table 17. Summary of the main results of the stride's coordination parameters measured on a track, comparing before and at 30 min after exercise sessions on a WT at different water levels (WW, without water; FET: water at the level of the fetlock; CAR: water at the level of the carpus; STF: water at the level of the stifle), with the accelerometer in pectoral (PECT) and sacrum midline (SML) position……………………………………………………………………………………………………..125

Table 18. Summary of the main results of the stride spatiotemporal parameters measured on a track, comparing before and at 30 min after exercise sessions on a WT at different water levels (WW, without water; FET: water at the level of the fetlock; CAR: water at the level of the carpus; STF: water at the level of the stifle),

with the accelerometer in pectoral (PECT) and sacrum midline (SML) position……………………………………………………………………………………………………..128

FIGURE INDEX:

Figure 1. Example of dorsoventral acceleration recording of the approach, jump and move off strides. From Barrey and Galloux (1997)…………………………………….30

Figure 2. Schematic representation of the positions of the accelerometer (left: on the pectoral region; right: on the sacrum midline). Accelerometer represented in orange……………………………………………………………………………………………………97

Figure 3. Means and standard deviations (indicated in whiskers) of the total accelerometric activity measured in horses exercised on a WT at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05……………………………………………………………………………………………………….99

Figure 4. Means and standard deviations (in whiskers) of the dorsoventral accelerometric activity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05……………………………………………………………………………………………………100

Figure 5. Means and standard deviations (in whiskers) of the dorsoventral displacement in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05……………………………………………………………………………………………………….100

Figure 6. Means and standard deviations (in whiskers) of the longitudinal accelerometric activity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05……………………………………………………………………………………………………….101

Figure 7. Means and standard deviations (in whiskers) of the mediolateral accelerometric activity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05……………………………………………………………………………………………………….102

Figure 8. Means and standard deviations (in whiskers) of the stride frequency in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between

water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at $p<0.05$……………………………………………….105

Figure 9. Means and standard deviations (in whiskers) of the stride length in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at $p<0.05$……………………………………………….105

Figure 10. Median and quartiles of the percent of change from baseline of the total accelerometric activity, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) $p<0.05$……………………………………………………………………………………116

Figure 11. Median and quartiles of the percent of change from baseline of the total accelerometric activity, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) $p<0.05$…………………………………………………………………………………..117

Figure 12. Median and quartiles of the percent of change from baseline of the total accelerometric activity, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) $p<0.05$…………………………………………………………………………………..117

Figure 13. Median and quartiles of the percent of change from baseline of the total accelerometric activity, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05……………………………………………………………………………………….118

Figure 14. Median and quartiles of the percent of change from baseline of the dorsoventral, longitudinal and mediolateral accelerometric activity, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05………………………………..119

Figure 15. Median and quartiles of the percent of change from baseline of the dorsoventral, longitudinal and mediolateral accelerometric activity, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05………………………………..119

Figure 16. Median and quartiles of the percent of change from baseline of the dorsoventral, longitudinal and mediolateral accelerometric activity, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05………………………………..120

Figure 17. Median and quartiles of the percent of change from baseline of the dorsoventral, longitudinal and mediolateral accelerometric activity, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05………………………………..120

Figure 18. Median and quartiles of the percent of change from baseline of the velocity, stride frequency and length, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05……………………………………………………………………………………126

Figure 19. Median and quartiles of the percent of change from baseline of the velocity, stride frequency and length, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05……………………………………………………………………………..126

Figure 20. Median and quartiles of the percent of change from baseline of the velocity, stride frequency and length, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05……………………………………………………………………………………127

Figure 21. Median and quartiles of the percent of change from baseline of the velocity, stride frequency and length, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05……………………………………………………………………………………128

1. General introduction to the study

The horse is a super athlete, being one of the characteristics involved in this excellence, the locomotion. From a biological point of view, locomotion can be considered as the ultimate mechanical expression of exercise activity. In order to sustain an exercise activity, the horse requires a synergic and synchronic functionality of several systems that are functionally linked and regulated by the nervous system. Thus, the cardiovascular and respiratory systems provide nutrients and oxygen to the contracting muscles during exercise. Inside the muscle fibers, biochemical energy is transformed into mechanical work. The complex organization of the locomotor system under neurosensorial control makes possible to use the individual muscle contractions to move the limbs to support and to propel the body. Biochemically, locomotion involves moving all the body and limb segments in rhythmic and automatic patterns which define various gaits. Therefore, the movement of the horse can be explained by mechanical laws.

Many different papers for decades of research have focused on the relationship between locomotion and sport in a variety of equestrian competitions. Furthermore, in horse breeding, selection has traditionally been based on evaluation of gait. In racehorses, maximum gallop velocity is pivotal for success. It has been demonstrated that best gallopers have a higher stride frequency (SF), stride regularity (REG) and diagonal dissociation (Witte et al., 2006; Barrey, 2013). A quick gallop acceleration at the start of a race is achieved initially by a high rate of SF increase and after, by stride lengthening (Barrey, 2013). During a race, a gallop acceleration is required at the finish to win and consequently, horses that are able to increase their SF to the highest values have a better chance to win. Cheetham et al. (2010), in a cross-sectional study using racing performance data for Thoroughbred horses aged 2, 3, 4 and 5 years and raced in the United States found that gait was one factor associated with earnings.

Possibly, one of the equestrian disciplines in which the study of the locomotor pattern is more intrinsically linked to performance is dressage. Descriptive reports of the different types of gait, i.e., walk, different types of trot (collected, medium, working), collection, piaffé, passage, transitions between gaits…. have been extensively published (Argue and Clayton, 1993; Clayton, 1993; 1994; Hölstrom et al., 1995; Weishaupt et al., 2009; Clayton and Hobbs, 2019). This abundant information is because performance in dressage is measured by a score awarded by the judges in relation to the ability to perform determined movements by a horse-rider combination. An excellent scoping review of the locomotor determinants of performance in dressage has been recently published by Hobbs et al., (2020).

Because of the importance of the locomotion in overtaking an obstacle, jumping is another well-studied equine sport evaluated in this context (Santamaría et al., 2004; Hernlund et al., 2010; Clayton et al., 2021). In order to clear an obstacle without fault, the shape, height and width of the obstacle dictate the characteristics of the ballistic flight of the horse's center of gravity and the trajectories of the limbs and the hooves. The successful jump involved three main biomechanical factors: 1) The distance between the hindlimb hooves to the base of the obstacle at the take-off (Barrey et al., 1993; Van den Bogert et al., 1994); 2) The external force impulses developed by the fore- and hindlimbs at take-off (Schamhart et al., 1993); and 3) The rotational characteristics of the body around the center of gravity in the sagittal plane (Barrey and Galloux, 1997; Galloux and Barrey, 1997).

But the study of equine locomotion is not only essential in order to achieve the maximum sport performance. Lameness is arguably the most important medical problem in horses. In one survey, limb lameness accounted for the first most frequently reported health problems in horses (Keegan, 2007). According to Keegan (2007), lameness has the highest annual incidence density of all medical problems in horses, with one half of all horse operations of 5 or more horses experiencing one or more cases of lameness per year. Assuming approximately 9.2 million horses in the United States and an average of 432 $ spent on veterinary services per lameness episode, it can be estimated that horse owners spend between 325 and 544 million of $ per year on the diagnosis and treatment of lameness. In Spain, we have not such detailed data, but the study commissioned by the Spanish Equestrian Federation (FHE, Federación Hípica Española) to the Deloitte company in 2011, showed that the highest veterinary economic cost for the general Spanish horse population was made up of lameness, colic and reproductive problems, although lameness was the top economic cost for sport horses. In addition to the economic costs, lameness causes loss of performance, loss of days of training, disqualification from competitions and in extreme cases, they can even trigger the withdrawal of the animal from the competition (Dyson, 2000; Muñoz et al., 2006; Preston et al., 2008; Egenvall et al., 2013; Swor et al., 2019; Paris et al., 2021).

Until about 10 years ago, locomotor study was a complex task to do, it required very specific and expensive equipment, only available in specialized labs, and study methods were tedious and time- and personal-consuming. Therefore, the techniques to assess locomotor patterns were not directly available to the equine clinician, being restricted to specialized centers. In fact, the use of high-speed cameras tracking markers glued on the skin over standardized anatomical locations has been the most used procedure for studying locomotion (Fredrickson et al., 1980; Drevemo and Jonhson, 1993). A great advance was the development of these techniques to be used in the field, on a track, where the horse usually works, competes and trains (Degueurce et al., 1996).

After high-speed cameras, force and pressure platforms were developed, although force platforms are based on the study of kinetic or force parameters. Stationary force plates or platforms are accepted kinetic instruments for the quantitative evaluation of equine lameness (Bertone, 2003; Ishihara et al., 2009; Oosterlinck et al., 2009). These force platforms are nowadays considered the ‘gold standard’ for measuring lameness. However, despite their recognized accuracy, the use of the force platforms is restricted to gait analysis under laboratory environment as procedures are laborious and costly. Moreover, capturing enough valid (strike of the hoof in the center of the plate) and pure (no other limbs striking at the same moment) of the limb of interest with the horse moving within a small range of accepted velocities is difficult and time-consuming. Many attempted trials do not result in a valid strike (Keegan, 2007).

In an attempt to make easy and practical the evaluation of locomotion in horses, portable pressure plates have been evaluated (Oosterlinck et al., 2009). These authors concluded that stand-alone pressure plates can be used to measure some temporal variables of the stride and loading symmetry ratios. They offer to the equine clinician a mobile, cost-efficient and quick gait evaluation method for routine clinical use. Even so, the pressure plate cannot be used interchangeably with a force plate to measure most of the variables of the stride (Oosterlinck et al., 2009).

In 2004, Keegan et al. were the first authors to evaluate a sensor-based system of motion analysis for detection and quantification of forelimb and hindlimb lameness in horses. They compared a sensor-based accelerometer gyroscopic system with the traditional video-based motion analysis system, and found a good correlation between both methods (r= 0.954 for forelimbs and r=0.824 for hindlimbs). Since then, these authors have published more information about this system and have improved its benefits (Keegan et al., 2011; McCracken et al., 2012; Reed et al., 2020). Other similar systems have developed lastly or are in development at the moment, including smart-phone based systems (Pfau et al., 2017; Manurova et al., 2021). Most of these systems focused on symmetry of movement and therefore, they are a helpful tool to diagnose and follow-up of lameness.

However, the objectives of some studies (both practical and experimental) in equine sport medicine are the evaluation of the proper gait characteristics of a horse for a determined equine discipline. With this objective in mind, Eric Barrey and his team developed an accelerometer, which was fixed externally in the horse, in the sternal area and in the sacrum midline in order to assess locomotor pattern. They applied this system to evaluate the technique in jumping horses (Barrey and Galloux, 1997), galloping racehorses (Barrey et al., 2001), trotters (Leleu et al., 2002; 2004), dressage (Barrey et al., 2002), and endurance horses (Cottin et al., 2010). This accelerometer (Equimetrix®) has also been used for lameness detection (Barrey and Desbrosse, 1996; Weishaupt et al., 2001) and for quantification of ataxia induced by different drugs (López-Sanromán et al., 2012; 2013; 2014; 2015; 2021; Frigerio et al., 2019). We have used this device to quantify locomotion after the application of an electrophysical agent, radiofrequency (Becero et al., 2020).

The present research was designed in order to increase our knowledge about the use of this 3-D accelerometer device in horses. To reach this main objective, we have planned three main studies, related to the use of this accelerometer, in order to make a follow-up of a treatment for a determined locomotor problem (navicular disease/syndrome) and for assessment of the locomotor changes induced by the exercise on a water treadmill (WT) at different water depths and on a training track.

2. Objectives and Hypothesis

The present research has been performed in order to expand our knowledge regarding the use and applications of the accelerometry in the horse, using the device Equimetrix®, as a useful method to quantify locomotion patterns under different health and lameness situations.

The following specific objectives were proposed:

First objective. ®To assess the use of an accelerometric system in the objective evaluation of the intensity of lameness in horses diagnosed with navicular syndrome as well as in the quantification of the improvement experienced after treatment; in this case, by intramuscular administration of clodronate (first paper)

Second objective. To study how the accelerometric pattern varies in horses exercised on a water treadmill under four different situations (without water, and with the water at the levels of the fetlock (metacarpophalangeal joint), carpus and stifle and at two different walking velocities (second paper).

Third objective. To analyze if the changes in the accelerometric pattern that happen in horses exercised on a water treadmill are maintained during terrestrial locomotion, determining which of the water depth/s is/are better to simulate the locomotor adaptations occurred on the water treadmill (third paper).

The following hypotheses will be checked:

First hypothesis: That horses diagnosed with navicular syndrome and treated with clodronate would have an elongation of the stride, together with an increase in stride regularity and symmetry and accelerometric activities.

Second hypothesis: That horses exercised on a water treadmill would have a greater total accelerometric activity, directed mainly to the dorsoventral axis and a longer stride length, compared to exercise on a water treadmill without water; in addition, these increases would be greater with water depth.

Third hypothesis: That horses walking and trotting by-hand on a training track after performing exercise on a water treadmill, would maintain some of the adaptations that happen when the animals are exercised on the water, particularly after exercise at deep water depths.

3. Literature Review

3.1. INTRODUCTION TO THE LOCOMOTION IN THE HORSE

3.1.1. Terminology and definitions

Horses move naturally through a range of velocities by transitioning different gaits when it is energetically beneficial (Hoyt and Taylor, 1982), as a means of reducing limb forces (Farley and Taylor, 1991) or to preserve gait stability (Granatosky et al., 2018). A gait is defined as a complex and strictly coordinated rhythmic and automatic movement of the limbs and the entire body of the animal, resulting in the production of movements. Gaits are classified as symmetric or non-symmetrical or asymmetrical. Symmetrical gaits are those gaits in which each forelimb or hindlimb is considered to be employed equivalently with very similar movements and forces. Thus, left and right footfalls occur with a regular rhythm or at approximately equal time intervals. The most common symmetrical gaits are the walk, the trot and the pace. Asymmetrical gaits are those gaits in which individual limb function is considered to be different both in movement and forces. The common asymmetrical equine gaits in athletic horses are the gallop and the canter (collected gallop) (Clayton, 2004).

A stride is defined as a full cycle of limb motion. Since the pattern is repeated, the beginning of the stride can be at any point in the pattern and the end of that stride at the same place in the beginning of the next pattern (Barrey, 2013).

A complete limb cycle includes a stance phase, when the limb is in contact with the ground; a swing phase when the limb is not in contact with the ground and a suspension phase when none of the hooves is in contact with the ground. The stride duration, i.e. the duration of a complete cycle of locomotion, is composed of a stride stance phase (total duration of ground contact) and the suspension phase. It is equal, therefore, to the sum of the stance and swing phase duration of a limb. The SF corresponds to the number of strides performed per unit of time and it is equal to the inverse of the stride duration. SF is expressed in stride/s or in Hz. The distance covered by an individual limb during a stride, is represented by the stride length (SL), and it is usually expressed in m/stride (Barrey, 2013). Stride length was therefore defined as the distance between two successive hoof placement of the same limb. These parameters are usually named stride spatiotemporal parameters or linear and temporal parameters (Galisteo et al., 1997; Muñoz et al., 1997; 1999; Cano et al., 1999; Santamaría et al., 2002; Waldern et al., 2019).

Table 1 shows the definition of some terms used in equine gait analysis.

Terminology	**Definitions and units of measurements**
Gait	Complex and strictly coordinated rhythmic and automatic movement of the limbs and the entire body of the animal
Stride	A complete cycle of locomotion
Stride duration	Time for one complete gait cycle or period of time between any two identical events of a cycle. It is expressed in seconds. It decreases with velocity and is associated with a shortened stance phase
Stride frequency	Number of strides per unit of time. It is equal to the inverse of stride duration. It is expressed in strides/s or Hz. It increases non linearly with velocity and it is limited by the protraction time
Stride length	It is the linear distance covered by a limb during a stride cycle. It is expressed in m. It is deduced from the relationship between velocity and stride frequency. It increases linearly with velocity
Overtracking	Distance between the hindlimb and forelimb hoof strike. It is positive if the hindlimb lands in front of the forelimb. Otherwise, it is negative.

Table 1. Definitions of different terms used in equine locomotion (modified from Brownwyn, 2014).

3.1.2. Equine gait description

According to the aforementioned parameters, a variety of equine gaits have been described. The walk is a symmetric four-beat rhythm, which means that four hoof impacts happen during a stride. Each footfall occurs separately in a lateral sequence. The footfalls may be equally spaced in time or they may occur as lateral or diagonal couplets (Clayton et al., 1995). The walk is the slowest equine gait, but it is considered one of the most complex because of the overlap variability. There is an alternating tripedal (2 hindlimbs/ 1 forelimb or 2 forelimbs/1 hindlimb) and bipedal (lateral or diagonal pair) support sequence. It results in the following football sequence: left hindlimb- left forelimb- right hindlimb- right forelimb (Clayton, 1995). There is no suspension phase in this gait, so an increase in velocity is dependent on moving the center of mass of the horse further forward during the stance phase. As the walk velocity increases, the stance phase of individual limbs shortens. Thus, the large overlap of multilimbed support decreases and bipedal support increases (Clayton, 1995; Colborne et al., 1998; Khumsap et al., 2001; 2002).

Different types of walk have been described in dressage horses, increasing the velocity from collected walk (1.37 m/s) to the extended walk (1.82 m/s) and simultaneously there is a small increase in SF (Clayton et al., 1995). The change in velocity is primarily the result of the lengthening of the stride by increasing the over-tracking distance (Clayton et al., 1995). Overtracking or overreaching is defined as the distance between the hindlimb hoof impact and the ipsilateral forelimb hoof impact, being positive or negative. It is positive when the imprint of the hindlimb is in front of the forelimb imprint (Clayton et al., 1995).

The trot is a medium-velocity symmetric two-beat diagonal gait, with a primarily diagonal bipedal support. The footfalls of the contralateral limbs are evenly spaced in time. The diagonal limb pairs swing more or less synchronously, giving the trot a two-beat rhythm, although there may be a slight dissociation of the limbs both at impact and lift off, and the diagonal phases are usually separated by a period of suspension (Drevemo et al., 1980; Hölmström et al., 1994; 1995; Clayton, 1994; 1997). There are different types of trot, with the velocity of the gait increasing from collected to medium and extended trot. Passage and piaffe are two dressage exercises derived from collected trot (Clayton, 1994; Clayton et al., 2019), although the spatiotemporal variables are quite different between all of them. In fact, suspension phase is shorter in passage; and a positive diagonal advanced placement is measured at the collected trot in elite dressage horses (Clayton, 1994; Clayton et al., 2019).

The canter and gallop refer to the same gait at increasing velocity. Both of them are asymmetrical gaits in which the hindlimb pair and the forelimb pair move as couplets. The canter is a three-beat gait at slower velocity and the gallop is a four-beat gait at a higher velocity (Robilliard et al., 2007; Nauwelaerts et al., 2013; Barrey, 2014). The first limb of the couplet to contact the ground is the trailing limb, the second is the leading limb. Horses typically canter and gallop with the leading limb on the same side in the fore and hindlimb pairs (Clayton et al., 2019). Horses typically use the transverse gallop, where the limb placement crosses the body axis giving a footfall sequence of left hindlimb - right hindlimb - left forelimb - right forelimb. Occasionally, the horse will use a rotary gallop, the footfalls following a circular pattern: left hindlimb- right hindlimb- left forelimb- right forelimb on a right lead. Increasing velocity results in less stance duration for each individual limb, a higher SF, and a longer SL with consequent decreased overlap of multilimbed ground contact (Witte et al., 2006).

3.2. KINEMATIC ANALYSIS

Current knowledge and technology allow equine locomotion to be quantified in two main ways: kinematic and kinetic analysis. Kinematic analysis refers to the geometry of movement or description of the way body parts move in space without regard for the forces producing it. In other words, kinematic analysis of the horse in motion studies the changes in the position of the body segments in space during a specific time. This movement can be described from a quantitative point of view by linear, temporal and angular variables, which relate time, displacement, velocity and acceleration, without making any reference to the cause of the motion (forces). The kinematic approach is more commonly employed, probably because it is easier to measure and to visualize.

3.2.1. Techniques for kinematic analysis

Different methods for kinematic analysis have been employed during the evolution of the study of biomechanics in horses. High-speed cameras are used to film the locomotion of these animals with markers, small white spots or half spheres glued onto the skin over standard anatomical locations, with the aim of identifying the approximate instantaneous center of rotation (Barrey, 2013). However, there have been different investigations concerning the skin displacement over the skeleton, which could generate some artifacts, mainly in the proximal joints (Van den Bogert et al., 1990; Van Weeren et al., 1992; Khumsap et al., 2004). Lastly, optoelectrical systems based on the emission or detection of infrared light are the most effective collection systems for kinematic data. Current infrared based systems, used in conjunction with reflective markers of LEDs (light-emitting diode), have high framing rates and high resolution, enabling the capture of whole body movement (Rhodin et al., 2005; Alvarez et al., 2008; Hobbs et al., 2011). Up to 12 connected cameras can be used in these systems.

The processing of the films recorded with the high-speed cameras is undertaken using computers and specifically designed software. It is a time-consuming task, so data is not quickly available. The video signal can be treated by a video interface in order to digitize the images and the appropriate software is used to collect semi-automatically or automatically the marker coordinates in space and time. A more sophisticated motion analysis system uses active markers consisting of photodiodes (Cartesian Optoelectronic Dynamic Anthropometer, CODA-3). This system has good resolution in three dimensions, and it is one of the most used systems in kinematic studies in horses (Van Weeren et al., 1990; 1993; Barrey, 1999; 2014; Back et al., 2002). With 3D (three-dimensional) systems, the skin displacement of the markers have been reduced or different algorithms have been used to compensate for skin displacement. In addition, each marker must be visualized by at least two cameras throughout the movement. The main disadvantage is that the subject needs to be equipped with many photodiodes connected to wires.

Most equine kinematic studies were initially based on two-dimensional motion analysis, but systems including four or more video cameras make it possible to reconstruct motion in three dimensions and to analyze the limb motions of both sides (Chateau et al., 2004; Clayton et al., 2007; Miró et al., 2009). One problem with this system is the limited field of view. Only about five meters which corresponds to several walking strides or one trotting stride can be analyzed. In order to analyze sporting exercise over greater distances, up to 30 m, a camera panning technique has been developed. It has been successfully used to study locomotion in real exercise conditions, as competitions. This technique has been used to characterize kinematics in dressage and jumping horses (Rogers et al., 1999; Cassiat et al., 2004; Nankervis et al., 2005) or horses exercised on a track (Kallings et al., 1999).

After filming, the operator needs to track manually, semi automatically or automatically the coordinates of the markers on each image. This is the most time-consuming task, because the number of images to measure is usually huge and sometimes the markers fixed on the skin are not easy to detect automatically, and therefore, a manual supervision is needed. Nowadays, the use of specific algorithms, such as direct linear transformation is an efficient and time-consuming way to automatically detect the trajectory of the skin markers (Poucelot et al., 2000; Cassiat et al., 2004).

Once the coordinates of the markers have been collected, the linear and angular velocities can be obtained by computing the first derivative of the trajectories and angles in relation to time. The advantages of the kinematic methods is that we can obtain all the kinematic parameters (displacement, velocity, angle of ration, angular velocity….) of the identified body segments (Barrey, 1999; 2013).

3.2.2. Kinematic parameters in the evaluation of equine lameness

Kinematic gait analysis has also been applied to diagnose and follow-up of lameness. As we said before, one of the concerns of these techniques is that the acquisition of meaningful data for large numbers of successive strides requires the horse to be filmed on a treadmill. Thus, the horses must be trained to walk on a treadmill. On the other hand, the velocity of movement can be controlled, which helps to decrease variation of movement between examinations. Motion pattern depends greatly on velocity and to detect small differences in kinematic parameters with mild lameness it should be consistent. Individual horses exhibit lameness better at trots of different velocities and levels of effort. These velocities are not possible to be reached without riding the horse or using a treadmill, which also highlights one potential limitation of evaluating lameness with the horse viewed in hand with an operator. It has been demonstrated that 12 of 18 horses had the most consistent kinematic asymmetry, indicative of lameness, at a moderate-velocity trot, whereas of the 6 remaining horses, asymmetric locomotor pattern was highest at high-velocity trot (Peham et al., 1998). However, we have to keep in mind that the horse moves differently on a treadmill compared to overground (Buchner et al., 1994; Álvarez et al., 2009; Weishaupt et al., 2010). Despite the differences in movement, they are not reflected in the most likely parameters of interest for detecting and quantifying lameness in the horse (Keegan, 2007).

Some kinematic parameters have been associated with the severity of lameness in the horse. Asymmetry of displacement, usually expressed as the ratio of the duration or range of motion between the right and left sides, is generally of more interest for the evaluation of lameness in the individual horse at a specific time (Church et al., 2009; Parkes et al., 2009; Byström et al., 2018; Rhodin et al., 2018). Changes in kinematic parameters are potentially useful to objectively measure the change in lameness from baseline examination, for example, before and after a diagnostic block or before and after a treatment, shoeing procedure, etc. in an individual horse.

A variety of kinematic parameters differ between soundness and lameness in horses. Lameness in the forelimbs causes an increase in the asymmetry of vertical head movement (acceleration and displacement) between left and right forelimb strides (Buchner et al., 1996; Keegan et al., 2001). Lameness in the hindlimb causes increases in the asymmetry of whole-pelvic vertical acceleration and displacement between left and right hind limb strides and in total displacement of the left and right tuber coxae relative to each other (Buchner et al., 1996; Kramer et al., 2000; 2004). Other changes include decreased maximum fetlock extension and distal interphalangeal joint flexion at midstance, decreased retraction of the forelimb for forelimb lameness, decreased protraction of the hindlimb in hindlimb lameness, decreased tarsal flexion during the stance phase and increased stance phase duration for mild to moderate lameness (Back et al., 1993; Buchner et al., 1995; 1996; Galisteo et al., 1997; Keegan et al., 1997; 1998; 2000; Kramer et al., 2000; Audigé et al., 2001).

For the purpose of using kinematic studies as a measure of lameness in a clinical scenario, it is clear that an index or threshold of asymmetry should be established. Kinematic parameters in horses with natural asymmetries or with a high stride-by-stride variability would be less sensitive indicators of lameness. For instance, one study using 6 horses with induced carpal lameness by injection of amphotericin B , trotting on a treadmill at 4 m/s found that head movement asymmetry changed after treatment, but there was not significant changes in SL, stance phase duration, forelimb abduction, or carpal and fetlock range of motion (Peloso et al., 1993). In other study evaluating frog pressure-induced transitory forelimb lameness, maximum fetlock extension at midstance and vertical head movement asymmetry were sensitive indications of lameness, whereas stride duration, stance duration and carpal joint angle extension during stance were insensitive (Keegan et al., 2000). Because no horse moves perfectly symmetrically, i.e. a natural 'leggedness' or asymmetry in equine gait is a normal finding, some authors have tried to established a kinematic symmetry index (KSI), with defined deviations from the symmetry, taken into account a certain degree of asymmetry. Thus, Audigé et al. (2001) calculated KSI in 13 clinically sound horses and 24 lame horses and found that KSI for proximal limb displacements were the most sensitive for determination of weight-bearing soundness and lameness. Similarly, Muñoz et al. (2006) evaluated the asymmetry of locomotion in endurance horses during competition, in order to differentiate between loss of asymmetry of kinematic parameters in fatigued horses and those disqualified from competitions because of lameness. In this study, the KSI was calculated by comparing stride duration in the diagonal pairs of limbs at trot during vet-gates (veterinary examinations) in endurance competitions. Horses were classified as symmetrical, when KSI was lower than the mean + 2 standard deviations of the values obtained before competition and asymmetrical, when KSI was higher than mean + 2 standard deviations. It was found that this KSI became more asymmetrical with fatigue, and this asymmetry was more evident in the faster horses and those with lameness. In fact, the KSI showed negative correlations with the velocities, both in competition and during the vet-gates. It was concluded that trot asymmetry increases during endurance events, but this asymmetry comparing stride duration in the two diagonal pairs of limbs at trot did not allow to differentiate between successful, fatigued and lame horses (Muñoz et al., 2006).

It has been highlighted that changes in kinematic parameters depend on the specific lameness model or condition. This idea has relevance for future research using kinematic analysis to locate lameness within a limb. For example, in one kinematic study, investigators found a decrease in forelimb protraction in horses with induced lameness in the forelimb heel but a significant increase in forelimb protraction in horses with induced lameness in the toe region of the forelimb hoof (Keegan et al., 2000). Similarly, in a model of induced hoof lameness, a significant increase in proximal limb joint flexions (carpus for forelimb and hock for hindlimb) during swing was found (Buchner et al., 1996). On the contrary, another study with a carpal-induced lameness described a reduced flexion of the carpus during swing (Back et al., 1993).

3.3. KINETIC ANALYSIS

Kinetics studies the cause of the motion, which can be explained by the force applied to the body, its mass distribution and its dimensions. In fact, kinetics is concerned with forces, energy, and work, which are also in relation to kinematic variables such as acceleration and velocity. The main aims of kinematic studies are to explain the influences of forces on movement, maximal performance, metabolic cost of locomotion, and triggers for gait transition (Weishaupt, 2008). As a summary, we can see that kinematics is concerned with the study of the description of movement and kinetics is concerned with the study of the action of forces. Kinetic analysis is not as intuitive or easy to visualize as kinematic analysis, because the human eye cannot see forces (Keegan, 2007; Barrey, 2009; 2013).

3.3.1. Techniques for kinetic analysis

The most used techniques for kinetic analysis included the measurement of ground reaction forces (GRFs) and acceleration analysis. The external forces can be measured using electronic force sensors which record GRFs when the hooves of the horse are in contact with the ground. The sensors can be installed either on the ground, in a stationary force platform or in a shoe attached to the hoof.

Stationary force plates provide a dynamic, noninvasive, quantitative assessment of the amplitude and orientation of ground contact forces transmitted through one limb during the stance phase. This assessment represents the sum of the trunk and limb forces generated by and resulting from the stance and reflects the acceleration of the body mass of the horse. The orientation of these forces is measured by deflection of sensing elements (strain gauges or piezoelectric quartz crystals) in the three orthogonal components of the GRFs: vertical, longitudinal, and transverse (Barrey, 2013; Witte et al., 2014). Vertical GRF (vGRF) is of the greatest magnitude, and it most directly measures limb specific weight bearing. As a consequence, vGRFs are sensitive in grading lameness and the most commonly used measurement in force plate studies (Back et al., 2009; Erkert et al., 2005; Weishaupt, 2008; Donnell et al., 2015; Whitfield et al., 2016; Pitti et al., 2018; Schoonver et al., 2018). Craniocaudal GRF measures forces affecting forward progression, braking (deceleration) and propulsion (acceleration). Mediolateral GRF has the smallest amplitude, so few studies have used this variable.

After recording these data, analysis of the three parameters, peak forces, total impulses (total forces integrated over time) and average force or impulse can be determined. All of these parameters help describe the rate and pattern of limb loading and unloading. In addition, a force plate enables measurement of the moment value, vertical torque, and center of pressure (Hobbs et al., 2016; Clayton et al., 2017; Clayton and Hoobs, 2021). Force plates have been very useful in performing deep studies concerning specific gaits and postural balance under different pathological conditions, therapeutic interventions, and medical and surgical treatments (Clayton et al., 2013; Clayton and Nauwelaerts, 2014; King et al., 2017; Pitti et al., 2018; Schoonver et al., 2018; Young et al., 2020).

In human biomechanics, the treadmill has been used to measure vGRF in standardized exercise conditions. In equine biomechanics, vGRFs have been measured for all the four limbs simultaneously with a treadmill integrated with a force measuring system (Weishaupt et al., 2002; Waldern et al., 2013; Serra Bragança et al., 2021).

Pressure sensitive plates are an alternative noninvasive means of measuring and analyzing vGRF and pressures during the stance phase, enabling calculation of variables such as regional force or pressure distribution (i.e. force or pressure per unit area) and total surface contact area in a static or dynamic situation (Oosterlinck et al., 2012). The advantages of the pressure plate over force plate measurements are time efficiency, ease of use, portability for transportation, expense, and provision of immediate analysis of loading under the hoof in both clinical and research settings. However, the pressure plate cannot measure horizontal or vertical forces, and data analysis is restricted to vertical forces (Oosterlick et al., 2010; 2012).

In an attempt to measure GRF during locomotion, designed horseshoes or boots with one or more force sensors or dynamometric horseshoe attached around the circumference of the hoof, commonly at the toe and quarters, have been used (Setterbo et al., 2009; Chateau et al., 2010; Crevier-Denoix et al., 2010). The advantages of instrumented horseshoes are: ease of application in the hoof; ability for simultaneous data collection from multiple limbs over a large number of successive strides and ability to use on different track surfaces and at high-speed locomotion.

Accelerometers detect and measure the magnitude of acceleration and deceleration (gravity induced reaction forces) of the surface to which they are attached and are being increasingly used in equine biomechanical studies. Single or unidirectional and triaxial or three-orthogonal directions models are commercially available and after processing, these express voltage outputs as linear vector quantities (Viry et al., 2015; Kienapfel et al., 2018; Ricard et al., 2020). The benefits of accelerometers are multifold. The device is small and lightweight; is easy to apply and use; is cost effective; can capture data from many gait cycles; and because of its portability, may be applied in the field, during competitions and on different surfaces. The most significant potential source of error of accelerometry is the inconsistent orientation of the sensitive axis of the device. Therefore, anatomic location should be carefully considered, as contamination of accelerometric signals from body-mounted devices may also be caused by displacement of the device and muscle activation artifacts (Barrey, 2013). Direct comparison between force plate data and accelerometer data showed a high correlation (Witte et al., 2004).

In the last decade a development of wireless data acquisition systems, integrated by inertial sensors in conjunction with accelerometers has happened. Several devices, such as Lameness Locator®, Equigait® or EquiMoves® are commercially available nowadays even though they have been designed more focused on lameness diagnosis and follow-up (McCracken et al., 2012; Donnell et al., 2015; Rhodin et al., 2017; Coleridge et al., 2020; Reed et al., 2020).

3.3.2. Kinetic parameters in the evaluation of equine lameness

In most natural conditions and induced lameness models, increasing severity in a limb correlates with decreasing peak vGRFs acting on the limb. In addition, there is a negative correlation between severity of lameness and vertical impulse (area under the vertical force curve), braking (negative horizontal) force and impulse, and propulsive (positive horizontal) force and impulse (Donnell et al., 2015; Bell et al., 2016; Schoonover et al., 2018; Serra Bragança et al., 2021). For unilateral forelimb lameness, a decrease in mean peak vGRFs in the lame forelimb is accompanied by increases in mean peak vGRFs in the contralateral forelimb and both hindlimbs at the walk and in the contralateral forelimb and hindlimb at the trot (Merkens and Schamhardt, 1988a, b). For unilateral hindlimb lameness, a decrease in mean peak vGRFs in the lame hindlimb is accompanied by an increase in mean peak vGRFs in both forelimbs at the walk (Merkens and Schamhardt, 1988a, b) but little change in other limbs at the trot (Weishaupt et al., 2004; 2006). If the velocity of the walk and particularly trot can be strictly controlled and if enough strikes are collected, mean peak vGRFs asymmetry can even detect subclinical lameness (Ishihara et al., 2005).

Unfortunately, although force determination and particularly alterations in peak vGRF measurements are the ‘gold standard’ for lameness detection, some lameness conditions did not affect this parameter, but cause a change in the shape of vGRF signal. For example, mean forelimb peak vGRF asymmetry in trotting horses with mild (1 over 5-point American Association of Equine Practitioners (AAEP) scale), induced superficial digital flexor tendinopathy or naturally occurring navicular disease did not differentiate between soundness and lameness (Williams et al., 1999). On the contrary, the shape of the vGRF changes with a decrease in vGRF in the cranial (superficial digital flexor tendinopathy) or caudal (navicular disease) phase of stance (Weishaupt, 2008; Bell et al., 2016). Thus, the shape of the vGRF curve could potentially contain some meaningful information to assist in localization of lameness within the affected limb.

3.4. ACCELEROMETRY

3.4.1. Use of accelerometry to evaluate locomotion in performance horses

Sport horses are generally trained for a single competitive sport, each of which have different functional and anatomical requirements. Therefore, this fact predisposes the horse working for a single sport to increased load at specific anatomical sites, increasing the risk of suffering specific injuries (Murray et al., 2006). Therefore, it is not surprising that over time there has been a great interest in the analysis of the equine locomotor system and pathologies in which it becomes impaired. Furthermore, the economic constraints have also favored the development of early performance evaluation of young horses to improve their training and selection (Barrey, 2013).

Equine biomechanics research aiming for more accurate and practical results, has developed more refined analysis methodology and hardware to achieve these goals (Gómez Álvarez and Van Weeren, 2019). In the latest years, technical developments have permitted the introduction of gait analysis systems in clinical practice, ongoing to accuracy and reliability of these techniques, which have a lower limit of detection for asymmetry than the 25% reported for observation by the human eye (Parkers et al., 2009; Van Weeren and Gómez Álvarez, 2019).

A horse's body is composed of articulated rigid segments, so it follows the same mechanical laws as inanimate objects. These laws must be applied carefully because their motion is much more complicated than the motion of a single inanimate object

(Barrey, 2013). Qualitative gait evaluation, even though consistent scores, is highly variable since it depends on the clinician's experience (Clayton and Schamhardt, 2013). In consequence, in the last 40 years, scientific analysis requiring quantitative data describing movement and its associated forces and technological advances have provided a huge range of technology for quantitative gait analysis (Clayton and Schamhardt, 2013; Sepúlveda et al., 2017; Van Weeren and Gómez Álvarez, 2019).

As indicated before, accelerometry is a kinetic gait analysis method which measures instantaneous changes of velocity produced by applying a force to an object or body (Barrey, 2013). These measurements of acceleration are performed with small sensors that measure acceleration of the surface to which they are attached (Clayton and Schamhardt, 2001). Sensors are made of a small, suspended mass giving a signal which is proportional to the acceleration. In order to study a body in motion, the sensor has to be fixed on its center of gravity, so that it provides a convenient way of studying kinetics (Barrey, 2013).

As the devices are small, portable and non-invasive, they can be used to quantify duration and frequency of physical activity. In the past decade they have been extensively used in humans and more recently in animals. Accelerometry has been regarded as a reliable method with which to collect information of human movement (Morrison, 2015). In veterinary medicine, they have shown to be a valid, practical and reliable technique to measure physical activity and for objective evaluation of animal locomotion (Barrey, 2013; Morrison, 2015).

In human medicine, accelerometers have been always worn at the level of the hip, the closest part to the body's center of mass (Morrison, 2015). For horse locomotion analysis, an accelerometer named Equimetrix® has been developed. The transducer is placed near to the center of gravity, at the level of the girth, in the caudal part of the sternum, between right and left *pectoralis ascendens* muscles. Even small changes in velocity can give a high acceleration or deceleration. The acceleration vector is proportional to the resultant force applied to the body, providing a convenient way of studying kinetics of a body in motion. The acceleration signal is treated by signal analysis procedures to measure dynamic and temporal stride variables (Barrey, 2013). Accelerometric analysis provides a variety of parameters of the body in motion: velocity (m/s), SF (cycles/s or Hz), SL (m), regularity (REG) and symmetry (SYM) which are dimensionless, displacements and accelerometric activities at the three body axes: dorsoventral displacement DVD (cm), dorsoventral accelerometric activity DVAA (W/Kg), propulsion or longitudinal accelerometric activity LAA (W/Kg), mediolateral accelerometric activity MLAA(W/Kg) and total accelerometric activity TAA (W/Kg), which is the sum of the activities in the three body

Terminology	**Units of measurements and definition**
Velocity	m/s. Relation between distance over time. Calculated by timing the horses over a defined distance
Stride frequency, SF	strides/s or Hz. Number of strides per unit of time. It is equal to the inverse of stride duration. Calculated by the software as twice the fundamental frequency derived by the software from the dorsoventral acceleration signal, using fast Fourier Transformation
Stride length, SL	m/stride. Distance between two successive prints of the same foot. Calculated by the software by dividing the measured speed by SF
Regularity, REG	Dimensionless. Acceleration pattern similarity of successive strides over the course of time. Calculated by the software as the correlation coefficient corresponding to a peak in the autocorrelation function of the dorsoventral acceleration, measured at a time equal to stride duration
Symmetry, SYM	Dimensionless. Acceleration pattern similarity between right and left acceleration patterns.
Dorsoventral displacement, DVD	cm. Dorsoventral movement of the center of gravity. Calculated by the software as an estimation from the double integration of the dorsoventral acceleration signal
Dorsoventral accelerometric activity, DVAA, W/kg	W/kg. It represents the accelerometric activity of limb suspension and loading activity. Calculated by the software as the integral of the power spectrum obtained by the Fast Fourier Transform from the dorsoventral acceleration signal

Terminology	**Units of measurements and definition**
Velocity	m/s. Relation between distance over time. Calculated by timing the horses over a defined distance
Stride frequency, SF	strides/s or Hz. Number of strides per unit of time. It is equal to the inverse of stride duration. Calculated by the software as twice the fundamental frequency derived by the software from the dorsoventral acceleration signal, using fast Fourier Transformation
Longitudinal or propulsion accelerometric activity LAA	W/kg. It represents the amount of deceleration and acceleration along the longitudinal axis. Calculated by the software as the integral of the power spectrum obtained by the Fast Fourier Transform from the longitudinal acceleration signal
Mediolateral accelerometric activity MLAA	W/kg. It represents the amount of deceleration and acceleration along the lateral axis. Calculated by the software as the integral of the power spectrum obtained by the Fast Fourier Transform from the lateral acceleration signal
Total accelerometric activity TAA	W/kg. Calculated by the software as the sum of DVAA, LAA and MLAA

axes, i.e. DDVA, LAA, MLAA. More information is provided in table 2.

Table 2. Definitions of different accelerometric measurements performed by the accelerometer Equimetrix® (modified from Frigerio et al., 2019)

Although gait analysis methods have mainly been used to identify, measure, and monitor potential gait disturbances in horses with musculoskeletal and neurological disorders, they have further applications such as optimizing training, riding, competition or even rehabilitation programs (Gómez Álvarez and Van Weeren, 2019).

As it has a high sensitivity on the detection of slight asymmetries, at the beginning accelerometers were mostly used in objective evaluation of locomotion asymmetries in lameness examination (Barrey et al., 1994; Weishaupt et al., 2001; Ishihara et al., 2005; Keegan et al., 2007). Accelerometers have also been used in the evaluation of coordination and stability in horses with ataxia due to neurological disorders (Keegan et al., 2004). On our study (Argüelles et al., 2019) about the clinical efficacy of clodronic acid in horses with navicular syndrome, accelerometry was used not only for objective evaluation of lameness in horses, but also for the evaluation of the improvement after treatment with clodronic acid of lame horses with navicular syndrome.

Moreover, accelerometry has been widely used for early selection, performance evaluation and monitoring of the response to training programs at different equine disciplines, such as flat racing (Barrey et al., 1995; 2001; Mottini et al., 2006; Pfau et al., 2005), trot racing (Leleu et al., 2002; 2003), endurance (Metayer et al., 2004; Viry et al., 2014), jumping (Barrey and Galloux, 1997; Bobbert et al., 2005; Langlois et al., 2006; Ricard et al., 2020) and dressage (Barrey et al., 2002; Biau and Barrey, 2004).

More recently, accelerometry has also been used for the evaluation of the effect of different drugs in equine locomotion. As sedatives disrupt kinetics, it has been used for the detection of these changes in motion and to determine which of these drugs has the quickest effect and the longer lasting effect (Buchner et al., 1999; López-Sanroman et al., 2012, 2013, 2014; Frigerio et al., 2019; Calvo-Santesmases et al., 2021).

3.4.1.1. Use of accelerometry in racehorses (thoroughbreds and trotters)

Despite frequent and intense horse wastage and financial loss associated with the racing industry, considerable resources are expended to investigate causes of loss during training and growth of thoroughbred horses. A thoroughbred’s racing career is short, and it´s number of racing opportunities is limited. This is the reason why there is great interest in maximizing the health and racing potential of these horses by owners and trainers (Jeffcott et al., 1982; Bailey et al., 1997).

To maximize an individual horse´s potential for winning, races should be selected in accordance with its racing ability. Making optimal decisions about entering horses in a profile may be developed for each horse while in early training, and should include indications of individual speed, endurance, and preferred track conditions. These characteristics have been determined by the analysis of locomotor variables of the racing gallop while maximal velocity using accelerometry (Barrey et al., 1993).

Locomotion variables of the racing gallop during individual maximal speed can only be obtained on the racetrack because temporal stride variables should be natural (Barrey et al., 2001). Despite physiological parameters are more accurately measured on an inclined high-speed treadmill at submaximal speed (Rose et al., 1995), the test is not as valuable as on a racetrack when measuring stride temporal and kinetic variables because maximal speed of the belt is limited to 14-15 m/s and biomechanics of locomotion are modified by the driven belt (Barrey et al., 1993).

In order to measure gait variables during training sessions on a racetrack, Barrey et al. (2001) described a gallop test to determine the locomotory profile of thoroughbreds. In the study, horses were trained at maximal speed on a racetrack with the same accelerometric device that we have used in our studies (Equimetrix®), fixed in the girth. After a warm-up at trot and canter for 10 min and galloping at increasing speed on a dirt track 1942 m long, maximal speed was measured during the last 800 m. Locomotory variables measured were SL, SF, times elapsed between each hoof midstance phase, REG, mean vector propulsion, energy of propulsion and energy loading. Mean maximum speed was 15.26 m/s (8.8-18.4 m/s). Several variables were significantly correlated to the gallop speed such as SL, SF, REG,. These gait variables were related to the race performance which was evaluated by the percentage of wins, placings and the logarithm of average earning per start. Best performers had higher SF and REG. Stride length was negatively correlated with performance and explained most of the velocity increase. Dorsoventral displacement was negatively correlated to performance, it decreased linearly with velocity and reduced energy expenditure of locomotion in good performers. Most of the kinetic and temporal stride variables were influenced by the velocity of the gallop. Stride length and SF increased linearly with the velocity. Velocity was mainly produced by SL increase and secondly by the SF. However, SL was negatively, and SF was positively correlated to performance. A quick gallop acceleration is needed at the start of the race and at the finish to win (Barrey et al., 2001). This is achieved firstly by a high-rate SF increase and secondly by a SL (Hiraga et al., 1995). In consequence, horses that can increase SF to the highest value are better performers with a better chance to win. Furthermore, horses that won short distance races, had higher SF and a longer ground contact duration which provided more time for propulsion. An increase in propulsive activity (mean vector propulsion, energy of propulsion) resulted in a greater velocity. Horses reached the wheel gallop at the fastest speed by lowering and keeping their center of gravity at the same height during the whole stride duration. This gallop is optimized to produce maximum mean vector propulsion and energy of propulsion in the longitudinal axis while avoiding energy wastage for vertical oscillations of the body mass (representing by DVD). Regularity was affected by the increase in speed as also shown in harnessed trotters (Barrey et al., 1994) and it was highly correlated to racing performance (Barrey et al., 2001).

In conclusion, good racehorses are able to trot or gallop at high-speed using their optimal SL and that they can accelerate by increasing SF to finish the race. Although SL is negatively correlated with performance, SF is positively correlated. Best performers reached high SF of 2.52 strides/s at trot and 2.81 strides/s at gallop. Maximal SF is dependent on the height of the horse, weight distribution of the limbs, elasticity of the limbs and rib cage, and the percentage of fast-twitch muscle fibers (Barrey et al., 2001).

In another study of Barrey et al. (1995), a locomotor test was performed in harnessed French race-trotters to describe some basic stride characteristics. Two commercially available accelerometers attached to the sternum with an elastic belt fastened at the girth, between the *pectoralis ascendens* muscles, of the horse were used to evaluate temporal and kinematic stride variables that could predict potential performance. To control the movements, each horse was filmed with a video camera mounted on a car that moved parallel to the horse on the track. The exercise test was the same for all horses, after a warm-up, they performed three stages of increasing speed: stage 1 – 2500 m at 6.67 m/s, stage 2 – 1250 m at 10 m/s and stage 3 – at maximum individual speed. Acceleration measurements were made when the horses trotted regularly on a straightway 310 m long to record a steady state gait. For the statistical analysis, horses were divided into three classes of performance, depending on the race performance indexes obtained during the year 1993 and published by the French Steeplechase Society (Barrey et al., 1995).

In the study of Barrey et al. (1995), basic stride variables were extracted from the acceleration data using specific procedures. Compared variables were velocity, SF, SL, magnitude of the longitudinal acceleration-deceleration, REG, SYM, RATIO between REG and SYM (RATIO=100xSYM/REG) and SUM (SUM=SYM+REG). Most variables were influenced by the increase in speed at each stage. The highest speed measured was 14.22 m/s with a SF of 2.52 strides/s and SL of 5.65 m. Stride frequency ranged from 1.66 to 2.52 strides/s and SL from 2.42 to 5.92 m, both increasing linearly with the speed. The magnitude of acceleration-deceleration increased linearly with speed and ranged from 17.46 to 48.76 m/s^2. Performance criteria were moderately correlated with the maximum velocity, SF and SL, being significantly higher in the best performer group of horses (Barrey et al., 1995). Asymmetries in the locomotion pattern have been described in trotting and galloping horses by several authors (Drevemo et al., 1987). In this study by Barrey et al. (1995), REG and SYM values, although lower in the intermediate group, were not significantly different between performance groups. Nor correlation was established between these variables and the racing performance index.

In conclusion, the study revealed that the velocity, SF, SL measured at the individual maximal velocity for each horse were significantly related to performance. In a good performer at maximum speed, SF was greater than 2.4 strides/s, with the contribution of the SF to the speed representing more than 34%. Stride length appeared to be a secondary factor but should be greater than 5.45 m (Barrey et al., 1995). Good race trotters should be able to trot at high speed using an optimal SL and be able to accelerate by increasing their SF in order to finish the race (Barrey, 2013).

Several studies have demonstrated a close correlation between the limb cycle events and the curves of acceleration in the three body axes in fast racing trotter horses. Accelerometry is a useful tool for testing young trotters and finding locomotor factors related to racing performance, as it works well under racetrack conditions, providing data on basic temporal stride variables (Leleu et al., 2002, 2004). According to previous studies, a higher TAA appears to be related to a better sport performance in harnessed race trotters (Barrey et al., 1995, 2001, 2002). In addition, Standardbred racehorses have been described to be those with the highest SF and longer stance and propulsion durations at submaximal speed (Leleu et al., 2005).

3.4.1.2. Use of accelerometry in show jumpers

Show jumping is one of the most popular equestrian disciplines. The horse is supposed to clear a set of obstacles in a specific order, in a limited time. Penalties or faults are incurred when the horse knocks down, refuses to jump or fails to complete the course within the time limit (Bobbert et al., 2005a).

A successful jump is dependent of three main biomechanical factors: the distance from the hindlimb hooves to the base of the obstacle at take-off (Barrey et al., 1993), external force impulses generated by fore and hindlimbs at take-off (Schamhardt et al., 1993) and rotational characteristics of the body around the center of gravity in the sagittal plane (Galloux and Barrey, 1997). Jumping a fence is a demanding task for a horse, so it is necessary that the riders and horses negotiate the course at the correct angle, height and speed to clear the fences without incurring faults (Bobbert et al., 2005a).

The horse needs to estimate its distance from the fence and push off at an appropriate location, generating an enormous amount of work at a high rate to project the center of gravity in the air, while at the same time producing an appropriate angular momentum, and flex the limbs at the correct time so as not to knock over the fence (Bobbert et al., 2005a,b). The ballistic flight of the center of gravity is determined by the impulse of the external forces applied by the hooves on the ground at take-off, setting the magnitude and direction of the initial velocity at the beginning of the airborne phase. External forces applied by hindlimbs at take-off, also determine the total kinetic movement during airborne phase, influencing the characteristics of body rotation over the obstacle (Barrey and Galloux, 1997). Various methods have been used to investigate the range of external forces that influence the jump, since they appear to be important factors for clearing obstacles. Experimentally, direct methods such as force plates (Merkens et al., 1991; Schamhardt et al. 1993) and indirect methods, using accelerometers fixed to the saddle (Preuschoft, 1989) have been used.

Individual variations in technique and skills during the jump are assumed to be related to differences in the external forces developed by the forelimbs and hindlimbs during the take-off. Therefore, accelerometric measurements, obtained as close to the center of gravity as possible, should be able to detect the difference between good and poor jumping techniques (Barrey and Galloux, 1997). Indeed, accelerometry has been successfully used to determine jumping ability in elite show jumpers jumping obstacles with different height and width (Deuel and Park, 1991; Van den Bogert et al., 1994; Barrey and Galloux, 1997). Moreover, accelerometry has been used to calculate heritability and to perform a genome-wide association study of the gait traits positively and negatively associated with jumping ability (Ricard et al., 2020).

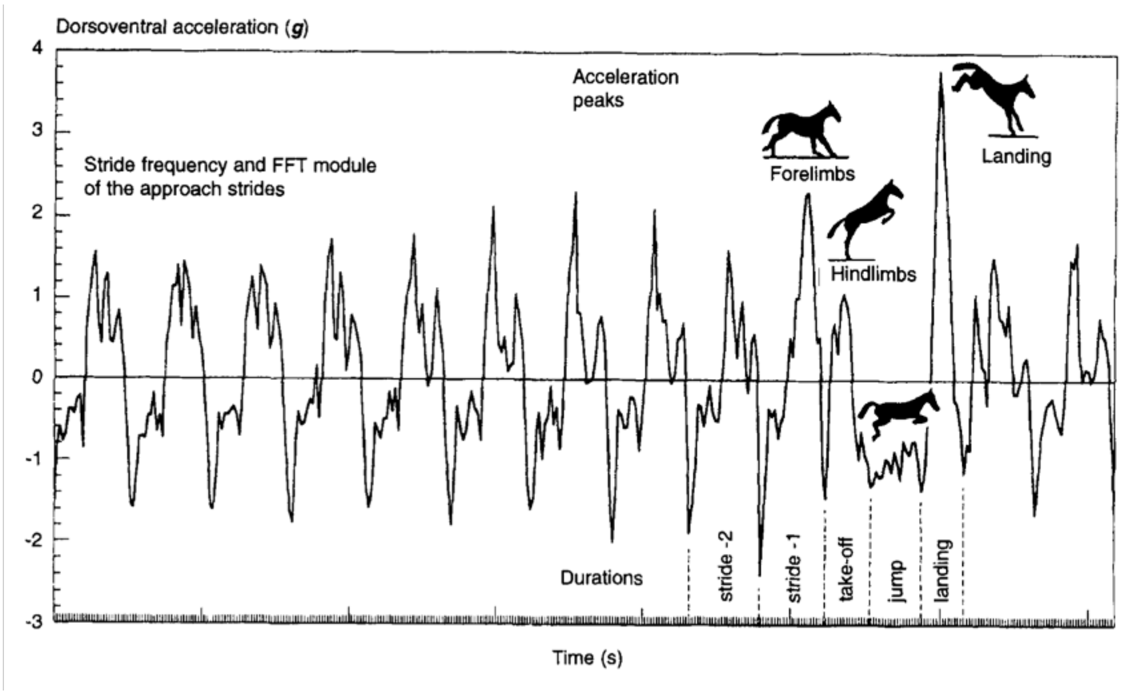

Figure 1. Example of dorsoventral acceleration recording of the approach, jump and move off strides. From Barrey and Galloux (1997).

Jumping technique was analyzed by Barrey and Galloux (1997), where show jumping horses completed a course with 14 obstacles of different height and width. Horses were equipped with the Equimetrix® system, fixed in a leather pocket over the caudal part of the sternum at the level of the girth. Dorsoventral acceleration peaks, SF, and SL were studied, with the aim of comparing and especially improving jumping techniques. These authors observed large individual differences in the ability and technique of the jump/rider binomial. The type of obstacle was a significant determinant of the acceleration of the pelvic limbs at the moment of take-off and of the acceleration peak at the support or landing. Horses with poor jumping technique, compared with more successful ones, were found to have a higher peak acceleration of the thoracic limbs at take-off, a higher thoracic/hind limb acceleration ratio value, and a higher SF in the approach to the obstacle. Hitting an obstacle was associated with a greatly reduced peak take-off acceleration, along with a high thoracic/hind limb acceleration ratio (Barrey and Galloux, 1997).

Deuel and Park (1991), performed an accelerometric study on 29 show jumping elite horses during the 1988 Olympic Games in Seoul. These authors described the relationship between horses jumping over an oxer (an obstacle that joins two verticals with a maximum separation of two meters between them), and the total number of knockdowns. Fewer total faults were associated with lower speed strides in the approach to the fence. Closer take-off hindlimb placements and closer landing forelimb placements were also associated with fewer penalties during the course (Deuel and Park, 1991).

In another study on elite horses jumping a high fence, it was demonstrated that the push-off produced by the hindlimbs at take-off was associated with the mechanical energy required to clear the fence (Van den Bogert et al., 1994). The action of the forelimbs should be limited to positioning the horse's body in the correct orientation to the jump before the last impulsion of the hindlimbs. Kinematic results described in this study were consistent with those found by Barrey and Galloux (1997), who reported that unsuccessful jumping horses had lower peak accelerations of the hind limbs in comparison to better performing horses. Unsuccessful jumpers braked too much with their forelimbs in the take-off impulse and the hindlimbs produced a weak acceleration to clear the obstacle. This force is one of the main factors that affects when making a jump cleanly, because it determines the flight's center of gravity, in addition to the characteristics of the rotation of the body over the obstacle during flight (Barrey and Galloux, 1997).

The moment of inertia and its influence on body rotation has also been studied in a group of jumping horses. Although a consistent relationship with the level of competition was not found, a higher number of knockdowns was recorded in horses that galloped with a low SF (less than or equal to 1.76 strides/s), and that significantly reduced SF at take-off (Galloux and Barrey, 1997).

3.4.1.3. Use of accelerometry in dressage horses

Dressage is an Olympic equestrian discipline that requires a high level of locomotor control of the horse by the rider (Barrey, 2013). In dressage, specific characteristics of the walk, trot and canter are required to have a good performance (Barrey et al., 2002). The horse must easily execute complex exercises, gait variations and gait transitions while maintaining a correct equilibrium and suppleness (Barrey, 2013). Gait characteristics have been thoroughly described in international dressage rules, so that the dressage test can be judged (Anon, 1999). Collective marks are awarded for paces, impulsion, submission, and the rider's position and seat. According to dressage rules, qualities of a dressage horse should be revealed by the regularity of paces, lightness, and cadence (Biau and Barrey, 2004).

One of the main limiting factors for dressage is the ability for collection. This high level of locomotor control of the horse by the rider is progressively achieved through exercise and collecting gaits. It is impossible to execute complex exercises correctly in competition without having attained good basic collection gaits (Barrey, 2013).

Collected gaits required for dressage, have been described in kinematic studies, in which stride characteristics have been studied in relation to results in competition (Biau and Barrey, 2004; Barrey, 2013). For this purpose, several studies have been performed in elite dressage horses during competition, such as the Olympic games in Seoul (Deuel and Park, 1990) and Barcelona (Clayton, 1994; 1997). Video recordings were performed during competition to study the movement of the best dressage horses. Deuel and Park (1990) found significant correlations between the speed of extended trot and the SF for the elite dressage horses. Clayton (1994) described that in advanced dressage horses, speed increases progressively through the collected, working, medium and extended canters as a result of an increase in SL while SF is constant. However, when comparing temporal stride kinematics of the canter pirouette and collected canter, it was concluded that the pirouette strides did not maintain the rhythm and timing of the collected canter (Clayton, 1997).

Biau and Barrey (2004) described the relationship between stride characteristics, measured by accelerometry, and scores at dressage tests in competition. The gait analysis was performed with the Equimetrix® accelerometer fixed in the sternal region. Nineteen horses divided into two groups of ten young and nine more experienced horses performed the test. Young horses performed a test according to dressage rules for young horses, while the test for the more experienced horses was a Prix St Georges middle level test. Gait variables measured during the test were correlated to judge´s marks and total scores. They concluded that the walk should be slow, regular, symmetric, with large DVAA and DVD. The trot should be slow with a large DVD and LAA. Finally, high LAA and DVAA are needed for the canter.

Traditionally, the ability of the dressage horse to perform has been predicted by its conformation. Moreover, the relationship between conformation criteria and dressage performance has been described (Holmström et al. 1990, 1994). Specific characteristics of the walk, trot and canter required for a good performing dressage horse, could be genetically selected. Barrey et al. (2002) compared walk and trot with conformational characteristics in young horses of different breeds used for dressage (German, French and Spanish breeds). In the study, a total of 142 horses aged three years, of three different breeds used for dressage were tested. Gait variables were measured with the Equimetrix® system and skeletal conformation measurements were performed by image analysis. Many gait and conformation variables were significantly different between the breeds. Differences in gait variables could be explained by conformational differences between breeds. Speed, SF and SL were related to the withers height, length of limb segments and angles of the proximal joints. It was observed that taller horses had a lower SF and lower SL, causing a smaller speed than for small horses (Barrey et al., 2002).

Although conformation characteristics were explained by breed variations, differences in dressage ability could not be explained. German and French horses were similar in terms of conformation, however German horses had gait characteristics more adapted for dressage competition. When comparing walk characteristics, German horses had good walk profiles according to the results of the test; while French horses had weak propulsion and high SF. Spanish horses had lower REG, low percentage of four-beat walk rhythm and lower DVD. At the trot, German horses showed a slow SF, high REG and large DVD and DVAA, which means elasticity and good propulsion, parameters similar to Fédération Equestre Internationale (FEI) dressage rules. On the contrary, French horses had a higher SF, smaller DVD and lower LAA. Spanish horses showed shorter SL, higher SF and lower DVD and DVAA than German horses. Movements of the Spanish horses were more elevated than extended. Spanish and German horses had high propulsion and LAA, which should be an advantage for performing passage and piaffe. Spanish horses could be considered as a reference for collected gaits used in farm work and old academic dressage.

The mean heritability of dressage performance described by Barrey et al. (2002) was rather low in accordance with values published by several authors. Nongenetic effects like training have an important influence on dressage performance (Langlois, 1980; Bruns, 1981; Huizinga and Van der Meij, 1989). Uphaus et al. (1992) described that subjective score of gait and conformation criteria were moderately correlated with future performance. Mean heritability was higher for trot than walk characteristics (Barrey et al. 2001). Some gait variables, such as SF, DVAA and LAA, which have moderate to high heritability, could be used for early genetic selection.

3.4.1.4. Use of accelerometry in endurance horses

Endurance races are competitions in which the speed and endurance ability of the horse are tested. The FEI regulates the rides, in which the rider and the horse must cover a specific course within a maximum time limit (Frazier, 2000). The ride is divided in several phases and at the end of each phase a veterinary inspection is performed. Demands imposed by the terrain and environmental conditions, make stress, and demands on horse and rider differ from other equine sports. Horses perform for many hours over uneven terrain and often in not ideal conditions (Nieto et al. 2004).

As endurance racing is an exercise of moderate intensity, but prolonged duration, the energy required for the effort mainly results from aerobic metabolism of fatty acids and glycogen in slow-twitch and intermediate contraction muscle fibers (Rivero and Piercy, 2008). Although aerobic capacity of a horse can be increased with training, it has been demonstrated that it is also influenced by the breed, being the Arabian breed the one with the highest aerobic capacity when compared to Anglo-Arabian and Andalusian horses (Rivero et al., 1993; López-Rivero, 1995; Castejón et al., 2006; Muñoz et al., 2017). Arabian horses have morphological, muscular and metabolic features designed for endurance races, being their energetic metabolism particularly suitable for this exercise (López-Rivero et al., 1989; Prince et al., 2002). When compared to Thoroughbreds, Arabian horses showed a lower respiratory exchange ratio (RER) and higher plasmatic concentration in free fatty acids, which makes them more suitable for endurance exercise than Thoroughbreds since these characteristics are associated with aerobic metabolism (Prince et al., 2002).

Endurance races are performed at submaximal speed; however, they result in a 7 to 10 hour metabolic and locomotor stress for the horse (Viry et al., 2014). Usually, horses that win the race have an average speed of 18 km/h, being their energetic metabolism mainly aerobic during most of the race to avoid rapid fatigue due to lactic acid accumulation (Cottin et al., 2010). Running economy (RE) is defined as the amount of oxygen consumption per distance covered. Those horses with the lowest oxygen consume, have a better RE (Cottin et al., 2010). In order to measure oxygen consumption and gait variables in Arabian endurance horses, and measure RER and RE at specific endurance competition winning speed, Cottin et al. (2010) tested five Arabian horses trained in endurance racing. Horses were ridden by the same experienced rider and trained in the same way for the test. Each horse was equipped with a homemade respiratory mask with a seal for expiratory flow. Horses had previously been trained to wear the mask at rest and exercise. Respiratory frequency, tidal volume, oxygen uptake and carbon dioxide production were measured, and ventilatory flow and RER were calculated. Gait variables were measured with Equimetrix®. Running speed and SF were measured. It was seen a strong locomotor-respiratory coupling in Arabian horses when galloping, as the respiratory frequency increased linearly with the SF at gallop. No locomotor-respiratory coupling was observed during trotting (Cottin et al., 2010). However, other studies have reported a strong locomotor-respiratory coupling at the fast trot, at speeds higher than 8,5 m/s (Barrey et al., 2000; Cottrell et al., 2006). Cottin et al. (2010) concluded that at the canter speed corresponding to endurance race winning speed (18 km/h), there was a linear relationship between speed and oxygen consumption volume (VO2), heart rate (HR) and gait variables.

Riders must continuously manage gait and speed to optimize and maintain horses' functional health and status during endurance races. It is important the interaction between horse and rider, as it influences the horses´ health status and it becomes determinant for the final outcome of the race (Viry et al., 2014). Studies describing fatigue due to prolonged riding are predominantly focused on either the horse or the rider, considering them as a separate system. The primary goal of the rider is the preservation of the functional status of the horse, so it is important to know the interactions between horse and rider during an endurance race (Nagy et al., 2010; Viry et al., 2014). To investigate patterns of horse-rider coordination, and describe the influence of expertise and fatigue on riding strategy in endurance races, Viry et al., (2014) evaluated five elite (>3 international podiums in the last 5 years) and five advanced (<3 international podiums in the last 5 years) dyads (rider + horse) during ten different international competitions varying from 91 to 124 km. Horse-rider coupling was evaluated by recording vertical movements using two triaxial accelerometers (Locometrix® and Equimetrix®). Locometrix® was worn by the rider, fixed in a neoprene belt. Equimetrix® was fixed to the horses´ caudal part of the sternal region. Horse-rider coupling patterns were evaluated by their spatiotemporal relationships over stride cycles. Predominantly, all dyads used two riding techniques per gait. The 2-point trot proportion was limited for both groups. Advanced dyads showed global stability in speed, in proportion of four combinations of gait and riding techniques and in mean relative phases. However, at the beginning of the race they had higher mean relative phase values, but from mid-race to the end, an increasing proportion of sitting canter with associated increases in racing speed and in mean heart rate was seen (Viry et al., 2014).

3.4.1.5. Use of accelerometry to evaluate lameness

Musculoskeletal injuries are a common health problem in the sport horse, being the main cause of loss of performance and training days, loss of the animal's economic level and withdrawal from competitive life (Jeffcott et al., 1982). Subclinical disorders of the locomotor system are the most frequent causes responsible for poor performance (Morris and Seeherman, 1991). Thus, prevention and early detection of locomotor inadequacies prior to appearance of clinical signs is a priority in equine sports medicine and welfare (Weishaupt et al., 2001).

Clinical assessment of subtle gait irregularities and their interpretation are often delicate and complex because the subjective visual examination is the clinician´s primary diagnostic tool. Lameness examination consists in identifying any lame limb and subsequently grading the severity of the lameness (Baxter et al., 2020). Despite being routinely performed, multiple studies have demonstrated low agreement for visual lameness examinations between equine clinicians. Additionally, in cases of mild lameness, a low repeatability for subjective evaluation of horses regardless of the conditions of evaluation has been described (Keegan et al., 2010; Thomsen et al., 2010).

In a study by Weishaupt et al. (2001), traditional orthopedic examination was compared with two different objective gait analysis techniques and the sensitivity of both methods tested. Twenty-two presumably sound horses were evaluated for gait symmetry, subjectively, by an orthopedic traditional work-up, performed by three experienced clinicians, and objectively by means of the measurement of vGRF and body-trunk acceleration. Lameness degree and limb involved were defined for each horse, so that they were classified as sound, fore- or hindlimb lame. Significant correlation was found between the clinical group assignment and the assignment based on GRF and acceleration variables. Correlation was also found between clinical and ground reaction forces when assessing affected limb. The synchronized time histories of vGRF and DVAA coincided. However, low correlations between these variables indicate that the GRF distribution within the four limbs and body segment accelerations, must be complex and variable depending on the lameness origin and cause. Nevertheless, significant correlation was found in the hindlimb between vGRF and DVAA, indicating that anatomical conformation of the hindquarters with the sacrum and pelvis more directly connected to the ground, hardly allow for dissipation of compensatory movements to closely located body segments. On the contrary, in the forelimbs, thorax is suspended between forelimbs and neck and head take a significant part on compensatory movements (Buchner et al., 1996). These anatomical differences are pretended to be the reason why no correlation was found between vGRF and DVAA on the forelimbs. However, kinetic and accelerometric variables studied, represent a helpful complementary tool in the lameness examination.

Thomsen et al. (2010) compared accelerometric symmetry scores and clinical lameness scores during experimentally induced transient distension of the metacarpophalangeal joint. Five sound horses were enrolled in the experiment. Horses were equipped with a triaxial 10G piezoresistive accelerometer firmly fixed by an elastic girth, in the lowest part of the back in the midline. Lameness was induced by injecting intra-articular saline which produced a varying and transient lameness ranging from 1-4 on the AAEP scale. Horses were sound two hours after the injection. Interobserver agreements were 70% and there was a statistically significant relationship between AAEP scores and symmetry scores. Symmetry score showed a strong statistical relationship with the visually scored degree of lameness, being low scores of lameness consistent with high degrees of SYM, which were perceived as soundness by the observers. All horses showed highest SYM scores at 3 min post injection and almost returned to start-values after 60 min post injection. Even though there was a marked decline in SYM score from 3 to 15 min post injection, some observers gave the same lameness score values. This indicates that the SYM score is valuable, especially when evaluating improvement or worsening of lameness as it can measure slight changes in lameness. The study concludes that SYM scores based on accelerometry may be an aid in the objective assessment and documentation of lameness.

3.4.1.6. Use of accelerometry to evaluate ataxia

Ataxia is a relatively common problem in equine practice. Clinical examination alone does not always allow differentiation between ataxic and neurologically sound horses. Moreover, exact biomechanical mechanisms of ataxic gaits are not completely understood (Strobach et al., 2006). Although different objective methods have been previously used for lameness evaluation in horses, as presented in previous sections, the movement pattern of clinically ataxic horses has not been evaluated. Sedation with alpha 2 agonist drugs changes movement patterns of neurologically sound horses (Buchner et al., 1999). Strobach et al. (2006) evaluated movement patterns of horses sedated with alpha 2 agonist drugs, as they had the hypothesis that movement pattern of clinically ataxic horses and ataxia induced by sedation could be similar. However, this hypothesis was refused because the motion pattern of sedated horses does not represent naturally occurring ataxia.

Although no investigation has been held out in objectively evaluating motion patterns of clinically ataxic horses, there has been great interest in studying movement patterns associated with the administration of sedative drugs. Lopez-Sanromán and his group has been studying the accelerometric pattern in horses at different times after administration of several types of sedative drugs, such as xylazine (López-Sanromán et al., 2012), using the Equimetrix® accelerometer. Many of the parameters investigated decreased after administration of xylazine. In this preliminary study, it was concluded that accelerometry offers an accurate, easy, low cost and portable method to objectively monitor gait abnormalities after xylazine sedation in horses.

Moreover, sedation is a standard procedure during equine lameness examination. Sometimes due to behavioral problems, there is no other choice than sedating the horse for being able to perform the evaluation (Ross, 2011). Therefore, in recent years different research has been done to evaluate gait pattern changes caused by the administration of different drugs and the accuracy of accelerometry to determine those changes. Studies have evaluated the use of different sedative drug administration and effects on gait patterns at different doses such as xylazine (López-Sanromán et al., 2012, 2013), detomidine (López-Sanromán et al., 2014, 2013; Calvo-Santesmases et al., 2021), romifidine (López-Sanromán et al., 2013), and acepromazine (López-Sanromán et al., 2015), within others. These studies demonstrated that accelerometry is a useful tool to monitor movement patterns in unsedated and sedated horses and to quantify differences between sedative drugs and doses administered (López-Sanromán et al., 2012, 2013, 2014, 2015; Calvo-Santesmeses et al., 2021).

4. INDIVIDUAL STUDIES

Three different studies have been included in the current doctoral thesis:

Study I

Clinical efficacy of clodronic acid in horses diagnosed with navicular syndrome: A field study using objective and subjective lameness evaluation

Argüelles D, **Saitua A**, Sánchez de Medina A, Muñoz JA, Muñoz A (2019). Research in Veterinary Journal 125, 298-304

Impact factor: 1.892 (year: 2019). Quartile Q1. Ranking: 31/141 (JCR Veterinary Sciences)

In this study, we assessed by accelerometry the effects of the administration of an bisphosphonate, clodronic acid, on 11 horses diagnosed with navicular syndrome and in relation to clinical evaluation. Horses were subjected to accelerometric evaluations before, and at 7, 30 and 90 days after treatment. Six of the 11 horses improved clinically. Accelerometric results of these horses revealed increased velocity, SL, REG and DVD, together with a reduction in SF. All of these accelerometric findings were associated with a gait improvement.

Study II

Combined effects of water depth and velocity on the accelerometric parameters measured in horses exercised on a water treadmill

Saitua A, Becero M, Argüelles D, Castejón-Riber C, Sánchez de Medina A, Satué K, Muñoz A (2020). Animals 10(2), 236.

Impact factor: 2.742 (year 2020). Quartile Q1. Ranking: 19/146 (JCR. Veterinary Sciences)

The use of the water treadmill (WT) in the fields of equine training and rehabilitation has expanded worldwide. In the second study, we evaluated the accelerometric changes during exercise on a WT, at different water depths (without water, and with the water at the level of fetlock, carpus and stifle) and at two different velocities at walk: 5 and 6 km/h. We found that TAA increased with water depth, but there were no differences when the water was at the depth of the carpus and stifle. This greater TAA was mainly distributed towards the DDVA. However, a reduction of the accelerometric activity in the other two body axes, LAA and MLAA, was found.

Study III

Previous exercise on a water treadmill at different depths affects the accelerometric pattern recorded on a track in horses

Saitua A, Castejón-Riber C, Requena F, Argüelles D, Calle-González N, Sánchez de Medina A, Muñoz A (2022). Animals. 12: 3086.

Impact factor: 3.231 (year: 2021). Quartile Q1. Ranking: 16/144 (JCR. Veterinary Sciences)

In the third study, we go further from the second study in order to elucidate whether the accelerometric changes found during an exercise on a WT at different depths persist on terrestrial locomotion. To achieve this, we evaluated the horses on a training track, at walk and at trot, before and at 30 min after different exercise sessions on the WT (without water, and with the water at the level of fetlock, carpus and stifle). We expressed the accelerometric parameters as percent changes from baseline values (values found on a track before exercise on the WT). As more relevant results, we found that some of the accelerometric changes described during an exercise session on the WT on study II persisted, but other parameters underwent a reduction when the horses were exercised on the WT at a depth of water at stifle, particularly when the accelerometer was fixed in the sacrum midline. We proposed that these findings could indicate fatigue when the animals were exercised in deep water.

5. STUDY I

CLINICAL EFFICACY OF CLODRONIC ACID IN HORSES DIAGNOSED WITH NAVICULAR SYNDROME: A FIELD STUDY USING OBJECTIVE AND SUBJECTIVE LAMENESS EVALUATION

(published in:
Research in Veterinary Science, 125, 298-304. 2019.
doi: https://doi.org/10.1016/j.rvsc.2019.07.018)

Impact factor: 1.892 (Year: 2019)
Quartil: Q1
Ranking: 31/141 (JCR. Veterinary Sciences)

The authors would like to express their gratitude to Osphos® for providing the clodronate for the study

5.1. Introduction

5.1.1. Navicular syndrome or disease

5.1.1.1. Anatomy of the navicular bone and associated structures

Navicular disease or syndrome is one of the most common and controversial causes of forelimb lameness in horses (Turner, 1989; Stashak, 1998; Dyson, 2003). It is estimated to be responsible for about one-third of all chronic forelimb lameness in horses (Colles, 1982; Stashak, 1998). Navicular syndrome is defined as a chronic lameness associated with pain arising from the navicular bone and the structures closely related, such as collateral sesamoidean ligaments (CSLs) of the navicular bone, distal sesamoidean impair ligament (DSIL), navicular bursa and the deep digital flexor tendon (DDFT) (Dyson, 2003; Sampson et al., 2008). The disease is characterized by degenerative changes in structure, composition and mechanical function of the cartilage, subchondral bone and surrounding soft tissues (Rijkenhuizen et al., 1989).

Navicular bone is a boat-shaped bone that lies on the palmar aspect of the distal interphalangeal joint (DIPJ). It develops from endochondral ossification with a single ossification center that is closed by 325 days of gestation (Colles, 1982). Dorsally, it articulates with the distal-palmar aspect of the middle phalanx, this articular surface is covered with hyaline cartilage (Rijkenhuizen et al., 1989). The palmar flexor surface has a prominent sagittal ridge covered by fibrocartilage; this provides a smooth surface for the opposing DDFT to glide during loading of the limb (Getty, 1975). Distally, it articulates with the distal phalanx with a small articular facet of hyaline cartilage. The distal border contains numerous foramina lined with synovium (Poulos and Smith, 1988; Rijkenhuizen et al., 1989).

The navicular bone is supported by three ligaments. A pair of CSLs maintain the bone in suspension, arising from depressions at both sides of the distal end of the proximal phalanx, extending palmaro-distally to insert on the extremities and proximal border of the navicular bone (Getty, 1975; Kainer, 1989). A branch from each CSL originates from the palmar process of the distal phalanx and inserts on the axial surface of the ipsilateral cartilage of the foot (Getty, 1975). The third ligament, the DSIL, originates at the distal margin of the navicular bone and after extending to the flexor surface of the distal phalanx, it inserts deep to the DDFT (Kainer, 1989).

Navicular bursa is located between the flexor surface of the bone and the DDFT, extending proximally 1-1.5 cm from the navicular bone and distally to the insertion of the DDFT on the distal phalanx (Getty, 1975). The DIPJ is a complex synovial structure near the navicular bone. It has dorsal and palmar extensions along the surfaces of the middle phalanx, with a small additional recess between the navicular bone and the distal phalanx (Sack and Habel, 1977).

Arterial supply to the navicular bone is given by the anastomosis formed by the medial and lateral palmar digital arteries (Colles and Hickman, 1977). On the proximal border, a transverse plexus joining the palmar digital arteries enter the foramina of the navicular bone. Distally, branches connecting from the palmar digital arteries from a distal navicular plexus, give off additional small arteries that enter the foramina along the distal border. Venous drainage occurs via the medial and lateral digital palmar veins (Waguespack and Hanson, 2010). Sensory innervation is supplied by the digital palmar nerves, which run distally through the CSL and are present within the DSIL (Bowker et al., 1994, 1995a,b). These nerves are also innervating synovial structures, such as DIPJ and navicular bursa (Bowker et al., 1995a,b; 1996; 1997).

5.1.1.2. Pathophysiological theories of the navicular syndrome

Three major pathophysiological theories have been proposed to be the cause of navicular syndrome. Vascular abnormalities, abnormal biomechanical forces and degenerative joint disease have been proposed regarding pathogenesis of the disease (Pool et al., 1989). The first theory, supported by Colles (1979), proposed a vascular etiology. Partial or complete occlusion of the digital arteries due to thrombosis, was cited to be the cause of ischemic necrosis of the bone and thus, pain (Coles, 1979). Different research failed to demonstrate the theory, by reproducing the disease altering the blood flow or identifying histological tissue changes compatible with the theory (Rijkenhuizen et al., 1989; Trotter, 2001; Rijkenhuizen, 2006). Later, other studies found evidence of hyperemia in navicular bones in clinically affected horses (Svalastoga and Smith, 1983; Ostblom et al., 1984). Increased vascularization is the result of a combination of active arterial hyperemia and passive venous congestion. Venous outflow obstruction in the result of venous congestion, was thought to cause bone marrow pressure increase and pain (Svalastoga and Smith, 1983; Pool et al., 1989; Pleasant et al., 1993).

The second theory, proposed by Trotter et al. (2001), involves biomechanical causes. This theory suggests that continual pressure between the DDFT and the flexor surface of the navicular bone leads to degenerative changes on the structures. It has also been shown that in response to increased pressure between the DDFT and the flexor surface of the navicular bone, remodeling of the spongiosa underlying the flexor fibrocartilage occurs (Ostblom et al., 1989). Abnormal forces on the navicular bone could arise from either excessive physiological load applied to a foot with a correct conformation or normal loads applied to a foot with abnormal conformation (Trotter, 2001). Poor hoof conformation and imbalances, particularly short heel and long toe hoof conformation accompanied with a broken-back hoof pastern axis, have been considered major risk factors for the development of the disease (Baxter et al., 2011). In addition, this conformation causes excessive and repetitive forces being applied to the distal third of the navicular bone by the DDFT (Baxter et al., 2011). The force exerted on the navicular bone is negatively correlated to both the angle between the third phalanx and the ground and the ratio between the heel and the toe height (Eliashar et al., 2004). This hoof conformation, known as reverse angle of the distal phalanx, increases the contact stress on the navicular bone by the DDFT (Baxter et al., 2011).

The third theory suggests that it is a process of degenerative joint disease. Changes in the fibrocartilage of the flexor surface of the navicular bone, subchondral bone, medullary cavity, and synovium bursa are like those observed in the hyaline cartilage and synovial membranes of joints with osteoarthritis (Pleasant et al., 1993; Wright et al., 1998). Additionally, surface fraying and core lesions of the DDFT, and adhesions between the navicular bone and the DDFT have been observed (Pleasant et al., 1993;

Pool et al., 1989; Wright et al., 1998). Research investigating changes in the navicular bone, the DDFT, CSL, DISL and navicular bursa demonstrated no age-related differences in horses between 4 and 15 years of age (Blunden et al., 2006a,b). This finding suggests that there may be an individual susceptibility to degenerative changes, and that non physiological biomechanical factors may promote susceptibility to suffer these changes (Dyson, 2011).

5.1.1.3. Clinical characterization of the navicular syndrome

Navicular syndrome affects horses of many breeds and ages. An early study indicated that breeds that appear to be in greater risk are Quarter Horses, Thoroughbreds and Warmbloods, in particular geldings (Lowe, 1976). It has been shown to have hereditary predisposition, probably due to limb conformation or to specific shape of the navicular bone (Dik and Van den Broek, 1995; Dik et al., 2001a,b). The shape of the navicular bone has been determined to be inherited in Dutch Warmbloods, which are predisposed to the disease and horses which proximal articular margin of the bone is concave appear to be at greater risk of developing the disease too (Dik and Van den Broek, 1995; Dik et al., 2001a,b; Rijkenhuizen, 2006). Whereas Finn Horses and Friesians rarely develop navicular syndrome, this may be because the proximal articular border of the bone tends to have a straight or convex contour (Dik and Van den Broek, 1995; Dik et al., 2001a,b; Rijkenhuizen, 2006). Furthermore, the syndrome is rarely diagnosed in ponies and Arabian horses (Verschooten et al., 1990; Dyson et al., 2003).

Horses with navicular syndrome, usually are presented with a history of progressive, chronic, unilateral or bilateral forelimb lameness, which normally has an insidious onset (Stashak, 1998). It may include a gradual performance loss, stiffness, shortening of the stride, loss action, unwillingness to turn and increased lameness when working on a hard surface (Dyson et al., 2003). The complaint from the owner may include stiffness, unwillingness to jump, drop fences and inability to lengthen stride (Dyson, 2011). Although chronic bilateral forelimb lameness is considered the norm, unilateral lameness can also occur, especially with lesions that involve the flexor surface of the navicular bone and/or the DDFT (Baxter and Stashak, 2011). Furthermore, although hindlimb lameness associated with navicular disease is unusual, it does occasionally occur (Valdez et al., 1978; Dyson, 2011).

Clinical signs usually become apparent between 7 and 10 years of age, although younger horses can be affected (Stashak, 1998; Dyson et al., 2003; Sampson et al., 2008). Lameness can affect horses as early as 2 to 3 years of age when they have developmental or congenital abnormalities affecting the navicular bone, such as bipartite navicular bones (Baxter et al., 2011). Abnormalities in hoof conformation may be present in horses with navicular syndrome, but it may be difficult to determine if these abnormalities are contributing to the disease or have been developed secondary to the lameness (Baxter et al., 2011). Low, underrun heels, contracted or collapsed heels, medial to lateral imbalances and long toes are common hoof problems in horses with navicular syndrome (Turner, 1986; Stashak, 1998; Wright, 1993). Positive response to hoof testers applied to the frog region, has been described as a consistent feature of navicular disease (Beeman, 1985). However, other authors described it may be sometimes negative (Wright, 1993; Dyson et al., 2003).

The disease usually affects both front feet, but it may initially appear as a unilateral lameness (Stashak, 1998). Wright (1993) reported an incidence greater than 95% of asymmetrical lameness. Most horses are lamer in one forelimb, both at a straight trot and when circled on a hard surface, but often demonstrate lameness on the opposite forelimb when circled with that limb on the inside (Baxter et al., 2011).

Lameness is sometimes apparent when the horse is walking on a hard surface in straight lines, but in some horses, there is only a slight shortening of the stride and reduced lift to the stride, making it difficult to detect (Dyson, 2011). Horses tend to exhibit a mild to moderate lameness at exercise, being worse on hard surfaces. Some clinicians feel the severity of lameness increases with the duration of the lameness (Stashak, 1998). However, the severity of the lameness may be more related to the specific cause rather than the duration of the lameness (Sampson et al., 2008). Horses with bilateral lameness tend to have a stiff, shuffling gait and they carry their head and necks rigidly when trotting. Furthermore, this gait is worsened when circled; becoming worse in either direction on a hard surface, as the lameness is usually exaggerated in the inside limb (Baxter et al., 2011). In some horses, the lameness is only evident under these circumstances (Dyson, 2011). While walking or trotting, some horses tend to land toe first and may occasionally stumble (Trotter, 2001; Rijkenhuizen, 2006).

Response to distal limb flexion can be variable, most horses show a transient, mild increase in lameness (Wright, 1983; Dyson et al., 2003). Distal limb flexion of one forelimb may increase lameness in the contralateral forelimb because of increased loading on the podotrochlear apparatus, but horses with primary injuries of the DDFT may also exhibit the same response (Dyson, 2011). Even though the response is not pathognomonic for navicular disease, elevation of the toe of the foot on a wedge or wooden board, with the contralateral limb picked up, may increase the lameness (Dyson, 2011).

5.1.1.4. Diagnosis of the navicular syndrome

Perineural anesthesia of the palmar digital nerves has been thought to only desensitize the palmar aspect of the foot, but different authors described that it is relatively nonspecific and alleviates pain in navicular bone, podotrochlear apparatus, navicular bursa, distal phalanx, middle phalanx, DIPJ, dorsal aspect of the hoof and possibly the proximal interphalangeal joint (PIPJ) and digital tendon sheath (Schoonover et al., 2005; Rijkenhuizen, 2006). Therefore, many clinical problems in the foot can be desensitized with a palmar digital nerve block (Ross, 1998; Dyson and Murray, 2007a,b,c; Sampson et al., 2008).

Intrasynovial anesthesia of the DIPJ and the navicular bursa can be performed to further localize the site of pain. Anesthesia in DIPJ has been demonstrated to alleviate pain in a large percentage of horses with navicular syndrome (Dyson et al., 1995; 2003; Stashak, 1998). However, several studies report a lack of specificity of DIPJ intrasynovial anesthesia due to diffusion of anesthetic and the location of the sensory nerves to the outpouching of the joint (Bowker et al., 1993; 1996; Dyson et al., 1995; Pleasant et al., 1997; Schumacher et al., 2003). Anesthesia of the navicular bursa is probably the most specific block that can be used to localize pain in horses with navicular syndrome (Baxter et al., 2011). A positive response to intrasynovial anesthesia of the navicular bursa indicates problems at the navicular bursa, navicular bone, DISL, CSLs, sole and/or hoof toe, and the distal aspect of the DDFT (Schumacher et al., 2001; 2003; 2004). However, this is not routinely performed because of the need for radiographic or fluoroscopic confirmation of the injection (Baxter et al., 2011).

Radiology remains the most commonly initial diagnostic tool for supporting clinical diagnosis of navicular syndrome, despite its limitations. Rijkenhuizen (2006) reported that a change of at least 40% of the bone density is required before degenerative changes can be identified in radiographs. Furthermore, in a study by Sampson et al. (2008) it was described that horses with navicular syndrome without radiographic abnormalities on the navicular bone were considered abnormal in most horses after magnetic resonance imaging (MRI). Therefore, it is understood that lack of radiographic abnormalities in the navicular bone, does not necessarily reject the clinical diagnosis of navicular bone syndrome. The complete radiographic evaluation of the navicular bone requires lateromedial, 60º dorsoproximal-palmarodistal oblique and palmaroproximal-palmarodistal oblique views (Stashak, 1998; Dyson, 2008). Significant radiographic abnormalities include enthesophytes at the proximo-medial and proximo-lateral aspect of the bone, proximal or distal extension of the flexor border of the bone, distal border fragments, large and variably shaped distal border radiolucent zones, discrete radiolucent areas in the spongiosa with or without detectable communication with the flexor cortex, new bone at the sagittal ridge, increased thickness of the flexor cortex, sclerosis of the spongiosa, and a bipartite bone (Dyson, 2008).

Although keratinized hoof wall, frog and sole limit the contact with the ultrasound probe, limiting good images to be obtained, ultrasound can be used to help diagnose potential soft tissue injuries within the foot of horses with navicular bone disease (Busoni and Denoix, 2001; Rijkenhuizen, 2006). A transcutaneous approach is usually used, either between the heel bulbs or though the frog, after softening it by soaking (Baxter et al., 2011). The technique can be used to assess the flexor surface of the navicular bone, distal part of the DDFT podotrochlear apparatus, DISL, CSLs, CL of the DIPJ and the entheses of the distal phalanx (Busoni and Denoix, 2001; Grewal et al., 2004; Rijkenhuizen, 2006). Negative findings on the ultrasound scan, do not rule out the presence of abnormalities in the navicular region (Baxter et al., 2011).

Scintigraphy is able to identify early alterations in bone metabolism, so it is thought to be able to identify early pathologic changes within the navicular bone (Baxter et al., 2011). Increased radiopharmaceutical uptake has been documented in horses with navicular bone disease. Scintigraphic evaluation of the foot can be helpful to identify the potential source of pain, but false positive results can occur, especially in horses with low heel conformation (Dyson and Murray, 2007a,d). Furthermore, negative scintigram of the foot does not preclude significant injuries (Dyson and Murray, 2007d).

Computed tomography (CT) is the best option to detect pathology within the cortex and trabeculae of the navicular bone (Tiejke, 1995; Rijkenhuizen, 2006). Bone shape changes, distal border fragments and intramedullary changes, which are not radiographically evident, can be seen on CT (Tiejke, 1995; Rijkenhuizen, 2006). Although soft tissue abnormalities are better detected with MRI than CT, intra-arterial contrast enhanced CT improves the imaging of the soft tissue structures within the foot, being an alternative to MRI (Puchalski et al., 2007).

Magnetic resonance imaging (MRI) is the currently preferred diagnostic technique to assess most horses with navicular syndrome (Sampson et al., 2008). The different sequences allow accurate evaluation of soft tissues, cartilage, and bone within the digit in near anatomic detail (Baxter et al., 2011). Numerous pathologic changes have been described after MRI in horses with navicular syndrome, and many horses appear to have multiple abnormalities present within the same foot (Murray et al., 2006; Dyson and Murray, 2007c; Sampson et al., 2008). Most commonly found abnormalities on MRI involve navicular bone, DISL, CSLs, DDFT, collateral ligaments (CL) of the DIPJ, navicular bursa and DIPJ (Murray et al., 2006; Dyson and Murray, 2007b,c; Sampson et al., 2008). The current difficulty when interpreting the results of MRI is determining what may be the primary abnormality when multiple lesions are found (Baxter et al., 2011).

5.1.1.5. Management and treatment of the navicular syndrome

Navicular syndrome is managed, as there is no cure for this complex condition currently (Dyson, 2003). Multiple factors are involved when making the treatment protocol for horses with navicular syndrome. It must be tailor-made for each horse based on the severity of the lameness, the intended use of the horse, hoof conformation, previous treatments and most likely diagnostic results or lack of diagnostics, such as MRI (Baxter et al., 2011). Developing a treatment protocol for horses without radiologic abnormalities without an MRI scan can be challenging because a definitive diagnosis is difficult to make in these cases (Baxter et al., 2011).

Medical treatment aims returning the horse to regular work as soon as possible, starting initially with work predominantly on straight lines. Horses should be exercised as much as possible daily, combining ridden exercise with hand walking or walking on a horse walker (Dyson, 2011). Horses with minimal radiographic abnormalities of the navicular bone are thought to respond better to medical management than those with radiographic abnormalities (Stashak, 1998). In some horses, use of corrective trimming and shoeing combined with isoxsuprine is sufficient, whereas in others additional analgesia is required using an NSAID (non-steroid anti-inflammatory drug) (Dyson, 2011). In horses with marked increased radiopacity of the medulla, lesions involving the flexor surface of the navicular bone or central osseous cystic lesions, response to medical treatment is often unsatisfactory and surgical treatment is indicated (Dyson, 2011). Moreover, horses with DDFT injuries with or without navicular bone abnormalities tend to do poorly regardless of treatment (Rijkenhuizen, 2006; Dyson and Murray, 2007).

Although a variety of treatment options are available for horses with navicular syndrome, the most important is corrective trimming and shoeing (Turner, 1986; Stashak, 1998; Rijkenhuizen, 2006). Rest and controlled exercise, drugs that improve blood flow, NSAIDs, bisphosphonates, intrasynovial medications and medications aimed in preventing osteoarthritis can be used (Baxter et al., 2011). Surgical treatment options include palmar/plantar digital neurectomy, desmotomy of the CSL, inferior check ligament desmotomy and endoscopy of the navicular bursa (Baxter et al., 2011).

The basis for managing horses with navicular syndrome is corrective trimming and shoeing (Turner, 1986; Dabareiner and Carter, 1993; Stashak, 1998; Rijkenhuizen, 2006). Response to correct shoeing alone is favorable for some horses, with no need for further medical or surgical therapy. Turner (1989) described an improvement in clinical signs three months after the initiation of treatment, with 86% of the horses remaining sound one year later. The goals of corrective trimming and shoeing are restoration of the normal foot balance, correct foot problems, reduce biomechanical forces on the navicular region, ease break-over, support the heels and protect injured areas of the foot (Dabareiner and Carter, 1993; Stashak, 1998). A variety of shoes have been used successfully in the management of horses with navicular disease, such as egg bar, egg bar-heart bar, straight bar shoes, natural balance shoe and the so-called Tennessee shoe (Dejardin et al., 2001; Dyson, 2011). Elevation of the heel, which can be achieved by using a wedge heel shoe or a rim or complete wedge pad, may relieve pressure from the DDFT on the palmar aspect of the navicular bone and thus, subsequent pain (Dyson, 2011).

The use of NSAIDs is a common adjunctive therapy for horses with navicular syndrome (Baxter et al., 2011). In horses a wide variety of NSAIDs is used, being flunixin meglumine, phenylbutazone or firocoxib the most commonly used ones (Baxter et al., 2011; Dyson, 2011). These drugs are administered at the lowest dose to achieve the desired effect. Although there are anecdotal reports of certain NSAIDs being better for horses with foot pain, there are no controlled studies that support these claims (Dyson, 2011). Their use is often reduced to periods that the horse is severely lame or through the initial treatment period when the horse needs to continue working (Baxter et al., 2011).

Although the clinical use of isoxsuprine has decreased in recent years, it has been commonly used to treat horses with navicular syndrome (Rose et al., 1983; Turner and Tucker, 1989; Stashak, 1998). Isoxsuprine is a ß-adrenergic agonist drug that is thought to have both vasodilatory and rheological properties. However, its mode of action in the treatment of navicular disease is unknown, because no measurable cardiovascular effects of isoxsuprine given orally were found in the horse (Ingle-Fehr and Baxter, 1999; Madison and Dyson, 2003). The drug is suggested to have some anti-inflammatory properties that could explain the favorable response to the therapy of some horses with navicular syndrome (Dyson, 2011). Nevertheless, the response to treatment in horses with major radiographic abnormalities is generally poor (Madison and Dyson, 2003).

Polysulfated glycosaminoglycans have been used in horses with navicular syndrome based on the assumption that the etiology may be similar to a degenerative joint disease (Baxter et al., 2011). Studies demonstrated a benefit of its administration intramuscularly in horses with navicular syndrome (Crisman et al., 1993; Dabareiner and Carter, 2003). It can also be injected intrasynovial, but there is no documented research that describes its benefit in horses with navicular disease (Baxter et al., 2011).

Bisphosphonates are drugs that reduce bone resorption and have been proven very effective in human medicine (Rijkenhuizen, 2006). Areas of increased bone resorption and formation are often typical in lesions within a diseased navicular bone. As they inhibit osteoclastic activity, they have been proposed for the treatment of navicular syndrome in the horse. It has been also labeled for use in horses with bone spavin and fetlock suspensory ligament enthesopathies (Baxter et al., 2011; Dyson, 2011). In recent years, several studies have been done with the aim of determining its efficacy in the treatment of horses with navicular disease. The effects of the bisphosphonates will be presented in more detail later.

Intrasynovial medications into the DIPJ and the navicular bursa are often used as an adjunctive to management of horses with navicular syndrome (Verschooten et al., 1990; Dabareiner and Carter, 2003; Dabareiner et al., 2003; Schoonover et al., 2005). Horses with more proximal lesions documented with MRI, may take advantage of the injection of medications into the digital tendon sheath. Decision making about whether to treat horses with navicular disease with intrasynovial medications, is empirically based on many clinical and diagnostic findings (Baxter et al., 2011). Medications used are similar to those used to treat osteoarthritis, synovitis and capsulitis, such as corticosteroids alone or combined with hyaluronan or polysulfated glycosaminoglycans (Baxter et al., 2011).

Regarding surgical treatment options palmar/plantar digital neurectomy, desmotomy of the CSL, inferior check ligament desmotomy, endoscopy of the navicular bursa and decompression of the navicular bone by surgical drilling have been described (Baxter et al., 2011; Dyson, 2011).

Palmar digital neurectomy is the most commonly performed surgical treatment in horses with navicular syndrome, and it is performed as a last resort when other treatment options fail (Baxter et al., 2011; Dyson, 2011). This technique has a better prognosis in those cases in which lameness associated with navicular disease is completely eliminated after perineural analgesia of the palmar digital nerves (Jackman et al., 1993; Maher, 2008; Oosterlinck et al., 2020).

Desmotomy of the CSLs has been described as a treatment option for navicular syndrome (Diehl, 1986; Wright, 1986). Wright (1986) reported 13 out of 16 horses with navicular syndrome, treated with this surgical technique, were able to work without lameness. Other studies reported similar results after the procedure (Wright, 1993; Bell et al., 1996). However, Diehl (1986) reported clinical improvement in 50% of the horses with navicular disease treated by desmotomy of the CSLs.

Turner (1993) described desmotomy of the accessory ligament of the DDFT (AL-DDFT) for treatment of navicular syndrome in horses with a marked upright conformation, as it is assumed that it will reduce the compressive forces generated by the DDFT on the navicular bone (McGuigan and Wilson, 2001; Wilson et al., 2001).

Endoscopy or bursoscopy of the navicular bursa can be performed in horses with navicular disease. Smith et al., (2007) described a modified approach to the navicular bursa bot as a diagnostic and treatment technique in horses with lameness associated with navicular syndrome.

5.1.2. Bisphosphonates in the horse

5.1.2.1 History and development of bisphosphonates

Bisphosphonates have been widely used in human medicine for treatment of different bone diseases. They were first discovered by the pharmacist Theodor Salzer in 1894 and used in textile and oil industries as corrosion inhibitors and complexing agents (Petroianu, 2011). In the 1960s, Fleisch (1982) found that inorganic pyrophosphate could prevent calcification by binding to hydroxyapatite and inhibiting its dissolution.

Pyrophosphates failed to influence bone resorption because they were rapidly hydrolyzed, but was then discovered that bisphosphonates, nonhydrolyzable analogs of pyrophosphate, inhibited bone resorption and were effective in vivo (Feisch et al., 1968; 1969; Francis et al., 1969).

Because of their affinity to hydroxyapatite, they have been notably investigated for the treatment of Paget's disease, osteoporosis, bone metastases, malignancy-associated hypercalcemia and pediatric bone diseases (Giger et al., 2013). Etidronate was the first bisphosphonate used in clinical trials for the treatment of FOP (fibrodysplasia ossificans progressiva) and Paget´s disease, and it has been shown to be notably more effective than drugs used previously (Basset et al., 1969; Smith et al., 1971).

During the 1970s, the bisphosphonates started to be used in oncology as therapeutic agents. Since then, they have become widely established as therapeutic and preventive agents of skeletal complications associated to bone metastases, multiple myeloma and postmenopausal osteoporosis. Bisphosphonates have unique pharmacological properties, such as selective uptake in the skeleton preferentially at the sites with increased bone remodeling and slow release from the bone. This ability to bind strongly to bone minerals gives them the property of selective uptake by their intended target organ, enabling their highly specific interaction with the relevant cellular sites of action, in particular osteoclast, but also with osteocytes (Russell et al., 2008; Cremers and Papapoulos, 2011).

There is emerging evidence of potential differences among bisphosphonates in terms of their clinical effects on speed of onset of fracture protection, sites of anti-resorption efficacy and duration of effect. Furthermore, a study suggested that each bisphosphonate has a distinct profile in terms of its mineral binding properties and biochemical actions within cells (Russell et al., 2008). Knowledge of their pharmacology as well as of differences among the various members of the class is essential for optimal clinical outcomes and minimization of the risk of adverse effects (Cremers and

Papapoulos, 2011). Some of the early examples of bisphosphonates developed in the 1970s and 1980s, for the treatment of diseases characterized by abnormal calcium metabolism, include etidronate, clodronate and pamidronate. Many members of this drug family are currently on the market including neridronate, risedronate, zoledronate, minodronate, alendronate and ibandronate (Ebetino et al., 2011).

5.1.2.2 Structure and biochemical features of bisphosphonates

Bisphosphonates are analogs of the naturally occurring pyrophosphate that can display the same physicochemical activity and can resist hydrolysis. In pyrophosphate, both atoms of phosphorus are bind by an oxygen (P-O-P), replacing the oxygen by a carbon atom stability is conferred to the molecule in bisphosphonates (P-C-P) (Russell et al., 2008; Socrates and Papapoulos, 2008). The carbon atom makes the P-C-P group resistant not only to chemical but also to enzymatic hydrolysis. As a result, bisphosphonates are not converted to metabolites and excreted unaltered. The two phosphonate groups have a dual function, they are required for both binding to bone mineral and for cell-mediated antiresorptive activity (Russell et al., 2008). Modifications to one or both phosphonate groups can dramatically reduce the affinity of bisphosphonate for bone mineral (Ebetino et al., 1998), as well as reducing biochemical potency (Ebetino and Dansereau, 1995; Luckman et al., 1998).

Two additional side chains are attached to the central carbon atom, which are not present in pyrophosphate, termed R1 and R2 respectively. The presence of these side chains allows the introduction of numerous substitutions, leading in the synthesis of a large number of compounds with different properties (Socrates and Papapoulos, 2008). R1 substituents such as hydroxyl or amino enhance chemisorption to mineral (Benedict, 1982; Van BeeKet al., 1996). This may be due to the tridentate binding of hydroxyl-substituted bisphosphonates to calcium. In contrast, bisphosphonates lacking an R1 substitution, or compounds with other substitutions such as chlorate or hydrogen, that provide bidentate binding to calcium crystals, have significantly lower binding affinities (Socrates and Papapoulos, 2008).

On the other hand, varying the R2 substituents results in differences in antiresorptive potency of several orders of magnitude (Ebetino et al., 1998). This higher antiresorptive potency is linked to the ability to affect biochemical activity (e.g. inhibition of the farnesyl pyrophosphate synthase), and is thought to also be linked to the ability to bind to hydroxyapatite (Nancollas et al., 2006; Henneman et al., 2008). So there is evidence of contribution of the R2 structure not only to the cellular but also to the physicochemical actions of bisphosphonates which may affect their uptake, distribution and long-term retention in bone (Russell et al., 2008).

5.1.2.3 Pharmacodynamics

Bisphosphonates have many different therapeutic effects: antiresorptive, chondroprotective, analgesic, anti-inflammatory and anti-angiogenic, but also some adverse effects. However, their main effect is the decrease of bone resorption.

Pharmacological properties of bisphosphonates are unique, and this is what makes them different to other therapies. Knowledge of their pharmacology and the differences between the different groups is essential for optimal clinical outcome and minimization of adverse effects (Cremers and Papapoulos, 2011). The most interesting property of these compounds is the selective uptake by the skeleton preferentially at sites with increased bone remodeling and slow release from bone (Cremers and Papapoulos, 2011). The pharmacological effect of bisphosphonates is related to both their binding to bone mineral and their biochemical effect on cells, predominantly osteoclasts (Russel et al., 2008).

After accumulation in bone tissues by specific release mechanisms, bisphosphonates are delivered to osteoclasts. The acidic environment of the resorption space between the bone and osteoclasts, resorption lacunae, triggers the release of bound bisphosphonates, resulting in their accumulation and uptake by fluid-phase endocytosis (Sato et al., 1991; Thompson et al., 2006). A proton pump in the osteoclast ruffled membrane that mediates bone resorption creates the acidic environment in the resorption lacunae (Baron. Et al., 1985; Blair et al., 1989). Osteoclasts can take up large amounts of bisphosphonates due to their ability to release the bisphosphonate from the bone surface during resorption, while non-resorbing cells take up small amounts that become available due to natural desorption from the bone surface (Coxon et al., 2008). Upon uptake by osteoclasts, bisphosphonates exert their inhibitory activity through two structure-dependent main mechanisms (Cremers and Papapoulos, 2011).

Bisphosphonates act on osteoclast inhibiting their activity either by a direct toxic effect on these cells, an action relevant for non-nitrogen containing bisphosphonates, or by altering their cytoskeleton, an action relevant for nitrogen containing bisphosphonates (Rogers, 2003; Reszka and Roldan, 2004).

First generation bisphosphonates or non-nitrogen-containing bisphosphonates, such as clodronate, act as analogs of pyrophosphates (Giger et al., 2013). Once up taken by the osteoclasts, they are metabolized to a cytotoxic analog of ATP, adenosine-5´-(β, γ-dichloromethylene)-triphosphate, which inhibits the mitochondrial adenine nucleotide translocase and eventually triggers apoptosis (Frith et al., 1997; Lehenkari et al., 2002).

Bisphosphonates that contain a nitrogen atom in an alkyl chain, such as alendronate, are between 10 and 100 folds more potent than first-generation ones and were shown to inhibit bone resorption through the mevalonate pathway (Van Beek et al., 1999). They alter mevalonate pathway, responsible of the production of cholesterol, other sterols and isoprenoid lipids (isoprenyl diphosphate, farnesyl diphosphate and geranylgeranyl diphosphate) affecting cell activity and survival by interfering with a posttranslational modification process, known as prenylation, of key regulatory proteins necessary for signaling (Rogers, 2003). Prenylation, which leads to the transfer of either a farnesyl or geranylgeranyl moiety to target proteins is required for the correct functioning of small ATPases, such as Rho, Rac, Ras or Rab (Luckman et al., 1998). These hydrolase enzymes can bind and hydrolyze guanosine triphosphate and thereby regulate a variety of important processes in osteoclasts, inducing changes in the cytoskeleton cell morphology such as raffled border loss, disruption of altered actin rings and vesicular trafficking, leading to their inactivation and apoptosis (Coxon and Rogers, 2003; Rogers, 2004).

The loss of osteoclast prenylation capacity may result in the blockade of intracellular signaling processes, required for osteoclast function. However recent studies have suggested that inhibited resorption is because accumulation of non-prenylated GTPases in an active rather than to the loss of protein prenylation capacity (Dunford et al., 2006; Rogers et al., 2011).

5.1.2.4. Pharmacokinetics

Bisphosphonates have been administered in human medicine most commonly orally (PO) or intravenously (IV). Numerous delivery systems are being investigated to circumvent the poor bioavailability of bisphosphonates, by delivering them more efficiently to target sites or altering their biodistribution (Giger et al., 2013).

When administered orally, bisphosphonates exhibit an average bioavailability of only 1-3%. Small differences in absorption rates have been demonstrated among bisphosphonates, which are very low, the nitrogen containing bisphosphonates investigated, such as alendronate, risendronate and ibandronate, have an absorption of about 0.7%; whereas, non-nitrogen containing bisphosphonates, clodronate and etidronate, seem to have a higher absorption of 2-2.5% (Cremers et al., 2005). The low oral absorption results from their negative charge and hydrophilic nature, which hamper their diffusion through cell membranes, as absorption is thought to occur via paracellular pathway (Boulenc et al., 1993). Poor bioavailability of these compounds when administered orally is also due to that despite they are absorbed through the hole gastrointestinal tract, as they chelate calcium, they form unabsorbable complexes with calcium (Gertz et al., 1993; Cremers and Papapoulos, 2011). Gastrointestinal absorption is decreased by food and drinks, as well as divalent cations, as magnesium and aluminum, and may increase in presence of elevated gastric pH (Gertz et al., 1994; Porras et al., 1999; Erza and Golomb, 2000; Dunn and Goa, 2001).

Due to their low oral bioavailability, these drugs are often administered parenterally. However, this route has important limitations, such as lower acceptance. When administering bisphosphonates IV, slow infusion is necessary to avoid kidney damage, taking into account that approximately 50% of the absorbed dose is rapidly eliminated by the kidney. Care must be taken when using bisphosphonates in patients with impaired kidney function (Giger et al., 2013).

Intramuscular (IM) administration can lead to local tissue damage. Systemic administration of bisphosphonates in high doses can be detrimental because of their long half-life, especially in pediatrics. Therefore, efficient delivery systems for local treatment would be desirable, and that's why different administration routes have been investigated, such as pulmonary, intranasal and transdermal (Giger et al., 2013). In animal models, these administration routes showed a 5-fold (transdermal) and 25-fold (nasal) higher bioavailability when compared to oral intake (Katsumi et al., 2010; 2011; Kusamori et al., 2010). However, these alternative routes can also have side effects, such as local toxicity (Roemer-Bécuwe at al., 2003).

After absorption, approximately half of the absorbed dose is eliminated by the kidney, the rest is mainly taken up by bone tissue (Cremers and Papapoulos, 2011) and return to the bloodstream by physicochemical desorption, or by the osteoclastic resorption, and finally adhered again to the bone (Nancollas et al., 2006).

The P-C-P structural pattern of these drugs makes them resistant to hydrolysis. Only a few bisphosphonates, such as non-nitrogen containing bisphosphonates clodronate and etidronate, are metabolized into cytotoxic ATP analogs (Frith et al., 1997).

5.1.2.5. Bisphosphonates in equine medicine

Orthopedic diseases in horses are the most common performance-limiting problem in training and competition. This produces important economic losses to the equine industry. One of these orthopedic diseases is the navicular syndrome, which is a progressive inflammatory disease of the equine digit that results in degeneration of the distal sesamoid and associated soft tissue structures. There are limited effective treatment options for this pathology and traditionally the main aim of the therapy has been pain relief to minimize discomfort and control of the disease progression (Pool et al., 1989).

The use of bisphosphonates is an interesting alternative in pathologies characterized by intense bone remodeling, which leads to osteolysis because of high bone turnover and pain (Soto et al., 2014). The FDA (United States Food and Drug Administration) approved indication of bisphosphonate use in horses to treat navicular syndrome in 2014 (Sánchez and Robertson, 2014). Tiludronate and clodronate were approved for the control of clinical signs associated with navicular syndrome in horses (Duesterdieck-Zellmer, 2016).

To the date four bisphosphonates have been used in equine medicine, pamidronate, zoledronate, tiludronate and clodronate; being tiludronate and clodronate the most used bisphosphonates nowadays, as they were approved in 2014 by the FDA for controlling clinical signs associated to navicular syndrome in horses. The approval study of the FDA, supports the effectiveness of IV tiludronate (1 mg/kg) and IM clodronate (1.4 mg/kg), in horses aged at least 4 years old, to decrease lameness associated to navicular syndrome (FDA Freedom of Information Summary Tildren® and FDA Freedom of Information Summary Osphos®).

Recently, there has been some controversy over the use of bisphosphonates in horses regarding the differences in mechanism of actions and effects of each class, and their extra label use in some osteoarticular pathologies. The use of these drugs has become controversial between equine practitioners, with many having strong opinions for or against their use in equine medicine (Duesterdieck-Zellmner, 2016). This has made of great interest research regarding the best choice of drug, administration route and dosage, frequency of treatment, minimum age of the horse to be treated, how to select the best candidates for a successful treatment and conditions that may benefit from their use (Duesterdieck-Zellmner, 2016).

The FDA approval study included horses diagnosed with navicular syndrome, with at least a grade 2-3 on the AAEP scale, lame for less than 6 months and free from renal failure. Two months after treatment 64% of the horses treated showed an improvement of the lameness grade of at least 1 grade on their predominantly lame limb and having not worsened from the other limb, in comparison to the 48% of the horses on the placebo group. However, most horses in this study received corrective shoeing (FDA Freedom of Information Summary Tildren®).

In a double-blind placebo-controlled trial performed by Denoix et al. (2003), in which 22 horses diagnosed with navicular syndrome in a period lesser than 6 months before the initiation of the treatment, 70% of the treated horses showed an improvement in lameness and 50% became sound 6 ½ months after the administration of the treatment. Tiludronate was administered at a dose of 0.1 mg/kg four times daily for 10 days, resulting in a dose of 1 mg/kg. Horses that had been lame for longer than 6 months before the administration of the treatment, seemed to need two or three treatments 2 months apart from the first one (Denoix et al., 2003).

Another study reported an improvement in lameness between 120 and 200 days after the administration of tiludronate (1 mg/kg IV), but none became sound (Whitfield at al., 2016). However, in this study all horses were trimmed by an experienced farrier during the evaluated period for correction of the hoof balance (Whitfield at al., 2016).

Response to the treatment can be quite variable, taking a long time to provide pain relief in horses with navicular syndrome. Furthermore, it may be necessary the inclusion of appropriate shoeing to improve outcomes (Duesterdieck-Zellmner, 2016).

On the other hand, the FDA approval study supports the effectiveness in decreasing lameness score in horses with navicular syndrome, after the administration of IM clodronate 1.4 mg/kg, with a maximum dose of 900 mg. At 56 days after treatment, 75% of the treated horses versus 3% of the placebo group, showed an improvement of at least 1 grade in the AAEP lameness score in the predominant lame limb, not having worsened from the other limb. However, 180 days after treatment, 41% of these treated horses were lamer than at the previous evaluation on day 56. Overall success rate at 180 days was 65%. These results indicate that the treatment effect may decrease between 2 and 6 months after treatment (FDA Freedom of Information Summary Clodronate®).

In a study performed by Mitchell et al. (2019) that measured lameness score, osteocalcin and carboxyl-terminal cross-linking telopeptide of type I collagen (CTX-1), which is a bone turnover marker, in horses with navicular syndrome, it was noticed an improvement in lameness score 1 week after clodronate administration that remained 8 weeks after. Nevertheless, there was no change in osteocalcin and CTX-1 after treatment despite the improvement in lameness. Results of this research suggest that a single dose of clodronate does not increase the risk of influencing bone turnover or increasing fracture risk (Mitchell et al., 2019).

However, in another study by Knych et al., (2022a) in which clodronate concentrations in blood and urine and CTX-1 and tartrate resistant acid phosphatase 5B (TRAcP5B), protein biomarkers of bone remodeling were measured over a 6 month period, it was found that clodronate appears to influence osteoclasts at label doses. Moreover, bisphosphonates can be detected in bones and teeth up to 30 days after administration (Knych et al., 2022b). This indicates that bisphosphonates reside in bone and can be detected in both blood and urine for extended periods of time. Furthermore, this could have potential long term effects in athletic performance of horses that have been treated with bisphosphonates, increasing the potential risk of bone fractures (Knych et al., 2022a, b). Regarding this information, more research needs to be carried out in correlation to these findings.

Even though the most common use of these drugs is navicular syndrome, they have been administered, with different success rates, in the treatment of other orthopedic problems in horses. In this way, tiludronate has been administered in horses diagnosed with bone spavin (Gough et al., 2010), in the treatment of signs of pain associated with osteoarthritic lesions of the thoracolumbar vertebral column (Coudry et al., 2007) and the effects of its administration in horses after long-term immobilization have been studied (Delguste et al., 2007). An article regarding the use of zoledronate for the treatment of a bone fragility disorder in horses has been also published (Katzman et al., 2012).

5.1.2.6. Side effects and concerns of the use of bisphosphonates in equine medicine

A concern of the administration of bisphosphonates in human beings is an increased risk of a specific fracture configuration involving the femur. This fracture has been associated with the anti-bone resorptive activity of the bisphosphonate administered, with a greater risk following treatment with the most potent drugs. This risk is considered low with non-nitrogen bisphosphonates. In the rare human cases of atypical femoral fracture, it tends to appear only after long term high dose therapy for osteoporosis (Khosla et al., 2007; 2012; Adler, 2016). This condition has not been reported in horses and safety data from clodronate indicates that no negative effect on bone density or bone strength happens with repeated overdose.

However, a study investigating the pharmacokinetics, pharmacodynamics and safety of zoledronic acid in horses reported a significant decrease in the plasma concentrations of CTX-1 during a period of 1 year after the administration of this bisphosphonate (Nieto et al., 2013). Anyways, several concerns about the administration of this type of drugs have been raised in the last years (Mitchell et al., 2019; Kynch et al., 2022; Vergara-Hernández et al., 2022).

Several side-effects have been described in horses, such as head boggling, transient swelling/pain at the injection site (in case of IM administration), pawing the ground, pruritus… These clinical signs are usually mild and self-limiting. Other signs are nervousness, lip licking, yawning and mild colic that resolve with walking. Rarely, episodes of kidney insufficiency have been reported and are more commonly observed in horses treated concurrently with nephrotoxic drugs (Markell et al., 2020). Therefore, it has been recommended to avoid the use of these nephrotoxic drugs, such as NSAIDs at the same time that bisphosphonates and to monitor renal parameters if there is any concern (Markell et al., 2020).

Recently, a retrospective analysis of the safety of the use of tiludronate has been published (Tischmacher et al., 2022). These authors followed-up a total of 1804 horses administered tiludronate and found that 23 horses (1.3%) displayed possibly related-sided effects, 18 of them after first treatment and 5 after subsequent treatments. The most common clinical sign was the colic, but the authors reported that this side-effect could be undervalued because some horses were pre-medicated with flunixin in order to avoid colic discomfort. Polyuria/polydipsia was recorded in one horse and resolved within a week. Another horse developed laminitis 4 days after treatment and another was diagnosed with a proximal interphalangeal joint septic arthritis within a week after tiludronate treatment. Two horses presented traumatic fractures several months after tiludronate administration (Tischmacher et al., 2022). Therefore, although several side-effects have been described, in main lines, these drugs can be considered safe for the horse. However, it should be taken in mind that the important side effects described in humans, such as atypical fracture of the femur or osteonecrosis of the jaw, have been reported long time after treatment or after prolonged treatments, up to 5 years (Vargas-

Franco et al., 2018; Lorosso et al., 2021). These long-term investigations do not exist in horses yet, so prolonged use should be evaluated in the future.

5.2. Objectives and hypotheses

The current study has been designed to assess the efficacy of a bisphosphonate, clodronic acid, administered intramuscularly in horses diagnosed with navicular syndrome with the use of accelerometry.

We hypothesized that, intramuscular administration of clodronic acid in horses diagnosed with navicular syndrome, would result in: 1) greater velocities and longer strides lengths at walk and at trot; 2) higher values for regularity and symmetry of the stride, indicating an objective reduction of lameness.

5.3. Material and methods

5.3.1. Horses selection: inclusion and exclusion criteria. Diagnostic procedure

A total of privately-owned 37 horses with suspicion of navicular syndrome were recruited for this study. Inclusion criteria were: 1) Age of the animals, older than 4 years; 2) Lameness score, higher or equal than 2 over 5 according to the guidelines of the AAEP[1]; 3) Limbs affected: both forelimbs; 4) Response to analgesia: Positive response to distal palmar digital nerve, intra-articular DIPJ and podotrochlear bursa anesthesia, with an improvement greater than 80% in lameness score; no improvement until longer than 10 min after intra-articular DIPJ block; 5) Radiologic findings: including at least one of

[1] Guidelines for the grading system for lameness, American Association of Equine Practitioners. 0: Lameness not perceptible under any circumstances; 1: Lameness difficult to observed and not consistent, regardless of circumstances (e.g. under saddle, circling, inclines, hard surface…); 2: Lameness difficult to observe at a walk or when trotting in a straight line but consistent under certain circumstances (weight-carrying, circling, inclines, hard surface…); 3: Lameness observable at a trot under all circumstances; 4: Lameness obvious at a walk; 5: Lameness producing minimal weight bearing in motion and/or at rest or a complete inability to move.

these changes: more than 7 lucent areas (synovial invaginations), normal in shape; poor definition between the palmar compact bone and the spongiosa; enthesophyte formation on the proximal border of the navicular bone; cyst lesion of the navicular bone; 6) MRI findings: findings compatible with bone disease of the navicular bone: cyst lesions, changes on the palmar compact bone or spongiosa normal pattern.

Exclusion criteria were: 1) Characteristics of the animals: younger than 4 years, pregnant or lactating; 2) Clinical status: general illness, hind limb lameness, other orthopedic or neurological conditions that could confound lameness examination; 3) Radiological findings: lameness attributable to other conditions different from navicular syndrome; 4) Others: change in shoeing within the 2 weeks prior to the study or during the study; history of previous neurectomy; treatment with NSAIDs, corticoids or other drugs or application of physiotherapy (i.e. extracorporeal shock waves) 30 days before or during the study.

After applying inclusion and exclusion criteria, a total of 11 horses were selected. Confirmation of navicular syndrome was made by diagnostic anesthesia (11 horses), radiographic examination (11 horses) and MRI (5 horses). The protocols for confirmation of navicular syndrome were those:

- Diagnostic anesthesia:

  1) palmar digital perineural block in the more affected forelimb. At least 80% of clinical improvement in lameness and shift of lameness to the contralateral limb;

  2) palmar digital perineural block in the contralateral limb;

  3) intra articular anesthesia of the DIPJ. Horses that look longer than 10 min to achieve a positive response to this anesthesia in both forelimbs were for the next step of the diagnostic protocol;

  4) local analgesia of the navicular bursa. Horses included in the research responded positively to bilateral palmar digital perineural block, took longer than 10 min to achieve any response to DIPJ anesthesia and had a positive response to navicular bursa anesthesia.

- Radiographic protocol: these radiologic projections were obtained: lateromedial dorsoproximal palmarodistal oblique and palmaroproximal-palmarodistal oblique with weight bearing.
- MRI: performed only in 5 horses. With the horses standing, in a 0.25 T open magnet. This standard foot imaging protocol was followed: images obtained in sagittal, transverse and frontal planes using gradient echo (GRE), T1-weighted, fast spin echo (FSE), T2-weighted and short inversion recovery (STIR) sequences.

5.3.2. Accelerometric evaluation and accelerometric parameters

The accelerometric study was performed with a portable 3D gait analyzer (Equimetrix®), fixed in the caudal part of the sternum at the level of the girth. The device records acceleration data continuously, at a sampling rate of 100 Hz. The accelerometric recordings were made at walk and at trot, in hand, over similar hard surfaces, covering a distance of 80 m, four times for each evaluation. In all the cases, the same research (A.S.) led the horses by hand to maintain velocity as similar as possible. Four accelerometric evaluations were carried out: at the beginning of the study (day 0), and at days 7 (control-1), 30 (control-2) and 90 (control-3) after administration of a single dose of clodronic acid intramuscularly.

The following accelerometric parameters were included in this research: 1) spatiotemporal parameters of the stride: velocity (m/s), SF (strides/s or Hz) and SL (m); 2) coordination parameters: REG and SYM; 3) Accelerometric activities and parameters: DVD (cm), DVAA (DVAA, W/kg), LAA (W/kg), MLAA (W/kg) and TAA (W/kg)

5.3.3. Statistical analysis

A test of Kolmogorov-Smirnov and a test of Levene were used to check the normal distribution of the data and the equality of the variance respectively. Data were not adjusted to a normal distribution. A Friedman repeated measures analysis of variance was used to assess differences in lameness score and in accelerometric parameters in the different evaluations (i.e. day 0, control-1 at 7 days after administration; control-2 at 30 days after and control-3, at 90 days after). A Wilcoxon matched pairs test was used as post-hoc.

According to the accelerometric parameters, the horses were divided into two groups: those that improved after clodronate administration and those that did not improve. The differences between both groups at baseline or day-0 were evaluated with a Kruskal Wallis test.

Results were significant at level of $p<0.05$.

5.4. Results

5.4.1. Clinical examination

Median and quartiles (in brackets) for lameness score for the 11 horses selected after applying inclusion and exclusion criteria were those: baseline 3 (2-3), control-1, 2 (2-3), control-2, 2 (1-2) and control-3, 2 (1-2). Six horses experienced a clinical improvement and five did not, but they were not worse compared to baseline. Only one horse had a total resolution of the lameness. In the table 3 we present the results of the clinical score of lameness in the horses that improved compared to those that did not improve.

Lameness evaluation performed	Horses that improved (n=6)	Horses that did not improve (n=5)
Baseline	3 (3-3)	3 (3-3)
Control-1	2 (2-3)	3 (2-3)
Control-2	2 (1-2)	3 (2-3)
Control-3	1 (1-2)	3 (2-3)

Table 3. Median and quartiles of lameness scoring according to the criteria established by AAEP in 11 horses diagnosed with navicular syndrome

5.4.2. Radiographic results

The radiographic scores taken from Dyson (2008) were used. This score system classifies radiographic findings into 5 different categories (0, excellent; 1, good; 2, fair; 3, poor; 4, bad).

Seven of the 11 horses (63.6%) had a score of 3 (poor) consisting in: poor definition between compact and spongious bone due to increased opacity; poorly defined lucent areas in the palmar compact bone of the horse; many (more than 7) radiolucent zones along the distal horizontal or sloping borders of the navicular bone; lucent zones along the proximal border of the bone; large enthesophyte formation on the proximal border of the bone; discrete mineralization within a collateral ligament of the navicular bone; radiopaque fragment on the distal border of the navicular bone.

The remaining 4 horses (36.4%) had a radiographic score of 4 (bad), consisting in: large cyst lesion within the spongious bone; lucent region in the palmar compact bone of the navicular bone; new palmar compact bone on the flexor cortex of the navicular bone.

5.4.3. Magnetic resonance imaging results

Because of limitations with the facilities and economic constraints from the owners, only 5 horses were subjected to MRI, at the beginning of the study, with the objective of the selection of the horses. Main MRI for each of the 5 horses are presented in detail in the paper of Argüelles et al. (2019).

5.4.4. Accelerometric results

The accelerometric results found in the 6 horses diagnosed of navicular syndrome that improved after treatment, at walk, are presented in table 4.

Accelerometric parameter (unit)	**Baseline**	**Control-1**	**Control-2**	**Control-3**
Spatiotemporal parameters of the stride				
Velocity (m/s)	1.3 **A** (1.2-1.4)	1.4 **A** (1.2-1.5)	1.55 **B** (1.3-1.65)	1.45 **A** (1.3-1.49)
SF (strides/s)	0.88 **A** (1.48-0.93)	0.83 **B** (0.78-0.91)	0.83 **B** (0.78-0.88)	0.83 **B** (0.78-0.88)
SL (m)	1.59 **A** (1.48-1.72)	1.57 **A** (1.53-1.70)	1.67 **A** (1.57-1.82)	1.78 **B** (1.71-1.92)
Coordination parameters of the stride				
REG (dimensionless)	159.5 **A** (89-219)	173 **A** (133-205)	182.5 **B** (119-226)	176.5 **B** (150-204)
SIM (dimensionless)	153 **A** (109-198)	139 **B** (121-175)	155 **A** (134-200)	161.5 **A** (138-220)
Accelerometric parameters				
DVD (cm)	3 **A** (2-4)	3 **A** (2-3.5)	3 **A** (2-4)	4 **B** (4-5)
DVAA (W/kg)	0.7 **A** (0.5-0.9)	0.7 **A** (0.5-0.9)	0.9 **A** (0.7-1.0)	0.7 **A** (0.6-0.8)
LAA (W/kg)	2 **A** (1.6-2.5)	2 **A** (1.4-2.5)	1.8 **A** (1.4-2.6)	1.8 **A** (1.4-2.2)
MLAA (W/kg)	1.7 **A** (1.4-2.0)	1.55 **A** (1.2-1.9)	1.85 **A** (1.35-2.2)	1.7 **A** (1.3-2.1)
TAA (W/kg)	4.6 **A** (3.95-5.1)	4.1 **B** (3.7-4.5)	4.45 **A** (3.8-5.5)	4.4 **A** (3.7-4.7)

Table 4. Median and quartiles of the accelerometric parameters measured at walk in the 6 horses diagnosed of navicular syndrome that had clinical improvement , before treatment (baseline), and after 7 (control-1), 30 (control-2) and 90 (control-3) days after intramuscular administration of a single dose of clodronic acid (different letters indicate significant differences at $p<0.05$ between baseline and controls performed at defined time after treatment)

A brief description of the accelerometric results found at walk and presented in the table 4 is shown here (Table 5)

Spatiotemporal parameters of the stride	Velocity increased from baseline in control-2
	SF showed a reduction in control-1, -2 and -3 compared to baseline
	SL increased significantly in control-3, compared to the other accelerometric examinations
Coordination parameters of the stride	REG increased in control-2 and control-3, compared to baseline and control-1
	SYM decreased in control-1 compared to baseline examination, but reached the same values found in baseline in control-2 and control-3. An improvement was not observed in this parameter
Accelerometric parameters	DVD increased in control-3 compared to the other accelerometric evaluations
	DVAA, LAA and MLAA did not show any significant change at walk during the study
	A reduction in TAA was found in control-1 compared to baseline and controls-2 and -3.

Table 5. Brief summary of the results found at walk in six horses that had a clinical improvement during the study.

The following table shows the accelerometric results found in the 6 horses diagnosed of navicular syndrome that improved after treatment, at trot (Table 6).

Accelerometric parameter (unit)	Baseline	Control-1	Control-2	Control-3
Spatiotemporal parameters of the stride				
Velocity (m/s)	3.09 **A** (2.8-3.3)	2.7 **B** (2.1-3.8)	3.4 **C** (2.8-3.7)	3.6 **C** (2.6-3.9)
SF (strides/s)	1.46 **A**	1.42 **B**	1.42 **B**	1.42 **B**

	(1.42-1.51)	(1.37-1.46)	(1.37-1.46)	(1.37-1.46)
SL (m)	2.25 **A** (2.13-2.43)	2.19 **A** (2-2.25)	2.34 **A** (2.12-2.5)	2.50 **B** (2.41-2.71)
Coordination parameters of the stride				
REG (dimensionless)	316.5 **A** (266-369)	332 **A** (260.5-359)	321.5 **A** (302-365.5)	345.4 **B** (343-384)
SIM (dimensionless)	220 **A** (185-266)	242 **A** (212-281)	220 **A** (198-255)	235 **A** (212-259)
Accelerometric parameters				
DVD (cm)	10 **A** (12.8-16.7)	9 **A** (8-10)	11 **A** (9-12)	11 **B** (9-12)
DVAA (W/kg)	15.2 **A** (12.8-16.7)	14.6 **A** (12.1-16.4)	17.3 **B** (14.9-19)	17.8 **B** (16.6-19.2)
LAA (W/kg)	6.75 **A** (5.8-7.4)	6.55 **A** (4.3-7.4)	6.7 **A** (5.3-7.5)	8.7 **B** (6.4-9.3)
MLAA (W/kg)	6.3 **A** (5.6-7.7)	5.8 **A** (4.8-6.5)	6.5 **A** (4.4-7.8)	5.6 **A** (4.8-7.8)
TAA (W/kg)	28 **A** (25.9-31.7)	24.5 **B** (23.5-28.6)	27.1 **A** (25-33.4)	28 **A** (28-33.8)

Table 6. Median and quartiles of the accelerometric parameters measured at trot in the 6 horses diagnosed of navicular syndrome that had clinical improvement , before treatment (baseline), and after 7 (control-1), 30 (control-2) and 90 (control-3) days after intramuscular administration of a single dose of clodronic acid (different letters indicate significant differences at p<0.05 between baseline and controls performed at defined time after treatment).

A brief summary of these results is presented in the table 7.

Spatiotemporal parameters of the stride	A reduction of velocity was found in control-1, with a later increase in control-2 and control-3, compared to baseline
	SF decreased in control-1, -2 and -3, compared to baseline
	SL increased in control-3

Coordination parameters of the stride	A significant increase in REG was found in control-3, whereas the values found in baseline, control-1 and control-2 were no significant different
	SYM changes were no detected comparing the four accelerometric examinations
Accelerometric parameters	DVD was significantly greater in control-2 and -3, compared to baseline and control-1
	DDVA increased in control-2 and control-3 compared to baseline and control-1
	LAA increased in control-3
	MLAA did not change during the study
	TAA did not show any significant difference when comparing the various accelerometric evaluations

Table 7. Brief summary of the results found at trot in six horses that had a clinical improvement during the study.

5.5. Main conclusions

The administration of intramuscular clodronic acid in horses diagnosed with navicular syndrome resulted in a reduction of the lameness score in 6 of the 11 recruited horses (55%). A mean decrease of two degrees in lameness was found, considering the AAEP scoring. From an accelerometric point of view, the improvement at walk consisted of increased velocity, SL, REG, DVD and reduced SF. At trot, increases in velocity, SL, REG, DVD, DDVA, LAA and decreases in SF were found in the horses that improved clinically.

6. STUDY II

COMBINED EFFECTS OF WATER DEPTH AND VELOCITY ON THE ACCELEROMETRIC PARAMETERS MEASURED IN HORSES EXERCISED ON A WATER TREADMILL

(published in:
Animals, 10, 236. 2020.
doi: https://doi.org/10.3390/ani10020236)

Impact factor: 1.2.752 (Year: 2020)
Quartil: Q1
Ranking: 19/146 (JCR. Veterinary Sciences)

6.1. Introduction

6.1.1. A brief overview of the exercise on the water in the sport horse

Different systems for exercise on water are used increasingly in different equine research, training and rehabilitation facilities. Although there is not much information about their use in training and rehabilitation aspects, in the last years there have been some papers considering different aspects of exercise on the water in the horse.

A recent paper, published by Tranquille et al. (2018) showed the results of an international survey concerning the use of exercise on a water treadmill (WT). They reported that WTs were found mostly at educational and rehabilitation centers, with only four in private training yards. All centers reported that horses needed between 2-3 sessions to acclimatize to the exercise on a WT. Habituation session ranged between 10-30 min of duration with the water depth increasing slowly to the level of the hock by the end of the second or third session. The average walk velocity was 1.6 m/s (range 0.7-2.8 m/s) at walk and 4.4 m/s (range 3.9-4.9 m/s) at trot.

More interestingly, 60% of the responses indicated that the WT was used for training, 32% for dressage, 16% eventing and 8% show jumping. Hock depth was most frequently used (24% of responders), with a mean session duration of 23 min, ranging between 5 and 54 min. The two main outcomes of a session were an impression of increased strength and fitness (62%) and an improvement in general performance (57%) as perceived by the owner/rider (Tranquille et al., 2018).

The main reported applications for WT exercise in the field of rehabilitation were for injuries of suspensory ligament and tendon injuries (41%). Mid-cannon (25%) and above the fetlock (24%) were the water depths most commonly used. When comparing training and rehabilitation sessions, a significantly greater proportion of training sessions used deep water compared to the rehabilitation session. Mean session duration was longer for training sessions (Tranquille et al., 2018).

Similarly, Wilson et al. (2018) published data derived from a questionnaire regarding biological, electrophysical and other modalities used in horses for rehabilitation of injuries or performance issues. These authors evaluated 305 responses, belonging to 10 geographic regions, from the USA, Europe, and Canada. An 86.5% of the responders used cold water hydrotherapy. 39% used WT, 30.4% used a swimming pool and 21.1% a saltwater spa.

There are different ways to use water and hydrotherapy in the sport horse, being the mainly, WT, above ground or in ground units; swimming pools, circular or straight, aqua walkers and standing saltwater spas or whirlpools. The in-ground WT has the capacity to have a greater amount of water, providing more buoyancy compared to the above-ground WT units. The above-ground underwater treadmill units are able to change the depth of water between each patient, allowing for targeted rehabilitation protocols designed to improve joint range of motion (King, 2013; Méndez-Angulo et al., 2013). Both WT units can have installed hydro jets creating additional turbulent fluid flow, increasing the resistance of limb movement through the water-enhancing muscle strength. Additionally, underground WT have the ability to vary the treadmill velocity, water temperature and solute concentration (King, 2016).

The aqua walkers are mechanical walkers fitted within a circular pool that contains a consistent depth of water. The diameter of the aqua walker determines the number of horses that can be exercised at a time (King, 2016; Muñoz et al., 2018; 2019). Most systems are able to exercise 6-8 horses at the same time. Horses are not completely buoyant and are separated from each other by dividers creating an individual pen, such as the same that land walkers. The depth of the water depends on the design of the system: some have just a shallow trough with water maintained no higher than the fetlock joint, whereas others maintain the water height at the level of the stifle. As happens with the land walkers, some horses choose to slow down and then rush forward as the divider approaches them from behind only to slow down again once they catch up to the divider in front of them.

Swimming typically takes place in linear or circular shaped pools with ramps installed for ease of entry and exit. Equine pools should be designed so that handlers on each side of the horse's head can walk alongside during the exercise. Linear pools may decrease cardiorespiratory stress as the horse is allowed to recover on exiting while being walked back to the entry point (Bromiley, 2007; King, 2016; Muñoz et al., 2018; 2019). Continuous lap swimming in circular pools does not allow for cardiorespiratory recovery until completion of the exercise session.

Horses are not natural swimmers and often use their thoracic limbs to maintain balance while the pelvic limbs are primarily used for propulsion. The explosive nature of the pelvic limb propulsion often results in extreme ranges of motion through the hip, stifle, and hock joints. Furthermore, on entry into the water, the horse adopts a posture that results in cervical, thoracolumbar, and pelvic extension (Santusuosso et al., 2021; 2022).

6.1.2. Proposed mechanisms of action of the exercise on water: physical properties of the water

The physical properties of the water provide a medium where the mechanisms of buoyancy (partial or complete), hydrostatic pressure, and viscosity, together with the ability to alter temperature, osmolarity, velocity of the WT and depth of water, can be applied in different combinations to train or to rehabilitate different injuries or conditions. Although we are going to focus on these fields of equine medicine, there are other possible applications of the exercise on WT in horses, such as neurological rehabilitation, weight control or regulation of insulin-glucose metabolism (Bonelli et al., 2017). In this aspect, the potential use of water exercise in horses diagnosed of metabolic syndrome, insulin resistance and particularly for these horses with endocrinopathic laminitis deserve future studies.

The **buoyancy** can be defined as the upward force exerted on the horse equivalent to the volume of water displaced, following the Archimedes principle (King, 2016; Nankervis et al., 2017; Muñoz et al., 2018; 2019). In other words, buoyancy is a lifting force that acts to reduce axial loading of the joints by minimizing vGRF (Greco-Otto et al., 2019). Using underwater force platform analysis in human beings, it has been demonstrated a significant reduction in vGRF during walking, which was inversely correlated with the depth of the water (Miyoshi et al., 2004). In humans walking at a slow velocity in water at the level of the manubrium, a reduction of 75% in weight bearing has been reported, with only a reduction of 25% in weight bearing when walking in water at the level of the pelvis (Harrison et al., 1992). An early study by McClintock et al. (1987) pursued to calculate the reduction in weight bearing in horses in flotation tanks, following the Archimedes principle. Therefore, the effective weight of a horse in the flotation tank was estimated considering the actual weight of the horse and the weight of the saline displaced. These authors estimated a reduction in body weight of 10-30% with the saline slightly above the elbow, and a reduction of 70% with the water at the level of the tuber coxae.

Increased buoyancy, i.e. deeper water, has been associated with a reduction of weight-bearing stress placed on the distal aspects of the limbs, helping in the reduction of pain and inflammation in association with loading exercises. Progressive loading of the limbs throughout a training or rehabilitation program can be obtained by progressively decreasing the water depth (Nankervis et al., 2017; Muñoz et al., 2018; 2019).

Although the reduction in weight-bearing during exercise on water is desirable in injured horses, it has raised some concerns about how it might affect training-associated tissue remodeling, particularly in young animals. Cartilage adaptation early in life is essential for a well-structured articular cartilage collagen network, and although bone undergoes remodeling during the whole life of the animal, it is only capable of modeling during the early stages of life. In mice exposed to partial weight bearing, decreases in bone mineral density, bone architecture and muscle mass were reported (Ellman et al., 2013). Under these premises, Silver et al. (2020) studied how training on land and on a WT could affect bone and cartilage metabolism in yearlings. They concluded that there were no negative effects of buoyancy on joint development in yearling horses, transitioned to an advanced workload when water was set at 60% of wither' height.

Further, in human beings, increased buoyancy resulted in significant improvements in joint range of motion. Thus, humans with lower extremity osteoarthritis show increased limb flexion when walking in water compared to the relatively lower joint range of motion when walking on land (Dixon and Howe, 2005).

Although improvements in joint motion have been also reported in horses in experimentally induced osteoarthritis in the carpus, the effect of different water depths were not specifically investigated (King et al., 2017).

The **viscosity** of the water, i.e. the fluid's resistance to flow, is much greater than that of the air. Viscosity results in a drag force which represents a resistance to forward progression. That means that increased effort is required to move through water and greater muscle activation, strength, motor control and joint stability are needed (Nankervis et al., 2017). Furthermore, drag forces increase with velocity of movement and this is the reason that the comfortable walk velocity for a horse on a WT is much lower (up to 50% lower according to Nankervis et al., 2017) than that on terrestrial locomotion. Electromyographic analysis in human beings has confirmed that exercise on water increases the activation of the agonist muscles, which are pivotal to accelerate the limb in the direction of movement (Poyhonen et al., 2001). In the same way, King et al. (2017) described better and more uniform activation patterns of selected thoracic limb muscles in horses with experimentally induced osteoarthritis of the middle carpal joint when they were rehabilitated on a WT compared to exercise on a land treadmill. In line with the effect of the viscosity, in the paper of Tranquille et al. (2018), owners had the perception that horses required more intense muscle activity during exercise on the WT and this contributed to increasing muscle mass and development, being this one the reason for including this modality of exercise into their training programs.

Another important physical property of the water is the **hydrostatic pressure**, which is regulated by Pascal's law, which states that a fluid exerts a pressure over all surfaces of an immersed body. The circumferential compression of the water on the tissues increased extravascular hydrostatic pressure, which in turn promotes venous return and lymphatic drainage, resulting in a reduction of edema and soft tissue swelling (Clayton, 2018). Because of that, exercise on water has been recommended for human patients with a variety of heart diseases, for instance stable coronary artery disease (Volaklis et al., 2007; Scheer et al., 2021). In addition, it is well known that increased intra-articular pressure in an osteoarthritic joint leads to decreased proprioception and impaired muscle activity with loss of stability of the affected joint. Kamioka et al. (2010) suggested that afferent excitation of joint mechanoreceptors induced by increased intra-articular pressure, as happens in osteoarthritis, may be dampened by the effects of increased hydrostatic pressure. Although these results have been reported in human beings, similar data have been found experimentally in horses with experimentally induced osteoarthritis (King et al., 2013; 2017). According to King (2016), changes in hydrostatic pressure during exercise on water enhance muscle spindle activity through the stimulation of skin surface sensory nerves and joint mechanoreceptors, organs that act as proprioceptors and modifiers of muscle activity to increase joint stability and to protect joint structures from excessive or abnormal loading.

These properties of the water can be changed by modifying water temperature and osmolality, as well as the velocity of the movement. Both cryotherapy and thermotherapy on water can be used. Cryotherapy is used with the aim of reducing soft tissue pain, inflammation and swelling, producing peripheral vasoconstriction and reduced soft tissue perfusion (Worster et al., 2000). The reduction of blood flow to the extremities together with the cold temperature decreases tissue metabolism, restricting the inflammatory processes and slowing down the nerve conduction velocity. As a consequence, decreased inflammatory mediator release, tissue metabolism, cellular oxygen demands, hypoxic injury and pain are found (Algafly et al., 2007). On the contrary, thermotherapy is used following the acute inflammatory phase. Warm water immersion at 36 ºC causes vasodilatation, which reduces peripheral vascular resistance and increases tissue perfusion (Yamazaki et al., 2000). Water temperature during exercise on the WT may also play a role in nociception by acting on local thermal receptors and increasing the release of endogenous opioids (Coruzzi et al., 1988). Water temperature appears to affect the heart rate of horses exercised on a WT (Nankervis et al., 2008), with greater HR values at increased water temperature (13, 16 and 19 ºC). Water temperature should be controlled in horses exercised on a WT, because these animals depend on evaporation of sweat for thermolysis. When exercised on the water, evaporative heat loss does not occur over the surface area of the skin covered by the water. This fact implies that a net heat gain during exercise with the water is at 19 ºC or higher, even if the intensity of this exercise is low might happen (Nankervis et al., 2008). In these cases, it is cautious to monitor the rectal temperature of the animals.

Depending on the unit of the WT used, the solute concentrations can be modified, with high osmolality associated with anti-inflammatory, osmotic, and analgesic effects (Bender et al., 2005). In humans, a reduction of the mechanical nociceptive threshold, i.e. a decrease in pain sensation, in knee osteoarthritis was described after a 2-week period of daily exercise in mineral water (Yurtkuran et al., 2006). In the same way, an improvement in human patients with fibromyalgia was reported, lasting up to 3 months following exercise in a sulfur pool (McVeigh et al., 2008). Even though less studied, there are some data concerning the effect of different osmolality of the water in the horse. Horses diagnosed with distal limb injuries, stood in hypertonic cold-water bath, at temperatures ranging between 5 and 9 ºC, for 10 min, 3 days a week for 4 weeks, experienced clinical and ultrasonographic evidence of faster healing of digital flexor tendon and suspensory ligament lesions, according to an early research (Hunt, 2001). Visual improvement in the degree of soft tissue swelling was also demonstrated within the first 8 days after starting the hypertonic cold-water therapy (Hunt, 2001). Ultrasonographic findings compatible with reduced peritendinous and periligamentous edema, decreased inflammatory infiltration and improved collagen fiber alignment after 4 weeks of the hypertonic cold-water therapy were described (Hunt, 2001). These effects were attributed to the added mineral components in water, providing an osmotic effect, which results in soft tissue inflammation, decreased pain and increased joint range of motion.

6.1.3. Physiological adaptations to exercise and training on water in the horse

6.1.3.1. Respiratory adaptations to exercise and training on water

The respiratory rate (RR) is considerably lower during summing (24-48 breaths/min) than during strenuous terrestrial exercise, approximately 120 breaths/min (Nicholl et al., 1978; Hobo et al., 1998; Jones et al., 2002). The breathing pattern differs from the usual 1:1 breath to stride ratio in galloping horses, although there are variations according to the reports (Thomas et al., 1980). The duration of expiration during free swimming has been reported to be approximately twice that of inspiration

(Thomas et al., 1980). Other authors described three phases of respiration during swimming based on poolside observations: a maximal short inspiration, then a prolonged period of apnea at full inspiratory volume and an attempted expiration against a closed glottis, followed by a final short explosive expiration (Thomas et al., 1980; Hobo et al., 1998).

During swimming, water pressure on the horse's body prevents adequate ventilation (McClintock et al., 1986; Hobo et al., 1998). Hobo et al. (1998) described increases in RR, an increase in both inspiratory and expiratory pressure and the expiratory time roughly doubled the inspiratory time. It was suggested that a longer expiratory time may limit sudden collapse of airways by water pressure during swimming and prevent a marked decrease in air space volume and thus maintain buoyancy (Hobo et al., 1998). Recently, Jones et al. (2019) described that horses experienced periods of post inspiratory apnea during swimming exercise, explained as a mechanism to aid buoyancy and, at least to some extent, be a manifestation of the mammalian dive reflex. This reflex has been reported in a variety of terrestrial vertebrates and is characterized by apnea, profound bradycardia and redirection of blood flow away from non-exercising muscles and organs (McCulloch et al., 1997; Oppenheimer, 2013). Because of these facts, swimming is not recommended in horses with impaired upper airway function, as they might be unable to fully protect their airways from water.

Recently, some ergoespirometric data have been reported in horses exercised on a WT, at three different velocities at a walk (1.11, 1.25 and 1.39 m/s) and at three different water depths (mid cannon, carpus and stifle), compared to a control situation or dry treadmill (Greco-Otto et al., 2017). Oxygen uptake was influenced by the velocity and the depth of the water, with mean values of 25.1 ml/kg/min with the water at the level of the stifle. These data are comparable with those found in land treadmills, without inclination, where VO2 values in Thoroughbred racehorses of 20.2 ml/kg/min at a walk velocity of 1.60 m/s. At the lowest water depths, horses were more erratic with their breathing pattern, probably because the work was insufficient to force them into a more rhythmic or controlled breathing strategy. In fact, as the workload increased, at the water depth of the stifle, horses began to breathe deeper and slower, with larger tidal volumes (Greco-Otto et al., 2017). This breathing strategy favors alveolar ventilation and gas exchange and reduces relative dead space ventilation.

All these data reflect that, nevertheless of the water depth, intensity of the exercise on a WT is low, and or submaximal nature. Some authors wondered if this intensity was enough to induce significant improvements associated with chronic exercise or training. In this sense, Greco-Otto et al. (2019) evaluated the effect of an 18-day WT training program on peak VO2 in nine Thoroughbred racehorses. The animals were divided into two different groups: six of them worked daily for 18 days on a WT at a water depth of the carpus (two first days) and stifle (the remaining days), whereas three horses were considered control and they were walked on a dry treadmill (i.e., without water). Before and after training, an ergoespirometric test was performed on a track. WT training resulted in an increase of 16.1% in peak VO2, but this change was not found in the group of horses exercised on the dry treadmill. It was concluded, therefore, that exercise on a WT, even its submaximal intensity, increases peak VO2 and endurance in thoroughbred racehorses, and the inclusion of this type of exercise into a training program for these horses may have a beneficial conditioning effect (Greco-Otto et al., 2019).

6.1.3.2. Cardiovascular adaptations to exercise and training on water

The cardiovascular changes during exercise on the water were the first physiological adaptations investigated in the horse. Swimming causes a significant increase in HR, but the maximum HR obtained during swimming is lower than this obtained during ground exercise (Thomas et al., 1989; Galloux et al., 1994). Additionally, there seems that there is not a significant relationship between HR and duration of swimming (Galloux et al., 1994).

Our research group has registered mean HR values of 66, 71, 82 and 81 beats/min in moderately fit horses exercised on a WT without water and with the water at the level of the fetlock, carpus and stifle, respectively (unpublished data). Our results are in agreement with those previously reported by other authors. Walking at 0.9 m/s at a depth of the ulna resulted in HRs up to 65 beats/min (Scott et al., 2010), which are quite similar to those measured during overland walking. Lindner et al. (2012) had 10 German Warmblood horses trotting on a WT at 5.5 m/s whilst the water depth was increased every five minutes from 20% of wither height to 77% of wither height in five increments. There was no significant increase in HR with water depth, as opposite as expected. In fact, HR at higher depths (50% wither height and above) was lower than in shallower water. This plateau in HR was associated with a plateau in workload due to increasing buoyancy and/or the contribution of increasing hydrostatic pressure in improving venous return and subsequent stroke volume (Lindner et al., 2012). To the authors' best knowledge, no studies have demonstrated mean HRs above 150 beats/min. Because of these results, we have indicated that other markers of exercise intensity, rather than HR should be used to monitor horses exercised on a WT (Muñoz et al., 2019). We observed that some unfit horses can experience increases in serum CK activities, and a mild muscle pain associated with daily exercises on a WT, but HR during the sessions were not higher than 150 beats/min. Caution about this should be taken.

With water immersion, there is a decrease in systemic vascular resistance and some changes in total peripheral resistance are dependent on water temperature in human beings (Yamazaki et al., 2000). However, swimming causes a significant increase in blood pressure in horses (Thomas et al., 1980).

6.1.3.3. Metabolic adaptations to exercise and training on water

Blood lactate (LA) is a common marker of fitness and exercise performance in sport horses, indicating both aerobic and anaerobic capacities, according to the exercise test performed. During submaximal exercises, blood LA is considered an indicator of the anaerobic contribution of exercise (Muñoz et al., 1999; 2002; Contreras-Aguilar et al., 2021). Blood LA has been measured in horses exercised on a WT. With differences between authors and horses (probably depending on fitness level), peak blood LA did not reach 3.0 mmol/l in horses trotting in water depths from 10-80% of wither' height (Lindner et al., 2012). Research performed by our group found mean blood LA concentrations of 1.01, 1.05, 1.05 and 1.11 mmol/l in unfit-moderately fit horses exercised on a WT without water, and with the water at the level of fetlock, carpus and stifle (Muñoz et al., 2019). Consequently, these data together with those presented for HR, indicated this type of exercise is aerobic and of submaximal intensity.

Because of these ideas, some researchers questioned if the use of the exercise on a WT could result in fitness improvement or maintenance in horses. In order to answer this question, Borgia et al. (2010) trained five healthy unfit horses on a WT for 5 days/week for 4 weeks, starting with 5 min per day and increasing progressively to 20 min per day. To assess the changes associated with this training regime, horses performed an incremental standardized exercise test on a land treadmill, before and after training. With this incremental exercise test, Borgia et al. (2010) obtained values of V200 (a marker of fitness, indicating the velocity at a HR of 200 beats/min). In addition, these authors obtained muscle biopsies of the *gluteus medius* muscle at rest, both before and after training. There were no significant differences in the resting concentrations of muscle substrates (ATP, glucose-6-P), metabolites (LA) and enzyme activities. V200 did not vary with WT training. The authors concluded that the WT exercise intensity was too low to induce evident physiological improvements. In fact, the WT program was the training regime recommended by rehabilitation of bowed tendons, consisting of 5 min per day for a week, progressing to 10 min per day the second week, 15 min the third week and 20 min the fourth week. The water depth was fixed at the level of the ventral abdomen.

The same team performed several years later another similar study (Firshman et al., 2015) to compare the effects of training on land treadmill vs. WT on the fiber properties and metabolic response of the gluteal muscle. In this second study, Firshman et al. (2015) walked 6 horses on a land treadmill and on a WT, following the same protocol and consisting of 5 days per week, 40 min at day, for 8 weeks. The horses underwent before and after training a standardized exercise test on a land treadmill. No training effect was identified on muscle fiber type composition, type II fiber diameter, muscle analyte concentrations, blood LA concentrations or HR responses to the test. Maximum diameters of type I fibers decreased significantly in gluteal muscle with conventional land training. No changes were identified in minimum fiber diameters.

The combined results of both studies led to the conclusion that 8 weeks of exercise on a WT, following the protocol recommended for tendon rehabilitation, did not result in a cardiocirculatory or metabolic training response in the gluteal muscle and consequently, a gradual introduction to conventional training is needed after WT rehabilitation.

Despite there is not an important improvement in fitness with the use of the WT in the sport horses, a recent study reported that training on a WT was superior to land treadmill to increase muscle diameter in the hindquarters, with a maximum effect after 4 weeks (De Meeus et al., 2021). These results support the inclusion of WT exercise in the training program for sport horses that require muscle building of the hindquarters.

6.1.3.4. Locomotor adaptations to exercise and training on water

Because locomotor pattern differs in swimming horses compared to horses exercised on a WT, this section will focus on exercise on a WT exclusively.

Physical properties of the water influence biomechanical responses to exercise on the WT. As the limbs get submerged in greater water depths, these effects in the locomotion pattern become more evident, mainly due to drag and buoyancy which have a significant effect in limb locomotion (Tranquille et al., 2017).

Walking on a WT results in a decrease in SF and an increase in SL compared to walking without water, at water depths between the proximal interphalangeal and the ulna joints (Scott et al., 2010; Méndez-Angulo et al., 2014). According to this finding, when evaluating locomotor pattern with a triaxial accelerometer fixed in two different positions (pectoral region/sternum and sacrum midline), a reduction in SF and an increase in SL was found with the water at the level of the fetlock and carpus compared to baseline conditions when the accelerometer was fixed in the sacrum midline. However, with the accelerometer in the sternal area, these changes were not observed until the water reached the carpus (Saitua et al., 2019; Muñoz et al., 2019). As fore- and hind-limbs differ in function, these findings suggest different changes in response to water depths between fore- and hind-limbs (Muñoz et al., 2019).

Stride features are expected to be different to overground locomotor patterns, as both drag and buoyancy increase with water depths, movements of the limbs are modified. Drag forces of water impede forward movement of the limbs as the water depth increases, meanwhile buoyancy assists upward movement of the limb (Edlich et al., 1978; Tranquile et al., 2017). Moreover, when walking both on a land and on a WT, the foot is drawn caudally by the belt during the stance phase, increasing the retraction of the limb (Buchner et al., 1994). During WT exercise, an increase in retraction with water depth has also been documented (Nankervis and LeFrancois, 2018). The stride pattern becomes longer and slower when walking at high water depth due to a reduction of the stance phase and an increase in swing phase duration, compared to WT exercise at low water depths (Mendez-Angulo et al., 2013).

Different studies have demonstrated significant changes in joint range of motion, depending on water depth in both horses and dogs exercised on a WT (Mendez-Angulo et al., 2013; Kathmann et al., 2006; Monk et al., 2006). In horses, Mendez-Angulo et al., (2013) determined the maximum amount of flexion and extension, describing a greater range of motion for all the evaluated joints (metacarpophalangeal, metatarsophalangeal, carpal and tarsal joints) when exercising in any amount of water compared to no water or baseline conditions. The greatest joint range of motion was found for metacarpophalangeal and metatarsophalangeal joints with the water at the metatarsophalangeal and the tarsal water levels, for the carpal joint with the water at the tarsal joint level and for the tarsal joint with the water at the stifle joint level. Tranquile et al., (2022) has recently reported a non-linear significant increase in carpal and tarsal joint flexion as the water depth increases. This increase on range of motion of the distal limb joints are reflected on pelvic vertical displacement as water depth increases both at walk and trot in horses exercised on a WT (Mooij et al., 2013; Nankervis et al., 2016; York and Walker, 2014).

It is believed that WT exercise is a high resistance and low impact exercise, but there is not much known about the effect of the therapy on limb loading. With the purpose of evaluating the effect of water depth and speed on segmental acceleration and impact attenuation, Greco-Otto et al. (2019) fixed three uniaxial accelerometers on the left forelimb (hoof, mid-cannon, and mid-carpus) to horses exercised at three water depths (mid-cannon, carpus and stifle) at two different speeds. In this study, peak acceleration at any location was higher at the dry control compared to any of the studied water depths. Water immersion decreased limb segmental accelerations and increased attenuation as the water depth was increased.

6.1.3.5. Changes in back kinematic associated with exercise on water

Biomechanical responses of the equine back to exercise and training include axial rotation, lateral bending, and pelvic flexion. These three movements have been studied in horses exercised on a WT at different water depths. Mooij et al. (2013) described the changes in these movements in horses exercised at different water depths (levels of hoof, fetlock, carpus, elbow, and shoulder joints), over a period of 10 days. It was found that the range of motion of the axial rotation increased significantly at each successive water depth. Relative to hoof depth, lateral bending decreased when water depths were at the levels of the elbow and shoulder joints, but not at the level of the fetlock and carpal joints. Pelvic flexion increased at each successive water depth (Mooij et al., 2013). These authors stated that WT training with water depths at the levels of the elbow and shoulder joints resulted in movement patterns of the back that were different to water depths at the levels of the hoof, fetlock, and carpal joint. Other interesting findings of this article were that there were no significant differences in the range of motion of the three movements between days 1 and 10 on the WT, nor between the left and right hindlimbs.

Some years later, Nankervis et al. (2017) measured the range of motion flexion-extension of the thoracolumbar spine and pelvic vertical displacement during exercise on a WT at three different depths (hoof, metatarsophalangeal and femoropatellar joints) compared to baseline (without water) in 14 horses. They used markers located on thoracic (T) locations T6, T10, T13, T18, lumbar (L) L3, L5, and sacrum (S), S3 to calculate flexion-extension at each location. In addition, two markers located on left and right tuber coxae were used to measure pelvic vertical displacement. It was found that walking on the WT was associated with a significantly greater flexion-extension of all but the most caudal region (L5), compared to control (hoof) depths. This fact was linked by the authors with the increase in SL with water depth, as back movement is largely passive at the walk and influenced by both limb movements and head and neck position.

The changes in flexion extension of the different parts of the back could be due to a change in flexion, extension or/and in both movements. Narkervis et al. (2017) found that changes in flexion extension were due to opposing changes within the thoracic and lumbar spine. T13 showed an increase in flexion-extension in deep water associated with a postural change towards increased extension compared to shallow water. In shallow water, the horse can lower the head and neck, causing cranial thoracic flexion. In contrast with this pattern in T13, L3 showed an increase in flexion extension on the WT as a result of increased flexion. Contrary to what it was expected, Nankervis et al. (2017) did not find significant association between pelvic vertical displacement and water depth, as opposed to what it was described previously by Mooij et al. (2013). The reason was that 6 of the 14 horses had the greatest pelvic vertical displacement in deep water, but 3 of them showed the greatest pelvic vertical displacement at medium depth, 3 at low depth and 1 at baseline. These results were interpreted as the horses adopt different hindlimb movement strategies in response to walking on the WT possibly due to differences in the strength of muscles activating the hindlimb and/or joint range of motion.

Because some of the changes in back kinematics were attributed to changes in hindlimbs kinematics, Tranquille et al. (2022) measured simultaneously back and limb kinematics in 6 horses at walk, at different water depths on a WT. They found that horses respond to an increase in water depth by increasing distal limb flexion, in line with the results previously presented by other authors (Méndez-Angulo et al., 2013). This limb flexion reached a threshold, when the response leveled off. This threshold was either below the carpus or above the carpus depending on the individual horse. The data provided by Tranquille et al. (2022) also demonstrated that changes in limb kinematics brought about the relatively modest increases in water depth at 1.6 m/s were of sufficient magnitude to induce significant changes in back and pelvic movements.

6.1.4. Use of the water treadmill to rehabilitate injured horses

Several disadvantages and risks of the use of the WT have been suggested, such as injury to the horse or handler during the process of introducing the horse to the WT exercise or skin problems (Mooij et al., 2013); uneven or over development of specific forelimb muscles (Tokuriki et al., 1999); potential to exacerbate injury as a result of overloading vulnerable structures (Nankervis et al., 2017) or the development of inappropriate extended thoracic posture (Nankervis et al., 2016). In spite of these suspected risks, the exercise on a WT is highly used for rehabilitation purposes. According to the survey performed by Tranquille et al. (2019), the main reported applications of WT exercise were for rehabilitation of suspensory ligament and tendon injuries (41% of the cases). The second group of conditions were articular disorders (osteoarthritis), poor performance, reintroduction to work, post-surgery exercise, weak core and conditioning after colic (40% of the cases). A 20% of the responders to the questionnaires use the exercise on the WT for back conditions, including kissing spines, sacroiliac weakness or injury, tuber coxae fracture, torn ligament and misaligned pelvis (Tranquille et al., 2019).

As indicated before, the drag force reduces the protraction of the forelimbs proportionally to the depth of water, whereas the protraction of the hindlimbs does not change and may even increase (Nankervis and LeFrancois, 2018). During overground locomotion, the protraction of the limbs is mainly passive because of the release of the elastic energy stored in the energy-storing structures, mainly superficial digital flexor tendon (SDFT) and suspensory ligament (SuspL). Some authors indicated that the greater activation of the brachiocephalic muscle during exercise on the WT, in association with the viscosity of the water, could modify the normal pendulum of the limb and consequently, the use of the WT in acute SDFT and SuspL injuries is not recommended at this moment (Adar, 2011; Nankervis et al., 2017; Muñoz et al., 2019). However, manufacturers of the WT recommend its use in this type of injuries using deep water, for a very short time, around 5 min per exercise session, increasing by 5 min every week.

However, the exercise on a WT is encouraged in subacute and chronic injuries of SDFT and SuspL, because of the reduced load supported by both structures in deep water and increased range of motion of the distal limb. However, the increased retraction of the limbs during exercise on a treadmill (both in land and water) can be counterproductive in the rehabilitation of proximal SuspL desmitis of the hindlimbs in acute and subacute phases. It is recommended to reduce the velocity of the treadmill in horses with this type of lesion in order to limit the load on the SL at the same time that the horse redevelops hindlimb musculature (Nankervis et al., 2017; Muñoz et al., 2019).

One important consideration is the introduction of riding exercises in horses with SDFT and SuspL injuries. The weight of the rider influences the kinematics of the limbs, increasing the relative duration of the stance phase, the maximum extension and the range of motion of the fetlock, particularly in the forelimbs (Van Oldruitenborgh-Oosterbaan et al., 1995). Because some of these changes have been described in horses exercised on a WT (Scott et al., 2010; Méndez-Angulo et al., 2013; Nankervis et al., 2017; 2018), it is recommended before riding exercises in horses with SDFT and SuspL injuries.

The deep digital flexor tendon (DDFT) and its accessory ligament (AL-DDFT) are maximally stretched during the last part of the stance phase (Denoix, 1994). Because of the greater retraction phase during locomotion on a treadmill, the use of this modality of exercise is not currently recommended. However, because of the effect of the water, due to buoyancy and mainly hydrostatic pressure, exercise on a WT could be recommended for horses with DDFT and/or AL-DDFT injuries (Muñoz et al., 2019).

Although commonly used in equine back disorders and stiffness, the exercise on WT should be used with caution, because of the thoracic extension (Mooij et al., 2013; Nankervis et al., 2017; Tranquille et al., 2022), which is mild with shallow water but increases with water depth. For this reason, exercise on a WT is contraindicated for horses with midthoracic impinging or overriding dorsal spinous processes (Nankervis et al., 2016).

The use of the WT is highly recommended in horses with joint injuries, osteoarthritis or after arthroscopy procedures (King, 2013; King et al., 2013; 2017; Potenza et al., 2020). In fact, water exercises are commonly prescribed for the management of joint diseases in human beings, mainly knee osteoarthritis and rehabilitation after arthroplasty (Bender et al., 2005; Kunduracilar et al., 2018; Sahin et al., 2019; Rewald et al., 2020). The therapeutic effects of water are particularly useful for management of disabled patients with significant joint pain associated with weightbearing. In these patients, land exercises are difficult to perform. Similarly, human patients undergoing arthroscopic surgery and joint replacement are also prescribed with water exercise therapy. This type of exercise decreases weight bearing stress and pain applied to the operated joint, which provides earlier and more intensive rehabilitation without risk of increasing pain or overloading injured tissues (Kim et al., 2012). Silva et al. (2008) demonstrated that human patients undergoing surgical reconstruction of the anterior cruciate ligament had improved knee range of motion and quadriceps muscle function after exercise on water, compared to traditional rehabilitation programs.

We have scientific evidence of the benefits of the exercise on a WT in horses with joint problems. King et al. (2013) induced carpal joint osteoarthritis in 16 horses and the animals were randomly assigned to two types of rehabilitation: land treadmill (or WW, without water) and WT, both at the same velocity, frequency and duration of exercise. The authors recorded postural sway under three different stance conditions (normal square stance, base-narrow placement of the thoracic limbs and blindfolded) using stationary force platforms. The main result of this study was that WT exercise significantly improved the postural stability of the horses compared to land treadmill exercise.

Several years later, a study performed with the same horses that those described by King et al. (2013), examined the biomechanical effects and histological changes of the horses (King et al., 2017). Rehabilitation on a WT resulted in greater clinical improvements with regard to symmetric thoracic limb loading, uniform activation patterns of select thoracic limb muscles, and return to baseline values for carpal joint flexion, compared to the results obtained in the group of horses rehabilitated on a treadmill without water. In the same way, exercise on the WT significantly reduced synovial membrane inflammation. As a conclusion, King et al. (2017) highlighted that WT exercise was a viable therapeutic option for the management of carpal osteoarthritis in horses.

The use of the exercise on WT is also a potent therapeutic option for recovering horses after arthroscopy (Potenza et al., 2020). In this paper, the authors investigated if the WT exercise assisted rehabilitation following arthroscopic surgery for natural osteochondral fragments of the metacarpophalangeal, metatarsophalangeal and carpal joints, compared to a conventional rehabilitation. The time to return to racing was 227 and 239 days in horses rehabilitated on a WT and with conventional rehabilitation respectively. In addition, the percentage of horses that raced after surgery was 83% and 61% for WT and conventional rehabilitation respectively. These data led to Potenza et al. (2020) to state that rehabilitation on a WT was superior in returning racehorses to racing following arthroscopic surgery.

6.2. Objectives and hypotheses

As other authors reported, horse owners perceived increased strength and muscle development after a WT training (Tranquille et al., 2019). In addition, increased flexion of the distal joints of the limbs in horses exercised at different water depths on a WT compared to land treadmill or a WT without water, has been described (Méndez-Angulo et al., 2014). Taking these results in consideration, the **main objective** of the present work is to describe the accelerometric adaptations to a WT exercise at different water depths and velocities.

We propose three hypotheses: 1) Because of the greater viscosity of the water in comparison with the air, the horses would need make a greater effort to advance, and therefore, TAA would increase with the depth of the water; 2) The increase of the TAA could result in increased DVAA and DVD, because of the greater flexion of the distal joints of the limbs reported by other authors (Méndez-Angulo et al., 2013; Tranquille et al., 2022); 3) The horses would have a greater stride SYM and REG, because of a greater gait stability as reported by King et al. (2013).

6.3. Material and methods

6.3.1. Horses

The present work consisted of two different trials, performed with two different horse groups. Trial A was performed with 6 adult horses, 2 mares and 4 geldings of different breeds (4 crossbred, 2 Andalusians). Trial B was performed with 5 adult horses, 2 mares and 3 geldings, 3 crossbreds and 2 Andalusians.

In both trials, horses were fully acclimatized to the WT exercise. Their fitness level was moderate and similar between the horses within each trial. However, horses of trial B were less fit than horses of trial A.

6.3.2. Exercise on the water treadmill

The horses in both trials were evaluated in the following situations: with the WT without water (WW, considered as baseline data), and with the water at the levels of the fetlock or metacarpophalangeal joint (FET), carpus (CAR) and stifle (STF). The order of these situations was established using a randomized design. Each exercise session has a duration of 40 min (including time needed for filling and discharge water).

Trial A: it was performed at a walk, at a velocity of 6.0 km/h. However, because horses were unable to perform the exercise session at this velocity with the water at the level of STF, the velocity was reduced to 5.0 km/h.

Trial B: in order to clarify if the accelerometric changes obtained in trial A derived from water depth or from velocity, a second trial (B) was designed. In this case, the horses perform all the exercise sessions at a velocity of 5 km/h.

6.3.3. Accelerometry

The accelerometer Equimetrix® was used, formed by three orthogonal accelerometers that permit measuring accelerations along the three body axes. In trial

A, the accelerometer was attached in two different locations: in the pectoral region (PECT) and over the midline of the sacrum region (SML). The positions of the accelerometer are indicated in the figure 2.

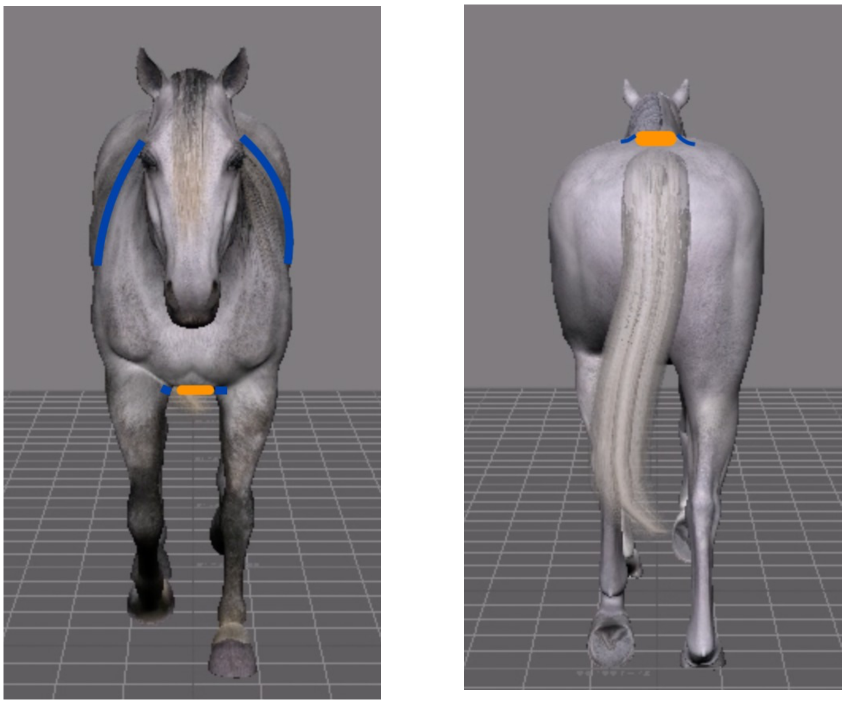

Figure 2. Schematic representation of the positions of the accelerometer (left: on the pectoral region; right: on the sacrum midline). Accelerometer represented in orange.

Data were continuously registered during exercise on the WT at a rate of 100 Hz. Data were downloaded and processed with a software program (Equimetrix®, Centaure 3D). More information about this accelerometer has been published in many different papers and it has been validated for horses (Barry et al., 2002; Biau et al., 2002; 2022; Biau and Barrey, 2004; Leleu et al., 2005; López-Sanromán et al., 2012; 2013; 2014; 2015; 2021; 2022; Viry et al., 2013; Ricard et al., 2020a,b; Calvo-Santesmases et al., 2021).

The same parameters described in the study I were measured: stride accelerometric parameters (TAA, DVAA, LAA, MLAA); coordination parameters (REG and SYM; spatiotemporal parameters (velocity, SF, SL). Accelerometric activities in the three body axes were also expressed as a percentage of the TAA, in order to investigate if a redistribution of TAA happened during exercise on the WT.

In trial A, horses wore the accelerometer only in PECT position at STF water level, because in this case, the accelerometer would have been submerged in water.

6.3.4. Statistics

Normality of the data was evaluated with a Shapiro-Wilk's W test. Data did not adjust to a normal distribution. Comparison of the accelerometric variables for each trial and position of the accelerometer were made between the different water situations (i.e. WW, and water at the level of FET, CAR and STF) with a Friedman repeated measurements analysis of variance on ranks. A Wilcoxon rank test was later performed when the Friedman test revealed significant differences. In order to assess the differences in the accelerometric activities in the three body axes expressed as percentage of the TAA, a Chi-square test was conducted.

Data are presented as means and standard deviations. $P < 0.05$ were considered significant.

6.4. Results

The values of TAA in both trials are presented in figure 3. Total accelerometric activity was higher at the CAR level in trial A with the accelerometer in PECT position. A progressive increase in TAA was observed in trial A with the accelerometer in SML position, up to the CAR level, showing a significant decrease at SFT level when the velocity was reduced. In trial B, with the accelerometer in SML position and compared to WW, TAA was significantly greater in the three conditions with water, without significant differences between them (Figure 3).

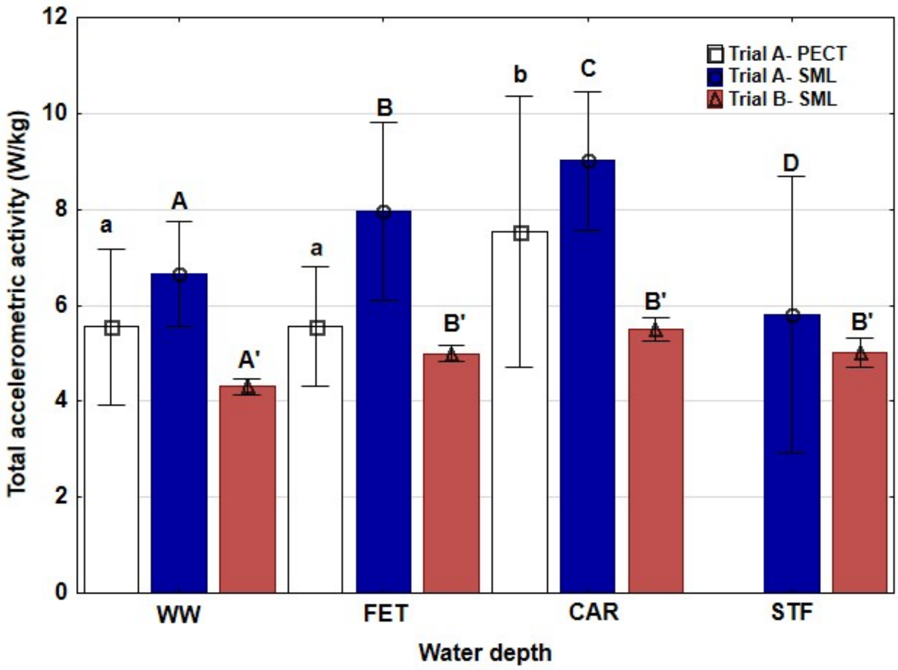

Figure 3. Means and standard deviations (indicated in whiskers) of the total accelerometric activity measured in horses exercised on a WT at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

Values of DVAA and DVD are presented in figures 4 and 5. In trial A, with the accelerometer in PECT position, a progressive increase from WW-FET-CAR was found for both DVAA and DVD. In trial A, with the accelerometer in SML position, DVAA increased at water level of CAR, but decreased with the water at the level of STF. In the same trial, DVD increased from WW with the water at the levels of FET and CAR, but decreased with the water at the level of STF. In trial B, with the accelerometer in SML position, DVD was lower at WW compared to other water depths, without significant differences between FET, CAR and STF. Also, in this trial, DVAA was similar at WW and FET, increasing at CAR and STF levels, without significant differences between both water depths (Figures 4 and 5).

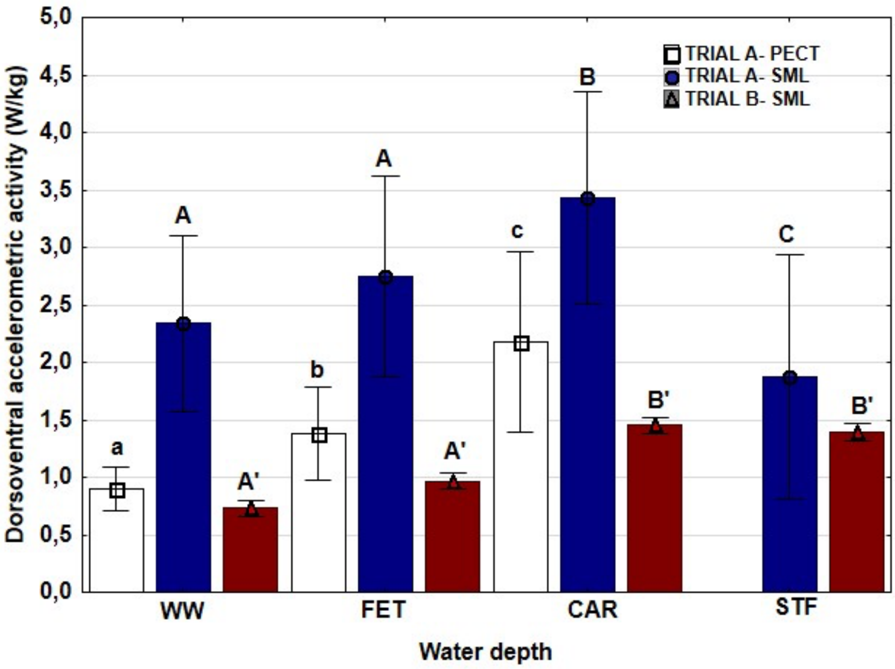

Figure 4. Means and standard deviations (in whiskers) of the dorsoventral accelerometric activity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

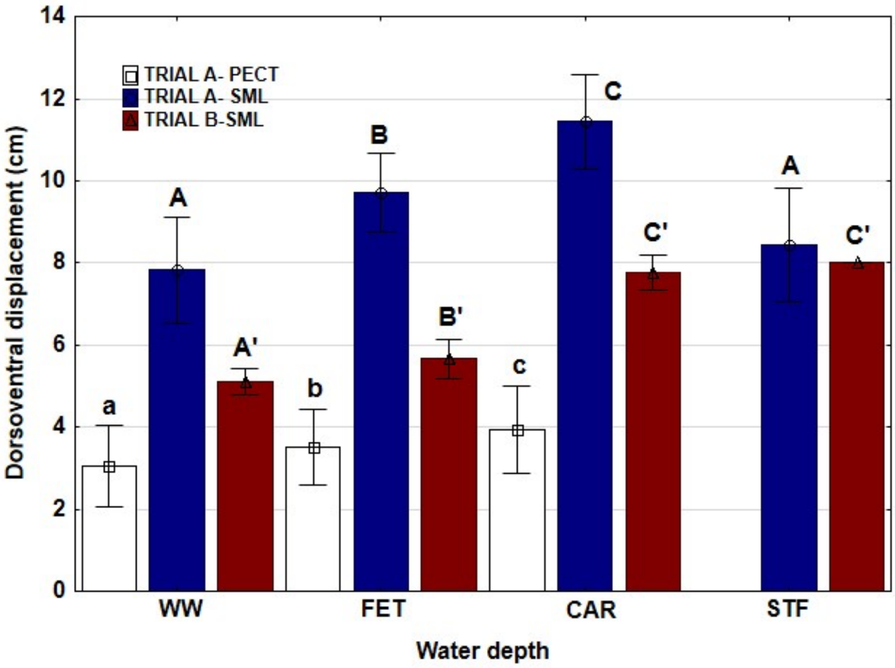

Figure 5. Means and standard deviations (in whiskers) of the dorsoventral displacement in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

The data of LAA and MLAA are presented in figures 6 and 7. Longitudinal accelerometric activity reached the greatest values with the water at the level of CAR in trial A with the accelerometer in PECT position. This parameter had the same values at water levels of WW, FET and CAR in trial A with the accelerometer in SML position. but showed a significant reduction with the water at the level of STF. In trial B, with the accelerometer in SML, LAA increased at FET level (Figure 6). Mediolateral accelerometric activity decreased at FET level in trial A with the accelerometer in PECT position but reached values similar to those found in WW with the water at the level of CAR. A progressive increase in MLAA was found in trial A with the accelerometer in SML position from WW to CAR, decreasing at STF level, with values similar to those found at the level of WW. In trial B, MLAA was not significantly different from WW to FET and CAR levels, but decreased at STF water level despite the same velocity (Figure 7).

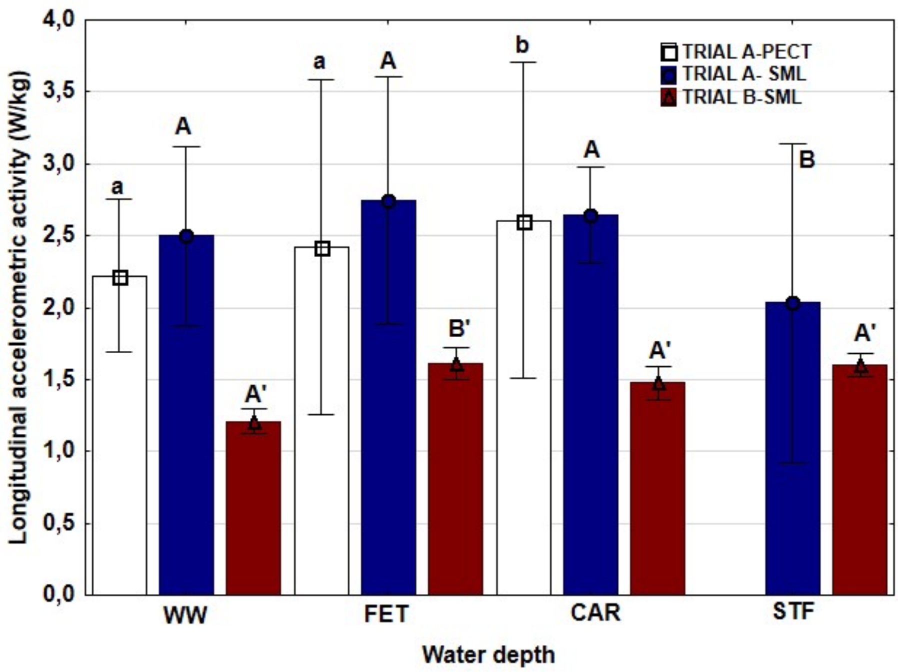

Figure 6. Means and standard deviations (in whiskers) of the longitudinal accelerometric activity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

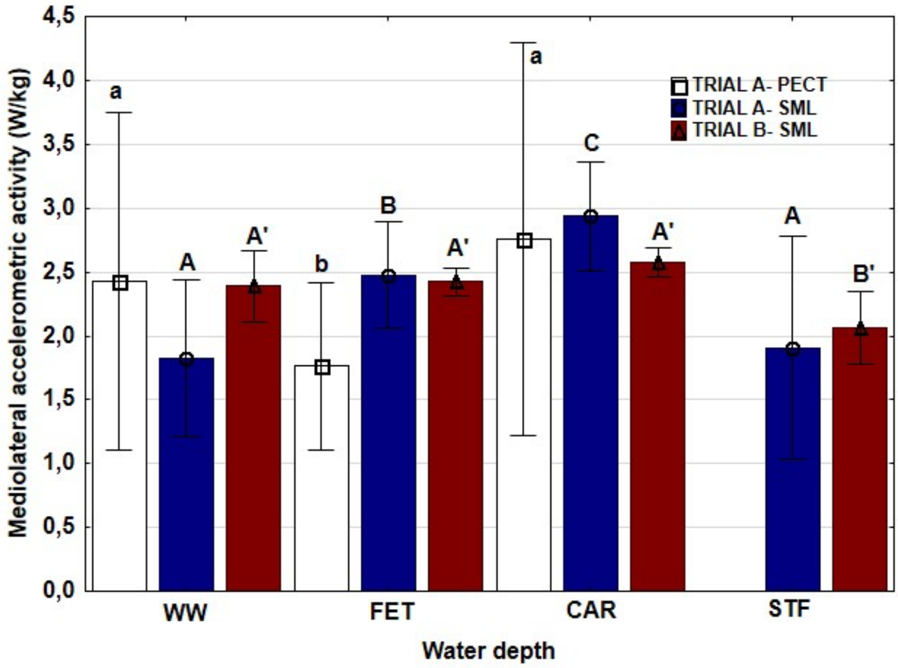

Figure 7. Means and standard deviations (in whiskers) of the mediolateral accelerometric activity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

Table 8 shows the accelerometric activities in the three body axes expressed as percent of the TAA. In main lines, a redistribution of the TAA was found compared to WW, consisting of increases in DVAA% at expense of LAA% and MLAA%.

	WW	**FET**	**CAR**	**STF**

% Dorsoventral accelerometric activity (% of the total accelerometric activity)				
Trial A- accelerometer in PECT position	16.87±3.43 **a**	24.95±5.90 **b**	29.96±8.06 **c**	
Trial A- accelerometer in SML position	34.38±7.36 **A**	34.12±5.57 **A**	37.525.08 **A**	31.84±5.09 **A**
Trial B- accelerometer in SML position	17.09±2.01 **A'**	19.34±1.33 **A'**	26.48±1.11 **B'**	27.77±1.61 **B'**
% Longitudinal accelerometric activity (% of the total accelerometric activity)				
Trial A- accelerometer in PECT position	41.43±9.31 **a**	42.31±12.60 **a**	34.99±7.64 **b**	
Trial A- accelerometer in SML position	37.14±4.74 **A**	34.00±4.28 **A**	29.70±3.57 **B**	35.01±4.33 **A**
Trial B- accelerometer in SML position	28.18±2.43 **A'**	32.19±1.36 **B'**	25.83±1.24 **C'**	31.99±1.86 **B'**
% Mediolateral accelerometric activity (% of the total accelerometric activity)				
Trial A- accelerometer in PECT position	41.70±10.03 **a**	32.74±12.93 **b**	35.05±10.22 **b**	
Trial A- accelerometer in SML position	28.48±11.30 **A**	31.88±5.28 **A**	32.78±2.94 **A**	33.15±7.97 **A**
Trial B- accelerometer in SML position	54.62±4.95 **A'**	48.47±1.54 **B'**	47.69±1.20 **B'**	40.33±3.53 **C'**

Table 8. Means and standard deviations of the dorsoventral, longitudinal y mediolateral accelerometric activities expressed as percent of the total accelerometric activity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

Regularity and SYM values were not affected by the water depth or by the velocity, as presented in table 9.

	WW	**FET**	**CAR**	**STF**
Stride symmetry (dimensionless)				

Trial A-accelerometer in PECT position	174.8±43.08 **a**	192.2±53.64 **a**	169.9±43.62 **a**	
Trial A-accelerometer in SML position	258.2±50.29 **A**	225.9±37.98 **A**	237.3±40.67 **A**	213.6±44.10 **A**
Trial B-accelerometer in SML position	204.1±32.63 **A'**	182.9±48.10 **A'**	192.7±38.74 **A'**	302.2±37.30 **A'**
Stride regularity(dimensionless)				
Trial A-accelerometer in PECT position	191.9±56.84 **a**	182.9±48.10 **a**	192.7±38.74 **a**	
Trial A-accelerometer in SML position	354.2±39.07 **A**	331.4±38.32 **A**	337.2±27.31 **A**	302.2±37.30 **A**
Trial B-accelerometer in SML position	310.2±38.91 **A'**	304.7±26.14 **A'**	289±32.28 **A'**	321.7±32.65 **A'**

Table 9. Means and standard deviations of the stride symmetry and regularity in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

Data obtained from spatiotemporal parameters of the stride are presented in figures 8 and 9. As expected, SF decreased and SL increased with water depth in the three trials. When the velocity was reduced in trial A, with the accelerometer in SML position, SL decreased with the water at the level of STF, achieving values similar to those found at WW. However, when the velocity was the same, in trial B with the accelerometer in SML position, SL increased at CAR level and further at STF water depth (Figure 9).

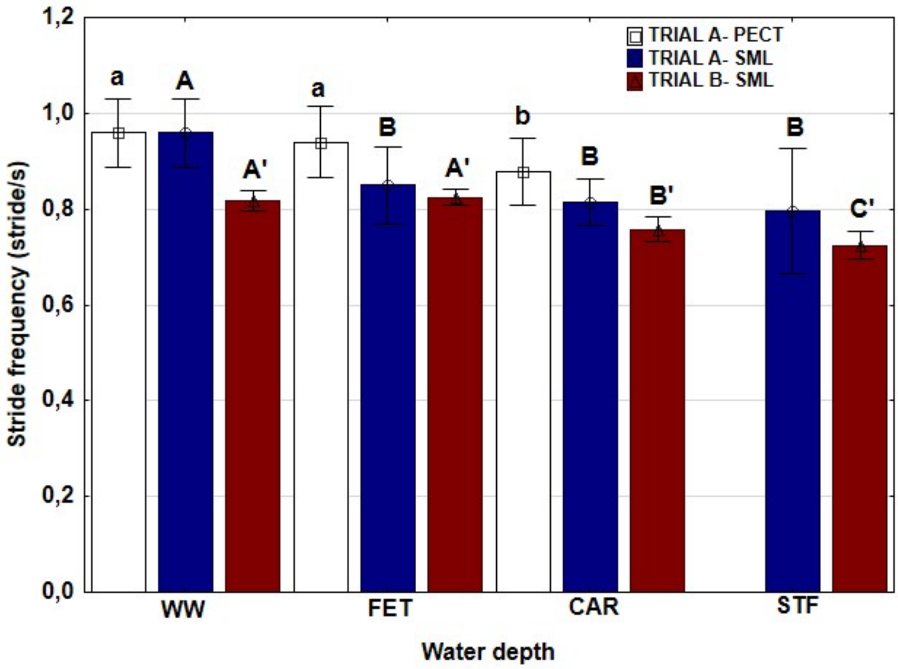

Figure 8. Means and standard deviations (in whiskers) of the stride frequency in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

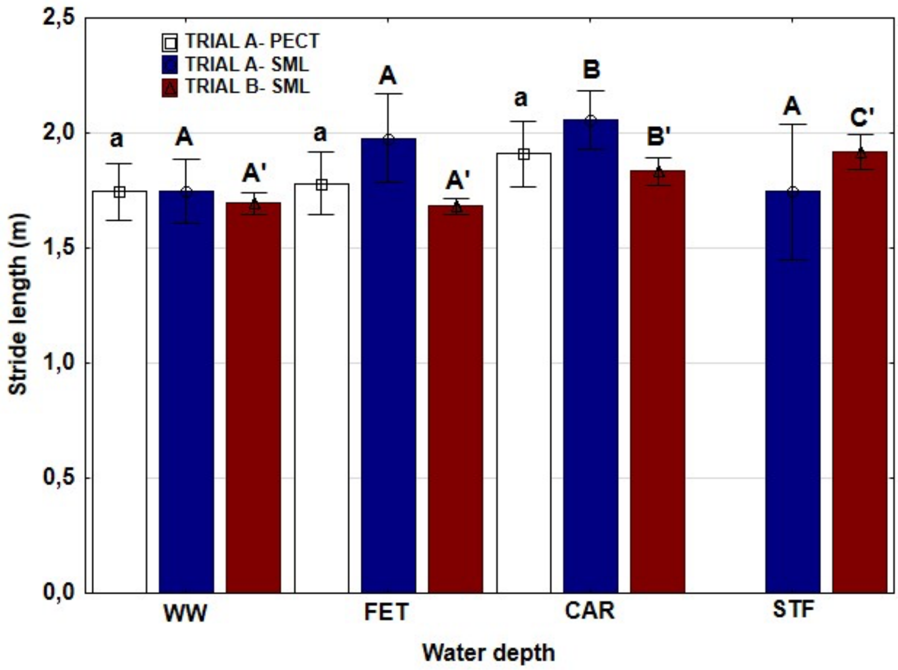

Figure 9. Means and standard deviations (in whiskers) of the stride length in horses exercised on a water treadmill at different water depths and velocities (Lowercase letters: significant differences between water

depths in trial A with the accelerometer in PECT position; capital letters: significant differences between water depths in trial A with the accelerometer in SML position; capital letters with apostrophes: significant differences between water depths in trial B with the accelerometer in SML position). Different letters indicate significant differences between water depths at each trial at p<0.05.

6.4.1. Summary of the results of trial A

In order to make clearer the results obtained in this study, we have summarized the main results separated by trials, according to the information provided in section 6.4. The most relevant results with the accelerometer in PECT position are summarized in table 10.

Accelerometric parameters	
Total accelerometric activity	TAA reached the highest values with the water at the level of CAR.
Dorsoventral displacement	A progressive increase from WW to FET and CAR.
Dorsoventral accelerometric activity DVAA (in absolute number)	A progressive increase from WW to FET and CAR.
Longitudinal accelerometric activity LAA (in absolute number)	LAA reached the greatest values with the water at the level of CAR.
Mediolateral accelerometric activity MLAA (in absolute number)	MLAA decreased at FET water level.
Dorsoventral accelerometric activity DVAA (%)	A progressive increase was found from WW to FET and CAR.
Longitudinal accelerometric activity LAA (%)	A reduction was found with the water at the level of FET and CAR.
Mediolateral accelerometric activity MLAA (%)	A reduction was found with the water at the level of FET and CAR.
Coordination parameters of the stride	
Regularity, REG	No significant differences in REG was found comparing the four water depths.

Symmetry, SYM	No significant differences in SYM was found comparing the four water depths.
Spatiotemporal parameters of the stride	
Stride frequency SF	It decreased at a water level of CAR compared to WW and FET.
Stride length SL	No significant differences were found when comparing the four water depths.

Table 10. Summary of the results found in trial A with the accelerometer in PECT position (p<0.05).

The results found in trial A with the accelerometer in SML position are summarized in table 11.

Accelerometric parameters	
Total accelerometric activity	A progressive increase in TAA was observed up to the water level of CAR. A significant decrease was found in TAA at STF level when the velocity was reduced.
Dorsoventral displacement	DVD increased from WW and FET levels to the water level of the CAR, but decreased at the water level of STF.
Dorsoventral accelerometric activity DVAA (in absolute number)	DVAA increased from WW and FET levels to the water level of the CAR, but decreased at the water level of STF.
Longitudinal accelerometric activity LAA (in absolute number)	LAA was no significant different at the water levels of WW, FET and CAR, but decreased at the water level of STF.
Mediolateral accelerometric activity MLAA (in absolute number)	MLAA increased from WW to the water level of FET and CAR, but decreased at the water level of STF.
Dorsoventral accelerometric activity DVAA (%)	DDVA % was not significantly different when comparing the four different water levels.
Longitudinal accelerometric activity LAA (%)	LAA % was not different when comparing the four different water levels.

Mediolateral accelerometric activity MLAA (%)	MLAA % decreased with the water at the level of CAR.
Coordination parameters of the stride	
Regularity, REG	There were no significant differences in REG between the four water levels.
Symmetry, SYM	There were no significant differences in SYM between the four water levels.
Spatiotemporal parameters of the stride	
Stride frequency SF	SF decreased at the water level of FET, CAR and STF compared to WW.
Stride length SL	SL was greater at the water level of CAR compared to WW, FET and STF.

Table 11. Summary of the results found in trial A with the accelerometer in SML position (p<0.05).

6.4.2. Summary of the results of trial B

In trial B, with the accelerometer in SML position, the main results are presented in table 12.

Accelerometric parameters	
Total accelerometric activity	It increased with the water at the level of FET, CAR and STF compared to WW. Differences between water levels of FET, CAR and STF were not found.
Dorsoventral displacement	It increased with the water at the levels of CAR and STF, without significant differences between them.
Dorsoventral accelerometric activity DVAA (in absolute number)	It increased at CAR and STF levels, without significant differences between them
Longitudinal accelerometric	It showed the highest values at FET level.

activity LAA (in absolute number)	
Mediolateral accelerometric activity MLAA (in absolute number)	MLAA did not change from WW level at FET and CAR water levels, but decreased at STF level.
Dorsoventral accelerometric activity DVAA (%)	It increased at water levels of CAR and STF.
Longitudinal accelerometric activity LAA (%)	It increased at water levels of FET and STF, but decreased at water level of CAR.
Mediolateral accelerometric activity MLAA (%)	It decreased at all water depths compared to WW, further at level of CAR.
Coordination parameters of the stride	
Regularity, REG	Significant differences between water depths were not found.
Symmetry, SYM	Significant differences between water depths were not found.
Spatiotemporal parameters of the stride	
Stride frequency SF	It decreased at the water level of CAR and presented a further decrease at the water level of STF.
Stride length SL	SL increased at the water level of CAR compared to WW and FET and showed a second increase at the water level of STF.

Table 12. Summary of the results found in trial B with the accelerometer in SML position (p<0.05).

6.5. Main conclusions

The present work demonstrated that TAA increased with water depth in horses exercised on a WT, up to the level of CAR. However, at STF level, supposedly as a consequence of buoyancy, TAA was not greater than at a water level of CAR. This result appears to indicate that horses could be exercised with the water at STF level increasing strength but with a greater reduction in limb load compared to CAR level.

In addition, the velocity during the exercise on a WT leads to a reduction in TAA, and therefore, the combination of variations in velocity and depth can be designed in order to achieve different training or rehabilitation goals.

During a WT exercise session, DVD and DVAA increased, results that have important applications for training dressage, jump and event horses. Stride SYM and REG did not change during exercise on the WT. As expected, SF decreased, and SL increased with depth of the water.

7. STUDY III

PREVIOUS EXERCISE ON A WATER TREADMILL AT DIFFERENT DEPTHS AFFECTS THE ACCELEROMETRIC PATTERN RECORDED ON A TRACK IN HORSES

(published in:
Animals, 12, 3086. 2022.
DOI: https://doi.org/10.3390/ani12223086)

Impact factor: 3.231 (Year: 2021)
Quartil: Q1
Ranking: 16/144 (JCR. Veterinary Sciences)

7.1. Introduction

Exercise in water is being increasingly used in the sport horse, either for rehabilitation or within a training program, due to the recognition of the benefits associated to the physical properties of water and to the increased commercial availability of water exercise systems for horses. However, despite the recommendations performed by equine clinicians and trainers of this modality of exercise, there are few scientific studies describing longitudinal changes through rehabilitation programs for specific musculoskeletal injuries. Similarly, to the authors' knowledge, there is a paucity of scientific descriptions of changes associated to training and performance when an exercise on a WT is introduced within a training program.

In our previous work (Study II) we found significant changes in accelerometric parameters when the horses were exercised on a WT, comparing four different situations: without water (WW, considering as baseline conditions) and with the water at levels of fetlock (FET), carpus (CAR) and stifle (STF). We found that TAA reached the greatest values with the water at the level of the CAR and STF, without significant differences between both water depths. We would like to highlight this result, because there is a belief between trainers that deeper water is more useful to increase muscle size and strength (Tranquille et al., 2019). However, the results of our previous work did not confirm that. In fact, there were no significant differences between water levels of CAR and STF, probably as a consequence of the greater buoyancy with the water at the level of STF.

Total accelerometric activity represents the sum of the accelerometric activities in the three body axes, i.e. dorsoventral (up-down), longitudinal (cranio-caudal) and mediolateral. In our previous study, we found that DVD and DVAA increased progressively with the depth of the water, with the lowest values when the horses were exercised WW and the highest values with the water at the levels of CAR and STF, without significant differences between these last water depths. These results were attributed to the greater flexion of the distal limb's joints when the horse is exercised on a WT compared to WW (Méndez-Angulo et al., 2014).

Increased TAA, DVD and DVAA values have been considered accelerometric characteristics positive for dressage and jumping horses. In fact, collection which is one of the most important factors determining success and training progression in a dressage horse (Barrey et al., 2002; Biau and Barrey, 2004), consisting in an increase of the vertical component of the acceleration (DDVA) together with a reduction of the forward component of the acceleration (LAA). Biau and Barrey (2004) demonstrated that horses having greater TAA, DVD and DVAA reached higher scores by judges in dressage competitions. Furthermore, greater values of the three mentioned parameters are also favorable for jumpers, representing a greater muscle activity to overtake the obstacle (Galloux and Barrey, 1997).

In summary, our previous data demonstrated that the accelerometric pattern of horses exercised on a WT, particularly at water levels of CAR and STF, appear to be advantageous for some sport horses. However, we have to keep in mind that all these changes happen during an exercise on a WT, but they do not have to be maintained during terrestrial locomotion. This idea led us to the design of the third study of the present thesis.

7.2. Objectives and hypotheses

It is well established that during the exercise on the WT, and according to the depth of the water, the horse changes its locomotor pattern. Because of that, the **main objective** of the present study is to elucidate whether, after performing a WT exercise session at different water depths, accelerometric modifications persist during subsequent terrestrial locomotion.

The hypotheses proposed are: 1) That TAA evaluated on a track after performing a WT exercise session, with the water at the levels of CAR and STF, would be greater in comparison with TAA after WW or water level of FET; 2) That DVAA and DVD on the track would be greater after WT exercise at the level of CAR and STF, because of the greater flexion of the joints of the limbs that have been described at these water depths

(Méndez-Angulo et al., 2014); 3) That LAA would be lower on the track after exercise on the WT.

7.3. Material and methods

7.3.1. Horses

Six privately-owned horses of both genders (2 mares and 4 geldings), aged between 10-15 years, with a mean of 12.6±1.6 years, and of different breeds (1 crossbred, 1 Arabian, 1 Anglo-Arabian and 3 Andalusian) were recruited for the study. The horses had no previous history of recent health issues, poor performance or lameness. The fitness level of the horses was moderate, according to the results of a brief exercise test performed on a track, measuring HR and blood LA accumulation.

Owners of the horses accepted the inclusion of the animals in the study, after being informed about the procedure, potential risks and derived benefits. During the study, horses were kept in a medium size paddock. In addition to the exercise performed on the WT, the horses were walked one hour per day in a walker.

7.3.2. Exercise on the water treadmill

The horses performed four different trials on the WT (Activo-Med®, Germany), following a randomized design (www.random.org). The four trials consisted of an exercise session at four different water depths: without water (WW), and with the water at the levels of the fetlock or metacarpophalangeal joint (FET), carpus (CAR) and stifle (STF). Each exercise session had a total duration of 40 min, including the time needed to fill and drain the treadmill. The velocity was fixed at 5 km/h, because our previous research (study II) demonstrated that it was a comfortable velocity for all the water depths.

Horses were fully acclimatized to the WT before starting the research. The horses were being exercised daily for the two weeks previous to the start of the study.

7.3.3. Accelerometric device, evaluation and parameters

The same accelerometer than in studies I and II was used (Equimetrix®, Centaure-Metrix, France). More information about this accelerometer is presented in the previous studies. The accelerometer was fixed at two different locations, in the pectoral region (PECT), fixed with a girth, and in the sacrum mid-line (SML), fixed with a tape. In these locations, the accelerometer provides more information concerning the accelerometric changes of the forelimbs and center of gravity and in the hindlimbs respectively.

In each WT exercise session, the horses were subjected to two accelerometric evaluations on a track, before WT exercise (considered as baseline) and after 30 min of finishing the WT exercise. Therefore, each horse was subjected to four baseline accelerometric evaluations (before WW, FET, CAR and STF) and four evaluations after WT exercise (after WT exercise at the water level of WW, FET, CAR and STF).

The accelerometric evaluations were performed by the same researcher (A.S.), in the same track, at the walk and at the trot, with the horses led by hand. The horses covered a distance of 80 m, four times for each accelerometric evaluation (two at walk and two at trot).

Three groups of accelerometric parameters have been included: 1) Accelerometric activities (TAA, DVAA, LAA, MLAA) and DVD; 2) Stride coordination parameters: REG and SYM; 3) Stride spatiotemporal parameters: velocity, SF and SL.

7.3.4. Statistics

Values obtained before each WT exercise session were considered baseline and values obtained at 30 min after each exercise session were expressed as percent of changes from baseline values. Data are presented as the median and quartiles.

Obtained percent of changes did not adjust to a normal distribution, assessed with a Shapiro-Wilk's W test and with the visualization of the histograms. The differences in the percent of variations in the track were evaluated with a Kruskal-Wallis test and when significant differences were found a Mann-Whitney test was applied as post-hoc.

The level of significance was fixed at $p<0.05$.

7.4. Results

7.4.1. Accelerometric parameters

The percent of change from baseline of TAA on the track is presented in the figures 10-13. This percent was greater after WT exercise at the level of CAR, both at walk and at trot and with the accelerometer in the two positions. Further, and with the accelerometer in SML position, a reduction of the percent of change in TAA was found after WT exercise at STF level, at both gaits (Figures 12 and 13).

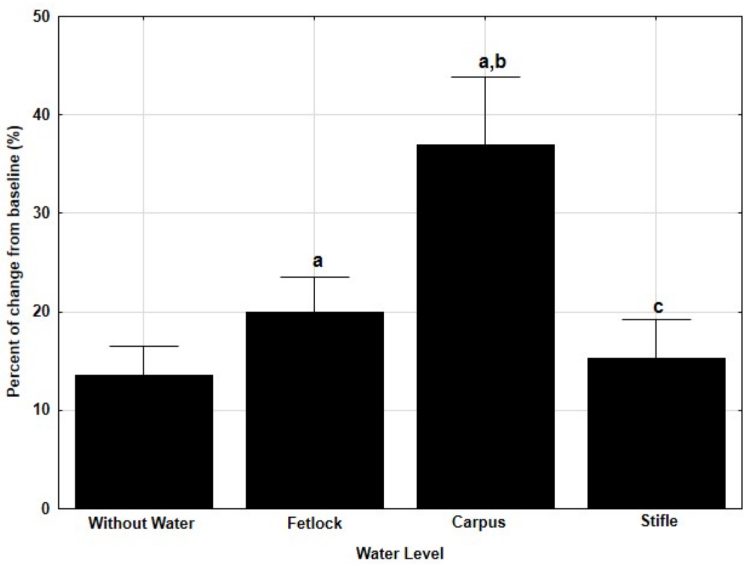

Figure 10. Median and quartiles of the percent of change from baseline of the total accelerometric activity, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

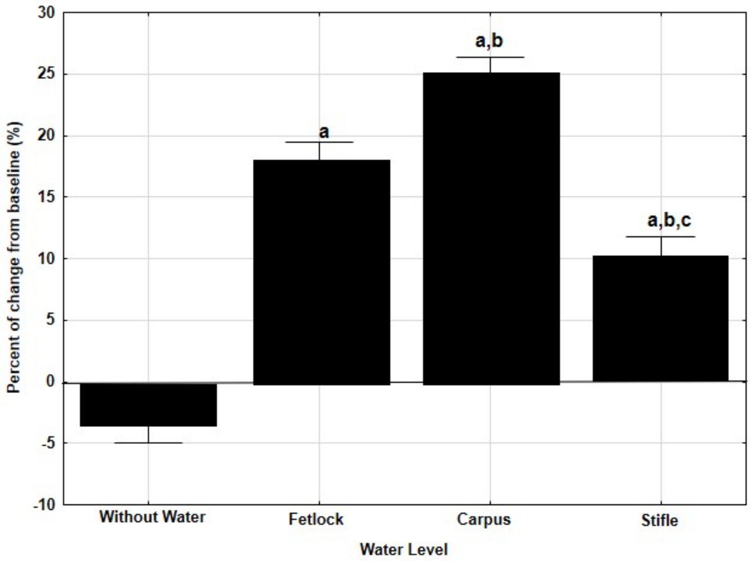

Figure 11. Median and quartiles of the percent of change from baseline of the total accelerometric activity, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

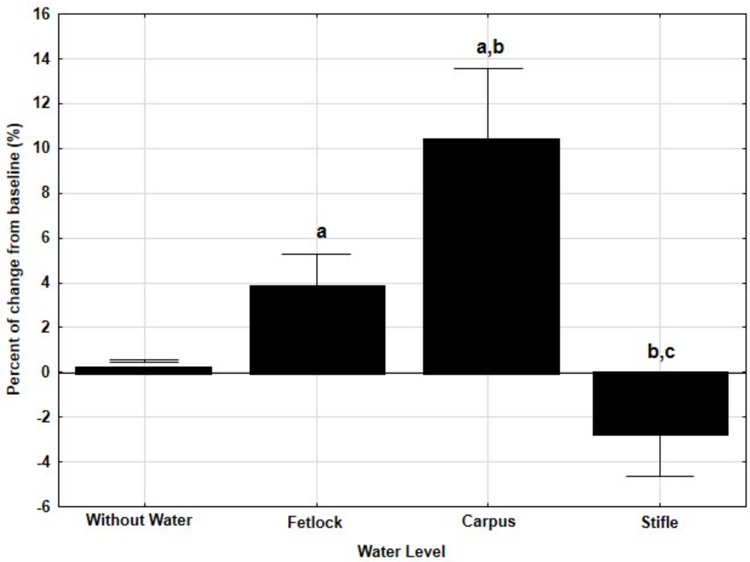

Figure 12. Median and quartiles of the percent of change from baseline of the total accelerometric activity, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

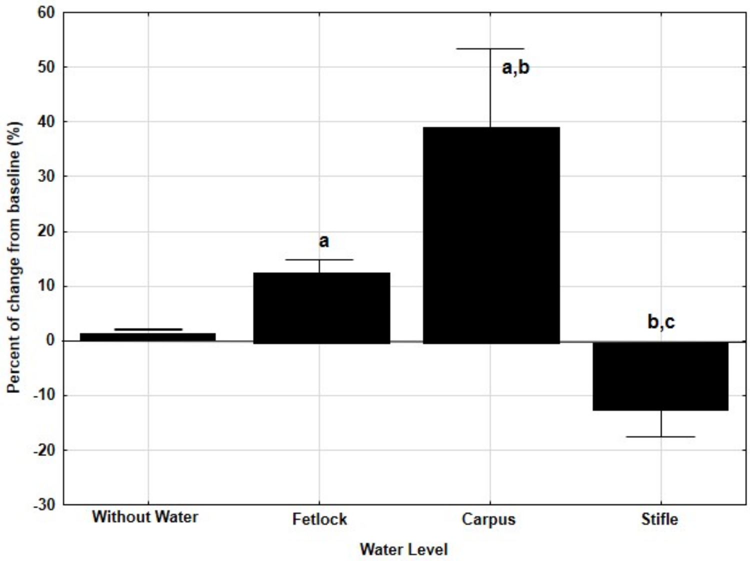

Figure 13. Median and quartiles of the percent of change from baseline of the total accelerometric activity, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

With the accelerometer in PECT position, DDVA increased significantly on the track, with the most evident increase found after WT exercise at the level of CAR at a walk and after WT exercise at levels of FET, CAR and STF at a trot. With the accelerometer in SML position, DDVA increased after WT exercise at the CAR and STF levels at walk, and at CAR level trot. Longitudinal accelerometric activity on the track, with the accelerometer in PECT position, increased after WT exercise at the level of FET and CAR at walk and trot. However, it decreased after WT exercise at the level of STF at trot. With the accelerometer in SML position, LAA decreased after exercise at the level of STF, both at walk and at trot, but increased after WT exercise at the level of CAR at trot. In main lines, MLAA increased independently of gait and position of the accelerometer after WT exercise at the water levels of FET and CAR. With the accelerometer in PECT position, MLAA increased also after WT exercise at the level of STF, both at trot and walk. On the contrary, with the accelerometer in SML position, MLAA decreased after WT exercise at the levels of STF, at walk and at trot (Figures 14 to 17).

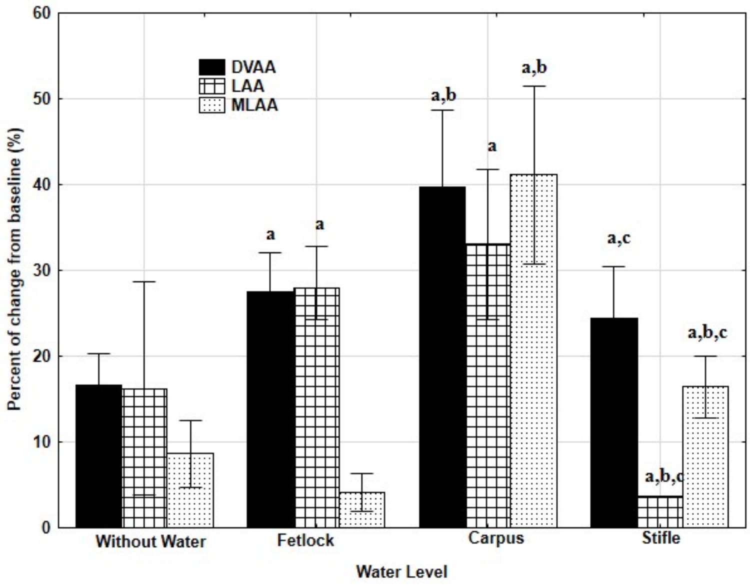

Figure 14. Median and quartiles of the percent of change from baseline of the dorsoventral, longitudinal and mediolateral accelerometric activity, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

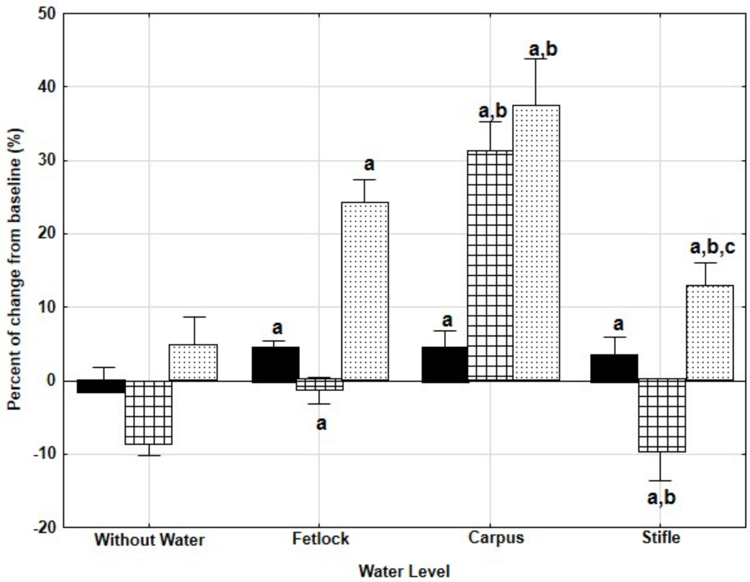

Figure 15. Median and quartiles of the percent of change from baseline of the dorsoventral, longitudinal and mediolateral accelerometric activity, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

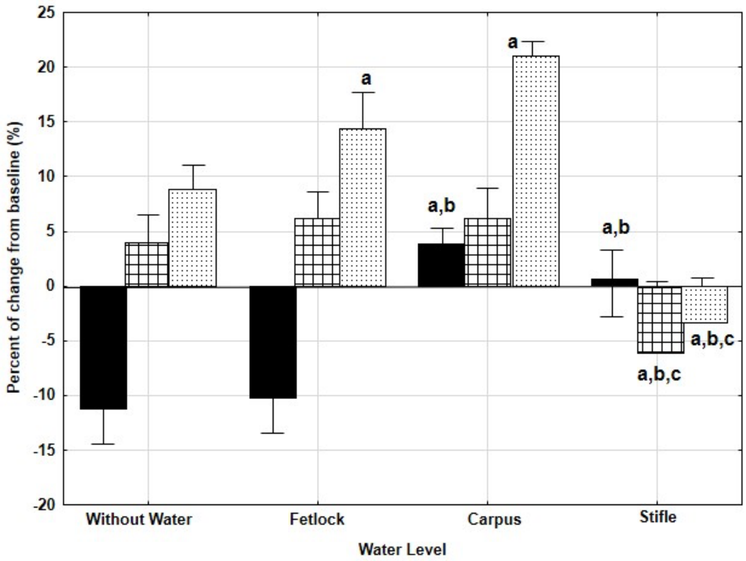

Figure 16. Median and quartiles of the percent of change from baseline of the dorsoventral, longitudinal and mediolateral accelerometric activity, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

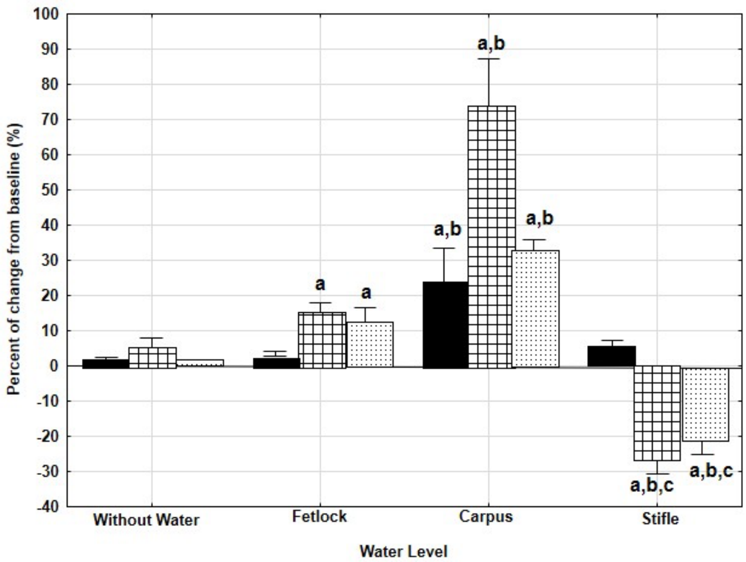

Figure 17. Median and quartiles of the percent of change from baseline of the dorsoventral, longitudinal and mediolateral accelerometric activity, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

In relation to DVD, the percent of change from baseline increased with the accelerometer in PECT position, with the greatest rise at walk after WT exercise at the level of the CAR. On the contrary, DVD decreased at walk after WT exercise with the water at the level of CAR and STF and at trot after WT exercise at the levels of STF with the accelerometer in SML position (Table 13).

WW	FET	p	CAR	p	STF	p
Accelerometer in PECT position at walk						
7.17	8.77		16.98	0.020 **a**	4.52	0.030 **b**
(4.91)(10.43)	(5.00)(11.10)		(13.3)(20.21)	0.010 **b**	(2.23)(6.81)	0.010 **c**
Accelerometer in PECT position at trot						
1.00	6.03		7.860		3.23	
(-0.72)(2.72)	(4.39)(7.67)		(6.20)(9.52)		(1.53)(4.94)	
Accelerometer in SML position at walk						
0.23	4.09	0.020 **a**	-10.04	0.010 **a**	-11.58	0.020 **a**
(-1.77)(2.23)	(-5.13)(13.39)		(-6.61)(-13.47)	0.030 **b**	(-8.54)(-14.61)	0.040 **b**
Accelerometer in SML position at trot						
0.78	0.78		-0.25		-1.88	0.030 **a**
(-0.49)(2.07)	(-0.49)(2.07)		(-1.64)(1.13)		(-0.25)(-2.51)	0.020 **b**

Table 13. Median and quartiles (in brackets) of the percent of change from baseline of the dorsoventral displacement measured on a track after WT exercise at four different water depths, at walk and at trot and the accelerometer in two different positions (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

A brief summary of the results of the accelerometric parameters is presented in table 14.

Total accelerometric activity TAA	Accelerometer in PECT position: The percent of change from baseline of TAA on the track was significantly greater after WT exercise at the level of the CAR, at walk and at trot.
	Accelerometer in SML position: A significant reduction in TAA was found after WT exercise at STF level, at both gaits: walk and trot.
Dorsoventral accelerometric activity DVAA	Accelerometer in PECT position: The percent of change from baseline of DDVA increased significantly on the track, showing the greatest increase after WT exercise at the level of the CAR at walk and after WT exercise at the levels of FET, CAR and STF at trot.
	Accelerometer in SML position: The percent of change from baseline of DVAA increased after WT exercise at the level of CAR and STF at walk and trot; but decreased after WT exercise at the level of STF in comparison to CAR.
Longitudinal accelerometric activity LAA	Accelerometer in PECT position: The percent of change from baseline of LAA on the track at both walk and trot, increased after WT exercise at the level of FET and CAR, but decreased after WT exercise at the level of STF.
	Accelerometer in SML position: The percent of change from baseline of LAA decreased at walk and at trot after WT exercise at a water level of STF. At trot, there was a significant increase in the percentage of change from baseline in LAA at FET and CAR water levels.
Mediolateral accelerometric activity MLAA	Accelerometer in PECT position: The percent of change from baseline of MLAA increased, independently of gait and position of the accelerometer after WT exercise at the level of FET, CAR and STF with the greatest increase after CAR exercise.
	Accelerometer in SML position: The percent of change from baseline of MLAA increased in FET and CAR water levels, but decreased after WT exercise at the level of STF, at walk and at trot.
Dorsoventral displacement DVD	Accelerometer in PECT position: The percent of change from baseline of DVD increased, with the maximum increase at walk after WT exercise at the level of CAR; but decreased significantly after WT exercise at the level of STF in comparison to WW, FET and CAR.
	Accelerometer in SML position: The percent of change from baseline of DVD after WT exercise at walk increased at FET water level, but decreased at the level of the CAR and STF. The percent of change

	from baseline of DVD after WT exercise at trot decreased at CAR and STF water levels, being significantly lower at STF water level.

Table 14. Summary of the main results of the accelerometric parameters measured on a track, comparing before and at 30 min after exercise sessions on a WT at different water levels (WW, without water; FET: water at the level of the fetlock; CAR: water at the level of the carpus; STF: water at the level of the stifle), with the accelerometer in pectoral (PECT) and sacrum midline (SML) position.

7.4.2. Stride coordination parameters

The percent of change from baseline of REG and SYM are presented in table 15 and 16. Regularity increased after WT exercise at the water level of CAR, with the accelerometer in PECT position and at a walk. By contrast, significant reductions were found with the accelerometer in SML position after WT exercise at STF level at walk and after CAR and STF levels at trot (Table 15). Stride SYM remained similar after WT at the different water depths with the accelerometer in PECT position. It was reduced after WT exercise at the level of the STF with the accelerometer in SML position in both gaits. Furthermore, SYM was also lower at trot after WT exercise at the level of CAR with the accelerometer in SML position, as shown in table 16.

WW	**FET**	**p**	**CAR**	**p**	**STF**	**p**
Accelerometer in PECT position at walk						
2.64	4.56		10.92		1.25	
(-5.07)(6.57)	(-5.07)(6.57)		(5.65)(14.56)	0.020 **a**	(5.46)(11.34)	
Accelerometer in PECT position at trot						
-0.67	-0.43		-1.42		-1.09	
(-3.09)(4.45)	(-3.48)(5.61)		(-4.68)(4.59)		(-5.52)(6.03)	
Accelerometer in SML position at walk						
0.00	-1.97		-3.23		-6.07	0.020 **a**

(-5.91)(6.92)	(-5.64)(3.40)		(-6.57)(2.39)		(-14.39)(1.23)	
Accelerometer in SML position at trot						
-1.85	-4.49		-11.01	0.020 **a**	-24.50	0.002 **a**
(-9.99)(4.40)	(-9.22)(4.54)		(-15.63)(-1.32)	0.010 **b**	(-29.02)(-18.34)	0.010 **b** 0.030 **c**

Table 15. Median and quartiles (in brackets) of the percent of change from baseline of the stride regularity measured on a track after WT exercise at four different water depths, at walk and at trot and the accelerometer in two different positions (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

WW	**FET**	**p**	**CAR**	**p**	**STF**	**p**
Accelerometer in PECT position at walk						
-5.59	0.48		-3.08		-7.72	
(-10.29)(10.80)	(-11.36)(9.72)		(-8.62)(7.84)		(-1.35)(12.72)	
Accelerometer in PECT position at trot						
-2.46	-2.31		-5.78		-5.67	
(-9.38)(5.13)	(-8.47)(3.25)		(-10.54)(9.34)		(-11.23)(8.93)	
Accelerometer in SML position at walk						
-1.55	-4.58		-6.70		-12.38	0.000 **a**
(-5.62)(4.45)	(-6.73)(-2.34)		(-11.32)(-3.45)		(-24.5)(-15.46)	0.020 **b** 0.010 **c**
Accelerometer in SML position at trot						
0.00	-4.50		-18.48	0.003 **a**	-29.82	0.000 **a**
(-7.35)(9.61)	(-11.46)(7.51)		(-22.45)(-8.37)		(-35.21)(-23.71)	0.010 **b** 0.020 **c**

Table 16. Median and quartiles (in brackets) of the percent of change from baseline of the stride symmetry measured on a track after WT exercise at four different water depths, at walk and at trot and the

accelerometer in two different positions (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

The main results of the parameters of coordination of the stride are summarized in table 17.

Regularity REG	Accelerometer in PECT position: The percent of change from baseline of REG on a track increased after WT exercise at the level of CAR at walk.
	Accelerometer in SML position: The percent of change from baseline of REG on a track decreased after WT exercise at the level of STF at walk and after WT exercise at the levels of CAR and STF at trot.
Symmetry SYM	Accelerometer in PECT position: The percent of change from baseline of SYM on a track remained statistically similar after WT at the different water depths
	Accelerometer in SML position: The percent of change from baseline of SYM on a track decreased after WT exercise at the level of the STF, at walk and at trot. In addition, the percent of change from baseline was lower at trot after WT exercise at the level of CAR.

Table 17. Summary of the main results of the stride's coordination parameters measured on a track, comparing before and at 30 min after exercise sessions on a WT at different water levels (WW, without water; FET: water at the level of the fetlock; CAR: water at the level of the carpus; STF: water at the level of the stifle), with the accelerometer in pectoral (PECT) and sacrum midline (SML) position.

7.4.3. Spatiotemporal parameters of the stride

Very mild changes were found in velocity after WT exercise. It increased at walk after WT exercise at the level of the CAR and STF and at trot after WT exercise at the level of CAR, with the accelerometer in PECT position. It decreased at trot after WT exercise at the level of STF. With the accelerometer in SML position, the velocity was reduced after WT exercise at the level of STF in both gaits (Figures 18 to 21). Stride frequency only differed from baseline with the accelerometer in PECT position. At a walk, a significant increase was observed after WT exercise at the levels of FET and CAR and at trot, SF decreased after WT exercise at the level of CAR. In relation to SL, an increase at walk, after WT exercise at the levels of CAR and STF was found with the accelerometer in SML position. However, at trot and with the accelerometer in SML position, the percent of change from baseline was reduced after WT exercise at the level of STF (Figures 18 to 21).

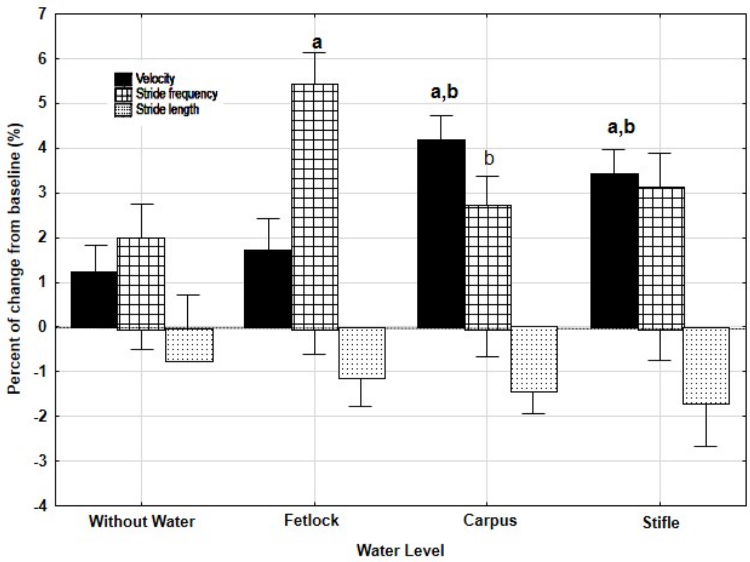

Figure 18. Median and quartiles of the percent of change from baseline of the velocity, stride frequency and length, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

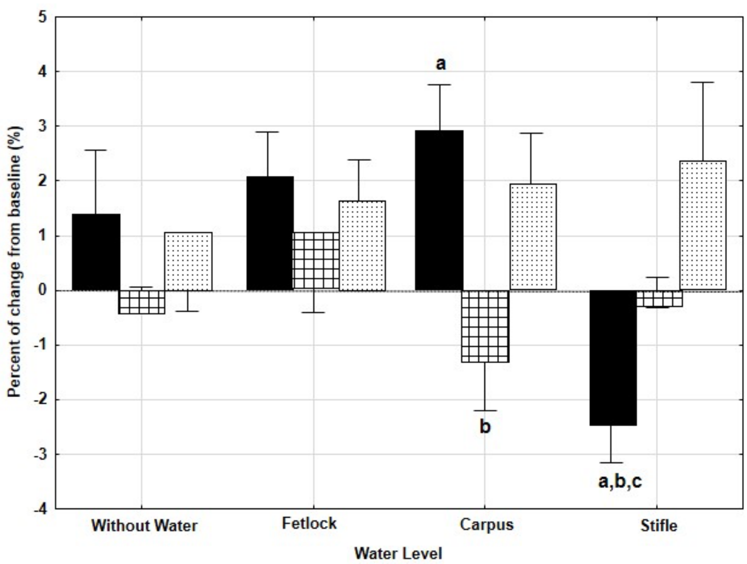

Figure 19. Median and quartiles of the percent of change from baseline of the velocity, stride frequency and length, measured on a track after WT exercise at different water depths, at trot and with the accelerometer in PECT position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

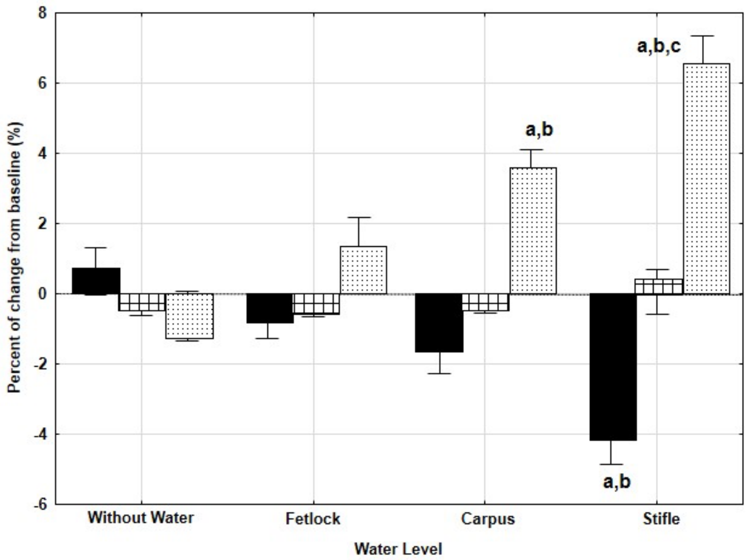

Figure 20. Median and quartiles of the percent of change from baseline of the velocity, stride frequency and length, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

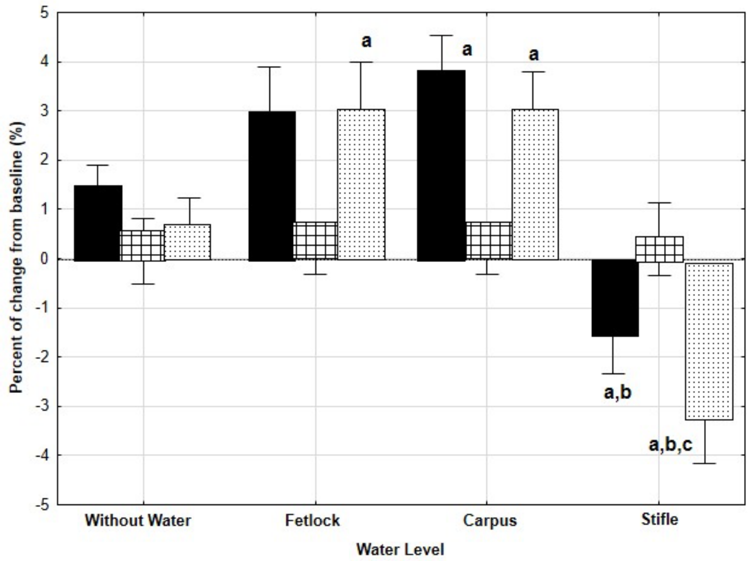

Figure 21. Median and quartiles of the percent of change from baseline of the velocity, stride frequency and length, measured on a track after WT exercise at different water depths, at walk and with the accelerometer in SML position (a: significant differences with treadmill without water; b: significant differences with water at the level of the fetlock; c: significant differences with water at the level of the carpus) p<0.05.

The main results of spatiotemporal parameters of the stride are presented in table 18.

Velocity	Accelerometer in PECT position: The percent of change from baseline increased after WT exercise at the level of the CAR and STF at walk, and also at the level of CAR at trot. However, it decreased at trot after WT exercise at the level of STF.
	Accelerometer in SML position: The percent of change from baseline was reduced after WT exercise at the level of STF, both at walk and at trot.
Stride frequency SF	Accelerometer in PECT position: The percent of change from baseline was greater after WT exercise at the levels of FET and CAR at walk, whereas this percent decreased after WT exercise at the level of CAR at trot.
	Accelerometer in SML position: The percent of change from baseline was not different when comparing values obtained in the four water depths.

Stride length SL	Accelerometer in PECT position: The percent of change from baseline of SL did not reach the statistical differences when comparing the different water depths.
	Accelerometer in SML position: The percent of change from baseline of SL increased after WT exercise at the level of CAR and STF at walk. However, at trot, the percent of change from baseline was reduced after WT exercise at the level of STF.

Table 18. Summary of the main results of the stride spatiotemporal parameters measured on a track, comparing before and at 30 min after exercise sessions on a WT at different water levels (WW, without water; FET: water at the level of the fetlock; CAR: water at the level of the carpus; STF: water at the level of the stifle), with the accelerometer in pectoral (PECT) and sacrum midline (SML) position.

7.5. Main conclusions

The present study has demonstrated that, when an exercise session is carried out on a WT, some of the accelerometric modifications found during the exercise on the water are maintained, at least for 30 minutes, during overground locomotion.

Findings to be highlighted were the reduction of TAA and LAA, DVD and velocity, particularly at trot and with the accelerometer in SML position. These reductions were found after exercise on WT at the level of STF. We believe that these changes can be indicative of fatigue after exercise at deep water, particularly in unfit or moderately fit horses. As a result of this, we consider that the velocity during exercise on a WT at the level of the STF, should be reduced to make a comfortable velocity for the horse. Therefore, the greater drag force at deep water depth counteracts the action of buoyancy.

8. GENERAL DISCUSSION

The locomotor characteristics of a horse are essential in the study of exercise physiology and equine sports medicine. Undoubtedly, the locomotor pattern is one of the main determinants of sporting success. In some equine competitions, such as dressage, locomotion is essential to have a successful horse and many different authors have tried to define which locomotor peculiarities are associated with a better score by the judges in dressage competitions (Deuel and Park, 1990; Back et al., 1994; Holmström et al., 1995; Clayton, 1997; Bertram and Clayton, 2016; Hobbs et al., 2020) or with better results in overtaking an obstacle in show jumpers or eventers or when comparing between different jumping fences or competitive categories (Van den Bogert et al., 1994; Moore et al., 1995; Hernlund et al., 2010; Clayton et al., 2021).

Furthermore, the relationship between mechanical work, energy expenditure and locomotion during exercise has been described in horses (Minetti et al., 1999). During locomotion, energy is used to move the horse's center of gravity. Running economy is essential in sport horses to avoid or reduce fatigue. Several investigations have tried to link some anatomical and physiological characteristics, i.e. muscle typology or responses of HR and LA to defined intensities of exercise with kinematic variables (Ronéus et al., 1995; Rivero and Clayton, 1996; Muñoz et al., 1999).

To this importance of the locomotor pattern in equine sports performance and its relationship with physiological adaptation to exercise, we must add that lameness and movement asymmetries are one of the most important causes of loss of performance or poor performance in sport horses (Dyson, 2000; Dyson et al., 2008; Preston et al., 2008; Swor et al., 2019; Paris et al., 2021).

Until about 15-20 years ago, the scientific study of equine locomotion was very restricted to labs, requiring highly specific, expensive material and tough laboratory data processing. Some of the studies that analyzed equine locomotion, given the need for this sophisticated equipment, evaluated only basic parameters, generally SF and SL, through measurements with 1-2 synchronized cameras. Knowing the number of frames per unit of time with these cameras, the duration of the stride was calculated, as well as its length, knowing the distance covered (Muñoz et al., 1997; 1999; Schuback et al., 1999; Vilar et al., 2008; Rose et al., 2009; Gunnarsson et al., 2017). Fortunately, the development of various systems such as accelerometers and inertial sensors has revolutionized the study of equine locomotion, which can be carried out under field conditions with easier and faster instruments.

Our research focuses on the study of a 3D accelerometry system, developed by Dr. Eric Barrey and his collaborators. As presented throughout this thesis, the use of this system has focused on two major themes. The first of them is the locomotor evaluation of horses from different sports disciplines, based on the relationship between the accelerometric parameters determined by the Equimetrix® system and the results in competition. In this aspect, the system has been used in many different types of equestrian competitions, including gallop racing (Barrey et al., 2001), trot racing (Leleu et al., 2002; 2004), dressage (Barrey et al., 2002; Barrey and Biau, 2004) and jumping (Barrey and Galloux, 1997; Galloux and Barrey, 1997).

A second aspect in which this accelerometer has been used is in the characterization of the locomotor pattern in horses sedated with different drugs. On many occasions, it is difficult to carry out a clinical diagnosis of lameness in nervous or in horses difficult to handle. Because of that, the team led by Dr. López-Sanromán has published numerous articles on how various sedatives affect the accelerometric pattern determined with the Equimetrix® system: xylazine (López-Sanromán et al., 2012), detomidine or romifidine (López-Sanromán et al., 2013), sublingual detomidine (López-Sanromán et al., 2014), acepromazine (López-Sanromán et al., 2015), detomidine alone or in combination with butorphanol (Frigerio et al., 2019), acepromazine after morphine (López-Sanromán et al., 2021), low doses of detomidine (Calvo-Santesmases et al., 2021) or morphine with or without acepromazine (López-Sanromán et al., 2022).

In our study, we have used the accelerometric device Equimetrix® for evaluation of lameness improvement after treatment and for assessment of locomotor changes in horses exercised on a water treadmill.

8.1. THE USE OF THE ACCELEROMETER EQUIMETRIX® TO MONITOR CHANGES IN LAMENESS IN HORSES DIAGNOSED OF NAVICULAR DISEASE BEFORE AND AFTER CLODRONIC ACID ADMINISTRATION

The Equimetrix® system has not had a very extensive use in the evaluation of lameness in horses. Barrey and Desbrosse (1996), however, applied this accelerometer to lameness detection in the horse. They studied 32 horses, 26 of them were presented for lameness diagnosis. They have different fore- and hindlimb causes of lameness, with degrees between 1 and 3. In that study, the authors used a previous Equimetrix® system, formed only by two accelerometers, measuring acceleration along the lateral and dorsoventral axes of the horse. The system was placed over the sternum. The horses performed a locomotor test consisting of walking and trotting on an asphalt road along a straight line of about 50 m.

Barrey and Desbrosse (1996) found that the shape of the dorsoventral acceleration signal was very repetitive when comparing between different strides in a sound horse, whereas the increasing degree of lameness affected the pattern of the dorsoventral and lateral acceleration curves. Additionally, in lameness grade 2 and 3, it was observed a reduction of the maximal acceleration amplitude every two half strides which appeared to indicate that the pain caused a decrease in the vertical hoof force during the support phase of the lame limb.

The degree of lameness affected the mean values of REG and SYM at walk and at trot, but the SF was not affected. However, it should be highlighted that the differences between the mean values of two continuous grades of lameness were not always significant. One important finding of the report of Barrey and Desbrosse (1996) was that the system was unable to differentiate between fore- and hindlimb lameness, but we have to remember that the accelerometric device was fixed on the sternum. Further, the mean REG and SYM values were smaller at walk than at trot.

On the other hand, the mediolateral acceleration time-curves were less repetitive than the dorsoventral ones. However, the mediolateral accelerometric pattern was also affected by lameness and it could be used to detect the side where the horse put more load. The horse reduced the lateral and medial acceleration during the support phase of the lame limb. The maximum mediolateral acceleration was greater on the contralateral side of the lame limb. Consequently, this previous report demonstrated that the Equimetrix® system could be used to monitor lameness, but it could not be used for diagnosis, since differences in the accelerometric pattern in horses with lameness of the fore vs hind limbs were not found.

In our study, we assessed the progression of lameness during the first 90 days after a single intramuscular dose of 765 mg de clodronic acid in horses diagnosed with navicular syndrome using the Equimetrix® accelerometer and in relation to the results of the clinical examination. The main results of our study were: 1) There was a clinical improvement after clodronic administration, with lameness scoring improving from 3 (baseline) to 2 (control-1, -2 and -3), indicating a reduction of one degree in lameness severity in the 11 horses studied; 2) If only the horses that improved after clodronic acid administration (n=6) were considered, an improvement of two degrees in lameness severity was found; 3) Significant changes in the accelerometric parameters were found in these 6 horses that presented clinical improvement.

8.1.1. Clinical improvement after clodronic acid administration in horses diagnosed with navicular syndrome

The reason for the clinical improvement after bisphosphonate administration in horses has been associated with direct effects on bone resorption, anti-inflammatory and/or analgesic effects (Mitchell et al., 2019; Silva et al., 2021; Vergara-Hernández et al., 2022). Bisphosphonates are considered important therapeutic tools to alleviate pain associated with bone metastasis and cancer in human patients in association with an inhibition of bone resorption (Hussain et al., 2022; Weit et al., 2022). The most commonly prescribed drugs for pain management in human patients with malignancy and cancer involvement are zoledronic acid, pamidronate, clodronate and ibandronate (Lebret et al., 2017). The study performed by Richbourg et al. (2018) using an experimental model, demonstrated that tiludronate and clodronate did not significantly impact formation or resorption parameters in horses after 60 days of administration. For that reason, they concluded that clinical improvement after administration might be attributed to the anti-inflammatory or analgesic effects rather than to the changes in bone remodeling. In fact, Mönkkönen et al. (1998) in human beings, confirmed that bisphosphonates inhibit pro-inflammatory cytokines and nitric oxide secretion from activated macrophages.

In our study, however, only 6 of the 11 studied horses showed clinical improvement after clodronate administration. As said before, a total improvement of one degree in lameness was found when considering the 11 horses. However, this improvement was greater (two degrees) when only horses that improved were included. Our results are in agreement with those previously reported by Frevel et al. (2017), who described an improvement in clinical lameness, of at least 1 grade in 77.72% of the horses treated with clodronate (vs. 3.30% in the control group).

We have no plausible explanation to justify why 5 horses did not show a clinical improvement. It could be due to additional causes of pain or because they had a very mild lameness grade. In fact, the horses that showed greater improvement after treatment were those that had a more severe lameness at the beginning of the study.

8.1.2. Changes in the parameters measured with the accelerometer Equimetrix® in horses diagnosed with navicular syndrome after clodronic acid administration

After treatment with clodronate, horses that improved presented increased values of DVD at walk and at trot and increased DVAA at trot, compatible with a greater vertical trunk movement of the center of gravity. We interpreted these results as indicative of a greater pressure of the lame hoofs during the stance phase, leading to higher DVD and DVAA during the swing phase. In most natural conditions and induced lameness models in horses, increasing severity of lameness in a limb correlates with decreasing pVGRF acting on that limb (Keegan, 2007). For unilateral forelimb lameness, a decrease in mean pVGRF in the lame forelimb is accompanied by increases in the mean pVGRF in the contralateral forelimb and both hindlimbs at the walk (Merkens et al., 1988) and in the contralateral force-limb and hindlimb at the trot (Weishaupt et al., 2006). If a clinical improvement happened after clodronate administration in 6 horses, they supposedly were having greater pVGRF and consequently, DVD and DVAA would increase. These improvements were found at control-2 and -3, therefore, at 30 and 90 days after treatment. In addition, from 30 to 90 days, the horses started a mild exercise program consisting in 20-40 daily ridden exercises at the different gaits. If the horses were not less painful, the onset of ridden exercises would result in negative changes in these accelerometric parameters.

Longitudinal accelerometric activity only increased in the control-3 at walk, MLAA did not change during the study and TAA decreased in control-1 at walk. Maybe the absence of significant findings in LAA and MLAA would be associated with the characteristics of the Equimetrix® device, because the dorsoventral accelerometric pattern is more repeatable than the longitudinal or mediolateral patterns. On the other hand, the lack of changes in TAA was unexpected, because after 90 days with a reduction of training in these horses, a greater reduction in TAA was supposed. We do not have scientific support to affirm that the slight exercise performed for the horses from 30 to 90 days was enough to maintain TAA. The authors are not aware of any longitudinal research evaluating accelerometric changes in horses subjected to various types and intensities of training using the Equimetrix® system.

The Equimetrix® system compares the shape of the dorsoventral acceleration curve between one stride to the following one (REG) and between left and right stances (SYM). Lameness during weight bearing in a limb leads to a reduction in the correlation coefficient (shape similarity) of the dorsoventral acceleration signals, decreasing REG and SYM. In our study, we found an increase in REG at walk at control-2 and -3 and at control-3 at trot. Consequently, we have demonstrated that clodronate administration improved the REG of the stride in lame horses with navicular syndrome. In addition, this improvement was detected earlier at a walk than at trot. Surprisingly, SYM was reduced at control-1 at walk. A hypothesis for this result could be that one limb improved less than the other limb and as a consequence, SYM was worse. However, we have no data to support this hypothesis.

Lame horses used to reduce their velocity of displacement. Horses with navicular syndrome usually had a reduction in SL and an increase in SF and these modifications are reversed after bilateral palmar digital nerve analgesia (McGuigan and Wilson, 2010).

These findings are in agreement with our results. We found an increase in velocity at control-2 at walk and at control-2 and -3 at trot, together with a significant reduction in SF, from control-1, both at walk and at trot. Stride length required a longer period, 90 days, to increase significantly after clodronic administration.

8.1.3. Limitations of the study

Our study, unfortunately, has some limitations in part associated with a field research performed with owned-client lame horses.

1) The same amount of clodronic acid was administered to all the horses, following the recommendations of the commercials of Osphos®. However, we do not know if it would have been better to calculate a specific amount for each horse considering its body weight. It could be that a greater amount of clodronate would be needed for some horses. In addition, only one dose was administered. Previous studies performed using tiludronate repeated administration in those horses that did not achieve clinical improvement (Denoix et al., 2003; Coudry et al., 2007; Gough et al., 2010). The reason for the need for a new administration of the drug was not suggested by these authors.

2) All the horses were shod and the type of shoeing when we started the study was maintained in order to differentiate the effect of clodronic acid from shoeing. We do not know how different types of shoeing affect the accelerometric parameters determined by the Equimetrix® system and if the combined effect of clodronic acid + shoeing could have a different accelerometric effect that clodronic acid administration alone

3) We were unable to make a control group with shoeing but without clodronic acid administration.

4) The horses had a reduction in their exercise training program, because of lameness. We do not know how much the lameness improved due to the reduction in training intensity. Furthermore, although the horses were exercised between 30 and 90 days after administration, the intensity of this exercise was low.

5) Some horses (n=5, representing 45.5% of the horses of the study) did not improve clinically or accelerometrically, but they did not get worse. One reason for this finding could be the duration of the onset of lameness prior to the enrolment of the study.

6) Despite trying to perform a clear diagnosis of navicular syndrome, some of the horses included in the present work could have had involvement of other anatomical structures. Resonance magnetic imaging was only performed in 5 of the studied horses, because this diagnostic modality was not available for animals in the University of Córdoba at the moment of the study. The owners of these horses accepted performing RMI in another Center. Therefore, we cannot affirm completely that there was an absence of other disorders. In fact, the 5 horses subjected to RMI had both bone and soft tissue injuries and clinical improvement was found in 2 of these horses and accelerometric improvement in 4 of them. Nevertheless, only those horses that demonstrated a positive response (i.e. a clinical improvement greater than 80% in lameness score) to bilateral distal palmar digital nerve block, intraarticular distal interphalangeal joint block longer than 10 min and podotrochlear bursa anesthesia were included.

7) The number of horses included in this study was low, only 11 animals, which could have limited the statistical significance. We were unable to recruit more animals because the owners had to commit to: a) Come to the University of Córdoba to carry out the controls; b) Do not apply any medication or change of shoeing during the 90 days of the study.

8.2. THE USE OF THE ACCELEROMETER EQUIMETRIX® TO ASSESS THE LOCOMOTOR PATTERN IN HORSES EXERCISED ON A WATER TREADMILL AT DIFFERENT WATER DEPTHS

8.2.1. Changes in total accelerometric activity during an exercise on a water treadmill at different water depths

The first hypothesis proposed in this study was that exercise on a WT at the level of the STF would result in greater values of TAA compared with the shallower water levels. We found that TAA was significantly greater at the water level of the CAR with the accelerometer fixed in the two studied positions, PECT and SML. However, with the water at the level of STF, we had to reduce the velocity because horses were unable to keep the same velocity as in the other water depths. The reduction of the velocity of exercise on the WT from 6 to 5 km/h makes the exercise more comfortable for the horses with the water at the level of STF, because drag force was reduced with the velocity. However, after velocity reduction, we found a decrease in TAA, with values that were even lower than those found in WW conditions.

Because we did not know if the reduction of TAA with the water at the level of STF was due to the depth of the water and/or the reduction of velocity, we designed a second trial (trial B), in which the horses were exercised at the same lower velocity (5 km/h) at all water depths. Despite the same velocity at all water levels, TAA increased progressively from WW, FET and CAR levels, but did not increase further with the water at the level of the STF, suggesting an important effect of the buoyancy at this water level.

A decreased TAA has been described after administration of different sedatives (Lopez-Sanromán et al., 2012; 2013; 2014; 2014; Frigerio et al., 2019), attributing this result to the myorelaxation induced by these drugs. It is interesting to speculate that, in our case, the increase of TAA from WW to FET and CAR would be indicative of greater muscle involvement and strength. Muscle strength derived from a combination of structural, neuromuscular, hormonal and metabolic factors, being the two first groups the most important. The structural factors associated with muscle strength are dependent on muscle histological and physiological properties, including angle of insertion of muscle fibers in tendons, thickness, length and orientation of tendons and points of application of the strength in relation to the centers of joint rotation and the direction of the resulting forces (Ronnestand et al., 2012; Castejón-Riber et al., 2017). These factors are modified by training, but we do not think that they varied in response to an isolated exercise session. Therefore, the variation in TAA in our study could be in part determined by the neuromuscular factors determining muscle strength. The pattern of activation of different agonists and antagonists muscles during an exercise on a WT at different velocities and water depths have been scarcely studied. Tokuriki et al. (1999) recorded the electromyographic activity of 7 skeletal muscles in the forelimbs and 1 in the hindlimbs during three different conditions: overground walking, swimming in a circular pool and walking and trotting on a WT. The depth of the water on the WT was not described. During exercise on a WT, the *brachiocephalicus* muscle showed increased activity, both at walk and at trot compared to overground walking, but lower activity compared to swimming. The extensor *digitorum communis* had the highest activity when horses were exercised on the WT at walk. Despite the authors mentioning that electrodes for electromyographic analysis were placed on the *vastus lateralis* and the *quadriceps femoris* of the hindlimbs, they did not provide data about these muscles, with only a graphic representation. Later, King et al. (2013; 2017) in an experimental model of osteoarthritis in the middle carpal joint found a delay in the activation of several muscles of the distal aspect of the forelimbs. After 8 weeks of rehabilitation on a WT with the water at the level of the shoulder, the horses had a change in the activation pattern of the deep digital flexor muscle, consisting in a reduction of the motor recruitment during stance and swing phases of the stride. These data appear to suggest that the recruitment of the motor units could be modified with exercise on a WT, even though we do not know if these changes could happen during/after only one session of exercise.

8.2.2. Changes in the accelerometric activity in the three body axes during an exercise on a water treadmill at different water depths

Previous studies demonstrated that the amount of flexion and extension of the joints of the distal aspects of the fore- and hindlimbs of horses exercised on a WT increased compared to exercise on a WT without water (Méndez-Angulo et al., 2014). Specifically, an increase in range of motion of carpal, tarsal, metacarpophalangeal and metatarsophalangeal joints was found, mainly due to an increase in flexion, in horses exercised on a WT. Because of these previous data, the second hypothesis of our work was that, as the water depth increases, DVD values would also increase. Furthermore, in order to achieve greater values of DVD, a greater DVAA would be needed and a greater percentage of TAA would be directed towards the dorsoventral axis.

Our data confirmed in part this second hypothesis. Dorsoventral displacement and DVAA progressively increased from WW-FET-CAR in absolute numbers, with the accelerometer in both positions, and an increase of DDVA% was also found in trial A. However, with the accelerometer in SML position, DVD and DVAA decreased with the water at the level of STF when the velocity was reduced from 6 to 5 km/h (trial A), but not when the velocity was 5 km/h (trial B). Our data demonstrated that DVD and DVAA depend both on the depth of the water and on the velocity of the exercise.

Both parameters appear to be very important in dressage horses, as revealed by various researches performed by Barrey and his team. Biau and Barrey (2004) found that young dressage horses increased DVD and DVAA with age and training, representing an increase in muscle activity and collection. In addition, when DVD and DVAA increased, the horses achieved better performance in dressage competitions. Further, Barrey et al. (2002) analyzed the accelerometric pattern of different horse breeds and they found that the accelerometric pattern associated with a higher score in competitions consisted in slow SF, high REG, large DVD and DVAA, which means more elasticity. Therefore, the fact that the exercise on the WT increases DVD and DVAA supports that this modality of exercise would be favorable for training horses for dressage. In addition, although there is little data available at this moment, high values of DVD and DVAA, measured with the Equimetrix® accelerometer appeared to be advantageous for jumping horses (Barrey and Galloux, 1997; Galloux and Barrey, 1997). Similarly, also in horses equipped with the Equimetrix® system, Ricard et al. (2020) found that the best horses had a mechanically higher height of the center of gravity (i.e. DVD) during jumping. Previously, Powers and Harrison (2002), using video recording, found that good horses showed higher values for the height of the center of gravity over the fence. However, we should indicate that there are some contradictory reports concerning these data. Bobbert et al. (2005), on the contrary, found that the best horses jumping vertical obstacles had lower height in the center of gravity. They justified their results with two reasons. Firstly, because horses were not required to jump maximally, there was no reason for differences in the values of the height in the center of gravity. Second, the optimum trajectory may be a better adjustment to the obstacle than a higher height jump. Nevertheless, a good score for trot and canter in jumpers was genetically linked to high DVD, together with low SF, LAA and MLAA (Ricard et al., 2020a,b). For walking, the link was with high DVAA, DVD, REG and low SF and LAA (Ricard et al., 2020a;b). Curiously, in a very recent report, Dugué et al. (2021) described that high MLAA and DVAA and low SF were moderately genetically correlated with longer functional longevity in jumping horses.

In our study, the increase shown by DVAA appears to act as a limiting factor for increasing LAA, which did not vary significantly with water depth and even it was reduced. In the same way, when LAA was expressed as a percent of TAA, a reduction was observed in both trial A and B, perhaps because of the increase in DVAA%.

8.2.3. Changes in the coordination parameters of the stride during an exercise on a water treadmill at different water depths

King et al. (2013) demonstrated that rehabilitation on a WT in horses with experimental osteoarthritis showed a greater stability compared to those horses rehabilitated conventionally. These previous data led us to our third hypothesis, that exercise on a WT would increase stride REG and SYM at shallow water levels (i.e. FET and CAR). We did not know what would happen at deeper water depths, because some authors, as Nankervis et al. (2019) described that deeper water (to the abdomen and above) can lead to loss of stability and 'rolling'. However, we were unable to confirm this hypothesis. We did not find significant differences in stride REG and SYM when comparing the different walking velocities or water depths on the WT. The only difference was found in trial B, when the SYM values were higher during the exercise with the water at the level of FET compared to WW. However, the reduction was very mild (even though statistically significant) and probably irrelevant from a practical point of view.

We can propose several explanations for the lack of differences in SYM and REG with the level of water. Firstly, we based our hypothesis on the study of King et al. (2013) that was carried out with lame horses, while in our case, we have studied sound horses.

In addition, our horses performed only several exercise sessions on the WT, whereas the horses of the study of King et al. (2013) were rehabilitated on the WT during 8 weeks, with daily exercises. Secondly, the devices used for evaluating these parameters were different. King et al. (2013) used force platforms whereas we have used accelerometry. Thirdly, the Equimetrix® accelerometer uses the dorsoventral accelerometric signal to calculate REG and SYM.

Another finding we would like to highlight is that the REG was not worse with the water at deep levels (STF level).

8.2.4. Changes in the spatiotemporal parameters of the stride during an exercise on a water treadmill at different water depths

As described by many different authors before (Scott et al., 2010; Méndez-Angulo et al., 2014; Nankervis et al., 2017), the increase of the water depth results in a reduction in SF together with an increase in SL, provided that the velocity is not changed. We have found that when the velocity is reduced with the water at the level of the STF, SL is the same as this found at the levels of WW, FET and CAR. However, when the velocity is the same in the four different water levels (WW, FET, CAR and STF), SL increased and SF decreased with the water at the level of STF compared to CAR. In our opinion, this is an important finding from a practical point of view. It means that we can use the combination of velocity and depth to reach specific locomotor goals. For instance, if we have an unfit horse, and we have to reduce the velocity of the WT when exercised at a water level of STF (because we want to increase flexion of the upper joints of the limbs or to limit as much as we can the load on the limbs), we must know that we are not obtaining an lengthening the stride.

8.2.5. Limitations of the study

Our study has some limitations:

1) This research has been carried out with a small number of animals and because of logistic reasons, the horses enrolled in both trials were different. Moreover, horses of trial B were less fit than horses of trial A and lower values for TAA were found in the horses of trial B.

2) Accelerometry was performed with the animals within the WT, but we don't know how the obtained results will influence terrestrial locomotion and performance in the sport horses.

8.3. THE USE OF THE ACCELEROMETER EQUIMETRIX® TO ASSESS HOW EXERCISE ON A WATER TREADMILL AT DIFFERENT WATER DEPTHS INFLUENCES LOCOMOTION ON A TRACK IN HORSES

As we have discussed in our previous research (8.2.), some of the accelerometric adaptations underwent by the horses during exercise on a WT at different water depths appear to be advantageous for some types of equine competition, such as dressage, jumping and three-day event. The basis of the affirmation is the results provided by researchers of other investigators that have tried to relate accelerometric peculiarities and performance in competitions: scoring by the judges in dressage (Barrey and Biau, 2004; Biau et al., 2004) and ability to overtake a jump in show jumping (Barrey and Galloux, 1997; Galloux and Barrey, 1997). We wonder if these supposedly advantageous accelerometric characteristics that appear on a WT are maintained during terrestrial locomotion.

8.3.1. Changes in total accelerometric activity on a track after water treadmill exercise at different water depths

The first hypothesis of our study was that TAA evaluated on a track would be greater after performing an exercise on a WT with the water at the level of CAR and STF, compared to WW or FET. We could demonstrate part of this hypothesis. Total accelerometric activity was higher on the track after WT exercise at CAR level, but it was not greater after WT exercise with the water at the level of STF, with the exception of the trot with the accelerometer fixed in PECT position. In this case, TAA increased from baseline, but decreased after WT exercise at the levels of FET and CAR. We did not expect to find this result. We think that fatigue could induce a reduction in TAA on the track when exercise on the WT was performed with the water at the level of the STF, if the velocity is not changed. Despite the fact that WT exercise at the level of STF implies a greater buoyancy, the deeper water would also increase drag force and this could promote an earlier onset of fatigue.

From our results, we can draw two main practical applications:

1) These results can help us with tailoring an appropriate rehabilitation program for injured horses, with low fitness levels if they have been with the injury for a medium or a long time. In this case, exercise on a WT with the water at the level of STF can be fatiguing.

2) Exercise on a WT at water levels of STF could be used to improve muscle strength, providing that velocity is maintained and not reduced. This idea is in line with the results of the survey performed by Tranquille et al. (2019) to trainers and horse owners that revealed that 60% of the responders to their questionnaire use the WT for training dressage, eventers and jumpers because of the main impression of increased strength, fitness and performance improvement perceived by the owner or rider. We could speculate that these subjective appreciations of the riders/trainers could be linked to the higher accelerometric activities during the WT exercise. Curiously, the results of the survey published by Tranquille et al. (2019) described that water at the levels of CAR and STF were used more frequently from training compared to rehabilitation.

8.3.2. Changes in the accelerometric activity in the three body axes on a track after water treadmill exercise at different water depths

Because during a WT exercise at the level of CAR, significant increases of DVD and DVAA were found, as demonstrated in our second study. Our second hypothesis was that these adaptations would persist in terrestrial locomotion. Our results confirmed this hypothesis. The depth of water that induced the most marked changes in DVAA on the tract was the CAR level, particularly at a walk with the accelerometer in PECT position and at trot with the accelerometric in SML position. We think these results could be attributed to the way the horses walk on the WT. At a water level of CAR, the horses tried to 'step on' the water, but this movement does not appear when the water is at the level of STF.

In the same way, DVD also increased on the track with the accelerometer in PECT position, and with the most evident increase after WT exercise at the water level of CAR. This parameter, however, decreased with the accelerometer in SML position and with the greatest reductions after WT exercise at the water levels of CAR and STF, probably as a result of fatigue.

Because in our second study we found an increase in DVAA together with a reduction in LAA, our third hypothesis was that this accelerometer pattern would persist on the track, particularly after WT exercise at the levels of CAR and STF. By contrast, LAA increased after WT exercise, with the largest increase after exercise at the water level of CAR. However, a decrease in LAA was observed on the track at trot, with the accelerometer in PECT position and at the walk and trot with the accelerometer in SML position. We interpreted these data as another indication that an exercise on a WT at a water level of STF can be fatiguing for an unfit horse, despite the buoyancy effect of deep water.

We also found increases of MLAA on the track after WT exercise, particularly when the exercise was performed at the level of CAR. This is an interesting result, since greater values for this parameter have been associated with a greater degree of flexibility (Biau and Barrey, 2002). However, a reduction was found in MLAA when the exercise on the WT was carried out at the level of STF.

8.3.3. Changes in the coordination parameters of the stride on a track after water treadmill exercise at different water depths

Stride REG and SYM are important parameters, indicative of a coordination of the horse and marked reductions have been associated with lameness (Barrey and

Desbrosse, 1996). We observed REG and SYM were either unchanged or reduced. The greatest reductions were observed with the accelerometer in SML position. There is an increase in REG after WT exercise at the CAR level, but only when the accelerometer was in PECT position. The reduction experienced by these parameters are not advantageous for the sport horse, since high values of REG and SYM have been associated with a better scores for the judges in dressage competitions (Biau and Barrey, 2004). We do not have an explanation for these results, with the exception of fatigue. More investigations should be done in order to elucidate the importance of these findings.

8.3.4. Changes in the spatiotemporal parameters of the stride on a track after water treadmill exercise at different water depths

The interpretation of the velocity in the present study is complex because the horses were led by hand on the track. However, the same handler (A.S.) led the horses and tried to keep the velocity as constant as possible. Even though we did not published results, in our experience, we have found marked variations in velocities when different handlers led the horses, particularly at trot. However, these variations are of minor intensity when a same handler performed several runs with the horses. However, we have to keep in mind that, there is a possibility that even small variations in velocity would affect spatiotemporal parameters of the stride.

The changes in SL and SF on the track after performing exercise on the WT have been less evident that those changes previously described for other parameters, such as accelerometric activities. Contrary to what happens during a WT exercise sessions, where a reduction of SF and an increase of SF have been described (Scott et al., 2010; Méndez-Angulo et al., 2013; Saitua et al., 2020), in our research, we found an increase in SF on the track at walk, with the accelerometer in PECT position. The maximum SF percent of change was observed after WT exercise at FET level. However, during a WT exercise session, the lowest value of SF has been described with the water at the level of the CAR (Saitua et al., 2020; Study II). A significant reduction in SF was found at trot after WT exercise at CAR level.

In relation to SL, a reduction at walk, but an increase at trot, was observed with the accelerometer in PECT position. With the accelerometer in SML position, an elongation of the stride was found at the walk and trot, after WT exercise at all water depths, with the exception of the trot after WT exercise at STF level.

8.3.5. Limitations of the study

Our study has the following limitations:

1) The accelerometric adaptations to an exercise session on a WT have been studied on the track shortly after exercise (30 min). It would be interesting to know how long these adaptations persist during overground locomotion, as well as their long-term effects.
2) We have studied horses with a moderate fitness level. We do not know to what extent these data can be extrapolated to horses with a better fitness level. However, we can consider that injured horses, in rehabilitation, can have a reduction in fitness and exercise capacity. Therefore, our study has importance in this sense.
3) This research was not designed with the goal of assessing how the inclusion of a WT exercise could affect adaptations to training or performance in competition. The achievement of this purpose would require a different experimental design, but it should be developed in a near future.

9. Conclusions

The current research focused on different aspects of the use of an accelerometric system, Equimetrix®, in sport horses. We attached this system in two different positions in the horses: in the PECT region, by a girth and in the SML midline, using a tape. The accelerometer was used both on a track and during an exercise on a WT. It has been used with two different objectives: as a method to quantify lameness and to objectively follow-up improvement of lameness after a treatment and as a tool to assess locomotor adaptations during and after exercise on a WT at different depths.

We did not have any significant problem with the use of this system, with the exception of some horses that required an adaptation to wear the accelerometer in SML position. One horse was reluctant to wear the accelerometer in this position and he started jumping when the accelerometer was positioned. He had to be removed from studies II and III.

We did not have any problem fixing the accelerometer in the PECT region when the horses were exercised on the WT at the level of the carpus. To do this, we put the device into a waterproof plastic bag. However, we did not dare to exercise the horses with the water at the stifle level, since in this case, the accelerometer could be completely submerged in the water.

Our research has led us obtain the following conclusions:

First conclusion. The accelerometer Equimetrix® was an useful and easy way to monitor the improvement shown by horses diagnosed with navicular syndrome after intramuscular administration of a bisphosphonate drug (clodronate or clodronic acid), as well as to assess locomotion on a water treadmill and on a track. Animals tolerated the device well and there were no problems with its use when it was in part submerged.

Second conclusion. From an accelerometric point of view, the improvement found in some horses diagnosed with navicular syndrome and treated with clodronic acid consisted of increased velocity, stride length, stride regularity and dorsoventral displacement together with a reduction in stride frequency. These accelerometric changes appeared together a clinical improvement, with a mean reduction of two degrees in lameness score.

Third conclusion. Total accelerometric activity in horses exercised on a water treadmill increased with water depth at the levels of fetlock and carpus compared to a situation of no-water, but it did not increased further with the water at the level of the stifle, probably because of the effect of buoyancy that assisted displacement on the treadmill. In addition, a reduction in velocity caused a decrease in total accelerometric activity with the water at the level of the stifle.

Fourth conclusion. The exercise on the water treadmill led to a redistribution of the total accelerometric activity into the three body axes, with significant increases in dorsoventral accelerometric activity and dorsoventral displacement, but at expenses of a reduction in the accelerometric activities in the other two body axes: longitudinal and mediolateral.

Fifth conclusion. Some of the accelerometric modifications that happened when the horses were exercised on the water treadmill persisted short-time during overground in-hand walking and trotting locomotor patterns. However, we do not know how long these adaptations persist in terrestrial locomotion.

Sixth conclusion. Some accelerometric changes found in overground locomotion at 30 min after finishing a session of water treadmill exercise at a level of the stifle, including a reduction in total and longitudinal accelerometric activities, dorsoventral displacement and velocity, were compatible with fatigue, probably attributed to the greater drag force that counteracted buoyancy. These results must be taken into account when tailoring a rehabilitation program in unfit horses.

10. Resumen

EVALUACIÓN DE LA BIOMECÁNICA DE LA LOCOMOCIÓN DEL CABALLO DE DEPORTE MEDIANTE ACELEROMETRÍA

INTRODUCCIÓN. En los últimos años se ha producido una expansión del desarrollo de diversos sistemas de estudio biomecánico que pueden ser aplicados de forma fácil y práctica en situaciones de campo y clínicas. Uno de estos sistemas es el acelerómetro Equimetrix®, utilizado fundamentalmente en la evaluación locomotora funcional de diversos caballos de deporte y en la valoración del grado de sedación inducida por diversos sedantes. El uso de este sistema en la monitorización de cojeras es muy limitado. El síndrome del navicular constituye una de las causas de cojeras más frecuente de miembros anteriores en caballos. Uno de los tratamientos médicos es la administración de bifosfonatos. Si bien la evaluación clínica del bifosfonato tiludronato ha sido publicada previamente, los estudios clínicos sobre la administración de clodronato son escasos y no se han utilizado sistemas objetivos de evaluación de la posible mejoría que inducen. Por otro lado, cada vez se utiliza más el ejercicio en cinta rodante o *treadmill* acuático (WT) para el entrenamiento y rehabilitación del caballo de deporte. Este hecho contrasta con la falta de conocimiento científico sobre esta modalidad de ejercicio.

OBJETIVOS. El objetivo principal de esta investigación es evaluar la posibilidad del uso de la acelerometría en varios aspectos del caballo de deporte. Se proponen tres objetivos particulares: 1) Analizar los cambios acelerométricos que experimentan los caballos con cojera asociada a síndrome de navicular en respuesta a la administración del bifosfonato clodronato y su relación con la evaluación clínica subjetiva; 2) Describir los cambios acelerométricos en caballos ejercitados en WT, a diferentes profundidades de agua y velocidades; 3) Investigar si, los cambios acelerométricos que los caballos ejercitados en WT muestran, persisten durante la locomoción terrestre en pista.

MATERIAL Y MÉTODOS. **Estudio I.** Se han estudiado 11 caballos con hallazgos clínicos y radiográficos compatibles con síndrome del navicular. Estos animales fueron tratados con una dosis única intramuscular de ácido clodrónico (765 mg/caballo). Se hicieron controles subjetivos de cojera y acelerometría, antes del tratamiento (basal), a los 7, 30 y 90 días deL tratamiento. **Estudio II.** Se han realizado dos experimentos: A. Se han estudiado 6 caballos sin cojera, ejercitados en WT durante 40 min, en 4 situaciones diferentes (sin agua WW; con el agua a nivel de la articulación metacarpofalangiana FET; a nivel del carpo CAR; a nivel de la babilla, STF). La velocidad fue de 6 km/h a profundidades WW, FET y CAR y a 5 km/h a profundidad STF. Experimento B. Otros 5 caballos realizaron las mismas sesiones de ejercicio, pero todas ellas a una velocidad de 5 km/h. **Estudio III.** Se han estudiado 6 caballos, ejercitados en WT a una velocidad de 5 km/h, durante 40 min, a 4 profundidades de agua (WW, FET, CAR y STF). Se realizaron registros acelerométricos antes (basal) y a los 30 min después de cada sesión de ejercicio, en pista, al paso y al trote. Se obtuvo el porcentaje de variación de los parámetros acelerométricos registrados en pista, tras el ejercicio en WT en relación al basal. **Acelerometría**. Se ha utilizado el acelerómetro triaxial Equimetrix®. En el estudio I se colocó en la región pectoral, mientras que en los estudios II y III se colocó también en la línea media del sacro. En el estudio I y III, los registros acelerométricos se hicieron en asfalto (estudio I) y en una pista de entrenamiento (estudio III), al paso y al trote. En el estudio II, los registros se hicieron en el WT. Se han obtenido tres grupos de parámetros: parámetros acelerométricos (actividad acelerométrica total AAT; dorsoventral DVAA; longitudinal LAA; mediolateral MLAA; desplazamiento dorsoventral DVD); parámetros de coordinación del tranco (regularidad REG; simetría SIM) y parámetros espaciotemporales del tranco (frecuencia de tranco SF, longitud de tranco SL; velocidad).

RESULTADOS. **Estudio I.** Se observó una mejoría clínica en 6 de los 11 caballos diagnosticados de síndrome del navicular, consistente en una reducción media de 2 grados en la valoración subjetiva de la cojera. Desde un punto de vista acelerométrico, estos 6 caballos presentaron un incremento de velocidad, SL, REG y DVD, y una reducción de SF. **Estudio II.** La TAA se incrementó con la profundidad del agua, desde WT a FET y CAR, pero no aumentó con el agua a nivel de STF (experimento A). Se observó una reducción de TAA cuando la velocidad fue inferior (experimento B). Se han encontrado incrementos de DVAA y DVD durante el ejercicio en WT, aumentando con la profundidad y con la velocidad, a expensas de una reducción de LAA y MLAA. El incremento en la profundidad de agua dio lugar a una SL mayor junto con una SF inferior. **Estudio III.** Se ha encontrado un aumento de TAA, DVAA, LAA, MLAA y DVD tras el ejercicio en WT a profundidades de WW, FET y CAR, correspondiendo las elevaciones más evidentes a la profundidad CAR y con el acelerómetro situado en el esternón. Sin embargo, se ha observado una reducción de TAA, LAA, DVD y velocidad, particularmente al trote, con el acelerómetro en la línea media del sacro y tras el ejercicio en WT a una profundidad de STF.

CONCLUSIONES. El acelerómetro Equimetrix® permitió detectar las modificaciones asociadas con un menor grado de cojera en caballos diagnosticados de síndrome de navicular tratados en una única dosis del bifosfonato clodronato. Los caballos ejercitados en WT presentaron un incremento de TAA, sobre todo en el eje dorsoventral y una elongación del tranco, siendo estos cambios dependientes de la velocidad y de la profundidad del agua. Una vez superado el carpo, una mayor profundidad del agua no implicó una mayor actividad acelerométrica. Estas modificaciones persistieron durante la locomoción terrestre a corto plazo. Sin embargo, cuando el ejercicio se llevó a cabo con una profundidad de agua a nivel de babilla, el caballo mostró cambios acelerométricos en pista indicativos de fatiga.

RELEVANCIA CLÍNICA. El acelerómetro Equimetrix® es un método fácil, que se puede usar en condiciones clínicas y de campo para seguir la evolución de diversos tratamientos en caballos con cojera. El ejercicio en WT mostró adaptaciones acelerométricas asociadas con un mejor rendimiento deportivo en caballos de salto y de doma clásica. Estas adaptaciones persistieron en la locomoción terrestre a corto plazo. Sin embargo, debe tenerse un cuidado especial del ejercicio en WT a nivel de STF en caballos con lesiones o poco entrenados, ya que puede condicionar fatiga.

PALABRAS CLAVE. Acelerometría. Bisfosfonatos. Caballos. Entrenamiento. Treadmill acuático. Rehabilitación. Síndrome navicular.

11. Summary

EVALUATION OF THE BIOMECHANICS OF LOCOMOTION IN THE ATHLETIC HORSE BY ACCELEROMETRY

INTRODUCTION. In recent years there has been an expansion in the development of various systems to study equine locomotion that can be applied easily and practically in field and clinical situations. One of these systems is the Equimetrix® accelerometer, used fundamentally in the functional evaluation of various sport horses and in the assessment of the degree of sedation induced by various sedatives. The use of this system in monitoring lameness is very limited. Navicular syndrome is one of the most common causes of lameness of the equine forelimbs. One of the medical treatments is the administration of bisphosphonates. Although the clinical evaluation of the bisphosphonate tiludronate has been previously published, clinical studies on the administration of clodronate are scarce and objective systems for evaluating the possible improvement they induce have not been used. On the other hand, the exercise on a water treadmill (WT) is increasingly used for training and rehabilitation of the sport horse. This fact contrasts with the lack of scientific knowledge about this form of exercise.

OBJECTIVES. The main objective of this research is to evaluate the possibility of using accelerometry in various clinical and research scenarios in the sport horse. Three particular objectives are proposed: 1) To analyze the accelerometric changes of horses with lameness associated with navicular syndrome in response to the administration of the bisphosphonate clodronate and its relationship with subjective clinical evaluation; 2) To describe the accelerometric changes in horses exercised on a WT, at different water depths and speeds; 3) To investigate whether the accelerometric changes that horses on a WT have, persist during overground locomotion.

MATERIAL AND METHODS. **Study I.** Eleven horses with clinical and radiographic findings compatible with navicular syndrome have been studied. These animals were treated with a single intramuscular dose of clodronic acid (765 mg/horse). Subjective lameness

and accelerometry controls were made before treatment (baseline), at 7, 30 and 90 days after treatment. **Study II.** Two trials have been carried out: Trial A. Six horses without lameness, exercised on the WT for 40 min, in 4 different situations (without water WW; with water at the level of the metacarpophalangeal joint FET; carpus CAR; at stifle level, STF). The velocity was 6 km/h at depths WW, FET and CAR and 5 km/h at depth STF. Trial B. Another 5 horses performed the same exercise sessions, but at a speed of 5 km/h. **Study III**. Six horses have been studied, exercised on a WT at a velocity of 5 km/h, for 40 min, at 4 water depths (WW, FET, CAR and STF). Accelerometric recordings were made before (baseline) and at 30 min after each exercise session, on the track, at walk and at trot. The percent of change of the studied accelerometric parameters after exercise on the WT in relation to baseline was obtained. **Accelerometry**. The triaxial accelerometer Equimetrix© has been used. In study I it was placed in the pectoral region, while in studies II and III it was also placed in the midline of the sacrum. In studies I and III, the accelerometric recordings were made on asphalt (study I) and on a training track (study III), at walk and at trot. In study II, the accelerometric recordings were made on the WT. Three groups of parameters have been obtained: accelerometric parameters (total accelerometric activity, TAA; dorsoventral DVAA; longitudinal LAA; mediolateral MLAA accelerometric activities; dorsoventral displacement DVD); stride coordination parameters (REG regularity; SIM symmetry) and stride spatiotemporal parameters (SF stride frequency, SL stride length; velocity).

RESULTS. **Study I.** Clinical improvement was observed in 6 of the 11 horses diagnosed with navicular syndrome, consisting of a mean reduction of 2 degrees in the lameness evaluation. From an accelerometric point of view, these 6 horses had increases in velocity, SL, REG and DVD, and reductions in SF. **Study II**. Total accelerometric activity increased with water depth, from WT to FET and CAR, but did not increase with water at the STF level (Trial A). A reduction in TAA was observed when the velocity was lower (Trial B). Increases in DVAA and DVD were found during exercise on the WT, increasing with depth and velocity, at the expense of a reduction in LAA and MLAA. The increase in water depth resulted in a higher SL and in a lower SF. **Study III**. An increase in TAA, DVAA, LAA, MLAA and DVD were found after exercise on the WT at levels of WW, FET and CAR. The most evident elevations corresponding to CAR level and with the accelerometer

located in the pectoral region. However, a reduction in TAA, LAA, DVD, and velocity was observed, particularly at trot, with the accelerometer at the sacrum midline, and after exercise on the WT at level of STF.

CONCLUSIONS. The Equimetrix® accelerometer allowed the detection of changes associated with a lower score of lameness in horses diagnosed with navicular syndrome treated with a single dose of the bisphosphonate clodronate. Horses exercised on a WT showed an increase in accelerometric activity, especially in the dorsoventral axis and an elongation of the stride, these changes being dependent on velocity and water depth. Above the carpus, greater water depth did not imply a greater accelerometric activity. These modifications persisted short-term in terrestrial locomotion. However, when the exercise was carried out at the level of STF, the horse showed accelerometric changes on the track compatible of fatigue.

CLINICAL RELEVANCE. The Equimetrix® accelerometer is an easy device, which can be used in clinical and field conditions to monitor the progress of treatments in lame horses. Water treadmill exercise led to some accelerometric adaptations associated with a better athletic performance in show jumping and dressage horses. These adaptations persisted at least for 30 min, in terrestrial locomotion. However, special care must be taken when exercising on a WT at the STF level in horses with injuries or poorly trained, since it can cause fatigue.

KEYWORDS. Accelerometry. Bisphosphonates. Horses. Navicular syndrome. Rehabilitation. Training. Water treadmill.

12. References

Adair III, H. S. (2011). Aquatic therapy for conditioning and treatment of tendon and ligament injuries. In *Proceedings of the 57th Annual Convention of the American Association of Equine Practitioners, San Antonio, Texas, USA, 18-22 November 2011*, 181-185.

Adler, R. A. (2016). Bisphosphonates and atypical femoral fractures. *Current Opinion in Endocrinology, Diabetes and Obesity*, *23*(6), 430-434.

Algafly, A. A., & George, K. P. (2007). The effect of cryotherapy on nerve conduction velocity, pain threshold and pain tolerance. *British journal of sports medicine*, *41*(6), 365-369.

Alvarez, C. G., Bobbert, M. F., Lamers, L., Johnston, C., Back, W., & Van Weeren, P. R. (2008). The effect of induced hindlimb lameness on thoracolumbar kinematics during treadmill locomotion. *Equine Veterinary Journal*, *40*(2), 147-152.

Alvarez, C. G., Rhodin, M., Byström, A., Back, W., & Van Weeren, P. R. (2009). Back kinematics of healthy trotting horses during treadmill versus over ground locomotion. *Equine veterinary journal*, *41*(3), 297-300.

Anon (1999). Rules for Dressage. Fédération Equestre Internationale (FEI). Articles 403, 404, 414, 415, 416.

Argue, C. K., & Clayton, H. M. (1993). A preliminary study of transitions between the walk and trot in dressage horses. *Cells Tissues Organs*, *146*(2-3), 179-182.

Argüelles, D., Saitua, A., de Medina, A. S., Muñoz, J. A., & Muñoz, A. (2019). Clinical efficacy of clodronic acid in horses diagnosed with navicular syndrome: A field study using objective and subjective lameness evaluation. *Research in veterinary science*, *125*, 298-304.

Audigié, F., Pourcelot, P., Degueurce, C., Geigert, D., & Denoix, J. M. (2001). Kinematic analysis of the symmetry of limb movements in lame trotting horses. *Equine Veterinary Journal*, *33*(S33), 128-134.

Back, W., Barneveld, A., Van Weeren, P. R., & Van den Bogert, A. J. (1993). Kinematic gait analysis in equine carpal lameness. *Cells Tissues Organs*, *146*(2-3), 86-89.

Back, W., MacAllister, C. G., Van Heel, M. C. V., Pollmeier, M., & Hanson, P. D. (2009). The use of force plate measurements to titrate the dosage of a new COX-2 inhibitor in lame horses. *Equine veterinary journal*, *41*(3), 309-312.

Bailey, C. J., Reid, S. W. J., Hodgson, D. R., Suann, C. J., & Rose, R. J. (1997). Risk factors associated with musculoskeletal injuries in Australian Thoroughbred racehorses. *Preventive Veterinary Medicine*, *32*(1-2), 47-55.

Baron, R., Neff, L., Louvard, D., & Courtoy, P. J. (1985). Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. *The Journal of cell biology*, *101*(6), 2210-2222.

Barrey, E. (1999). Methods, applications and limitations of gait analysis in horses. *The veterinary journal*, *157*(1), 7-22.

Barrey, E. (2013). Biomechanics of locomotion in the athletic horse. *Equine Sports Medicine and Surgery-Basic and Clinical Sciences of the Equine Athlete*, 189-211.

Barrey, E., & Desbrosse, F. (1996). Lameness detection using an accelerometric device. *Pferdeheilkunde*, *12*, 617-622.

Barrey, E., & Galloux, P. (1997). Analysis of the equine jumping technique by accelerometry. *Equine Veterinary Journal*, *29*(S23), 45-49.

Barrey, E., Auvinet, B., & Couroucé, A. (1995). Gait evaluation of race trotters using an accelerometric device. *Equine Veterinary Journal*, *27*(S18), 156-160.

Barrey, E., Desliens, F., Poirel, D., Biau, S., Lemaire, S., Rivero, J. L., & Langlois, B. (2002). Early evaluation of dressage ability in different breeds. *Equine Veterinary Journal*, *34*(S34), 319-324.

Barrey, E., Evans, S. E., Evans, D. L., Curtis, R. A., Quinton, R., & Rose, R. J. (2001). Locomotion evaluation for racing in thoroughbreds. *Equine Veterinary Journal*, *33*(S33), 99-103.

Barrey, E., Galloux, P., & Valette, J. P. (1993). Relationships between accelerometric and kinematic measurements in a running horse. Proceedings of XIVth Congress International society of Biomechanics. Paris. 148-149.

Barrey, E., Galloux, P., Valette, J. P., Auvinet, B., & Wolter, R. (1993). Stride characteristics of overground versus treadmill locomotion in the saddle horse. *Cells Tissues Organs*, *146*(2-3), 90-94.

Barrey, E., Hermelin, M., Vaudelin, J. L., Poirel, D., & Valette, J. P. (1994). Utilisation of an accelerometric device in equine gait analysis. *Equine Veterinary Journal*, *26*(S17), 7-12.

Bassett, C. A. L., Donath, A., Macagno, F., Preisig, R., Fleisch, H., & Francis, M. D. (1969). Diphosphonates in the treatment of myositis ossificans. *The Lancet*, *294*(7625), 845.

Baxter, G. M., Stashak, T. S., & Keegan, K. G. (2020). Examination for Lameness: History, Visual Exam, and Conformation. *Adams and Stashak's Lameness in Horses*, 67-188.

Baxter, G. M., Stashak, T. S., Belknap, J. K., & Parks, A. (2011). Lameness in the Extremities. *Adams & Stashak's Lameness in Horses. 6de Editie, Wiley-Blackwell, Iowa*, 475-832.

Becero, M., Saitua, A., Argüelles, D., Sánchez de Medina, A. L., Castejón-Riber, C., Riber, C., & Muñoz, A. (2020). Capacitive resistive electric transfer modifies gait pattern in horses exercised on a treadmill. *BMC veterinary research*, *16*(1), 1-12.

Bell, B. T. L., Bridge, I. S., & Sullivan, S. T. K. (1996). Surgical treatment of navicular syndrome in the horse using navicular suspensory desmotomy. *New Zealand Veterinary Journal*, *44*(1), 26-30.

Bell, R. P., Reed, S. K., Schoonover, M. J., Whitfield, C. T., Yonezawa, Y., Maki, H., Pai, P. F., & Keegan, K. G. (2016). Associations of force plate and body-mounted inertial sensor measurements for identification of hind limb lameness in horses. *American Journal of Veterinary Research*, *77*(4), 337-345.

Bender, T., Karagülle, Z., Bálint, G. P., Gutenbrunner, C., Bálint, P. V., & Sukenik, S. (2005). Hydrotherapy, balneotherapy, and spa treatment in pain management. *Rheumatology international*, *25*(3), 220-224.

Benedict, J. J. (1982). The physical chemistry of the diphosphonates—its relationship to their medical activity. In *Symposium CEMO (Centre d'Etude des Maladies Ostéoarticulaires de Genève) IV. Diphosphonates and bone. Editions Médecine at Hygiène, Geneva* (pp. 1-19).

Bertone, A. L. (2003). Gait analysis for the quantification of lameness. In *Diagnosis and management of lameness in the horse* (pp. 222-225). WB Saunders.

Biau, S., & Barrey, E. (2004). Relationship between stride characteristics and scores in dressage tests. *Pferdeheilkunde*, *20*(2), 140-144.

Biau, S., & Barrey, E. (2004). The trot characteristics during the first years of dressage training. *Pferdeheilkunde*, *20*(2), 135-139.

Biau, S., Lemaire, S., & Barrey, E. (2002). Analysis of gait transitions in dressage horses using wavelet analysis of dorsoventral acceleration. *Pferdeheilkunde*, *18*(4), 343-350.

Blair, H. C., Teitelbaum, S. L., Ghiselli, R., & Gluck, S. (1989). Osteoclastic bone resorption by a polarized vacuolar proton pump. *Science*, *245*(4920), 855-857.

Blunden, A., Dyson, S., Murray, R., & Schramme, M. (2006a). Histopathology in horses with chronic palmar foot pain and age-matched controls. Part 1: Navicular bone and related structures. *Equine veterinary journal*, *38*(1), 15-22.

Blunden, A., Dyson, S., Murray, R., & Schramme, M. (2006b). Histopathology in horses with chronic palmar foot pain and age-matched controls. Part 2: the deep digital flexor tendon. *Equine veterinary journal*, *38*(1), 23-27.

Bobbert, M. F., & Santamaría, S. (2005a). Contribution of the forelimbs and hindlimbs of the horse to mechanical energy changes in jumping. *Journal of Experimental Biology*, *208*(2), 249-260.

Bobbert, M. F., Santamaría, S., van Weeren, P. R., Back, W., & Barneveld, A. (2005b). Can jumping capacity of adult show jumping horses be predicted on the basis of submaximal free jumps at foal age? A longitudinal study. *The veterinary journal*, *170*(2), 212-221.

Bonelli, F., Sgorbini, M., Meucci, V., Sighieri, C., & Baragli, P. (2017). How swimming affects plasma insulin and glucose concentration in Thoroughbreds: a pilot study. *The Veterinary Journal*, *226*, 1-3.

Bos, H., Van Der Meij, G. J. W., & Dik, K. J. (1986). Heredity of navicular disease. *Veterinary Quarterly*, *8*(1), 68-72.

Boulenc, X., Marti, E., Joyeux, H., Roques, C., Berger, Y., & Fabre, G. (1993). Importance of the paracellular pathway for the transport of a new bisphosphonate using the human CACO-2 monolayers model. *Biochemical pharmacology*, *46*(9), 1591-1600.

Bowker, R. M., Linder, K., Sonea, I. M., & Guida, L. A. (1995a). Sensory nerve fibres and receptors in equine distal forelimbs and their potential roles in locomotion. *Equine Veterinary Journal*, *27*(S18), 141-146.

Bowker, R. M., Linder, K., Van Wulfen, K. K., & Sonea, I. M. (1997). Anatomy of the distal interphalangeal joint of the mature horse: relationships with navicular suspensory ligaments, sensory nerves and neurovascular bundle. *Equine veterinary journal*, *29*(2), 126-135.

Bowker, R. M., Rockershouser, S. J., Linder, K., Vex, K. B., Sonea, I. M., & Caron, J. P. (1994). A silver-impregnation and immunocytochemical study of innervation of the distal sesamoid bone and its suspensory ligaments in the horse. *Equine veterinary journal*, *26*(3), 212-219.

Bowker, R. M., Sonea, I. M., Vex, K. B., & Caron, J. P. (1993). Substance P innervation of equine synovial membranes: joint differences and neural and nonneural receptor localizations. *Neuroscience letters*, *164*(1-2), 76-80.

Bowker, R. M., Under, K., Sonea, I. M., & Holland, R. E. (1995b). Sensory innervation of the navicular bone and bursa in the foal. *Equine Veterinary Journal*, *27*(1), 60-65.

Bowker, R. M., Van Wulfen, K. K., & van Wulfen, K. K. (1996). Microanatomy of the intersection of the distal sesamoidean impar ligament and the deep digital flexor tendon: A preliminary report. *Pferdeheilkunde*, *12*, 623-627.

Bromiley, M. (2013). *Equine injury, therapy and rehabilitation*. John Wiley & Sons.

Bruns, E. (1981). Estimation of the breeding value of stallions from the tournament performance of their offspring. *Livestock Production Science*, *8*(5), 465-473.

Buchner, H. F. (2001). Gait adaptation in lameness. *Equine locomotion*, 251-279.

Buchner, H. H. F., Kübber, P., Zohmann, E., & Peham, C. H. (1999). Sedation and antisedation as tools in equine lameness examination. *Equine Veterinary Journal*, *31*(S30), 227-230.

Buchner, H. H. F., Savelberg, H. H. C. M., Schamhardt, H. C., & Barneveld, A. (1996). Head and trunk movement adaptations in horses with experimentally induced fore-or hindlimb lameness. *Equine veterinary journal*, *28*(1), 71-76.

Buchner, H. H. F., Savelberg, H. H. C. M., Schamhardt, H. C., & Barneveld, A. (1995). Temporal stride patterns in horses with experimentally induced fore-or hindlimb lameness. *Equine veterinary journal*, *27*(S18), 161-165.

Buchner, H. H. F., Savelberg, H. H. C. M., Schamhardt, H. C., Merkens, H. W., & Barneveld, A. (1994). Kinematics of treadmill versus overground locomotion in horses. *Veterinary Quarterly*, *16*(sup2), 87-90.

Buchner, H. H. F., Savelberg, H. H. C. M., Schamhardt, H. C., Merkens, H. W., & Barneveld, A. (1994). Habituation of horses to treadmill locomotion. *Equine veterinary journal*, *26*(S17), 13-15.

Busoni, V., & Denoix, J. M. (2001). Ultrasonography of the podotrochlear apparatus in the horse using a transcuneal approach: technique and reference images. *Veterinary radiology & ultrasound*, *42*(6), 534-540.

Byström, A., Egenvall, A., Roepstorff, L., Rhodin, M., Bragança, F. S., Hernlund, E., Van Weeren, P. R., & Clayton, H. M. (2018). Biomechanical findings in horses showing asymmetrical vertical excursions of the withers at walk. *PLoS One*, *13*(9), e0204548.

Calvo-Santesmases, A., Manso-Díaz, G., Fores, P., & López-Sanromán, J. (2021). Kinematic Patterns in Horses Sedated With Low Doses of Detomidine: An Accelerometric Evaluation. *Journal of Equine Veterinary Science*, *107*, 103777.

Cano, M. R., Miró, F., Vivo, J., & Galisteo, A. M. (1999). Comparative biokinematic study of young and adult Andalusian horses at the trot. *Journal of Veterinary Medicine Series A*, *46*(2), 91-102.

Castejon-Riber, C., Riber, C., Rubio, M. D., Agüera, E., & Muñoz, A. (2017). Objectives, principles, and methods of strength training for horses. *Journal of Equine Veterinary Science*, *56*, 93-103.

Castejón, F., Rubio, D., Tovar, P., Vinuesa, M., & Riber, C. (1994). A comparative study of aerobic capacity and fitness in three different horse breeds (Andalusian, Arabian and Anglo-Arabian). *Journal of Veterinary Medicine Series A*, *41*(1-10), 645-652.

Castejon, F., Trigo, P., Muñoz, A., & Riber, C. (2006). Uric acid responses to endurance racing and relationships with performance, plasma biochemistry and metabolic alterations. *Equine Veterinary Journal*, *38*(S36), 70-73.

Chateau, H., Degueurce, C., & Denoix, J. M. (2004). Effects of 6 elevation of the heels on 3D kinematics of the distal portion of the forelimb in the walking horse. *Equine veterinary journal*, *36*(8), 649-654.

Chateau, H., Holden, L., Robin, D., Falala, S., Pourcelot, P., Estoup, P., Denoix, J. M., & Crevier-Denoix, N. (2010). Biomechanical analysis of hoof landing and stride parameters in harness trotter horses running on different tracks of a sand beach (from wet to dry) and on an asphalt road. *Equine Veterinary Journal*, *42*, 488-495.

Cheetham, J., Riordan, A. S., Mohammed, H. O., McIlwraith, C. W., & Fortier, L. A. (2010). Relationships between race earnings and horse age, sex, gait, track surface and number of race starts for Thoroughbred and Standardbred racehorses in North America. *Equine veterinary journal*, *42*(4), 346-350.

Church, E. E., Walker, A. M., Wilson, A. M., & Pfau, T. (2009). Evaluation of discriminant analysis based on dorsoventral symmetry indices to quantify hindlimb lameness during over ground locomotion in the horse. *Equine veterinary journal*, *41*(3), 304-308.

Clayton, H. M. (1993). The extended canter: a comparison of some kinematic variables in horses trained for dressage and for racing. *Cells Tissues Organs*, *146*(2-3), 183-187.

Clayton, H. M. (1994). Comparison of the stride kinematics of the collected, working, medium and extended trot in horses. *Equine Veterinary Journal*, *26*(3), 230-234.

Clayton, H. M. (1997). Classification of collected trot, passage and piaffe based on temporal variables. *Equine Veterinary Journal*, *29*(S23), 54-57.

Clayton, H. M. (2004). *The dynamic horse: A biomechanical guide to equine movement and performance*. Sport Horse Pub.

Clayton, H. M., & Hobbs, S. J. (2019). A review of biomechanical gait classification with reference to collected trot, passage and piaffe in dressage horses. *Animals*, *9*(10), 763.

Clayton, H. M., & Hobbs, S. J. (2021). Ground reaction forces of dressage horses performing the piaffe. *Animals*, *11*(2), 436.

Clayton, H. M., & Nauwelaerts, S. (2014). Effect of blindfolding on centre of pressure variables in healthy horses during quiet standing. *The Veterinary Journal*, *199*(3), 365-369.

Clayton, H. M., & Schamhardt, H. C. (2001). Measurement techniques for gait analysis. *Equine locomotion*, *55*, 76.

Clayton, H. M., Buchholz, R., & Nauwelaerts, S. (2013). Relationship between morphological and stabilographic variables in standing horses. *The Veterinary Journal*, *198*, e65-e69.

Clayton, H. M., George, L. S., Sinclair, J., & Hobbs, S. J. (2021). Characteristics of the Flight Arc in Horses Jumping Three Different Types of Fences in Olympic Competition. *Journal of Equine Veterinary Science*, *104*, 103698.

Clayton, H. M., Schamhardt, H. C., & Hobbs, S. J. (2017). Ground reaction forces of elite dressage horses in collected trot and passage. *The Veterinary Journal*, *221*, 30-33.

Clayton, H. M., Sha, D. H., Stick, J. A., & Robinson, P. (2007). 3D kinematics of the interphalangeal joints in the forelimb of walking and trotting horses. *Veterinary and Comparative Orthopaedics and Traumatology*, *20*(01), 01-07.

Colborne, G. R., Lanovaz, J. L., Sprigings, E. J., Schamhardt, H. C., & Clayton, H. M. (1998). Forelimb joint moments and power during the walking stance phase of horses. *American journal of veterinary research*, *59*(5), 609-614.

Coleridge, M., Schumacher, J., & DeGraves, F. (2020). Comparison of lameness scores after a low 4-point nerve block to lameness scores after additional desensitisation of the dorsal metatarsal nerves in horses with experimentally induced pain in the metatarsophalangeal joint. *Equine Veterinary Education*, *32*(4), 199-203.

Colles, C. (1982). Navicular disease and its treatment. *In Practice*, *4*(2), 29-36.

Colles, C. M. (1979). Ischaemic necrosis of the navicular bone and its treatment. *The Veterinary Record*, *104*(7), 133-137.

Colles, C. M., & Hickman, J. (1977). The arterial supply of the navicular bone and its variations in navicular disease. *Equine veterinary journal*, *9*(3), 150-154.

Contreras-Aguilar, M. D., Cerón, J. J., Muñoz, A., & Ayala, I. (2021). Changes in saliva biomarkers during a standardized increasing intensity field exercise test in endurance horses. *Animal*, *15*(6), 100236.

Coruzzi, P., Ravanetti, C., Musiari, L., Biggi, A., Vescovi, P. P., & Novarini, A. (1988). Circulating opioid peptides during water immersion in normal man. *Clinical science*, *74*(2), 133-136.

Cotrel, C., Leleu, C., & Courouce-Malblanc, A. (2006). Factors influencing variation in locomotor-respiratory coupling in Standardbred Trotters in the field. *Equine Veterinary Journal*, *38*(S36), 562-566.

Cottin, F., Metayer, N., Goachet, A. G., Julliand, V., Slawinski, J., Billat, V., & Barrey, E. (2010). Oxygen consumption and gait variables of Arabian endurance horses measured during a field exercise test. *Equine Veterinary Journal*, *42*, 1-5.

Coudry, V., Thibaud, D., Riccio, B., Audigié, F., Didierlaurent, D., & Denoix, J. M. (2007). Efficacy of tiludronate in the treatment of horses with signs of pain associated with osteoarthritic lesions of the thoracolumbar vertebral column. *American journal of veterinary research*, *68*(3), 329-337.

Coxon, F. P., & Rogers, M. J. (2003). The role of prenylated small GTP-binding proteins in the regulation of osteoclast function. *Calcified tissue international*, *72*(1), 80.

Coxon, F. P., Thompson, K., Roelofs, A. J., Ebetino, F. H., & Rogers, M. J. (2008). Visualizing mineral binding and uptake of bisphosphonate by osteoclasts and non-resorbing cells. *Bone*, *42*(5), 848-860.

Cremers, S. C., Pillai, G. C., & Papapoulos, S. E. (2005). Pharmacokinetics/pharmacodynamics of bisphosphonates. *Clinical pharmacokinetics*, *44*(6), 551-570.

Cremers, S., & Papapoulos, S. (2011). Pharmacology of bisphosphonates. *Bone*, *49*(1), 42-49.

Crevier-Denoix, N., Robin, D., Pourcelot, P., Falala, S., Holden, L., Estoup, P., Desquilbet, L., Denoix, J. M., & Chateau, H. (2010). Ground reaction force and kinematic analysis of limb loading on two different beach sand tracks in harness trotters. *Equine veterinary journal*, *42*, 544-551.

Crisman, M. V., Wilcke, J. R., Correll, L. S., & Irby, M. H. (1993).

Pharmacokinetic disposition of intravenous and oral pentoxifylline in horses. *Journal of veterinary pharmacology and therapeutics*, *16*(1), 23-31.

Dabareiner, R. M., & Carter, G. K. (2003). Diagnosis, treatment, and farriery for horses with chronic heel pain. *Veterinary Clinics: Equine Practice*, *19*(2), 417-441.

Dabareiner, R. M., Carter, G. K., & Honnas, C. M. (2003). Injection of corticosteroids, hyaluronate, and amikacin into the navicular bursa in horses with signs of navicular area pain unresponsive to other treatments: 25 cases (1999–2002). *Journal of the American Veterinary Medical Association*, *223*(10), 1469-1474.

Dalin, G., & Jeffcott, L. B. (1985). Locomotion and gait analysis. *Veterinary Clinics of North America: Equine Practice*, *1*(3), 549-572.

Degueurce, C., Dietrich, G., Pourcelot, P., Denoix, J. M., & Geiger, D. (1996). Three-dimensional kinematic technique for evaluation of horse locomotion in outdoor conditions. *Medical and Biological Engineering and Computing*, *34*(3), 249-252.

Dejardin, L. M., Arnoczky, S. P., Cloud, G. L., & Stick, J. A. (2001). Photoelastic stress analysis of strain patterns in equine hooves after four-point trimming. *American journal of veterinary research*, *62*(4), 467-473.

Delguste, C., Amory, H., Doucet, M., Piccot-Crezollet, C., Thibaud, D., Garnero, P., Detilleux, J., & Lepage, O. M. (2007). Pharmacological effects of tiludronate in horses after long-term immobilization. *Bone*, *41*(3), 414-421.

Denoix, J. M., Thibaud, D., & Riccio, B. (2003). Tiludronate as a new therapeutic agent in the treatment of navicular disease: a double-blind placebo-controlled clinical trial. *Equine Veterinary Journal*, *35*(4), 407-413.

Deuel, N. R., & Park, J. (1990). Canter lead change kinematics of superior Olympic dressage horses. *Journal of Equine Veterinary Science*, *10*(4), 287-298.

Deuel, N. R., & Park, J. (1991). Kinematic analysis of jumping sequences of Olympic show jumping horses. *Equine exercise physiology*, *3*, 158-166.

Deuel, N. R., & Park, J. J. (1990). The gait patterns of Olympic dressage horses. *Journal of Applied Biomechanics*, *6*(2), 198-226.

Diehl, M., Schmitt, K., Huerlimann, J., Girard, P., & Vingerhoets, M. (1986). Description and preliminary results on desmotomy of the medial and lateral collateral ligaments as a means of therapy of navicular disease in the horse. *Pferdeheilkunde*, *2*, 123-125.

Dik, K. J., & Van den Broek, J. (1995). Role of navicular bone shape in the pathogenesis of navicular disease: a radiological study. *Equine veterinary journal*, *27*(5), 390-393.

Dik, K. J., Belt, A. V. D., & Broek, J. V. D. (2001a). Relationships of age and shape of the navicular bone to the development of navicular disease: a radiological study. *Equine Veterinary Journal*, *33*(2), 172-175.

Dik, K. J., van den Belt, A. J. M., Enzerink, E., & van Weeren, P. R. (2001b). The radiographic development of the distal and proximal double contours of the equine navicular bone on dorsoproximal-palmarodistal oblique (upright pedal) radiographs, from age 1 to 11 months. *Equine veterinary journal*, *33*(1), 70-74.

Dixon, J., & Howe, T. E. (2005). Quadriceps force generation in patients with osteoarthritis of the knee and asymptomatic participants during patellar tendon reflex reactions: an exploratory cross-sectional study. *BMC Musculoskeletal Disorders*, *6*(1), 1-6.

Donnell, J. R., Frisbie, D. D., King, M. R., Goodrich, L. R., & Haussler, K. K. (2015). Comparison of subjective lameness evaluation, force platforms and an inertial-sensor system to identify mild lameness in an equine osteoarthritis model. *The Veterinary Journal*, *206*(2), 136-142.

Drevemo, S., & Johnston, C. (1994). The use of a panning camera technique in equine kinematic analysis. *Equine Veterinary Journal*, *26*(S17), 39-43.

Drevemo, S., Dalin, G., Fredricson, I., & Hjerten, G. (1980). Equine locomotion: 1. The analysis of linear and temporal stride characteristics of trotting Standardbreds. *Equine Veterinary Journal*, *12*(2), 60-65.

Drevemo, S., Fredricson, I., Hjerten, G., & McMiken, D. (1987). Early development of gait asymmetries in trotting Standardbred colts. *Equine Veterinary Journal*, *19*(3), 189-191.

Duesterdieck-Zellmer, K. F. (2016). Review of bisphosphonate use in horses. In *Proceedings of the 62nd Annual Convention of the American Association of Equine Practitioners, Orlando, Florida, USA, 3-7 December 2016* (pp. 371-378). American Association of Equine Practitioners (AAEP).

Dugué, M., Dumont Saint Priest, B., Crichan, H., Danvy, S., & Ricard, A. (2021). Genomic correlations between the gaits of young horses measured by accelerometry and functional longevity in jumping competition. *Frontiers in genetics*, *12*, 619947.

Dunn, C. J., & Goa, K. L. (2001). Risedronate. *Drugs*, *61*(5), 685-712.

Dyson, S. (2000). Lameness and poor performance in the sports horse: dressage, show jumping and horse trials (eventing). In *Proceedings of the Annual Convention of the AAEP* (Vol. 46, pp. 308-315).

Dyson, S. (2008). Radiological interpretation of the navicular bone. *Equine Veterinary Education*, *20*(5), 268-280.

Dyson, S. (2011). Radiological interpretation of the navicular bone. *Equine Veterinary Education*, *23*(2), 73-87.

Dyson, S. J. (2003). Navicular disease and other soft tissue causes of palmar foot pain. In *Diagnosis and Management of Lameness in the Horse* (pp. 286-299). WB Saunders.

Dyson, S. J. (2011). Lesions of the equine neck resulting in lameness or poor performance. *Veterinary Clinics: Equine Practice*, *27*(3), 417-437.

Dyson, S. J., & Murray, R. (2007c). Lameness and diagnostic imaging in the sports horse: recent advances related to the digit. In *Proc. Am. Ass. equine Practnrs* (53), 262-275.

Dyson, S. J., Arthur, R. M., Palmer, S. E., & Richardson, D. (1995). Suspensory ligament desmitis. *Veterinary Clinics of North América: Equine Practice*, *11*(2), 177-215.

Dyson, S., & Murray, R. (2007a). Use of concurrent scintigraphic and magnetic resonance imaging evaluation to improve understanding of the pathogenesis of injury of the podotrochlear apparatus. *Equine veterinary journal*, *39*(4), 365-369.

Dyson, S., & Murray, R. (2007b). Magnetic resonance imaging evaluation of 264 horses with foot pain: the podotrochlear apparatus, deep digital flexor tendon and collateral ligaments of the distal interphalangeal joint. *Equine veterinary journal*, *39*(4), 340-343.

Dyson, S., & Murray, R. (2007d). Verification of scintigraphic imaging for injury diagnosis in 264 horses with foot pain. *Equine veterinary journal*, *39*(4), 350-355.

Dyson, S., Murray, R., Schramme, M., & Branch, M. (2003). Lameness in 46 horses associated with deep digital flexor tendonitis in the digit: diagnosis confirmed with magnetic resonance imaging. *Equine veterinary journal*, *35*(7), 681-690.

Ebetino, F. H., & Dansereau, S. M. (1995). Bisphosphonate antiresorptive structure–activity relationships. *Bisphosphonate on bones*, 139-153.

Ebetino, F. H., Francis, M. D., Rogers, M. J., & Russell, R. G. G. (1998). Mechanisms of action of etidronate and other bisphosphonates. *Reviews in Contemporary Pharmacotherapy*, *9*(4), 233-243.

Ebetino, F. H., Hogan, A. M. L., Sun, S., Tsoumpra, M. K., Duan, X., Triffitt, J. T., ... & Russell, R. G. G. (2011). The relationship between the chemistry and biological activity of the bisphosphonates. *Bone*, *49*(1), 20-33.

Egenvall, A., Tranquille, C. A., Lönnell, A. C., Bitschnau, C., Oomen, A., Hernlund, E., Montavon, S., Franko, M.A., Murray, R. C., Weishaupt, M. A., Weeren, v.R., & Roepstorff, L. (2013). Days-lost to training and competition in relation to workload in 263 elite show-jumping horses in four European countries. *Preventive veterinary medicine*, *112*(3-4), 387-400.

Eliashar, E., McGuigan, M. P., & Wilson, A. M. (2004). Relationship of foot conformation and force applied to the navicular bone of sound horses at the trot. *Equine Veterinary Journal*, *36*(5), 431-435.

Ellman, R., Spatz, J., Cloutier, A., Palme, R., Christiansen, B. A., & Bouxsein, M. L. (2013). Partial reductions in mechanical loading yield proportional changes in bone density, bone architecture, and muscle mass. *Journal of Bone and Mineral Research*, *28*(4), 875-885.

Erkert, R. S., MacAllister, C. G., Payton, M. E., & Clarke, C. R. (2005). Use of force plate analysis to compare the analgesic effects of intravenous administration of phenylbutazone and flunixin meglumine in horses with navicular syndrome. *American journal of veterinary research*, *66*(2), 284-288.

Ezra, A., & Golomb, G. (2000). Administration routes and delivery systems of bisphosphonates for the treatment of bone resorption. *Advanced drug delivery reviews*, *42*(3), 175-195.

Farley, C. T., & Taylor, C. R. (1991). A mechanical trigger for the trot-gallop transition in horses. *Science*, *253*(5017), 306-308.

FDA Freedom of Information Summary Osphos. (2014) NADA 141– 427. Accessed from: http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM406997.pdf.

FDA Freedom of Information Summary Tildren. (2014). NADA 141– 420. Accessed from: http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM408781.pdf.

Fleisch, H. (1989). Bisphosphonates: a new class of drugs in diseases of bone and calcium metabolism. *Bisphosphonates and tumor osteolysis*, 1-28.

Fleisch, H., Graham, R., Russell, G., & Francis, M. D. (1969). Diphosphonates inhibit hydroxyapatite dissolution in vitro and bone resorption in tissue culture and in vivo. *Science*, *165*(3899), 1262-1264.

Fleisch, H., Russell, R. G. G., Bisaz, S., Casey, P. A., & Mühlbauer, R. C. (1968). The influence of pyrophosphate analogues (diphosphonates) on the precipitation and dissolution of calcium phosphate in vitro and in vivo. *Calcified Tissue Research*, *2*(1), 10-10.

Francis, M. D., Graham, R., Russell, G., & Fleisch, H. (1969). Diphosphonates inhibit formation of calcium phosphate crystals in vitro and pathological calcification in vivo. *Science*, *165*(3899), 1264-1266.

Frazier, D. L. (2000). Who speaks for the horse-the sport of endurance riding and equine welfare. *Journal-American Veterinary Medical Association*, *216*(8), 1258-1260.

Fredricson, I., Drevemo, S., Dalin, G., Hjerten, G., & Björne, K. (1980). The application of high-speed cinematography for the quantitative analysis of equine locomotion. *Equine veterinary journal*, *12*(2), 54-59.

Frevel, M., King, B. L., Kolb, D. S., Boswell, R. P., Shoemaker, R. S., Janicek, J. C., Cole, R. C., Poole, H. M., & Longhofer, S. L. (2017). Clodronate disodium for treatment of clinical signs of navicular disease–a double-blinded placebo-controlled clinical trial. *Pferdeheilkunde*, *33*(3), 271-279.

Frigerio, M. A., Gómez Cisneros, D., Santiago Llorente, I., Manso-Díaz, G., & López-Sanromán, J. (2019). A kinematic comparison of the locomotor pattern of horses sedated with detomidine alone and in combination with low doses of butorphanol. *Equine veterinary journal*, *51*(6), 825-830.

Frith, J. C., Mönkkönen, J., Blackburn, G. M., Russell, R. G. G., & Rogers, M. J. (1997). Clodronate and liposome-encapsulated clodronate are metabolized to a toxic ATP analog, adenosine 5′-(β, γ-dichloromethylene) triphosphate, by mammalian cells in vitro. *Journal of Bone and Mineral Research*, *12*(9), 1358-1367.

Galisteo, A. M., Cano, M. R., Morales, J. L., Miro, F., Vivo, J., & Agüera, E. (1997). Kinematics in horses at the trot before and after an induced forelimb supporting lameness. *Equine Veterinary Journal*, *29*(S23), 97-101.

Galloux, P., & Barrey, E. (1997). Components of the total kinetic moment in jumping horses. *Equine Veterinary Journal*, *29*(S23), 41-44.

Galloux, P., Goupil, X., Vial, C., Auvinet, B., & Lepage, O. (1994). Heart rate and blood lactic acid concentration of the horse during swimming training. *The Equine athlete (USA)*.

Gertz, B. J., Holland, S. D., Kline, W. F., Matuszewski, B. K., & Porras, A. G. (1993). Clinical pharmacology of alendronate sodium. *Osteoporosis International*, *3*(3), 13-16.

Gertz, B. J., Holland, S. D., Kline, W. F., Matuszewski, B. K., Freeman, A., Quan, H., Kenneth, C., Lasseter, M. D., Muclow, M., D., & Porras, A. G. (1995). Studies of the oral bioavailability of alendronate. *Clinical Pharmacology & Therapeutics*, *58*(3), 288-298.

Getty, R., & Sisson, S. (1975). Equine osteology. *Sisson and Grossman's the Anatomy of the Domestic Animals*.

Giger, E. V., Castagner, B., & Leroux, J. C. (2013). Biomedical applications of bisphosphonates. *Journal of Controlled Release*, *167*(2), 175-188.

Gómez Álvarez, C. B., & van Weeren, P. R. (2019). Practical uses of quantitative gait analysis in horses. *Equine veterinary journal*, *51*(6), 811-812.

Gough, M. R., Thibaud, D., & Smith, R. K. W. (2010). Tiludronate infusion in the treatment of bone spavin: a double blind placebo-controlled trial. *Equine veterinary journal*, *42*(5), 381-387.

Granatosky, M. C., Bryce, C. M., Hanna, J., Fitzsimons, A., Laird, M. F., Stilson, K., Wall, C.E., & Ross, C. F. (2018). Inter-stride variability triggers gait transitions in mammals and birds. *Proceedings of the Royal Society B*, *285*(1893), 20181766.

Greco-Otto, P., Baggaley, M., Edwards, W. B., & Léguillette, R. (2019). Water treadmill exercise reduces equine limb segmental accelerations and increases shock attenuation. *BMC veterinary research*, *15*(1), 1-10.

Grewal, J. S., McClure, S. R., Booth, L. C., Evans, R. B., & Caston, S. S. (2004). Assessment of the ultrasonographic characteristics of the podotrochlear apparatus in clinically normal horses and horses with navicular syndrome. *Journal of the American veterinary medical association*, *225*(12), 1881-1888.

Gunnarsson, V., Stefánsdóttir, G. J., Jansson, A., & Roepstorff, L. (2017). The effect of rider weight and additional weight in Icelandic horses in tölt: part II. Stride parameters responses. *animal*, *11*(9), 1567-1572.

Henneman, Z. J., Nancollas, G. H., Ebetino, F. H., Russell, R. G. G., & Phipps, R. J. (2008). Bisphosphonate binding affinity as assessed by inhibition of carbonated apatite dissolution in vitro. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*, *85*(4), 993-1000.

Hernlund, E., Egenvall, A., & Roepstorff, L. (2010). Kinematic characteristics of hoof landing in jumping horses at elite level. *Equine Veterinary Journal*, *42*, 462-467.

Hiraga, A., Yamanobe, A., & Kubo, K. (1995). Relationships between stride length, stride frequency, step length and velocity at the start dash in a racehorse. *Journal of Equine Science*, *5*(4), 127-130.

Hobbs, S. J., Bertram, J. E., & Clayton, H. M. (2016). An exploration of the influence of diagonal dissociation and moderate changes in speed on locomotor parameters in trotting horses. *PeerJ*, *4*, e2190.

Hobbs, S. J., Licka, T., & Polman, R. (2011). The difference in kinematics of horses walking, trotting and cantering on a flat and banked 10 m circle. *Equine Veterinary Journal*, *43*(6), 686-694.

Hobbs, S. J., St George, L., Reed, J., Stockley, R., Thetford, C., Sinclair, J., Williams, J., Nankervis, K., & Clayton, H. M. (2020). A scoping review of determinants of performance in dressage. *PeerJ*, *8*, e9022.

Hobo, S., Yoshida, K., & Yoshihara, T. (1998). Characteristics of respiratory function during swimming exercise in thoroughbreds. *Journal of veterinary medical science*, *60*(6), 687-689.

Holmström, M., Fredricson, I., & Drevemo, S. (1994). Biokinematic analysis of the Swedish Warmblood riding horse at trot. *Equine Veterinary Journal*, *26*(3), 235-240.

Holmström, M., Fredricson, I., & Drevemo, S. (1995). Biokinematic effects of collection on the trotting gaits in the elite dressage horse. *Equine Veterinary Journal*, *27*(4), 281-287.

Hoyt, D. F., & Taylor, C. R. (1981). Gait and the energetics of locomotion in horses. *Nature*, *292*(5820), 239-240.

Huizinga, H. A., & Van der Meij, G. J. W. (1989). Estimated parameters of performance in jumping and dressage competition of the Dutch Warmblood horse. *Livestock Production Science*, *21*(4), 333-345.

Hunt E. (2001). Response of twenty-seven horses with lower leg injuries to cold spa bath hydrotherapy. *Journal of equine veterinary science*, 21, 188–93.

Hussain, M. A., Joseph, A., Cherian, V. M., Srivastava, A., Cherian, K. E., Kapoor, N., & Paul, T. V. (2022). Bisphosphonate-induced atypical femoral fracture in tandem: long-term follow-up is warranted. *Endocrinology, Diabetes & Metabolism Case Reports*, *2022*(1).

Ingle-Fehr, J. E., & Baxter, G. M. (1999). The effect of oral isoxsuprine and pentoxifylline on digital and laminar blood flow in healthy horses. *Veterinary Surgery*, *28*(3), 154-160.

Ishihara, A., Bertone, A. L., & Rajala-Schultz, P. J. (2005). Association between subjective lameness grade and kinetic gait parameters in horses with experimentally induced forelimb lameness. *American Journal of Veterinary Research*, *66*(10), 1805-1815.

Ishihara, A., Reed, S. M., Rajala-Schultz, P. J., Robertson, J. T., & Bertone, A. L. (2009). Use of kinetic gait analysis for detection, quantification, and differentiation of hind limb lameness and spinal ataxia in horses. *Journal of the American Veterinary Medical Association*, *234*(5), 644-651.

Jackman, B. R., Baxter, G. M., Doran, R. E., Allen, D., & Parks, A. H. (1993). Palmar digital neurectomy in horses 57 cases (1984–1990). *Veterinary surgery*, *22*(4), 285-288.

Jeffcott, L. B., Rossdale, P. D., Freestone, J., Frank, C. J., & Towers-Clark, P. F. (1982). An assessment of wastage in Thoroughbred racing from conception to 4 years of age. *Equine veterinary journal*, *14*(3), 185-198.

Jones, J. H., Cox, K. S., Takahashi, T., Hiraga, A., Yarbrough, T. B., & Pascoe, J. R. (2002). Heterogeneity of intrapleural pressures during exercise. *Equine Veterinary Journal*, *34*(S34), 391-396.

Jones, S., Franklin, S., Martin, C., & Steel, C. (2020). Complete upper airway collapse and apnoea during tethered swimming in horses. *Equine veterinary journal*, *52*(3), 352-358.

Kainer, R. A. (1989). Clinical anatomy of the equine foot. *Veterinary Clinics of North America: Equine Practice*, *5*(1), 1-27.

Kallings, P., Johnston, C., & Drevemo, S. (1999). Effects of flunixin on movement and performance of standardbred trotters on the track. *Equine Veterinary Journal*, *31*(S30), 270-273.

Kathmann, I., Cizinauskas, S., Doherr, M. G., Steffen, F., & Jaggy, A. (2006). Daily controlled physiotherapy increases survival time in dogs with suspected degenerative myelopathy. *Journal of veterinary internal medicine*, *20*(4), 927-932.

Katsumi, H., Nakatani, M., Sano, J. I., Abe, M., Kusamori, K., Kurihara, M., Shiota, R., Takashima, M., Fujita, T., Sakane, T., Hibi, T., & Yamamoto, A. (2010). Absorption asafety of alendronate, a nitrogen-containing bisphosphonate, after intrapulmonary administration in rats. *International journal of pharmaceutics*, *400*(1-2), 124-130.

Katsumi, H., Takashima, M., Sano, J. I., Nishiyama, K., Kitamura, N., Sakane, T., Hibi, T., & Yamamoto, A. (2011). Development of polyethylene glycol-conjugated alendronate, a novel nitrogen-containing bisphosphonate derivative: evaluation of absorption, safety, and effects after intrapulmonary administration in rats. *Journal of pharmaceutical sciences*, *100*(9), 3783-3792.

Katzman, S. A., Nieto, J. E., Arens, A. M., MacDonald, M. H., Puchalski, S. M., Galuppo, L. D., Snyder, J. R., Maher, O., & Bell, R. J. (2012). Use of zoledronate for treatment of a bone fragility disorder in horses. *Journal of the American Veterinary Medical Association*, *240*(11), 1323-1328.

Keegan, K. G. (2007). Evidence-based lameness detection and quantification. *Veterinary Clinics of North America: Equine Practice*, *23*(2), 403-423.

Keegan, K. G., Dent, E. V., Wilson, D. A., Janicek, J., Kramer, J., Lacarrubba, A., ... & Norris, T. (2010). Repeatability of subjective evaluation of lameness in horses. *Equine Veterinary Journal*, *42*(2), 92-97.

Keegan, K. G., Kramer, J., Yonezawa, Y., Maki, H., Pai, P. F., Dent, E. V., Kellerman, T.E., Wilson, R.A., & Reed, S. K. (2011). Assessment of repeatability of a wireless, inertial sensor–based lameness evaluation system for horses. *American journal of veterinary research*, *72*(9), 1156-1163.

Keegan, K. G., Pai, P. F., Wilson, D. A., & Smith, B. K. (2001). Signal decomposition method of evaluating head movement to measure induced forelimb lameness in horses trotting on a treadmill. *Equine Veterinary Journal*, *33*(5), 446-451.

Keegan, K. G., Wilson, D. A., Smith, B. K., & Wilson, D. J. (2000). Changes in kinematic variables seen with lameness induced by applying pressure to the frog and to the toe in adult horses trotting on a treadmill. *Am J Vet Res*, *61*(6), 612-9.

Keegan, K. G., Wilson, D. A., Wilson, D. J., Smith, B., Gaughan, E. M., Pleasant, R. S., Lillich, J. D., Kramer, J., Howard, R. D., Bacon-Miller, C., Davis, E. G., May, K. A., Cheramie, H. S., Valentino, W. L., & Van Harreveld, P. D. (1998). Evaluation of mild lameness in horses trotting on a treadmill by clinicians and interns or residents and correlation of their assessments with kinematic gait analysis. *American journal of veterinary research*, *59*(11), 1370-1377.

Keegan, K. G., Wilson, D. J., Wilson, D. A., Frankeny, R. L., Loch, W. E., & Smith, B. (1997). Effects of anesthesia of the palmar digital nerves on kinematic gait analysis in horses with and without navicular disease. *American journal of veterinary research*, *58*(3), 218-223.

Keegan, K. G., Yonezawa, Y., Pai, P. F., Wilson, D. A., & Kramer, J. (2004). Evaluation of a sensor-based system of motion analysis for detection and quantification of forelimb and hind limb lameness in horses. *American journal of veterinary research*, *65*(5), 665-670.

Khosla, S., Bilezikian, J. P., Dempster, D. W., Lewiecki, E. M., Miller, P. D., Neer, R. M., Recker, R. R., Shane, E., Shoback, D., & Potts, J. T. (2012). Benefits and risks of bisphosphonate therapy for osteoporosis. *The Journal of Clinical Endocrinology & Metabolism*, *97*(7), 2272-2282.

Khosla, S., Burr, D., Cauley, J., Dempster, D. W., Ebeling, P. R., Felsenberg, D., Gagel, R. F., Gilsanz, V., Guise, T., Koka, S., McGowan, J., Shum, L., Silverman, L. S., Van Poznak, C. H., Watts, N., Woo S., & Shane, E. (2007). Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. *Journal of bone and mineral research*, *22*(10), 1479-1491.

Khumsap, S., Clayton, H. M., & Lanovaz, J. L. (2001). Effect of walking velocity on hindlimb kinetics during stance in normal horses. *Equine Veterinary Journal*, *33*(S33), 21-26.

Khumsap, S., Clayton, H. M., Lanovaz, J. L., & Bouchey, M. (2002). Effect of walking velocity on forelimb kinematics and kinetics. *Equine Veterinary Journal*, *34*(S34), 325-329.

Khumsap, S., Lanovaz, J. L., & Clayton, H. M. (2004). Verification of skin-based markers for 3-dimensional kinematic analysis of the equine tarsal joint. *Equine veterinary journal*, *36*(8), 655-658.

Kienapfel, K., Preuschoft, H., Wulf, A., & Wagner, H. (2018). The biomechanical construction of the horse's body and activity patterns of three important muscles of the trunk in the walk, trot and canter. *Journal of animal physiology and animal nutrition*, *102*(2), e818-e827.

Kim, B. H., Lee, S. M., Bae, Y. H., Yu, J. H., & Kim, T. H. (2012). The effect of a task-oriented training on trunk control ability, balance and gait of stroke patients. *Journal of Physical Therapy Science*, *24*(6), 519-522.

Kim, I. S., Chung, S. H., Park, Y. J., & Kang, H. Y. (2012). The effectiveness of an aquarobic exercise program for patients with osteoarthritis. *Applied Nursing Research*, *25*(3), 181-189.

King, M. R. (2016). Principles and application of hydrotherapy for equine athletes. *Veterinary Clinics: Equine Practice*, *32*(1), 115-126.

King, M. R., Haussler, K. K., Kawcak, C. E., McIlwraith, C. W., & Reiser II, R. F. (2013). Effect of underwater treadmill exercise on postural sway in horses with experimentally induced carpal joint osteoarthritis. *American journal of veterinary research*, *74*(7), 971-982.

King, M. R., Haussler, K. K., Kawcak, C. E., McIlwraith, C. W., Reiser, R. F., Frisbie, D. D., & Werpy, N. M. (2017). Biomechanical and histologic evaluation of the effects of underwater treadmill exercise on horses with experimentally induced osteoarthritis of the middle carpal joint. *American Journal of Veterinary Research*, *78*(5), 558-569.

Knych, H. K., Finno, C. J., Katzman, S., Ryan, D., McKemie, D. S., Kass, P. H., & Arthur, R. M. (2022). Clodronate detection and effects on markers of bone resorption are prolonged following a single administration to horses. *Equine Veterinary Journal*.

Knych, H. K., Janes, J., Kennedy, L., McKemie, D. S., Arthur, R. M., Samol, M. A., Uzal, F. A., & Scollay, M. (2022). Detection and residence time of bisphosphonates in bone of horses. *Journal of Veterinary Diagnostic Investigation*, *34*(1), 23-27.

Kramer, J., Keegan, K. G., Kelmer, G., & Wilson, D. A. (2004). Objective determination of pelvic movement during hind limb lameness by use of a signal decomposition method and pelvic height differences. *American*

Journal of Veterinary Research, *65*(6), 741-747.

Kramer, J., Keegan, K. G., Wilson, D. A., Smith, B. K., & Wilson, D. J. (2000). Kinematics of the hind limb in trotting horses after induced lameness of the distal intertarsal and tarsometatarsal joints and intra-articular administration of anesthetic. *American journal of veterinary research*, *61*(9), 1031-1036.

Kunduracilar, Z., Sahin, H. G., Sonmezer, E., & Sozay, S. (2018). The effects of two different water exercise trainings on pain, functional status and balance in patients with knee osteoarthritis. *Complementary therapies in clinical practice*, *31*, 374-378.

Kusamori, K., Katsumi, H., Abe, M., Ueda, A., Sakai, R., Hayashi, R., Hirai,Y., Quan, Y. S., Kamiyama, F., Sakane, T., & Yamamoto, A. (2010). Development of a novel transdermal patch of alendronate, a nitrogen-containing bisphosphonate, for the treatment of osteoporosis. *Journal of Bone and Mineral Research*, *25*(12), 2582-2591.

Langlois, B., Poirel, D., & Bresch, J. L. (1980). Estimation de la valeur génétique des chevaux de sport d'après les sommes gagnées dans les compétitions équestres françaises. In *Annales de génétique et de sélection animale* (Vol. 12, No. 1, pp. 15-31). EDP Sciences.

Lebret, T., Casas, A., Cavo, M., Woll, P. J., Deleplace, C., Kennedy, C., Schoen, C., & Jackisch, C. (2017). The use of bisphosphonates in the management of bone involvement from solid tumours and haematological malignancies–a European survey. *European journal of cancer care*, *26*(4), e12490.

Lehenkari, P. P., Kellinsalmi, M., Näpänkangas, J. P., Ylitalo, K. V., Mönkkönen, J., Rogers, M. J., Azhayeb, A., Väänaänen, H. K., & Hassinen, I. E. (2002). Further insight into mechanism of action of clodronate: inhibition of mitochondrial ADP/ATP translocase by a nonhydrolyzable, adenine-containing metabolite. *Molecular pharmacology*, *61*(5), 1255-1262.

Leleu, C., Bariller, F., Cotrel, C., & Barrey, E. (2004). Reproducibility of a locomotor test for trotter horses. *The Veterinary Journal*, *168*(2), 160-166.

Leleu, C., Cotrel, C., & Barrey, E. (2005). Relationships between biomechanical variables and race performance in French Standardbred trotters. *Livestock Production Science*, *92*(1), 39-46.

Leleu, C., Gloria, E., Renault, G., & Barrey, E. (2002). Analysis of trotter gait on the track by accelerometry and image analysis. *Equine Veterinary Journal*, *34*(S34), 344-348.

Lindner, A., Wäschle, S., & Sasse, H. H. L. (2012). Physiological and blood biochemical variables in horses exercising on a treadmill submerged in water. *Journal of animal physiology and animal nutrition*, *96*(4), 563-569.

López-Rivero, J. L. (1995). Efecto del entrenamiento sobre el músculo esquelético del equino (revisión bibliográfica). *Agro-Ciencia*, *11*(1), 71-85.

López-Rivero, J. L., Agüera, E., Monterde, J. G., Rodríguez-Barbudo, M. V., & Miró, F. (1989). Comparative study of muscle fiber type composition in the middle gluteal muscle of Andalusian, Thoroughbred and Arabian horses. *Journal of Equine Veterinary Science*, *9*(6), 337-340.

López-Sanromán, F. J., Cisneros, G. D., del Arco, V. M., Llorente, S. I., & González, S. M. (2015). The use of low doses of acepromazine as an aid for lameness diagnosis in horses: An accelerometric evaluation. *Veterinary and Comparative Orthopaedics and Traumatology*, *28*(05), 312-317.

López-Sanromán, F. J., De La Riva Andrés, S., Holmbak-Petersen, R., Pérez-Nogués, M., Jackson, P. F., & González, M. S. (2014). An accelerometric measure of the gait pattern in horses after the administration of sublingual detomidine. *Research in veterinary science*, *97*(2), 391-396.

López-Sanromán, F. J., Freilich, G. M., Gomez-Cisneros, D., Varela, M., Santiago, I., & Manso-Díaz, G. (2021). Accelerometric Evaluation of the Locomotor Pattern After Administration of Morphine in Conscious Healthy Horses. *Journal of Equine Veterinary Science*, *104*, 103701.

López-Sanromán, F. J., Holmbak-Petersen, R., Santiago, I., de Segura, I. G., & Barrey, E. (2012). Gait analysis using 3D accelerometry in horses sedated with xylazine. *The Veterinary Journal*, *193*(1), 212-216.

López-Sanromán, F. J., Holmbak-Petersen, R., Varela, M., del Alamo, A. M., & Santiago, I. (2013). Accelerometric comparison of the locomotor pattern of horses sedated with xylazine hydrochloride, detomidine hydrochloride, or romifidine hydrochloride. *American journal of veterinary research*, *74*(6), 828-834.

Lorusso, L., Pieruzzi, L., Gabriele, M., Nisi, M., Viola, D., Molinaro, E., Bottici, V., Elisei, R., & Agate, L. (2021). Osteonecrosis of the jaw: a rare but possible side effect in thyroid cancer patients treated with tyrosine-kinase inhibitors and bisphosphonates. *Journal of Endocrinological Investigation*, *44*(12), 2557-2566.

Lowe, J. E. (1975). Sex, breed and age incidence of navicular disease. In *Proceedings of the American Association Equine Practitioners annual convention*.

Luckman, S. P., Coxon, F. P., Ebetino, F. H., Russell, R. G. G., & Rogers, M. J. (1998). Heterocycle-containing bisphosphonates cause apoptosis and inhibit bone resorption by preventing protein prenylation: Evidence from structure-activity relationships in J774 macrophages. *Journal of Bone and Mineral Research*, *13*(11), 1668-1678.

Luckman, S. P., Hughes, D. E., Coxon, F. P., Russell, R. G. G., & Rogers, M. J. (2005). Nitrogen-containing biphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *Journal of Bone and Mineral Research*, *20*(7), 1265-1274.

Maher, O., Davis, D. M., Drake, C., Myhre, G. D., Labbe, K. M., Han, J. H., & Lejeune, S. S. (2008). Pull-through technique for palmar digital neurectomy: forty-one horses (1998–2004). *Veterinary surgery*, *37*(1), 87-93.

Markell, R., Saviola, G., Barker, E. A., Conway, J. D., & Dujardin, C. (2020). What do we know about clodronate now? A medical and veterinary perspective. *Journal of equine veterinary science*, *88*, 102874.

Marunova, E., Dod, L., Witte, S., & Pfau, T. (2021). Smartphone-based pelvic movement asymmetry measures for clinical decision making in equine lameness assessment. *Animals*, *11*(6), 1665.

McClintock, S. A., Hutchins, D. R., Laing, E. A., & Brownlow, M. A. (1986). Pulmonary changes associated with flotation techniques in the treatment of skeletal injuries in the horse. *Equine veterinary journal*, *18*(6), 462-466.

McCracken, M. J., Kramer, J., Keegan, K. G., Lopes, M., Wilson, D. A., Reed, S. K., LaCarrubba, A., & Rasch, M. (2012). Comparison of an inertial sensor system of lameness quantification with subjective lameness evaluation. *Equine Veterinary Journal*, *44*(6), 652-656.

McCulloch, P. F., Ollenberger, G. P., Bekar, L. K., & West, N. H. (1997). Trigeminal and chemoreceptor contributions to bradycardia during voluntary dives in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *273*(2), R814-R822.

McGuigan, M. P., & Wilson, A. M. (2001). The effect of bilateral palmar digital nerve analgesia on the compressive force experienced by the navicular bone in horses with navicular disease. *Equine Veterinary Journal*, *33*(2), 166-171.

McVeigh, J. G., McGaughey, H., Hall, M., & Kane, P. (2008). The effectiveness of hydrotherapy in the management of fibromyalgia syndrome: a systematic review. *Rheumatology international*, *29*(2), 119-130.

Mendez-Angulo, J. L., Firshman, A. M., Groschen, D. M., Kieffer, P. J., & Trumble, T. N. (2013). Effect of water depth on amount of flexion and extension of joints of the distal aspects of the limbs in healthy horses walking on an underwater treadmill. *American journal of veterinary research*, *74*(4), 557-566.

Mendez-Angulo, J. L., Firshman, A. M., Groschen, D. M., Kieffer, P. J., & Trumble, T. N. (2014). Impact of walking surface on the range of motion of equine distal limb joints for rehabilitation purposes. *The Veterinary Journal*, *199*(3), 413-418.

Merkens, H. W., & Schamhardt, H. C. (1988). Distribution of ground reaction forces of the concurrently loaded limbs of the Dutch Warmblood horse at the normal walk. *Equine Veterinary Journal*, *20*(3), 209-213.

Merkens, H. W., Schamhardt, H. C., Van Osch, G. J. V. M., & Van den Bogert, A. J. (1991). Ground reaction force analysis of Dutch Warmblood horses at canter and jumping. *Equine exercise physiology*, *3*, 128-135.

Metayer, N., Biau, S., Cochet, J. L., & Barrey, E. (2004). Stduy of locomotion and morphological factors in the performance of the horse specialized in endurance tests. *30ème journée de la recherche équine, 3 mars 2004*, 67-76.

Minetti, A. E., Ardigo, L. P., Reinach, E., & Saibene, F. (1999). The relationship between mechanical work and energy expenditure of locomotion in horses. *Journal of Experimental Biology*, *202*(17), 2329-2338.

Miró, F., Santos, R., Garrido-Castro, J. L., Galisteo, A. M., & Medina-Carnicer, R. (2009). 2D versus 3D in the kinematic analysis of the horse at the trot. *Veterinary research communications*, *33*(6), 507-513.

Mirzaie, A., Halaji, M., Dehkordi, F. S., Ranjbar, R., & Noorbazargan, H. (2020). A narrative literature review on traditional medicine options for treatment of corona virus disease 2019 (COVID-19). *Complementary therapies in clinical practice*, *40*, 101214.

Mitchell, A., Watts, A. E., Ebetino, F. H., & Suva, L. J. (2019). Bisphosphonate use in the horse: what is good and what is not?. *BMC veterinary research*, *15*(1), 1-7.

Miyoshi, T., Shirota, T., Yamamoto, S. I., Nakazawa, K., & Akai, M. (2004). Effect of the walking speed to the lower limb joint angular displacements, joint moments and ground reaction forces during walking in water. *Disability and Rehabilitation*, *26*(12), 724-732.

Mönkkönen, J., Similä, J., & Rogers, M. J. (1998). Effects of tiludronate and ibandronate on the secretion of proinflammatory cytokines and nitric oxide from macrophages in vitro. *Life Sciences*, *62*(8), PL95-PL102.

Mooij, M. J. W., Jans, W., Den Heijer, G. J. L., De Pater, M., & Back, W. (2013). Biomechanical responses of the back of riding horses to water treadmill exercise. *The Veterinary Journal*, *198*, e120-e123.

Morris, E. A., & Seeherman, H. J. (1991). Clinical evaluation of poor performance in the racehorse: the results of 275 evaluations. *Equine veterinary journal*, *23*(3), 169-174.

Morrison, R., Sutton, D. G. M., Ramsoy, C., Hunter-Blair, N., Carnwath, J., Horsfield, E., & Yam, P. S. (2015). Validity and practical utility of accelerometry for the measurement of in-hand physical activity in horses. *BMC veterinary research*, *11*(1), 1-8.

Mottini, V., Leleu, C., & Cotrel, C. (2006). Harnessed vs. mounted Standardbreds on the track: Changes in gait and physiological variables. *Equine Veterinary Journal*, *38*(S36), 468-472.

Muñoz, A., Santisteban, R., Rubio, M. D., Riber, C., Agüera, E. I., & Castejon, F. M. (1999). Locomotor response to exercise in relation to plasma lactate accumulation and heart rate in Andalusian and Anglo-Arabian horses. *Veterinary research communications*, *23*(6), 369-384.

Muñoz, A., Santisteban, R., Rubio, M. D., Vivo, R., Agüera, E. I., Escribano, B. M., & Castejón, F. M. (1997). Training as an influential factor on the locomotor pattern in Andalusian horses. *Journal of Veterinary Medicine Series A*, *44*(1-10), 473-480.

Muñoz, A., Castejón-Riber, C., Riber, C., Esgueva, M., Trigo, P., & Castejón, F. (2017). Current knowledge of pathologic mechanisms and derived practical applications to prevent metabolic disturbances and exhaustion in the endurance horse. *Journal of Equine Veterinary Science*, *51*, 24-33.

Muñoz, A., Cuesta, I., Riber, C., Gata, J., Trigo, P., & Castejón, F. M. (2006). Trot asymmetry in relation to physical performance and metabolism in equine endurance rides. *Equine Veterinary Journal*, *38*(S36), 50-54.

Muñoz, A., Riber, C., Santisteban, R., Lucas, R. G., & Castejón, F. M. (2002). Effect of training duration and exercise on blood-borne substrates, plasma lactate and enzyme concentrations in Andalusian, Anglo-Arabian and Arabian breeds. *Equine Veterinary Journal*, *34*(S34), 245-251.

Muñoz, A., Saitua, A., Becero, M., Riber, C., Satué, K., de Medina, A. S., Argüelles, D., & Castejón-Riber, C. (2019). The use of the water treadmill for the rehabilitation of musculoskeletal injuries in the sport horse. *Journal of veterinary research*, *63*(3), 439-445.

Muñoz, A., Santisteban, R., Rubio, M. D., Agüera, E. I., Escribano, B. M., & Castejón, F. M. (1999). Locomotor, cardiocirculatory and metabolic adaptations to training in Andalusian and Anglo-Arabian horses. *Research in Veterinary Science*, *66*(1), 25-31.

Murray, R. C., Dyson, S. J., Tranquille, C., & Adams, V. (2006). Association of type of sport and performance level with anatomical site of orthopaedic injury diagnosis. *Equine veterinary journal*, *38*(S36), 411-416.

Murray, R. C., Schramme, M. C., Dyson, S. J., Branch, M. V., & Blunden, T. S. (2006). Magnetic resonance imaging characteristics of the foot in horses with palmar foot pain and control horses. *Veterinary Radiology & Ultrasound*, *47*(1), 1-16.

Nagy, A., Murray, J. K., & Dyson, S. (2010). Elimination from elite endurance rides in nine countries: a preliminary study. *Equine veterinary journal*, *42*, 637-643.

Nancollas, G. H., Tang, R., Phipps, R. J., Henneman, Z., Gulde, S., Wu, W., Mangood, A., Russell R. G. G., & Ebetino, F. H. (2006). Novel insights into actions of bisphosphonates on bone: differences in interactions with hydroxyapatite. *Bone*, *38*(5), 617-627.

Nankervis, K. J., & Lefrancois, K. (2018). A comparison of protraction-retraction of the distal limb during treadmill and water treadmill walking in horses. *Journal of Equine Veterinary Science*, *70*, 57-62.

Nankervis, K. J., Launder, E. J., & Murray, R. C. (2017). The use of treadmills within the rehabilitation of horses. *Journal of Equine Veterinary Science*, *53*, 108-115.

Nankervis, K., Dumbell, L., Herbert, L., Winfield, J., Guire, R., & Launder, E. (2015). A comparison of the position of elite and non-elite riders during competitive show jumping. *Comparative Exercise Physiology*, *11*(2), 119-125.

Nankervis, K., Tranquille, C., McCrae, P., York, J., Lashley, M., Baumann, M., King, M., Sykes, E., Lambourn, J., Miskimmin, K. A., Allen, D., Van Mol, E., Brooks, S., Willingham, T., Lacey, S., Hardy, V., Ellis, J., & Murray, R. (2021). Consensus for the General Use of

Equine Water Treadmills for Healthy Horses. *Animals*, *11*(2), 305.

Nauwelaerts, S., Aerts, P., & Clayton, H. (2013). Spatio-temporal gait characteristics during transitions from trot to canter in horses. *Zoology*, *116*(4), 197-204.

Nicholl, TK, Fregin, GF & Gerber, N. H. (1978). Swimming-a method to study the physiologic response of the horse to exercise. *Journal of the South African Veterinary Association*, *49*(4), 313-315.

Nieto, E. J., Salinas, J. R., & Torres, A. R. (2013). Manejo terapéutico con ácido zoledrónico de la osteoporosis primaria en mujeres posmenopáusicas. Mérida, Venezuela. *Revista Metabolismo Óseo y Mineral*, *11*(3), 64-68.

Nieto, J. E., Snyder, J. R., Beldomenico, P., Aleman, M., Kerr, J. W., & Spier, S. J. (2004). Prevalence of gastric ulcers in endurance horses–a preliminary report. *The Veterinary Journal*, *167*(1), 33-37.

Oosterlinck, M., Pille, F., Back, W., Dewulf, J., & Gasthuys, F. (2010). Use of a stand-alone pressure plate for the objective evaluation of forelimb symmetry in sound ponies at walk and trot. *The Veterinary Journal*, *183*(3), 305-309.

Oosterlinck, M., Pille, F., Lubbers, C., Haspeslagh, M., & Martens, A. (2020). Guillotine versus pull-through technique for palmar digital neurectomy: A retrospective study on 40 horses. *Equine Veterinary Education*, *32*, 37-41.

Oosterlinck, M., Pille, F., Sonneveld, D. C., Oomen, A. M., Gasthuys, F., & Back, W. (2012). Contribution of dynamic calibration to the measurement accuracy of a pressure plate system throughout the stance phase in sound horses. *The Veterinary Journal*, *193*(2), 471-474.

Oppenheimer, S. (2013). Human's association with water bodies: The 'Exaggerated Diving Reflex'and its relationship with the evolutionary allometry of human pelvic and brain sizes. *Hum Evol*, *28*(3-4), 137-170.

Østblom, L. C., Lund, C., & Melsen, F. (1984). Navicular bone disease: results of treatment using egg-bar shoeing technique. *Equine Veterinary Journal*, *16*(3), 203-206.

Østblom, L., Lund, C., & Melsen, F. (1989). Navicular bone disease: a comparative histomorphometric study. *Equine Veterinary Journal*, *21*(6), 431-433.

Papapoulos, S. E. (2008). Bisphosphonates: how do they work?. *Best practice & research clinical endocrinology & metabolism*, *22*(5), 831-847.

Paris, A., Beccati, F., & Pepe, M. (2021). Type, prevalence, and risk factors for the development of orthopedic injuries in endurance horses during training and competition. *Journal of the American Veterinary Medical Association*, *258*(10), 1109-1118.

Parkes, R. S. V., Weller, R., Groth, A. M., May, S., & Pfau, T. (2009). Evidence of the development of 'domain-restricted'expertise in the recognition of asymmetric motion characteristics of hindlimb lameness in the horse. *Equine Veterinary Journal*, *41*(2), 112-117.

Peham, C., Licka, T., Mayr, A., Scheidl, M., & Girtler, D. (1998). Speed dependency of motion pattern consistency. *Journal of Biomechanics*, *31*(9), 769-772.

Peloso, J. G., Stick, J. A., Soutas-Little, R. W., Caron, J. C., DeCamp, C. E., & Leach, D. H. (1993). Computer-assisted three-dimensional gait analysis of amphotericin-induced carpal lameness in horses. *American journal of veterinary research*, *54*(9), 1535-1543.

Petroianu, G. A. (2011). Pharmacist Theodor Salzer (1833–1900) and the discovery of bisphosphonates. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *66*(10), 804-807.

Pfau, T., & Weller, R. (2017). Comparison of a standalone consumer grade smartphone with a specialist inertial measurement unit for quantification of movement symmetry in the trotting horse. *Equine Veterinary Journal*, *49*(1), 124-129.

Pfau, T., Witte, T. H., & Wilson, A. M. (2005). A method for deriving displacement data during cyclical movement using an inertial sensor. *Journal of Experimental Biology*, *208*(13), 2503-2514.

Pitti, L., Oosterlinck, M., Díaz-Bertrana, M. L., Carrillo, J. M., Rubio, M., Sopena, J., Santana, A., & Vilar, J. M. (2018). Assessment of static posturography and pedobarography for the detection of unilateral forelimb lameness in ponies. *BMC veterinary research*, *14*(1), 1-8.

Pleasant, R. S., Baker, G. J., Foreman, J. H., Eurell, J. A., & Losonsky, J. M. (1993). Intraosseous pressure and pathologic changes in horses with navicular disease. *American journal of veterinary research*, *54*(1), 7-12.

Pleasant, R. S., Moll, H. D., Ley, W. B., Lessard, P., & Warnick, L. D. (1997). Intra-articular anesthesia of the distal interphalangeal joint alleviates lameness associated with the navicular bursa in horses. *Veterinary Surgery*, *26*(2), 137-140.

Pool, R. R., Meagher, D. M., & Stover, S. M. (1989). Pathophysiology of navicular syndrome. *Veterinary Clinics of North America: Equine Practice*, *5*(1), 109-129.

Porras, A. G., Holland, S. D., & Gertz, B. J. (1999). Pharmacokinetics of alendronate. *Clinical pharmacokinetics*, *36*(5), 315-328.

Poulos, P. W., & Smith, M. F. (1988). The nature of enlarged “vascular channels” in the navicular bone of the horse. *Veterinary Radiology*, *29*(2), 60-64.

Pourcelot, P., Audigié, F., Degueurce, C., Geiger, D., & Denoix, J. M. (2000). A method to synchronise cameras using the direct linear transformation technique. *Journal of Biomechanics*, *33*(12), 1751-1754.

Powers, P., & Harrison, A. (2002). Show-Jumping: Effects of the rider on the linear kinematics of jumping horses. *Sports Biomechanics*, *1*(2), 135-146.

Pöyhönen, T., Keskinen, K. L., Kyröläinen, H., Hautala, A., Savolainen, J., & Mälkiä, E. (2001). Neuromuscular function during therapeutic knee exercise under water and on dry land. *Archives of physical medicine and rehabilitation*, *82*(10), 1446-1452.

Preston, S. A., Trumble, T. N., Zimmel, D. N., Chmielewski, T. L., Brown, M. P., & Hernandez, J. A. (2008). Lameness, athletic performance, and financial returns in yearling Thoroughbreds bought for the purpose of resale for profit. *Journal of the American Veterinary Medical Association*, *232*(1), 85-90.

Preuschoft, H. (1989). The external forces and internal stresses in the feet of dressage and jumping horses. *Zeitschrift für Säugetierkunde*, *54*(3), 172-190.

Prince, A., Geor, R., Harris, P., Hoekstra, K., Gardner, S., Hudson, C., & Pagan, J. (2002). Comparison of the metabolic responses of trained Arabians and Thoroughbreds during high-and low-intensity exercise. *Equine Veterinary Journal*, *34*(S34), 95-99.

Puchalski, S. M., Galuppo, L. D., Hornof, W. J., & Wisner, E. R. (2007). Intraarterial contrast-enhanced computed tomography of the equine distal extremity. *Veterinary Radiology & Ultrasound*, *48*(1), 21-29.

Reed, S. K., Kramer, J., Thombs, L., Pitts, J. B., Wilson, D. A., & Keegan, K. G. (2020). Comparison of results for body-mounted inertial sensor assessment with final lameness determination in 1,224 equids. *Journal of the American Veterinary Medical Association*, *256*(5), 590-599.

Reszka, A.A., Roldan, G.A. (2004) Nitrogen-containing bisphosphonate mechanism of action. *Mini Reviews in Medicinal Chemistry* 4, 711-719.

Rewald, S., Lenssen, A. T., Emans, P. J., de Bie, R. A., van Breukelen, G., & Mesters, I. (2020). Aquatic cycling improves knee pain and physical functioning in patients with knee osteoarthritis: a randomized controlled trial. *Archives of physical medicine and rehabilitation*, *101*(8), 1288-1295.

Rhodin, M., Bergh, A., Gustås, P., & Álvarez, C. G. (2017). Inertial sensor-based system for lameness detection in trotting dogs with induced lameness. *The Veterinary Journal*, *222*, 54-59.

Rhodin, M., Johnston, C., Holm, K. R., Wennerstrand, J., & Drevemo, S. (2005). The influence of head and neck position on kinematics of the back in riding horses at the walk and trot. *Equine veterinary journal*, *37*(1), 7-11.

Rhodin, M., Persson-Sjodin, E., Egenvall, A., Serra Bragança, F. M., Pfau, T., Roepstorff, L., Weishaupt, M. A., Thomsen, M. H., Van Weeren, P. R., & Hernlund, E. (2018). Vertical movement symmetry of the withers in horses with induced forelimb and hindlimb lameness at trot. *Equine veterinary journal*, *50*(6), 818-824.

Ricard, A., Dumont Saint Priest, B., Chassier, M., Sabbagh, M., & Danvy, S. (2020). Genetic consistency between gait analysis by accelerometry and evaluation scores at breeding shows for the selection of jumping competition horses. *PloS one*, *15*(12), e0244064.

Ricard, A., Dumont Saint Priest, B., Danvy, S., & Barrey, E. (2020a).

Accelerometers Provide Early Genetic Selection Criteria for Jumping Horses. *Frontiers in Genetics*, *11*, 448.

Richbourg, H. A., Mitchell, C. F., Gillett, A. N., & McNulty, M. A. (2018). Tiludronate and clodronate do not affect bone structure or remodeling kinetics over a 60 day randomized trial. *BMC veterinary research*, *14*(1), 1-11.

Rijkenhuizen, A. B. M. (2006). Navicular disease: a review of what's new. *Equine veterinary journal*, *38*(1), 82-88.

Rijkenhuizen, A. B., Nemeth, F., Dik, K. J., & Goedegebuure, S. A. (1989). The arterial supply of the navicular bone in adult horses with navicular disease. *Equine Veterinary Journal*, *21*(6), 418-424.

Rivero, J. L. L. (1996). Discrimination between endurance horses with different performance records. *Pferdeheilkunde*, *12*(4), 510-513.

Rivero, J. L. L., & Clayton, H. M. (1996). The potential role of the muscle in kinematic characteristics. *Pferdeheilkunde*, *12*, 635-640.

Rivero, J. L. L., Ruz, M. C., Serrano, A. L., & Diz, A. M. (1995). Effects of a 3 month endurance training programme on skeletal muscle histochemistry in Andalusian, Arabian and Anglo-Arabian horses. *Equine veterinary journal*, *27*(1), 51-59.

Rivero, J. L., & Piercy, R. J. (2008). Muscle physiology: responses to exercise and training. *Equine exercise physiology: the science of exercise in the athletic horse*, 463.

Rivero, J. L., Serrano, A. L., Henckel, P. O. U. L., & Aguera, E. (1993). Muscle fiber type composition and fiber size in successfully and unsuccessfully endurance-raced horses. *Journal of Applied Physiology*, *75*(4), 1758-1766.

Robilliard, J. J., Pfau, T., & Wilson, A. M. (2007). Gait characterisation and classification in horses. *Journal of Experimental Biology*, *210*(2), 187-197.

Roemer-Bécuwe, C., Vigano, A., Romano, F., Neumann, C., Hanson, J., Quan, H. K., & Walker, P. (2003). Safety of subcutaneous clodronate and efficacy in hypercalcemia of malignancy: a novel route of administration. *Journal of pain and symptom management*, *26*(3), 843-848.

Rogers, C. W., Davies, A. S., Pfeiffer, D. U., & Davie, P. S. (1999). Temporal changes in the trot between the first and third horse inspections at a CCI*** 3-day event. *Equine Veterinary Journal*, *31*(S30), 254-257.

Rogers, M. J. (2003). New insights into the molecular mechanisms of action of bisphosphonates. *Current pharmaceutical design*, *9*(32), 2643-2658.

Rogers, M. J. (2004). From molds and macrophages to mevalonate: a decade of progress in understanding the molecular mode of action of bisphosphonates. *Calcified tissue international*, *75*(6), 451-461.

Rogers, M. J., Crockett, J. C., Coxon, F. P., & Mönkkönen, J. (2011). Biochemical and molecular mechanisms of action of bisphosphonates. *Bone*, *49*(1), 34-41.

Ronéus, N., Essén-Gustavsson, B., Lindholm, A., & Persson, S. (1999). Muscle characteristics and plasma lactate and ammonia response after racing in Standardbred trotters: relation to performance. *Equine Veterinary Journal*, *31*(2), 170-173.

Rønnestad, B. R., Kojedal, Ø., Losnegard, T., Kvamme, B., & Raastad, T. (2012). Effect of heavy strength training on muscle thickness, strength, jump performance, and endurance performance in well-trained Nordic Combined athletes. *European journal of applied physiology*, *112*(6), 2341-2352.

Rose, N. S., Northrop, A. J., Brigden, C. V., & Martin, J. H. (2009). Effects of a stretching regime on stride length and range of motion in equine trot. *The Veterinary Journal*, *181*(1), 53-55.

Rose, R. J., Allen, J. R., Hodgson, D. R., & Kohnke, J. R. (1983). Studies on isoxsuprine hydrochloride for the treatment of navicular disease. *Equine Veterinary Journal*, *15*(3), 238-243.

Rose, R. J., King, C. M., Evans, D. L., Tyler, C. M., & Hodgson, D. R. (1995). Indices of exercise capacity in horses presented for poor racing performance. *Equine Veterinary Journal*, *27*(S18), 418-421.

Ross, M. W. (1998). Observations in horses with lameness abolished by palmar digital analgesia. In *Proc. Am. Ass. equine Practnrs* (Vol. 44, pp. 230-232).

Ross, M. W. (2011, November). Fetlock lameness. In *Proceedings from the American College of Veterinary Surgeons Veterinary Symposium* (pp. 93-102).

Rossdale, P. D., Hopes, R., & Digby, N. J. (1985). Epidemiological study of wastage among racehorses 1982 and 1983. *The Veterinary Record*, *116*(3), 66-69.

Russell, R. G. G., Watts, N. B., Ebetino, F. H., & Rogers, M. J. (2008). Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. *Osteoporosis international*, *19*(6), 733-759.

Sack, W. O., & Rooney, J. R. (1991). Rooney's Guide to the Dissection of the Horse.

Sahin, H. G., Kunduracilar, Z., Sonmezer, E., & Ayas, S. (2019). Effects of two different aquatic exercise trainings on cardiopulmonary endurance and emotional status in patients with knee osteoarthritis. *Journal of back and musculoskeletal rehabilitation*, *32*(4), 539-548.

Sampson, S. N., Schneider, R. K., & Gavin, P. R. (2008). Magnetic resonance imaging findings in horses with recent and chronic bilateral forelimb lameness diagnosed as navicular syndrome. *Proceedings Am. Assoc. Equine Practice*, *54*, 419-434.

Santamaría, S., Back, W., Van Weeren, P. R., Knaap, J., & Barneveld, A. (2002). Jumping characteristics of naive foals: lead changes and description of temporal and linear parameters. *Equine Veterinary Journal*, *34*(S34), 302-307.

Santamaría, S., Bobbert, M. F., Back, W., Barneveld, A., & van Weeren, P. R. (2004). Variation in free jumping technique within and among horses with little experience in show jumping. *American journal of veterinary research*, *65*(7), 938-944.

Santosuosso, E., Leguillette, R., Vinardell, T., Filho, S., Massie, S., McCrae, P., Johnson, S., Rolian, C., & David, F. (2021). Kinematic analysis during straight line free swimming in horses: Part 1-Forelimbs. *Frontiers in veterinary science*, *8*, 752375.

Sato, M., Grasser, W., Endo, N., Akins, R., Simmons, H., Thompson, D. D., Golub, E., & Rodan, G. A. (1991). Bisphosphonate action. Alendronate localization in rat bone and effects on osteoclast ultrastructure. *The Journal of clinical investigation*, *88*(6), 2095-2105.

Schamhardt, H. C., Van den Bogert, A. J., & Hartman, W. (1993). Measurement techniques in animal locomotion analysis. *Cells Tissues Organs*, *146*(2-3), 123-129.

Scheer, A., Shah, A., de Oliveira, B. I. R., Moreno-Suarez, I., Jacques, A., Green, D., & Maiorana, A. (2021). Twelve weeks of water-based circuit training exercise improves fitness, body fat and leg strength in people with stable coronary heart disease: a randomised trial. *Journal of Physiotherapy*, *67*(4), 284-290.

Schoonover, M. J., Jann, H. W., & Blaik, M. A. (2005). Quantitative comparison of three commonly used treatments for navicular syndrome in horses. *American journal of veterinary research*, *66*(7), 1247-1251.

Schoonover, M. J., Whitfield, C. T., Young, J. M., Sippel, K. M., & Payton, M. E. (2018). Quantitative assessment of intravenous regional limb perfusion of tiludronate as an adjunctive treatment for lameness caused by navicular syndrome in horses. *American journal of veterinary research*, *79*(12), 1313-1320.

Schuback, K., Essén-Gustavsson, B., & Persson, S. G. B. (1999). Incremental treadmill exercise until onset of fatigue and its relationship to metabolic response and locomotion pattern. *Equine Veterinary Journal*, *31*(S30), 337-341.

Schumacher, J., Livesey, L., De Graves, F. J., Schumacher, J., Schramme, M. C., Hathcock, J., Taintor, J. & Gomez, J. (2004). Effect of anaesthesia of the palmar digital nerves on proximal interphalangeal joint pain in the horse. *Equine veterinary journal*, *36*(5), 409-414.

Schumacher, J., Schumacher, J., Gillette, R., DeGraves, F., Schramme, M., Smith, R., Perkins, J., & Coker, M. (2003). The effects of local anaesthetic solution in the navicular bursa of horses with lameness caused by distal interphalangeal joint pain. *Equine veterinary journal*, *35*(5), 502-505.

Schumacher, J., Schumacher, J., Graves, F. D., Schramme, M., Smith, R., Coker, M., & Steiger, R. (2001). A comparison of the effects of local analgesic solution in the navicular bursa of horses with lameness caused by solar toe or solar heel pain. *Equine Veterinary Journal*, *33*(4), 386-389.

Scott, R., Nankervis, K., Stringer, C., Westcott, K., & Marlin, D. (2010). The effect of water height on stride frequency, stride length and heart rate during water treadmill exercise. *Equine Veterinary Journal*, *42*, 662-664.

Serra Bragança, F. M., Hernlund, E., Thomsen, M. H., Waldern, N. M., Rhodin, M., Byström, A., ... & Weishaupt, M. A. (2021). Adaptation strategies of horses with induced forelimb lameness walking on a treadmill. *Equine veterinary journal*, *53*(3), 600-611.

Setterbo, J. J., Garcia, T. C., Campbell, I. P., Reese, J. L., Morgan, J. M., Kim, S. Y., Hubbard, M., & Stover, S. M. (2009). Hoof accelerations and ground reaction forces of Thoroughbred racehorses measured on dirt, synthetic, and turf track surfaces. *American Journal of Veterinary Research*, *70*(10), 1220-1229.

Silva, L. E., Valim, V., Pessanha, A. P. C., Oliveira, L. M., Myamoto, S., Jones, A., & Natour, J. (2008). Hydrotherapy versus conventional land-based exercise for the management of patients with osteoarthritis of the knee: a randomized clinical trial. *Physical therapy*, *88*(1), 12-21.

Silvers, B. L., Leatherwood, J. L., Arnold, C. E., Nielsen, B. D., Huseman, C. J., Dominguez, B. J., Glass, K. G., Martinez, R. E., Much, M. L., & Bradbery, A. N. (2020). Effects of aquatic conditioning on cartilage and bone metabolism in young horses. *Journal of Animal Science*, *98*(8), skaa239.

Smith, M. R. W., Wright, I. M., & Smith, R. K. W. (2007). Endoscopic assessment and treatment of lesions of the deep digital flexor tendon in the navicular bursae of 20 lame horses. *Equine veterinary journal*, *39*(1), 18-24.

Smith, R. L., & Lawrence Jr, J. C. (1985). Insulin action in denervated skeletal muscle. Evidence that the reduced stimulation of glycogen synthesis does not involve decreased insulin binding. *Journal of Biological Chemistry*, *260*(1), 273-278.

Smith, R., Russell, R. G. G., & Bishop, M. (1971). Diphosphonates and Paget's disease of bone. *The Lancet*, *297*(7706), 945-947.

Soto, S. A., & Barbará, A. C. (2014). Bisphosphonates: pharmacology and clinical approach to their use in equine osteoarticular diseases. *Journal of equine veterinary science*, *34*(6), 727-737.

Stashak, T. S. (1998). Navicular syndrome (navicular disease). *Current techniques in equine surgery and lameness*, 537-543.

Strobach, A., Kotschwar, A., Mayhew, I. G., Peham, C., & Licka, T. (2006). Gait pattern of the ataxic horse compared to sedated and nonsedated horses. *Equine Veterinary Journal*, *38*(S36), 423-426.

Svalastoga, E., & Smith, M. (1983). Navicular disease in the horse. The subchondral bone pressure. *Nordisk Veterinaermedicin*, *35*(1), 31-37.

Swor, T. M., Dabareiner, R. M., Honnas, C. M., Cohen, N. D., & Black, J. B. (2019). Musculoskeletal problems associated with lameness and poor performance in cutting horses: 200

cases (2007–2015). *Journal of the American Veterinary Medical Association*, *254*(5), 619-625.

Thomas, D. P., Fregin, F., Gerber, N. H., & Ailes, N. B. (1980). Cardiorespiratory adjustments to tethered-swimming in the horse. *Pflügers Archiv*, *385*(1), 65-70.

Thompson, K., Rogers, M. J., Coxon, F. P., & Crockett, J. C. (2006). Cytosolic entry of bisphosphonate drugs requires acidification of vesicles after fluid-phase endocytosis. *Molecular pharmacology*, *69*(5), 1624-1632.

Thomsen, M. H., Persson, A. B., Jensen, A. T., Sørensen, H., & Andersen, P. H. (2010). Agreement between accelerometric symmetry scores and clinical lameness scores during experimentally induced transient distension of the metacarpophalangeal joint in horses. *Equine veterinary journal*, *42*, 510-515.

Tietje, S. (1995). Computed tomography of the navicular bone region in horse: a comparison with radiographic documentation. *Pferdeheilkunde*, *11*(1), 51-61.

Tischmacher, A., Wilford, S., Allen, K., Mitchell, R. D., Parkin, T., & Denoix, J. M. (2022). Retrospective analysis of the use of tiludronate in equine practice: safety on 1804 horses, efficacy on 343 horses. *Journal of Equine Veterinary Science*, *115*, 104007.

Tokuriki, M., Ohtsuki, R., KAl, M., Hiraga, A., Oki, H., Miyahara, Y., & Aoki, O. (1999). EMG activity of the muscles of the neck and forelimbs during different forms of locomotion. *Equine Veterinary Journal*, *31*(S30), 231-234.

Tranquille, C. A., Nankervis, K. J., Walker, V. A., Tacey, J. B., & Murray, R. C. (2017). Current knowledge of equine water treadmill exercise: what can we learn from human and canine studies?. *Journal of Equine Veterinary Science*, *50*, 76-83.

Tranquille, C. A., Tacey, J. B., Walker, V. A., Nankervis, K. J., & Murray, R. C. (2018). International Survey of Equine Water Treadmills—Why, When, and How?. *Journal of Equine Veterinary Science*, *69*, 34-42.

Trotter, G. (2001). The biomechanics of what really causes navicular disease. *Equine veterinary journal*, *33*(4), 334-336.

Turner, A. S., & Tucker, C. M. (1989). The evaluation of isoxsuprine hydrochloride for the treatment of navicular disease: a double blind study. *Equine Veterinary Journal*, *21*(5), 338-341.

Turner, T. A. (1986). Shoeing principles for the management of navicular disease in horses. *Journal of the American Veterinary Medical Association*, *189*(3), 298-301.

Turner, T. A. (1989). Diagnosis and treatment of the navicular syndrome in horses. *Veterinary Clinics of North America: Equine Practice*, *5*(1), 131-144.

Uphaus, H., Preisinger, R., & Kalm, E. (1992, September). Comparison of performance test and progeny test in Hannoverian Warmblood mares. In *Book of abstracts of the 43rd* *meeting of the European Association of Animal Production* (pp. 548-549).

Valdez, H., Adams, O. R., & Peyton, L. C. (1978). Navicular disease in the hindlimbs of the horse. *Journal of the American Veterinary Medical Association*, *172*(3), 291-292.

Van Beek, E., Löwik, C., Que, I., & Papapoulos, S. (1996). Dissociation of binding and antiresorptive properties of hydroxybisphosphonates by substitution of the hydroxyl with an amino group. *Journal of Bone and Mineral Research*, *11*(10), 1492-1497.

Van Beek, E., Pieterman, E., Cohen, L., Löwik, C., & Papapoulos, S. (1999). Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates. *Biochemical and biophysical research communications*, *264*(1), 108-111.

Van den Bogert, A. J., Jansen, M. O., & Deuel, N. R. (1994). Kinematics of the hind limb push-off in elite show jumping horses. *Equine Veterinary Journal*, *26*(S17), 80-86.

Van den Bogert, A. J., Van Weeren, P. R., & Schamhardt, H. C. (1990). Correction for skin displacement errors in movement analysis of the horse. *Journal of Biomechanics*, *23*(1), 97-101.

Van Oldruitenborgh-Oosterbaan, M. M. S., Barneveld, A., & Schamhardt, H. C. (1995). Effects of weight and riding on workload and locomotion during treadmill exercise. *Equine Veterinary Journal*, *27*(S18), 413-417.

Van Weeren, P. R., & Álvarez, C. G. (2019). Equine gait analysis: The slow start, the recent breakthroughs and the sky as the limit?. *Equine veterinary journal*, *51*(6), 809-810.

Van Weeren, P. R., Van den Bogert, A. J., & Barneveld, A. (1992). Correction models for skin displacement in equine kinematics gait analysis. *Journal of Equine Veterinary Science*, *12*(3), 178-192.

Van Weeren, P. R., Van den Bogert, A. J., Back, W., Bruin, G., & Barneveld, A. (1993). Kinematics of the standardbred trotter measured at 6, 7, 8 and 9 m/s on a treadmill, before and after 5 months of prerace training. *Cells Tissues Organs*, *146*(2-3), 154-161.

Van Weeren, P. R., Van den Bogert, A. J., Barneveld, A., Hartman, W., & Kersjes, A. W. (1990). The role of the reciprocal apparatus in the hind limb of the horse investigated by a modified CODA-3 opto-electronic kinematic analysis system. *Equine Veterinary Journal*, *22*(S9), 95-100.

Vargas-Franco, J. W., Castaneda, B., Rédiní, F., Gómez, D. F., Heymann, D., & Lézot, F. (2018). Paradoxical side effects of bisphosphonates on the skeleton: What do we know and what can we do?. *Journal of Cellular Physiology*, *233*(8), 5696-5715.

Vergara-Hernandez, F. B., Nielsen, B. D., & Colbath, A. C. (2022). Is the Use of Bisphosphonates Putting Horses at Risk? An Osteoclast Perspective. *Animals*, *12*(13), 1722.

Verschooten, F., Ooms, L. A. A., Desmet, P., & Peremans, K. (1990). Metrenperone treatment of navicular disease in horses compared with isoxsuprine: a clinical study. *Journal of*

Equine Veterinary Science, *10*(3), 230-233.

Vilar, J. M., Spadari, A., Billi, V., Desini, V., & Santana, A. (2008). Biomechanics in young and adult italian standardbred trotter horses in real racing conditions. *Veterinary research communications*, *32*(5), 367-376.

Viry, S., De Graaf, J. B., Frances, J. P., Berton, E., Laurent, M., & Nicol, C. (2015). Combined influence of expertise and fatigue on riding strategy and horse–rider coupling during the time course of endurance races. *Equine Veterinary Journal*, *47*(1), 78-82.

Viry, S., Frances, J. P., Berton, E., Laurent, M., & Nicol, C. (2014). Preliminary study on the influence of the riding «desert style» on the horse-rider coupling endurance race. In *40ème journée de la recherche équine. Actes de colloque, epidémiologie et pathologie, Paris, France, 18 March 2014* (pp. 168-170). Institut Français du Cheval et de l'Équitation.

Volaklis, K. A., Spassis, A. T., & Tokmakidis, S. P. (2007). Land versus water exercise in patients with coronary artery disease: effects on body composition, blood lipids, and physical fitness. *American heart journal*, *154*(3), 560-e1.

Waguespack, R., & Hanson, R. R. (2010). Navicular syndrome in equine patients anatomy, causes, and diagnosis. *Compend Contin Educ Vet*, *32*(12), E7.

Waldern, N. M., Wiestner, T., Ramseier, L. C., Amport, C., & Weishaupt, M. A. (2013). Effects of shoeing on limb movement and ground reaction forces in Icelandic horses at walk, tölt and trot. *The Veterinary Journal*, *198*, e103-e108.

Waldern, N. M., Wiestner, T., von Peinen, K., Álvarez, C. G., Roepstorff, L., Johnston, C., Meyer, H., & Weishaupt, M. A. (2009). Influence of different head-neck positions on vertical ground reaction forces, linear and time parameters in the unridden horse walking and trotting on a treadmill. *Equine veterinary journal*, *41*(3), 268-273.

Weishaupt, M. A. (2008). Adaptation strategies of horses with lameness. *Veterinary Clinics of North America: Equine Practice*, *24*(1), 79-100.

Weishaupt, M. A., Byström, A., von Peinen, K., Wiestner, T., Meyer, H., Waldern, N., Johnston, C., & Roepstorff, L. (2009). Kinetics and kinematics of the passage. *Equine Veterinary Journal*, *41*(3), 263-267.

Weishaupt, M. A., Hogg, H. P., Auer, J. A., & Wiestner, T. (2010). Velocity-dependent changes of time, force and spatial parameters in Warmblood horses walking and trotting on a treadmill. *Equine Veterinary Journal*, *42*, 530-537.

Weishaupt, M. A., Hogg, H. P., Wiestner, T., Denoth, J., Stüssi, E., & Auer, J. A. (2002). Instrumented treadmill for measuring vertical ground reaction forces in horses. *American journal of veterinary research*, *63*(4), 520-527.

Weishaupt, M. A., Wiestner, T., Hogg, H. P., Jordan, P., & Auer, J. A. (2004).

Compensatory load redistribution of horses with induced weightbearing hindlimb lameness trotting on a treadmill. *Equine veterinary journal*, *36*(8), 727-733.

Weishaupt, M. A., Wiestner, T., Hogg, H. P., Jordan, P., & Auer, J. A. (2006). Compensatory load redistribution of horses with induced weight-bearing forelimb lameness trotting on a treadmill. *The Veterinary Journal*, *171*(1), 135-146.

Weishaupt, M. A., Wiestner, T., Hogg, H. P., Jordan, P., Auer, J. A., & Barrey, E. (2001). Assessment of gait irregularities in the horse: eye vs. gait analysis. *Equine Veterinary Journal*, *33*(S33), 135-140.

Whitfield, C. T., Schoonover, M. J., Holbrook, T. C., Payton, M. E., & Sippel, K. M. (2016). Quantitative assessment of two methods of tiludronate administration for the treatment of lameness caused by navicular syndrome in horses. *American Journal of Veterinary Research*, *77*(2), 167-173.

Williams, G. E., Silverman, B. W., Wilson, A. M., & Goodship, A. E. (1999). Pathology-specific changes in equine ground reaction force data demonstrated by principal component analysis. *American journal of veterinary research*, *60*(60), 549-555.

Wilson, A. M., McGuigan, M. P., Fouracre, L., & MacMahon, L. (2001). The force and contact stress on the navicular bone during trot locomotion in sound horses and horses with navicular disease. *Equine Veterinary Journal*, *33*(2), 159-165.

Wilson, J. M., McKenzie, E., & Duesterdieck-Zellmer, K. (2018). International survey regarding the use of rehabilitation modalities in horses. *Frontiers in Veterinary Science*, *5*, 120.

Witte, T. H., Hirst, C. V., & Wilson, A. M. (2006). Effect of speed on stride parameters in racehorses at gallop in field conditions. *Journal of Experimental Biology*, *209*(21), 4389-4397.

Witte, T. H., Knill, K., & Wilson, A. M. (2004). Determination of peak vertical ground reaction force from duty factor in the horse (Equus caballus). *Journal of Experimental Biology*, *207*(21), 3639-3648.

Worster, A. A., Gaughan, E. M., Hoskinson, J. J., Sargeant, J., & Erb, J. H. (2000). Effects of external thermal manipulation on laminar temperature and perfusion scintigraphy of the equine digit. *New Zealand Veterinary Journal*, *48*(4), 111-116.

Wright, I. M. (1986). Navicular suspensory desmotomy in the treatment of navicular disease: technique and preliminary results. *Equine veterinary journal*, *18*(6), 443-446.

Wright, I. M. (1993). A study of 118 cases of navicular disease: clinical features. *Equine veterinary journal*, *25*(6), 488-492.

Wright, I. M. (1993). A study of 118 cases of navicular disease: clinical features. *Equine veterinary journal*, *25*(6), 488-492.

Wright, I. M., Kidd, L., & Thorp, B. H. (1998). Gross, histological and histomorphometric features of the navicular bone and related structures in the horse. *Equine veterinary journal*, *30*(3), 220-234.

Yamazaki, F., Endo, Y., Torii, R., Sagawa, S., & Shiraki, K. (2000). Continuous monitoring of change in hemodilution during water immersion in humans: effect of water temperature. *Aviation, space, and environmental medicine*, *71*(6), 632-639.

York, J., & Walker, A. (2014). Vertical Displacement of the E quine Pelvis When Trotting on an Aqua Treadmill. *Equine Veterinary Journal*, *46*, 55-55.

Young, J. M., Schoonover, M. J., Kembel, S. L., Taylor, J. D., Bauck, A. G., & Gilliam, L. L. (2020). Efficacy of orally administered gabapentin in horses with chronic thoracic limb lameness. *Veterinary Anaesthesia and Analgesia*, *47*(2), 259-266.

List of Abbreviations

ABBREVIATION	MEANING
3D	Three-dimensional
AAEP	American Association of Equine Practitioners
AL-DDFT	Accesory ligament of the deep digital flexor tendon
ATP	Adenosine-triphosphate
C	Carbon atom
CAR	Carpus
CEI	Competition Equestre Internationale
CK	Creatin-kinase
CL	Collateral ligament
CODA-3	Cartesian Optoelectronic Dynamic Anthropometer
CS	Citrate-synthase
CSLs	Collateral sesamoidean ligaments
CT	Computed tomography
CTX-1	Carboxyl-terminal cross-linking telopeptide of type I collagen
DDFT	Deep digital flexor tendon
DIPJ	Distal interphalangeal joint
DSIL	Distal sesamoidean impair ligament
DVAA	Dorso-ventral accelerometric activity
DVAA%	Dorso-ventral accelerometric activity percentage of the total accelerometric activity
FDA	United States Food and Drug Administration
FEI	Fédération Equestre Internationale
FET	Metacarpophalangeal joint (fetlock)
FHE	Federación Hípica Española
FOP	Fibrodysplasia ossificans progressive
GRFs	Ground reaction forces
GRFz	Vertical components of the ground reaction forces
GTPases	Guanosin-triphosphatases
HAD	3-hydroxyacyl-coenzyme-A-dehydrogenase
HR	Heart rate
IV	Intravenously
KSI	Kinematic Symmetry Indices
LA	Lactate accumulation
LAA	Longitudinal/Propulsion accelerometric activity
LAA%	Longitudinal accelerometric activity percentage of the total accelerometric activity
LDH	Lactate dehydrogenase

LED	Light-emitting diode
MLAA	Medio-lateral accelerometric activity
MLAA%	Medio-lateral accelerometric activity percentage of the total accelerometric activity
MRI	Magnetic resonance imaging
NSAID	Non-steroidal anti-inflamatory drugs
O	Oxygen atom
P	Phosphorus atom
PECT	Pectoral region
PO	Orally
pVGRF	Peak vertical ground reaction forces
RE	Running economy
REG	Regularity
RER	Respiratory exchange ratio
ROM	Range of motion
RR	Respiratory rate
SDFT	Superficial digital flexor tendon
SF	Stride frequency
SL	Stride length
SML	Midline of the sacrum region
STF	Stifle
SUM	SYM+REG
SuspL	Suspensory ligament
TAA	Total accelerometric activity
TRAcP5B	Tartrate-resistant-acid-phosphatase-5B
V200	Velocity at a heart rate of 200 beats/min
vGRF	Vertical ground reaction forces
VO2	Oxygen consumption/uptake
WT	Water treadmill
WW	Without water

Published Papers

11.1. PAPER I

Clinical Efficacy of Clodronic Acid in Horses Diagnosed with Navicular Syndrome: A Field Study Using Objective and Subjective Lameness Evaluation

11.2. PAPER II

Combined Effects of Water Depth and Velocity on the Accelerometric Parameters Measured in Horses on a Water Treadmill

11.3. PAPER III

Previous Exercise on a Water Treadmill at Different Depths Affects the Accelerometric Pattern Recorded on a Track in Horses