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Osteopathic single manipulation of the sacroiliac joint and lower cervical spine improves selected gait parameters in sport horses

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List of abbreviations

IMU	Inertial Measurement Units
TG	Treatment Group
CG	Control Group
AAEP	American Association of Equine Practitioners
Min Diff	Minimum Difference
Max Diff	Maximum Difference
DAP	Diagonal Advanced Placement
R	Right
L	Left
LF	Left Front limb
R	Right Front limb
LH	Left Hindlimb
RH	Right Hindlimb
Max	Maximum
OM	Osteopathic Manipulation
GLMM	Generalized Linear Mixed Models
MT	Muscle Tone
OB	Osteopathic Blockage
ROM	Range of Motion
HMM	Head Minimum difference Mean
SDM	Stride Duration Mean
StanDM	Stance Duration Mean
MPM	Maximum Protraction Mean
MRM	Maximum Retraction Mean

HROM	Head Range of Motion
WROM	Withers Range of Motion
SROM	Sacrum Range of Motion
HHStanM	Hip-Hike Stance Mean
HHSM	Hip-Hike Swing Mean.
HROM	Head Range of Motion
SIJ	Sacroiliac Joint
SID	Sacroiliac Dysfunction
SI	Sacroiliac
RM	Repeated Measures
GLM	Generalized Linear Model
Std. Error	Standard Error
CI	Confidence Interval
FBM	Force-based manipulations
NIH	National Institutes of Health
SIJD	Sacroiliac Joint Dysfunction
CVCM	Cervical Vertebral Compressive Myelopathy

Resum

Títol:

Una única manipulació osteopàtica de l'articulació sacroilíaca i la columna cervical baixa milloren paràmetres seleccionats de la marxa en cavalls d'esport.

Antecedents: Els tractaments osteopàtics són àmpliament utilitzats en cavalls d'esport, però els seus efectes sobre la funció locomotora no s'han investigat a fons utilitzant mesures biomecàniques objectives. Aquest estudi pretenia demostrar que una única manipulació osteopàtica dirigida a l'articulació sacroilíaca o a la columna cervical baixa pot millorar la simetria de la marxa equina, l'amplitud de moviment, la protracció o retracció de les extremitats i la qualitat general del moviment avaluada mitjançant unitats de mesurament inercial (IMU).

Mètodes: Es van realitzar dos estudis diferents en un entorn clínic amb cavalls en entrenament. En un estudi es va manipular l'articulació sacroilíaca, mentre que en l'altre es va tractar la unió cervicotoràcica C6-C7. Es van utilitzar nou sensors IMU per a avaluuar la marxa equina abans, immediatament després, 3 dies després i 15 dies després de la manipulació osteopàtica. També es van realitzar puntuacions visuals del grau de coixesa i exàmens osteopàtics, incloses avaluacions del dolor.

Resultats: Es van observar millores significatives després de les manipulacions osteopàtiques cervicals i sacroilíiques. En el cas de la disfunció cervical, la interacció entre els factors grup i temps va reduir significativament les puntuacions de disfunció cervical ($p < 0,05$). Als 15 dies post tractament, el grup de tractament va mostrar una puntuació mitjana de 1,11 (*IC: 0,72-1,71) enfront de 2,30 (*IC: 1,41-3,74) en el grup de control. Es van trobar dos canvis significatius al trot: major protracció del membre posterior dret en línia recta i major protracció del membre anterior dret a la corda a la dreta. Aquesta última es va

veure influïda significativament per la interacció entre grup i tractament ($p <0,05$), mostrant el grup de tractament 27,11 (*IC: 26,34-27,86) enfront de 29,52 (*IC: 28,26-30,77) en el grup de control als 15 dies post-tractament.

Quant a la disfunció sacroilíaca, la manipulació osteopàtica va reduir significativament el tonus muscular del dors, el dolor d'esquena i el dolor sacroilíac, i els efectes van persistir almenys 15 dies després de la intervenció. L'anàlisi biomecànica va revelar una reducció significativa de la protracció dels membres posteriors ($F(1,3) < 3,432$, $p <0,05$) i un augment de la retracció ($F(1,3) < 3,681$, $p <0,05$), sobretot en galopar cap al costat afectat. Es va observar una major amplitud de moviment del cap al galop cap al costat no afectat. No es van trobar resultats concloents per a les passades en línia recta en l'estudi sacroilíac.

Conclusions: Tots dos estudis van demostrar petites però significatives millors en els paràmetres de la marxa i reducció del dolor després de la manipulació osteopàtica. L'estudi cervical va mostrar efectes duradors en les puntuacions de disfunció i en els canvis específics de la marxa, mentre que l'estudi sacroilíac va revelar una millora en els moviments de les extremitats posteriors durant el galop sobretot cap el costat afectat. No obstant això, l'alta variabilitat entre subjectes i la grandària limitada de les mostres, especialment en els grups de control, van plantejar problemes d'interpretació.

Les recerques futures haurien de donar prioritat a mostres de major grandària, incorporar anàlisi de subgrups basats en patrons de moviment i emprar evaluacions multidimensionals. Aquestes haurien d'incloure escales de dolor, expressions facials i paràmetres qualitatius per a captar millor l'espectre complet dels efectes del tractament osteopàtic. A més, en la recerca osteopàtica equina s'hauria de posar l'accent en la comprensió dels mecanismes neuromusculars en lloc de centrar-se únicament en conceptes biomecànics. Els períodes de seguiment més llargs i les sessions de tractament múltiples poden proporcionar una visió més completa de l'eficàcia de la manipulació osteopàtica en cavalls.

Resumen

Título:

Una única manipulación osteopática de la articulación sacroilíaca y la columna cervical baja mejoran parámetros seleccionados de la marcha en caballos de deporte.

Antecedentes: Los tratamientos osteopáticos son ampliamente utilizados en caballos de deporte, pero sus efectos sobre la función locomotora no se han investigado a fondo utilizando medidas biomecánicas objetivas. Este estudio pretendía demostrar que una única manipulación osteopática dirigida a la articulación sacroilíaca o a la columna cervical inferior puede mejorar la simetría de la marcha equina, la amplitud de movimiento, la protracción o retracción de las extremidades y la calidad general del movimiento evaluada mediante unidades de medición inercial (IMU).

Métodos: Se realizaron dos estudios distintos en un entorno clínico con caballos en entrenamiento. En un estudio se manipuló la articulación sacroilíaca, mientras que en el otro se trató la unión cervicotorácica C6-C7. Se utilizaron nueve sensores IMU para evaluar la marcha equina antes, inmediatamente después, 3 días después y 15 días después de la manipulación osteopática. También se realizaron puntuaciones visuales del grado de cojera y exámenes osteopáticos, incluidas evaluaciones del dolor.

Resultados: Se observaron mejoras significativas tras las manipulaciones osteopáticas cervicales y sacroilíacas. En el caso de la disfunción cervical, la interacción entre los factores grupo y tiempo redujo significativamente las puntuaciones de disfunción cervical ($p <0,05$). A los 15 días postratamiento, el grupo de tratamiento mostró una puntuación media de 1,11 (IC: 0,72-1,71) frente a 2,30 (IC: 1,41-3,74) en el grupo de control. Se encontraron dos cambios significativos al trote: mayor protracción del miembro posterior derecho en línea recta y mayor protracción del miembro anterior derecho a la cuerda a la derecha. Esta última se vio influida significativamente por la interacción

entre grupo y tratamiento ($p < 0,05$), mostrando el grupo de tratamiento 27,11 (IC: 26,34-27,86) frente a 29,52 (IC: 28,26-30,77) en el grupo de control a los 15 días post-tratamiento.

En cuanto a la disfunción sacroilíaca, la manipulación osteopática redujo significativamente el tono muscular del dorso, el dolor de espalda y el dolor sacroilíaco, y los efectos persistieron al menos 15 días después de la intervención. El análisis biomecánico reveló una reducción significativa de la protracción de las extremidades posteriores ($F(1,3) < 3,432$, $p < 0,05$) y un aumento de la retracción ($F(1,3) < 3,681$, $p < 0,05$), sobre todo al galopar hacia el lado afectado. Se observó una mayor amplitud de movimiento de la cabeza al galope hacia el lado no afectado. No se encontraron resultados concluyentes para los pasos en línea recta en el estudio sacroilíaco.

Conclusiones: Ambos estudios demostraron pequeñas pero significativas mejoras en los parámetros de la marcha y reducción del dolor tras la manipulación osteopática. El estudio cervical mostró efectos duraderos en las puntuaciones de disfunción y en los cambios específicos de la marcha, mientras que el estudio sacroilíaco reveló una mejora en los movimientos de las extremidades posteriores durante el galope sobretodo hacia el lado afectado. Sin embargo, la alta variabilidad entre sujetos y el tamaño limitado de las muestras, especialmente en los grupos de control, plantearon problemas de interpretación.

Las investigaciones futuras deberían dar prioridad a muestras de mayor tamaño, incorporar análisis de subgrupos basados en patrones de movimiento y emplear evaluaciones multidimensionales. Éstas deberían incluir escalas de dolor, expresiones faciales y parámetros cualitativos para captar mejor el espectro completo de los efectos del tratamiento osteopático. Además, en la investigación osteopática equina debería hacer hincapié en la comprensión de los mecanismos neuromusculares en lugar de centrarse únicamente en conceptos biomecánicos. Los periodos de seguimiento más largos y las sesiones de tratamiento múltiples pueden proporcionar una visión más completa de la eficacia de la manipulación osteopática en caballos.

Summary

Title:

Osteopathic single manipulation of the sacroiliac joint and lower cervical spine improves selected gait parameters in sport horses.

Background: Osteopathic treatments are widely used in sport horses, but their effects on locomotor function have not been thoroughly investigated using objective biomechanical measures. This study aimed to demonstrate that a single osteopathic manipulation targeting the sacroiliac joint or lower cervical spine can improve equine gait symmetry, range of motion, protraction or retraction of the limbs and overall movement quality as assessed by inertial measurement units (IMUs).

Methods: Two separate studies were conducted in a clinical setting using horses in training. In one study, the sacroiliac joint was manipulated, while in the other, the C6-C7 cervico-thoracic junction was treated. Nine IMU sensors were used to evaluate equine gait before, immediately after, 3 days after, and 15 days after the osteopathic manipulation. Visual lameness degree scoring and osteopathic examinations, including pain assessments, were also performed.

Results: Significant improvements were observed after both cervical and sacroiliac osteopathic manipulations. For cervical dysfunction, the interaction between group and time factors significantly reduced cervical dysfunction scores ($p < 0.05$). At 15 days post-treatment, the treatment group showed a mean score of 1.11 (CI: 0.72-1.71) compared to 2.30 (CI: 1.41-3.74) in the control group. Two significant changes were found at trot: increased protraction of right hindlimb in a straight line and increased right forelimb protraction when lunging to the right. The latter was significantly influenced by group and treatment interaction ($p < 0.05$), with the treatment group showing 27.11 (CI: 26.34-27.86) versus 29.52 (CI: 28.26-30.77) in the control group at 15 days post-treatment.

For sacroiliac dysfunction, osteopathic manipulation significantly reduced back muscle tone, back pain, and sacroiliac pain, with effects persisting at least 15 days post-intervention. Biomechanical analysis revealed significant reduction in hindlimb protraction ($F(1,3) < 3.432$, p

<0.05) and increase in retraction ($F(1,3) < 3.681$, $p < 0.05$), particularly when cantering towards the affected side. Increased head range of motion was observed when cantering towards the unaffected side. No conclusive results were found for passes in a straight line in the sacroiliac study.

Conclusions: Both studies demonstrated small but significant improvements in gait parameters and pain reduction following osteopathic manipulation. The cervical study showed lasting effects on dysfunction scores and specific gait changes, while the sacroiliac study revealed improvement in hindlimb movements during canter. However, high inter-subject variability and limited sample sizes, particularly in control groups, posed challenges in interpretation.

Future research should prioritize larger sample sizes, incorporate subgroup analyses based on movement patterns, and employ multidimensional assessments. These should include pain scales, facial expressions and qualitative parameters to better capture the full spectrum of osteopathic treatment effects. Additionally, understanding neuromuscular mechanisms rather than focusing solely on biomechanical concepts should be emphasized in equine osteopathic research. Longer follow-up periods and multiple treatment sessions may provide more comprehensive insights into the efficacy of osteopathic manipulation in horses.

Justification:

Osteopathic and chiropractic treatments in sport horses have been in constant development, have become widely accepted by the veterinary community [1,2] and they are especially valued by riders and well received by the horses. In humans, numerous studies have shown the improvement obtained by osteopathic treatment in athletes by measuring objective values such as range of motion, degrees of pain reduction, increased endurance and spatial awareness [3,4]. Empirically, manual therapy has the capacity to improve equine motor skills. There are studies that show that osteopathic therapy of the back of the horse [5–7] improves certain motor qualities; there are very few studies evaluating its effects on the cervical area and none about the effects of osteopathic manual therapy in distal limbs and other regions such as the pelvis[8].

It is very important to know the effects of osteopathic treatment of the sacroiliac joint and neck in horses, as this information is currently lacking. It is necessary to know the effects of a single osteopathic manipulation. Most studies treat the horse as a whole, which is the norm in equine [9] and human osteopathy [10], but it is important to know the isolated effects of a single manipulation on horses.

The use of objective tests to measure the effect of osteopathic manipulation in horses has been developed using high speed cameras and markers in equine biomechanics centres [11] or with semi-objective methods such as pressure algometry [9] but recently the use of force inertial sensors [12] has been incorporated for kinematic evaluation. Inertial measurement units IMUs have the advantage that they can be used out of the lab setting and on clinical patients and competition horses, which are the most common receptors of manual therapy and equine osteopathy.

Background:

Several studies show that at a purely locomotor level, vertebral fixations or pain in the vertebral areas are accompanied by compensations and irregularities in horse locomotion and that often, the primary cause of the compensation is the distal limb [13,14]. There is also evidence that osteopathic treatment of the horse's back modifies the biomechanics not only of the spine itself but also of the limbs [11]. Similarly, forelimb [15] and hindlimb [16] injuries modify the biomechanics of the spine. However, there is no information on the effects of osteopathic manipulation in the distal limbs, pelvis [17,18] and neck on the biomechanics of the horse.

Manual therapy is a broad term which may include osteopathy and chiropractic and to avoid terminology confusion, literature from both fields will be included in this study and referred to as osteopathy. Since in veterinary terms, "blockage" or "block" can be confused with a neural block, the term joint fixation will be used as a synonym of vertebral or osteopathic dysfunction.

There are studies that report good clinical results of osteopathic and chiropractic treatments by subjective or semi-objective examinations [18–20] and only a few have been done using inertial sensors or image capturing systems. These have not shown relevant biomechanical changes [18].

Other studies have looked at joint motion, range of motion of certain segments, especially the spine, before and after manipulations [21]. These studies reveal a clear difference in dorsal mobility that can be maintained for up to 3 weeks [22].

Clinical experience suggests that forelimb lameness is more closely associated with cervical pain and dysfunction and that horses with back pain may have concurrent hind limb lameness issues [23–25].

The effect of osteopathic manipulations on the reduction of back pain has also been determined, in comparison with massage or even phenylbutazone [19] or in comparison with other physiotherapeutic

treatments such as low frequency intensity laser therapy [20]. There are also studies that evaluate the effects of osteopathy on muscle function [26], and others assessing the effect of chiropractic on static bioimpedance and dynamic acoustic myography [27].

No studies that demonstrate a decrease in lameness, or an improvement in the symmetry of the horse's gait or movement have been reported. More research is necessary to investigate the effect and magnitude of axial skeleton pain and stiffness with inertial parameters [18], as a standard objective method. Even if riders feel an improvement in the horse's mobility and comfort with increases in symmetry, stride length, and more willingness to train, science has not been able to objectively measure the improvement reported by riders and observed by veterinarians in clinical evaluations at different gaits and performing specific dressage movements.

The efficacy of manual therapies in horses remains controversial, with variability in treatment outcomes reported across studies [8]. Objective evaluation of subtle changes in movement symmetry and performance with inertial measurement units (IMUs) before and after therapy may provide greater insight on treatment effects. IMUs incorporate accelerometers and gyroscopes to measure three-dimensional motion, allowing assessment of temporal, postural, and coordination variables that may be altered by neuromusculoskeletal dysfunction [28,29]. Recent equine studies have demonstrated the sensitivity of IMU sensors to detect induced lameness [30] as well as effects of diagnostic analgesia [31,32]. Applying similar IMU methods to analyse responses to manual treatment may offer more reliable quantification of changes in movement quality and consistency. The establishment of standardized IMU protocols for evaluation of therapies could improve understanding of optimal techniques for specific equine disorders. Studies with IMU sensors that objectively evaluate traditional manual therapies in horses are therefore warranted.

The term "somatic dysfunction" is commonly used in osteopathy to describe the findings or issues identified and treated by osteopaths. This diagnosis is based on the TART criteria, which stands for

Tenderness, Asymmetry, Restriction of mobility, and Texture changes. However, the diagnostic capacity of palpation and the inter-operator reliability of this model have been questioned. Some authors have raised concerns about the low inter-operator reliability, especially in terms of restriction of mobility and texture changes, as well as the high frequency and low clinical relevance of asymmetries [33]. Additionally, the poor specificity and clinical diagnostic accuracy has been highlighted, suggesting that these palpitory findings may be caused by different issues [33]. Despite the lack of consensus in the definition, somatic dysfunction is defined as the impaired or altered function of related components of the somatic system, including skeletal, arthrodial, and myofascial structures, and related vascular, lymphatic, and neural elements. It has been associated with disorders of muscles, tendons, and the skeleton, with common clinical manifestations of pain, reduced muscle movement, and decreased limb/spine mobility in patients with musculoskeletal conditions [34]. Furthermore, increased segmental muscle activity and sympathetic nervous system output at spinal levels associated with clinically detected segmental dysfunctions, support the concept of somatic dysfunction [35]. Osteopathic practitioners use manual techniques to both diagnose and treat somatic dysfunction [36]. The concept of somatic dysfunction has also been recognized by governments and medical institutions within the professional profile of osteopaths, indicating its significance in clinical practice [37]. Additionally, the correlation of palpitory findings of somatic dysfunction with objectively confirmed gastrointestinal abnormalities has been explored, suggesting a potential link between somatic dysfunction and visceral pathology [38].

Somatic dysfunction is not associated with a typical “veterinary injury”; often, horses with these dysfunctions do not show externally obvious pain or lameness or the changes may be very subtle, but expert riders may notice small alterations in locomotion that cause difficulty in certain exercises and decrease the horse's performance. Some osteopaths compare the treatment of horses with these symptoms with fine-tuning a piano. Osteopaths treat somatic dysfunctions in different axial and appendicular joints associated with fascial, muscular, and

visceral dysfunctions and explained by a reduction in nerve conduction and blood flow. These changes are difficult to objectify.

Hypothesis:

The main hypothesis of this PhD thesis is that a single osteopathic manipulation performed by a skilled and well-trained equine osteopath can improve the horse's locomotor pattern, as evaluated by inertial measurement units (IMUs) and classical veterinary and osteopathic examination. Specifically, it is hypothesized that osteopathic manipulations targeting the sacroiliac joint (SIJ) and the cervical-thoracic hinge (C6-C7-T1) can lead to measurable improvements in the horse's gait symmetry, protraction and retraction, and overall movement quality.

This hypothesis is based on the following premises:

1. Somatic dysfunctions, defined as impaired or altered function of the musculoskeletal system, can affect equine motor skills and performance, even in the absence of overt pain or lameness [34,39].
2. Osteopathic manipulations have been shown to improve spinal mobility and reduce back pain in horses [19,40]
3. Clinical observations suggest that forelimb lameness may be associated with cervical pain and dysfunction, while hindlimb lameness may be linked to back pain and pelvic dysfunctions [41,42]
4. IMUs have demonstrated sensitivity in detecting subtle changes in movement symmetry and compensatory patterns associated with lameness and musculoskeletal dysfunctions [12,43]

By objectively evaluating the effects of targeted osteopathic manipulations on equine biomechanics using IMUs, this thesis aims to provide evidence for the efficacy of manual therapies in improving equine locomotor function and performance.

Objectives:

The main objective of this thesis is to investigate if a single osteopathic manipulation performed by a skilled and well-trained equine osteopath improves the horse's locomotor pattern using objective gait analysis (IMU sensors) in addition to classical veterinary and osteopathic evaluation

Specific objectives include:

1. To assess the immediate, short-term (3 days), and longer-term (15 days) effects of a single osteopathic manipulation on the horse's movement symmetry, ROM, limb protraction and retraction and overall gait quality, as measured by IMU sensors.
2. To compare the sensitivity and reliability of IMU sensors in detecting subtle changes in equine gait following osteopathic interventions in comparison to other classical methods, like lameness evaluation and physical exam.
3. To establish standardized IMU protocols for the objective evaluation of manual therapies in horses, contributing to the understanding of optimal techniques for specific equine musculoskeletal disorders.
4. To explore the potential mechanisms by which osteopathic manipulations facilitate improved movement and performance in horses, considering factors such as pain reduction, increased range of motion, and neuromuscular coordination.

5. To suggest improvements for further studies in the objective evaluation of osteopathic techniques, both from the biomechanical point of view and from other possible aspects.

By achieving these objectives, this thesis aims to provide scientific evidence for the efficacy of osteopathic manipulations in improving equine locomotor function, contributing to the development of evidence-based practices in equine manual therapy and enhancing our understanding of the biomechanical effects of these interventions.

Original Article 1

Title

Effect of a single osteopathic manipulation of dysfunctional caudal cervical vertebrae in horses

Authors

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Abstract

Background: The equine neck plays a crucial role in posture and locomotion, and cervical dysfunction can lead to pain and abnormal movement patterns, which seems to improve with osteopathic treatments,

Objectives: To evaluate the effects of a single osteopathic manipulation (OM) of the caudal cervical spine on gait parameters, pain and muscle tone in sport horses with cervical vertebral dysfunction.

Study Design: Blinded, randomised, controlled trial.

Materials and Methods: 27 sport horses with dysfunctional C6-C7 were randomly allocated to a treatment group (TG) (n=19) receiving a single osteopathic manipulation or to a control group (CG) (n=8) without any treatment. Osteopathic evaluation (scoring of various variables), semi-objective lameness scoring, and objective gait analysis using inertial measurement sensors were performed at walk and trot in straight line and in circles and at canter during lunging, before, 15 minutes after, and 3 and 15 days after treatment. Results were analysed by generalised linear mixed models.

Results: Vertebral dysfunctions score was significantly reduced by the interaction between group and time factors ($p <0.05$ before OM, TG: (mean (confidence intervals)) 2.83 (2.10-3.81), CG: 2.23 (1.41-3.74); 3 days, TG: 1.06 (0.68- 1.67), CG: 1.80 (1.06-3-12); and 15 days, TG: 1.11 (0.72-1.71), CG: 2.30 (1.41-3.74) after OM, pain and muscle tone scores also decreased slightly. Two significant changes were found at trot. In straight line on protraction on the right hindlimbs, and in lunging to the right a significant increasing on the right forelimb protraction. The latter was significantly influenced by the interaction between group and treatment ($p <0.05$; before OM, TG: 28.24 (27.37-29.14), CG: 28.43 (26.97-29.82); 15 min, TG: 27.78 (26.98-28.59), CG: 28.82 (27.41-30.22); 3 days, TG: 26.53 (25.64-27.45), CG: 28.67 (27.23-30.10); and 15 days, TG: 27.11 (26.34-27.86), CG: 29.52 (28.26-30.77).

Main Limitations: The effects were relatively subtle, which may be difficult to detect even with the gait analysis tool used.

Conclusion: A single osteopathic cervical manipulation resulted in some small but positive changes in locomotion (protraction and retraction) and functional parameters including decreased pain and muscle tone. Further research with longer follow-up and more than one treatment is needed.

Key words:

Equine, Osteopathy, Chiropractic treatment, Manual therapy, Cervical dysfunction, Locomotion asymmetry

1. Introduction

The anatomy and biomechanics of the equine neck are complex, and its role is not only supporting the head, but contributing to maintaining posture and locomotion[1]. The neck is vulnerable to a range of pathologies that can cause pain and discomfort, thus, changing horses' movement patterns.

The relationship between poor performance in sport horses, abnormal locomotion and lower cervical dysfunction has been well established [2,3]; for example, forelimb lameness in horses has been sometimes associated with radiographic abnormalities of one or more cervical vertebrae [4] and with lower cervical radiculopathy [5]. The athletic demands of sport horses often result in pain in lower cervical vertebrae, the cervicothoracic junction, and related structures in middle aged, and even in younger horses [6] on which cervical pain is often overlooked. Exercise overload may also result in excessive muscular tension, which is described as a condition where muscles remain semi-contracted for a prolonged period, sometimes used as an indicator of acute stress in horses [6], resulting in neck stiffness, reducing range of motion and creating pain and loss of flexibility [7].

Similarly, pathologies such as cervical vertebral malformation and Wobbler Syndrome can cause arthritis of articular facets [8] and bursitis [9]; and vertebral misalignment and/or dynamic instability [11] may cause cervical spinal cord compression, neck pain and proprioceptive deficits leading to significant changes in locomotion and performance [2,3].

Early detection and treatment of neck disorders are essential to prevent further damage and dysfunction [10]. The importance of applying a more holistic approach to prevent cervical pain injuries from happening is paramount in horses in training and competition. In order to address these issues once present, various therapeutic approaches, together and/or besides conventional treatments can be used including physiotherapy and chiropractic and osteopathic approaches [11].

Somatic dysfunction from a vertebral segment is considered a central concept in the theory and practice of osteopathy, Somatic dysfunction can have different origins (e.g. articular, muscular or fascial origin) and different consequences at muscular, neuromuscular, fascial, visceral, neural, and even emotional level. Although the term is being reconsidered due to unclear

pathophysiology and poor reliability of detection[12]; other terms such as vertebral dysfunction, osteopathic dysfunction may be more straightforward and are more frequently used in veterinary medicine.

As well as the colloquial terms "block" or "fixation". Cervical dysfunction is defined by altered muscle tone, tenderness, pain, loss of function or subtle changes in gait, that occur in the absence of pathological lesions and are related to abnormal function of the neuromuscular system[13]. Some studies treating cervical and thoracic intervertebral joints using dynamic mobilisation exercises have shown successful results improving cervical flexion [14]. There are also positive effects of chiropractic [15] or osteopathic [16] manipulation on vertebral range of motion and on reducing pain in horses with back pain [17]. Colles reports significant improvements in the degree of chronic forelimb lameness after osteopathic treatment in a group of 51 horses with somatic dysfunction in the neck or back and no other diagnosed forelimb pathology and no improvement with standard veterinary treatment [13]. However, there are few reports of the effect of manual therapy on the equine neck region using chiropractic or osteopathic techniques [18,19]. In human medicine, there is a long-standing debate about the differences between

osteopathy and chiropractic, which have a common origin and are relatively similar because both have similar principles: both are applied over bones, muscles, and connective tissue, using the hands to diagnose and treat abnormalities of structure and function [20]. There are other terms that include manual techniques that were apparently very similar despite the efforts of certain groups to individualise them, such as manipulative therapy [20], forced-based manipulation [21] and spinal manipulation [22]. In comparative studies between different manual techniques, even with or without thrust, no important differences between the different techniques have been found [23].

Osteopathic treatment to alleviate mild discomfort in the horse's neck is a common practice in competition horses, even in the absence of documented pathology through diagnostic tests and imaging. In general, riders report good results from these practices [24], with an improvement of horses' back flexibility, lateral bending, ease of collection and overall locomotion. There is literature on this subject in humans that supports its use for treating vertebral dysfunction, muscle tension, reduced mobility and neck [25] and back pain [26,27] however

the mechanisms that result in these benefits remain yet to be fully understood.[12,28]. To the knowledge of the authors, there are no studies on the effect of osteopathic treatment in the neck of horses.

The aim of this study was to evaluate the effects of an isolated osteopathic manipulation of the caudal cervical spine on the locomotion, pain reduction and neck mobility in horses with history and clinical examination consistent with cervical dysfunction and presenting mild neck discomfort and pain. Gait (upper body symmetry: head, withers, and sacrum); stride length, stride duration and, protraction and retraction of the limbs; lameness, pain, muscle tone and vertebral dysfunction before and after applying the manipulation of the horses in the treatment group was analysed and compared with the horses in the control group.

2. Material and methods

2.1 Study design

This was a blinded, randomised, controlled clinical trial with two separate groups of horses (Figure 1).

2.2. Horses and inclusion/exclusion criteria

A total of 27 sport horses, with osteopathic cervical dysfunction of the caudal cervical region (C6-C7) were selected from several riding centres. The horses were randomly allocated into an osteopathic treatment group (TG) (n= 19) and a control group (CG) (n=8).

Inclusion Criteria:

- Active sport horses aged 6 to 14 years in a regular training program at medium-high level for the past 6-12 months, with a minimum of 3-5 days per week of directed work.
- Disciplines: dressage and show jumping.
- Rider complaint of discomfort during lateral bending, circling, and/or specific dressage exercises.
- Osteopathic dysfunction (fixation) of vertebrae cervical (C) 6-7.

Exclusion Criteria:

- Musculoskeletal injury diagnosed or treated in the last 3 months and any invasive medication in the last 3 months, such as intraarticular injection.
- Horses with clearly defective conformation of the back and/or limbs.
- Horses without rigorous monitoring of their work.
- Evidence of cervical joint arthrosis, fractures, or other injuries on radiographic or ultrasound imaging from their clinical history.
- Horses presenting obvious lameness of more than 2 out of 5 grades in the American Association of Equine Practitioners (AAEP) scale.

2.3. Intervention design

The TG received a single osteopathic manipulation (OM) using direct techniques, described below, targeting only the lower cervical segments, while the control group received no treatment. An expert licensed equine physiotherapist and osteopath performed all the

evaluations and delivered the manipulation to the treatment group, always utilising the same direct high-velocity, low-amplitude thrust technique, in the lower cervical segments C6-C7, consisting of, just at the end-field point of cervical rotation at the fixation point, a thrust on the transverse apophysis of the cervical vertebra to induce a rotation while inducing a latero-flexion of the neck towards the ipsilateral side of the cervical fixation with the one hand (video1). All evaluations and treatments were performed with the horses standing quietly in a familiar environment on a firm and even ground to minimise stress and variability.

During the initial evaluation, the direction of the osteopathic fixation (right or left rotation) presented by the horses was determined and it was manipulated accordingly. In the treatment group (n=19) 4 horses had a fixation in left rotation (21%) and 15 in right rotation (79%); in the control group (n=8) 2 horses had a fixation in left rotation (25%) and 6 in right rotation (75%).

2.4 Evaluation

Osteopathic physical evaluation parameters were recorded on days 1 (pre-treatment), 3, and 15 after treatment performed qualified

osteopath and physiotherapist. Videos for lameness examination and lameness scoring were recorded and objective biomechanical data was captured on day 1 (pre-treatment and 15 minutes post-treatment), day 3, and day 15 after treatment. The lameness examination was performed by two blinded, expert equine practitioners and the objective biomechanical analysis was performed by a trained member of the research team.

2.4.1. Osteopathic physical examination and scoring

The osteopathic physical evaluation included the following parameters: muscle tone, pain, vertebral dysfunction and manual cutaneous reflexes assessments. The neck, back and sacroiliac regions of each horse was individually scored from 0-3 for muscle tone, sensitivity/pain (by applying manual light pressure between 5 and 10 cm beside the midline of the spinous processes), vertebral dysfunction was determined by vertebral fixation score (hypomobility, sensitivity and/or tight joints) as reported in human [29,30]. The reflexes evaluated and scored (from 0-3) were both thoracic flexion and extension, lumbar extension, both lumbosacral flexion and extension, and both right and left latero-flexion as reported in horses [31–33]. These parameters were

evaluated using a functional evaluation scale (Table 1), with slight modifications of the scoring proposed in previous studies[34–36].

Table 1. Scoring for evaluation of muscle tone, pain, vertebral dysfunction of three areas: neck, back, and sacroiliac during osteopathic physical examination (modified from [34-36]).

Clinical Parameter	Scoring			
	0	1	2	3
Muscle tone				
Manual evaluation muscle tone of the muscular groups	Soft musculature with no avoidance	Stiff musculature with no avoidance	Stiff musculature with avoidance characterized by lordosis or movement away pressure	Stiff musculature with avoidance characterized by kicking or biting
Pain				
5-10 cm off midline, abaxial to spinous process	No obvious avoidance	Avoidance and lordosis of spine or movement away from pressure	Avoidance plus tossing of head and/or tail swish	Violent avoidance characterized by kicking biting or jumping
Vertebral Fixations				
Osteopathic assessment of spine	No fixation	1 segment fixated	2 segments fixated	3 or more segments fixated
Cutaneous reflexes				
Manual reflex evaluation	Normal reflex	Decreased reflex with no clear pain (avoidance)	Clear pain (avoidance) or complete absence of reflex	Violent avoidance characterized by kicking biting or jumping

2.4.2. Lameness semi-objective scoring

The lameness examination was carried out by evaluating video recordings in a straight line for 40 metres at walk and trot, and in circles to the left and right on a semi-firm surface (arena used for competition) at walk, trot and canter. Each limb of each horse was independently graded for lameness according to the AAEP lameness scale from 0 to 5, with the modification of considering 0.5 degrees of change.

Horses were lunged in an arena familiar to them, at times of the day when they could be calm to avoid overexcitement and to work in a way as close as possible to the horse's usual routine. In straight line passes, the aid of a Global Positioning System (Garmin Fenix V GPS^{a)} on the handler's wrist was used in order to maintain the same speed for each horse. At the lunge, the passes were performed by the same handler, maintaining the conditions as similar as possible in all passes.

2.4.3 Kinematic data collection

For the objective locomotion evaluation, 9 inertial measurement units (IMU's) (ProMove-mini, 200Hz Equimoves system[®]) ^c were used. IMUs were placed lateral to the cannon bones of the 4 limbs, top of the head, withers, sacrum, right tuber-coxae and left tuber-coxae (Picture 1); the results were calculated by the Equimoves validated system for equine

gait analysis [37] The following parameters were analysed: maximal protraction (MP) and maximal retraction (MR) of the limbs (left forelimb (LF), right forelimb (RF), left hindlimb (LH), right hindlimb (RH)), vertical displacement of the head minimum difference (HMindiff), head maximum difference H(Maxdiff), withers minimum difference (WMindiff), withers maximum difference (WMaxdiff), sacrum minimum difference (SMindiff), also known as pelvis (PMindiff), sacrum (pelvis) maximum difference (SMaxdiff), vertical head, withers and sacrum range of motion (ROM), stride duration, stance duration, swing/stance phases duration at walk and trot (in straight line and lunging to the left and right), and at canter (lunging to the left and right).



Picture 1. Video recording and Kinematic data collection of horse when cantering to the left. IMUs were placed lateral to the cannon bones of the 4 limbs, top of the head, withers, sacrum, right tuber-coxae and left tuber-coxae.

2.5. Statistical analysis

Data were analysed by using the free statistical software (JASP (JASP 0.18.3 (Intel) ^b). Data from osteopathic physical evaluation using functional evaluation scale, lameness scoring, and objective biomechanical analysis were evaluated by generalized linear mixed models (GLMMs). Discrete variables, particularly those derived from observations from the osteopathic physical evaluation were analysed by using the Poisson distribution family and the Log as link function, whereas variables from lameness scoring and objective biomechanical analysis (numeric continuous variables) were evaluated by using the Gaussian distribution family and the identity was used as a link function. For the osteopathic physical evaluation, the fixed factors included in the model were time (with 3 levels: 1= before OM, 2= 3 days after OM, and 3= 15 days after OM), treatment (with 2 levels: TG and CG), anatomical region (with 3 levels: neck, back and sacroiliac region), and their interaction.

For lameness scoring and biomechanical data the fixed factors included were time (with 4 levels: 1= before OM, 2= 15 min after OM, 3= 3 days after OM, and 4= 15 days after OM)), treatment (with 2 levels: TG and CG) and their interaction. In all the models, the horse ID was

declared as a random factor. To note, these variables were evaluated independently for each gait (walk, trot and canter) at straight, and circling to the right and left directions. In these cases, once the initial model was considered as significant, a Bonferroni post-hoc test was used to establish differences between the fixed factors and their interaction. A $p < 0.05$ was accepted as significant for all the tests.

In the initial validation of the models, two additional fixed factors were included: 1) anatomic side of the fixation (with two levels: left and right rotation), and 2) equestrian centre (with nine levels). Both fixed factors did not influence the models. Therefore, they were excluded from the modelling to simplify the study.

Sample size and power calculations were performed using the free online statistical software (GLIMMPSE (<https://glimmpse.samplesizeshop.org>)). Initially, preliminary data from 3 horses in each group were used to obtain this information. In general, the recommended sample size to evaluate each variable in the study was 8 horses per group with a β value >0.8 and a significance level (α) of 0.05.

3. Results

3.1. Osteopathic physical examination

At the initial evaluation, 21 of the 27 horses evaluated had right C6 or C7 fixations (78%) and 6 of the horses had left C6 or C7 fixations (22%). Muscle tone, pain and vertebral fixation score variables were significantly ($p < 0.001$) influenced by the anatomical region factor. The fixed factors time, group, and the interactions included in the model did not affect these variables supplementary Table 1 (Supplementary Table 1). Muscle tone score of neck and back were similar, but they were significantly reduced compared to the values registered for this variable in the sacroiliac region (Figure 2A). Pain and vertebral fixation scores ($p < 0.001$) were significantly different between the anatomic regions, with the highest values for neck, followed by back and sacroiliac region (Figure 2B &C). On the other hand, cutaneous reflexes were not influenced by the time, group, and the interactions between them evaluated in the study (Supp. Table 1).

Regarding the score for the osteopathic parameters, muscle tone was significantly affected ($p < 0.05$) by time factor (Figure 3A), pain was not affected by any of the fixed factors, whereas osteopathic fixation was significantly influenced by time factor (Figure 3B) ($p < 0.05$) and the interaction between time and group factors (before OM, TG: (mean

(confidence intervals)) 2.83 (2.10-3.81), CG: 2.23 (1.41-3.74); 3 days, TG: 1.06 (0.68- 1.67), CG: 1.80 (1.06-3-12); and 15 days, TG: 1.11 (0.72-1.71), CG: 2.30 (1.41-3.74) after OM, $p <0.05$ is shown in (Table 2) and (Figure 3C).

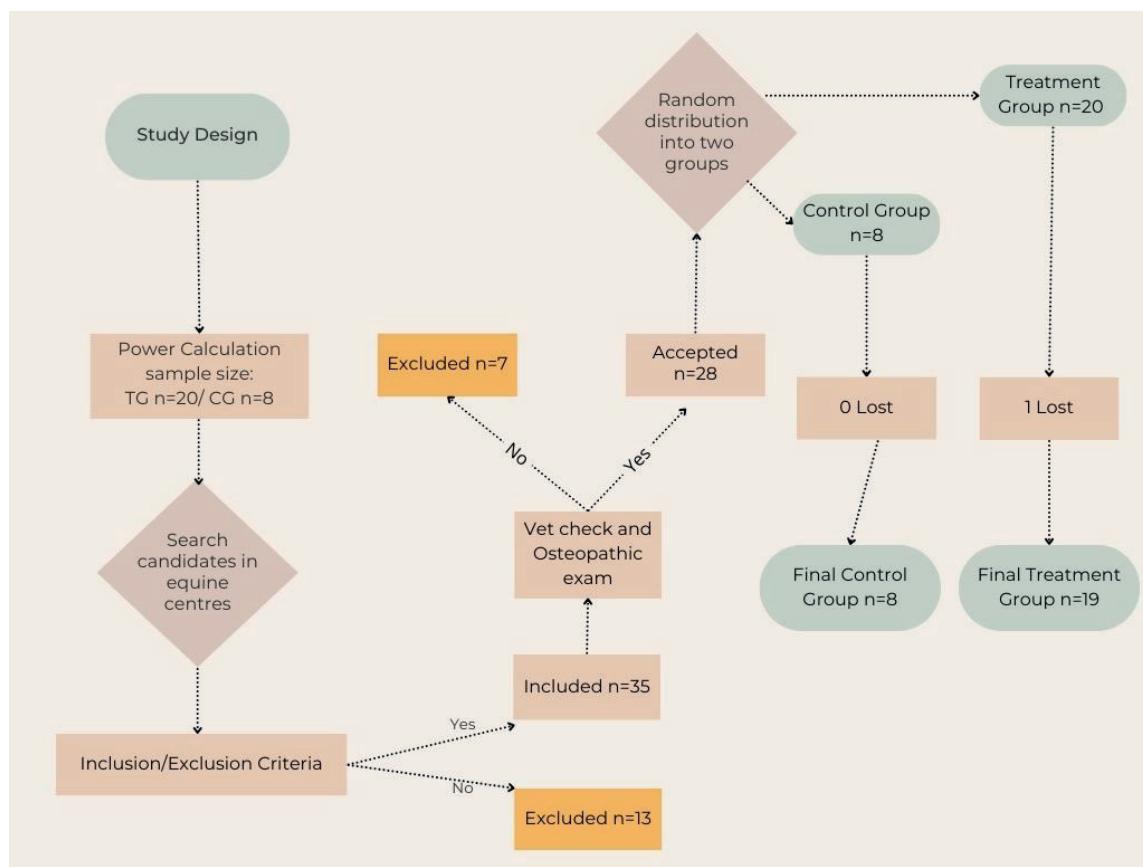


Figure 1. Flowchart horses' selection in a study describing the effect of a single osteopathic manipulation of dysfunctional caudal cervical vertebrae in horses.

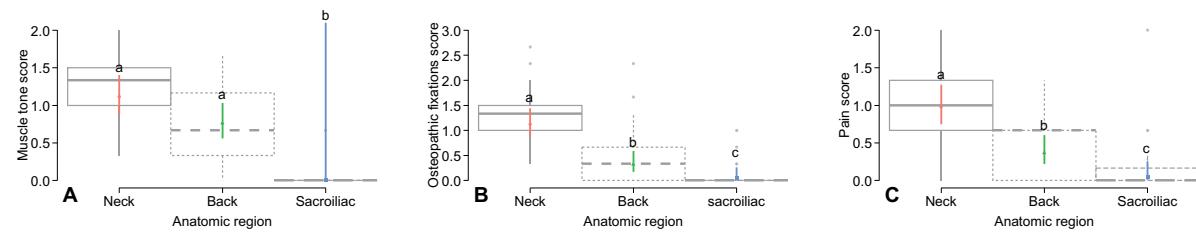


Figure 2. Box plots showing the means and their 95% confidence intervals (CIs) for (A) muscle tone (0-3), (B) Osteopathic fixations (score "n") and (C) pain (0-3) according to the osteopathic evaluation (table 1) performed before treatment in 27 number of horses (control and treatment group). ^{a-b}= different lowercase letters denote significant differences ($p < 0.001$) for the variables evaluated by the Bonferroni test between anatomic regions.

Table 2. P values of generalized linear mixed models evaluating the effects of the fixed factors individually and their interactions (*) with each other on osteopathic physical examination scoring.

Osteopathic clinical parameter	Effect	P value
Muscle tone	Group	0.778
	Time	0.988
	Anatomical region (AR)	< .001
	Group * Time	0.984
	Group * AR	0.647
	Time * AR	0.791
	Group * Time * AR	0.994
Pain	Group	0.641
	Time	0.153
	Anatomical region (AR)	< .001
	Group * Time	0.663
	Group * AR	0.442
	Time * AR	0.949
	Group * Time * AR	0.488
Osteopathic block	Group	0.168
	Time	0.393
	Anatomical region (AR)	< .001
	Group * Time	0.205
	Group * AR	0.903
	Time * AR	0.182
	Group * Time * AR	0.051

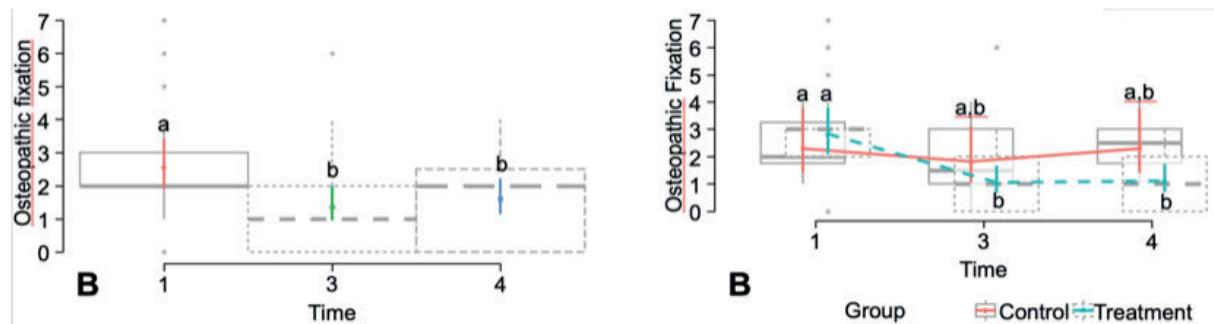


Figure 3. Box plots showing the means and their 95% confidence intervals (CIs) for (A) total osteopathic fixations according time factor in all horses n=27, (B) Total osteopathic fixations according to the interaction between treatment group n= 19 and control group n=8 and time factors 1: before, 3: day 3, 4: day 15. Osteopathic evaluation performed according to evaluation scale (table1) ^{a-b}= different lowercase letters denote significant differences ($p < 0.001$) for the variables evaluated by the Bonferroni test between anatomic regions.

3.2. Lameness scoring

Horses with a degree of lameness higher than 1.5 were excluded from the study, and the majority (>90%) exhibited no or minimal lameness (0.5/5). No statistically significant differences were observed in any of the evaluated limbs for any of the variables studied (time, group, and time-group interaction) across all gaits (walk, trot, and canter) in both straight lines and circles. (Supplementary Table 2).

3.3. Objective biomechanical analysis

All biomechanical variables were studied in all gaits. The majority of the parameters studied did not show significant differences including upper body symmetry parameters in relation to the treatment group factor, time factor, or their interaction. Statistically significant changes found are reported below.

3.3.1. Walk

The only variable significantly influenced by the time factor ($p < 0.001$) when walking on straight line was the MaxProtraction of the right hind limb (MPRH) (Figure 4A) and (Supplementary Table 3).

When walking in circles, the results differed slightly between left and right directions. On the left side, only vertical head ROM was significantly increased by the time factor ($p < 0.05$) (Supplementary Table 4).

In contrast, no significant differences were detected in any of the evaluated biomechanical variables when walking in circles to the right side (Supplementary Table 5).

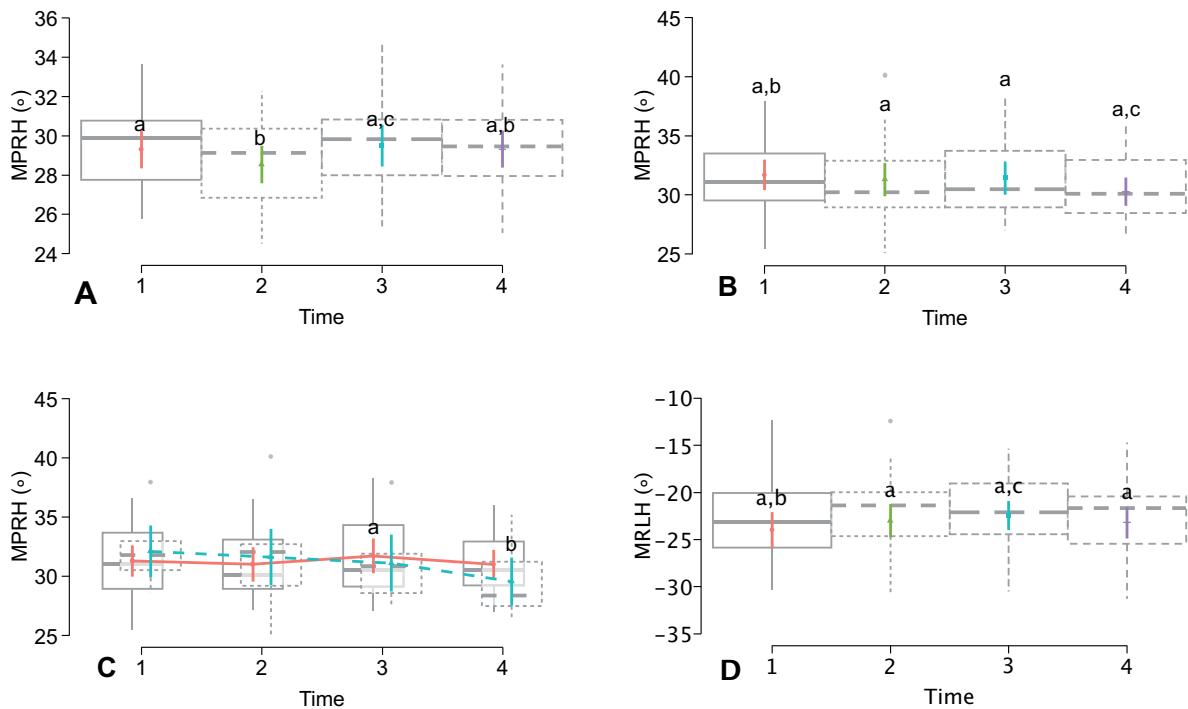


Figure 4. Generalized linear mixed models (GLMM) Box plots indicating means ($^{\circ}$) and their 95% Confidence intervals (CIs) for **A)** Maximal Protraction (MP) of the right hindlimb (RH) according to time factor at walking in straight line. **B)** MPRH according to time factor. **C)** MPRH according to treatment per time factors at trotting on straight line. **D)** Maximal retraction (MR) of left hindlimb (LH) values according to time factor at trotting on straight line. 1=before osteopathic manipulation (OM), 2= 15 min after OM, 3= 3 days after OM, and 4= 15 days after OM). $a-b$ = different low-case letters denote significant ($P < 0.01$) differences by the Bonferroni's test.

3.3.2. Trot

At trot in a straight line, several biomechanical parameters showed significant changes. The MaxProtraction of the right hind limb (MPRH) was significantly affected by both the time factor ($p < 0.001$) (Figure 3B) and the interaction between group and time factors ($p < 0.05$) (Figure

3C). Additionally, the MaxRetraction of the left hind limb (MRLH) was significantly affected by the time factor ($p < 0.05$) (Figure 3D). The stance duration of the left hind limb was also affected by the time factor ($p < 0.05$). The remaining evaluated biomechanical parameters were not significantly affected by the fixed factors or their interaction in the models (supplementary Table 6).

Trotting in circles revealed more pronounced differences between the treatment groups. When trotting to the left side, MaxProtraction of the left hind limb (MPLH) and MaxRetraction of the left hind limb (MRLH) were both significantly influenced by the group factor ($p < 0.001$ for both) (Figures 5A & 5B). Additionally, the vertical pelvic ROM was affected by the group factor ($p < 0.05$) (supplementary Table 7).

When trotting in circles to the right, several parameters showed significant changes. The MaxProtraction of the right front limb (MPRF) was significantly influenced by both the group factor ($p < 0.05$) (Figure 5C) and the interaction between group and time factors ($p < 0.05$; before OM, TG: 28.24 (27.37-29.14), CG: 28.43 (26.97-29.82); 15 min, TG: 27.78 (26.98-28.59), CG: 28.82 (27.41-30.22); 3 days, TG: 26.53 (25.64-27.45), CG: 28.67 (27.23-30.10); and 15 days, TG: 27.11 (26.34-27.86), CG: 29.52 (28.26-30.77) (Figure 4D). The MaxProtraction of the right hind

limb (MPRH) and MaxRetraction of the right hind limb (MRRH) were significantly affected by the group factor ($p = <0.001$ and $p < 0.05$), respectively) (Figures 6A & 6B). Similar to the left side, the sacrum ROM was also affected by the group factor ($p < 0.05$) when trotting to the right (supplementary Table 8)

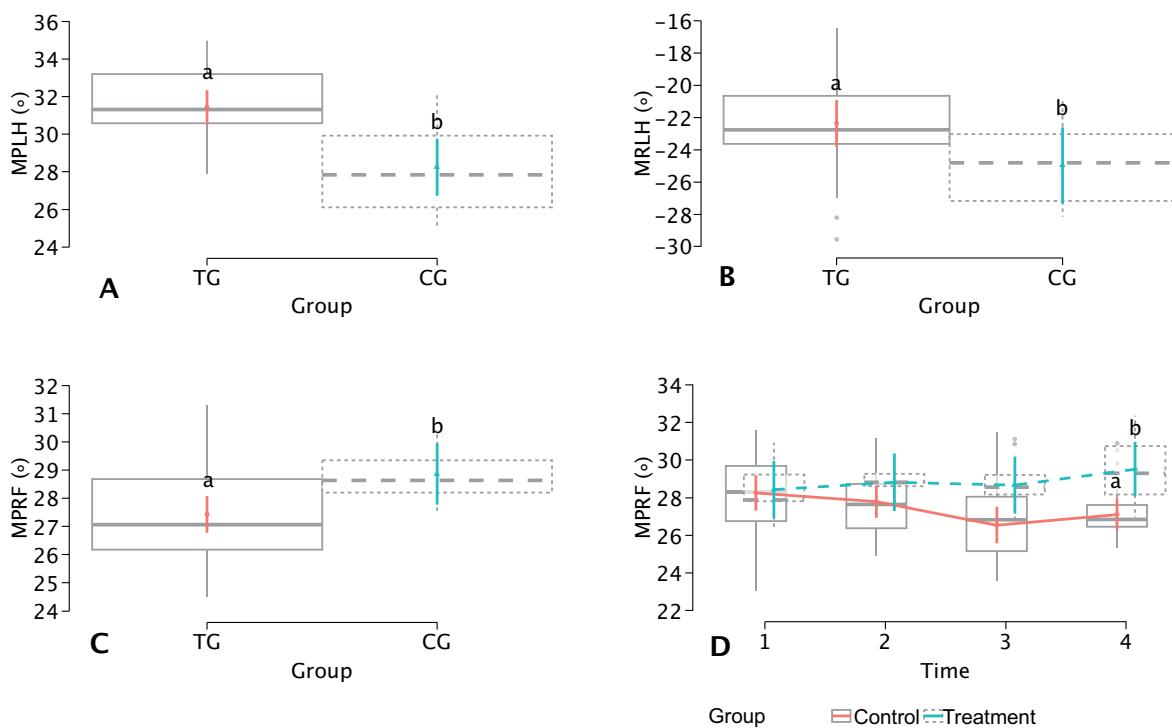


Figure 5. Generalized linear mixed models (GLMM) Box plots indicating means ($^{\circ}$) and their 95%Confidence intervals (CIs) for **A)** Maximal protraction (MP) Left Hindlimb (LH) according to group factor at trotting in circles to the left side. **B)** Maximal Retraction (MR) LH according to group factor at trotting in circles to the left side. **C)** MP Right Front (RF) according to group factor at trotting in right circles. **D)** MPRF according to group x time factor at trotting in right circles. 1= min before osteopathic manipulation (OM), 2= 15 min after OM, 3= 3 days after OM, and 4= 15 days after OM). TG=treatment group n=19, CG=control group n=8. a-b= different low-case letters denote significant ($P < 0.01$) differences by the Bonferroni's test.

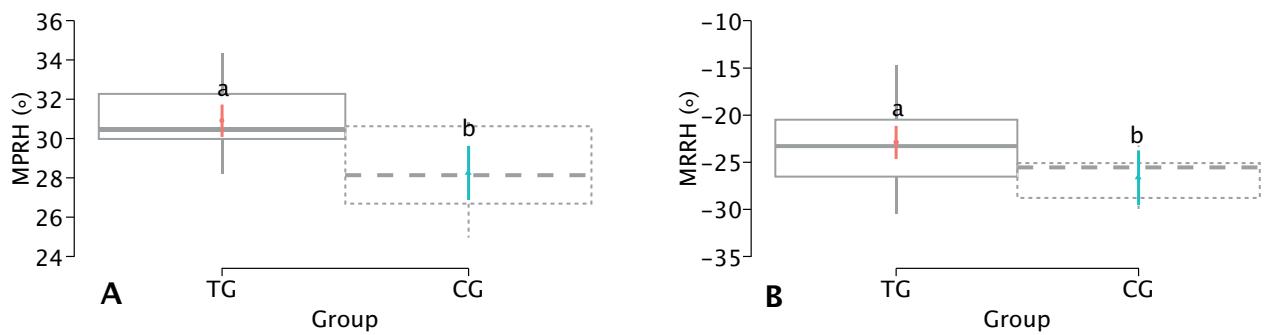


Figure 6. Generalized linear mixed models (GLMM) Box plots indicating means (°) and their 95% Confidence intervals (CIs) for **A**) Maximal protraction (MP) Right Hindlimb (RH) according to the group factor (control group and treatment group) when trotting in circles to the left. **B**) Maximal retraction (MR) RH according to treatment group factor when trotting in circles to the right. 1=min before osteopathic manipulation (OM), 2= 15 min after OM, 3= 3 days after OM, and 4= 15 days after OM). TG= treatment group n=19, CG= control group n=8. ^{a-b}= different low-case letters denote significant ($P < 0.01$) differences by the Bonferroni's test.

3.3.3. Canter

The biomechanical analysis of cantering showed fewer significant changes compared to walking and trotting, with differences observed mainly on the left side. When cantering to the left side, the MaxProtraction of the right front limb (MPRF) was significantly affected by the group factor ($p < 0.05$) (Figure 7A). The stance duration of the left front limb and the MaxRetraction of the left front limb (MRLF) were both significantly affected by the time factor ($p < 0.05$) (Figure 7B). Additionally, the MaxProtraction of the left front limb showed a significant effect of the time factor ($p < 0.05$) (supplementary Table 9).

In contrast, cantering to the right side did not reveal any significant changes in the evaluated biomechanical parameters (supplementary Table 10).

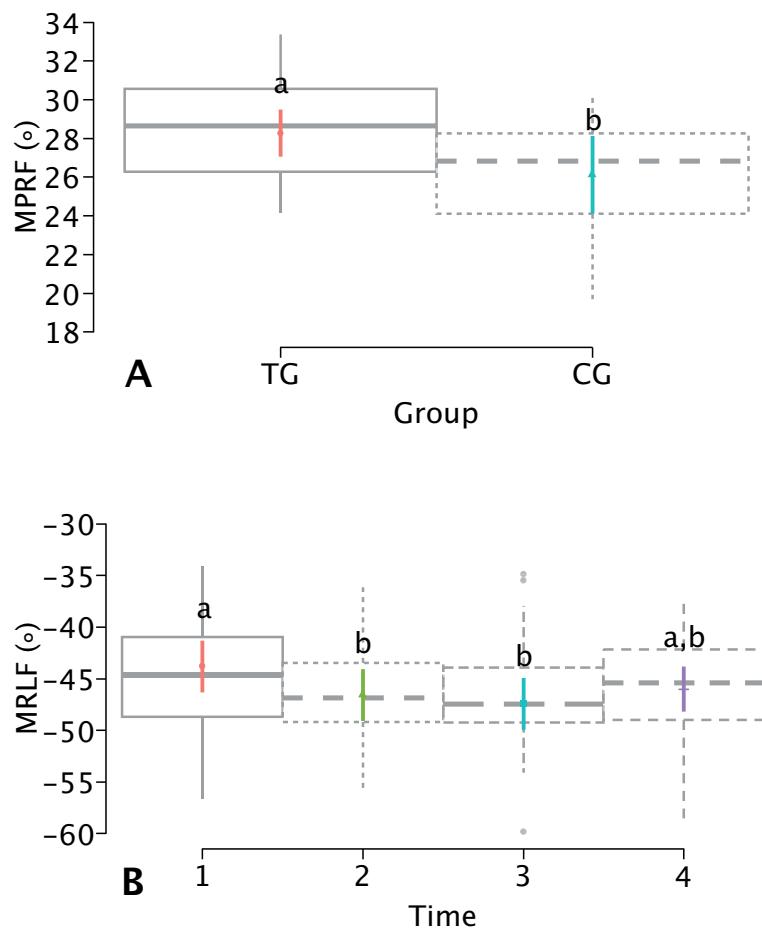


Figure 7. Generalized linear mixed models (GLMM) Box plots indicating means (°) and their 95% Confidence intervals (CIs) for **A**) Maximal protraction (MP) Right frontlimb (RF) according to group factor at cantering in circles to the left side. **B**) Maximal Retraction (MR) Left frontlimb (LF) according to time factor at cantering in circles to the left side. 1= 15 min before osteopathic manipulation (OM), 2= 15 min after OM, 3= 3 days after OM, and 4= 15 days after OM). TG= treatment group n=19, CG= control group n=8. ^{a-b}= different low-case letters denote significant (P< 0.01) differences by the Bonferroni`s test.

4. Discussion

The study's key findings were that a single osteopathic manipulation of the caudal cervical spine in sport horses with cervical dysfunction resulted in some significant, though slight changes in gait parameters and osteopathic variables.

At the walk, minimal effects were seen. This suggests that isolated cervical osteopathic manipulation in these horses did not have an impact on walk symmetry or stride variables. However, at the trot in a straight line and during lunging, max retraction and max protraction of the hindlimbs increased over time in the treatment group compared to the control group, although not always in both sides. This indicates that there is an effect of the treatment on in gait mechanics and suggests there is a positive effect of cervical osteopathic manipulation; especially on the forelimb of the side the horses were circling towards to during circling, suggesting improvement in flexibility and mobility. At the canter to the left side, retraction and stance duration of the left hindlimb increased over time, suggesting improved weight-bearing and hind limb ROM. These results indicate that changes in the neck (i.e. joints and adjacent structures) product of the osteopathic treatment performed in this study seem to positively affect protraction and

retraction of the hindlimbs. This could be explained as an improved or more natural neck positioning and therefore body posture, which has been shown affects weight-bearing distribution [38] and protraction and retraction [39] in ridden and thoracolumbar kinematics in unridden horses [40]. Extensive literature exists on the correlation between head and neck position and movement patterns in horses [40–42]. It can be argued that enhancing the mobility of the lower neck in horses with cervical dysfunction would be analogous to head and neck positions that permit greater freedom of movement in this region. Indeed, there is evidence to suggest that such an approach would have a positive impact on mobility. However, there is a lack of research on the impact of neck pathology on locomotion [19], and even less on the effects of osteopathic treatment of the neck on horse locomotion. Further research in this area is therefore warranted.

It is noteworthy that the majority of observed improvement in protraction and retraction of the fore limbs, both during the trot and canter in circles, occurred in the inside limbs. It has been demonstrated that horses exhibiting lameness in a straight line, increase lameness and vertical head displacement when lunging on the painful inside limb [43] suggesting that circling is a more sensitive exercise for

musculoskeletal pain than when moving in straight line, however, there may be other factors involved. Research in humans shows that axial loading of the cervical vertebrae is increased by latero-flexion, which can affect cervical motor control [44] and potentially increase nerve root compression [45], and subsequently may affect the movement of the inner limb even further when circling.

Although equestrians often seek chiropractic and osteopathic interventions with successful outcomes when reporting resistance issues in one rein (i.e. circling), scientific evidence supporting this improvement after using osteopathy was previously unavailable. This study provides some novel insights into the biomechanical implications of osteopathic manipulation in horses, such as increasing protraction of the forelimb, particularly during circular movement.

No difference was observed in upper body symmetry (head, withers and sacrum vertical displacement) which is a commonly used parameter to aid with diagnosing lameness or limb motion irregularities [46,47]. It is therefore not surprising that there was no significant difference either in the lameness scores recorded by two blinded veterinarians. As the level of lameness in the horses at the start of the study was minimal, it was expected that no major improvements would be observed.

The small but significant biomechanical changes observed in this study were as anticipated, as anecdotal evidence from riders and veterinary health professionals indicates that such improvements post-treatment are subtle. However, apart from being subtle, the changes were not consistent in both sides or for all gaits. This is due to several reasons. Firstly, the participating horses were naturally affected by neck dysfunction, where natural variability is expected, in contrast to experimental studies where most factors can be controlled. Secondly, the horses were assessed overground and not on a treadmill as in previous studies with similar objectives [48] Thirdly, only one treatment was performed and not repeated as it is usually done in these type of treatments [17,49]. Finally, the horses at baseline had subtle cervical dysfunction, consequently, leading to only subtle changes after treatment, which may be missed by the statistical analysis.

In spite of the subtle biomechanic changes found mentioned above, the osteopathic physical examination revealed notable changes, including a decrease in muscle tone and a reduction in palpation-induced pain. Pain and increased muscle tone are two of the typical signs of cervical

dysfunction, along with decreased performance, unwillingness to work with the bit, subtle hind limb gait abnormalities, lack of drive and possibly forelimb lameness [19]. Therefore, improving pain, reducing muscle tension, and increasing limb protraction and retraction is desired when treating cervical dysfunction. These findings align with other equine osteopathic study where biomechanical assessments proved inconclusive, yet semi-objective and subjective measures such as physical examinations, lameness evaluations, and pressure algometry yielded significant results [50]. This discrepancy underscores the pressing need to develop and implement alternative objective methodologies for assessing the efficacy of osteopathic manipulations in equine subjects. While biomechanical considerations remain relevant, their limitations in this context suggest that a more comprehensive approach is necessary. In human osteopathic research, there has been a shift towards employing the neuromotor model to evaluate the efficacy and elucidate the mechanisms of action of osteopathic manipulations [51,52]. This paradigm shift from a purely biomechanical model to a more integrative approach could provide valuable insights if adapted for equine osteopathy. Future research should focus on developing and validating such multifaceted

assessment tools that can capture the complex effects of osteopathic interventions in horses, potentially combining biomechanical, neuromotor, and physiological parameters.

This study acknowledges several inherent constraints common to manual therapy research. A primary challenge in the field is the lack of standardised approaches in interventions, often leading to variations in treatment techniques and protocols [53]. Additionally, manual therapy studies frequently face issues with sample size, difficulties in blinding, insufficient understanding of mechanisms of action [54], and barriers to integrating research evidence into practice [55]. To address these limitations, a rigorous methodology was implemented in this study. A power calculation was conducted to determine an appropriate sample size, and pre- and in-study tests were standardised using a functional evaluation scale. To mitigate bias, the veterinarians assessing lameness were blinded, and inertial measurement units were employed for objective gait analysis. In addition, a single manipulation technique was used to avoid compensations or cumulative effects that might arise from manipulating multiple structures simultaneously, as it is common in clinical practice. While the use of horses in active competition enhances the study's ecological validity and clinical relevance, it

potentially increased intrinsic variability, particularly in the control group. This approach, however, aligns with the aim to bridge the gap between research and clinical practice, albeit at the cost of some experimental control. Moreover, by employing this methodology in a real-world setting, this study contributes to a better understanding of the mechanisms of action underlying osteopathic manipulations, addressing one of the fundamental limitations in the field.

5. Conclusion

The observed changes in muscle tone, protraction and retraction, along with previous literature, supports that osteopathic manipulation of lower cervical vertebrae provides some slight positive biomechanical effects in horses with caudal cervical dysfunctions. Particularly on the same side towards which the horse is trotting or cantering, there was no information on this subject until now and it would be interesting to study this issue further.

Further research is required, including horses with more severe clinical signs, the evaluation of the effects of multiple manipulations and the inclusion of longer follow-up periods, with more than just one day's treatment and additional outcome measures. It may be of interest to conduct another similar study under laboratory conditions to minimise variability.

Author's declaration of interests

No competing interests have been declared.

Ethical animal research

This clinical trial did not imply pain, suffering or morbidity of the studied animals, therefore, this type of non-invasive animal study does not require a specific authorization by an ethical committee for animal experimentation.

Owner informed consent

Owners gave consent for their horse's inclusion in the study, they were informed about the nature of the clinical trial and signed an informed consent accordingly.

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Authorship

The study was designed by Toni Ramon, Dra Marta Prades, and Dra. Constanza B. Gómez Álvarez, who also contributed to the writing,

analysis, and interpretation of data. Dr. Jorge U Carmona was responsible for the statistical analysis and data analysis. Dra. Marta Prades conducted the initial physical examination and assessment of inclusion/exclusion criteria for the horses. Toni Ramon performed the osteopathic examination and manipulations.

Manufacturers' addresses

a Garmin International Inc. Olathe (Kansas, USA)

b Equimoves System (EquiMoves[®]-www.equimoves.nl) consisting of nine ProMove-mini IMUs (Inertia Technology B.V., Enschede, The Netherlands).

c JASP (JASP 0.18.3 (Intel), University of Amsterdam, The Netherlands) - R-Studio, Boston, Massachusetts, USA. and SPSS 24.0 (IBM, USA).

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Original Article 2

Title

Effect of an isolated osteopathic manipulation on the Sacroiliac joint in horses with sacroiliac dysfunction

Authors

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Abstract

Background: The application of osteopathic treatments in equine medicine has gained increasing interest for enhancing performance and well-being. However, objective assessment of equine locomotion parameters following osteopathic sessions, particularly in sport horses, remains limited.

Objective: This study aimed to evaluate the effects of a single osteopathic manipulation (OM) on the sacroiliac joint (SIJ) of sport horses using objective biomechanical analysis.

Study Design: Blinded, randomised, controlled clinical trial.

Methods: Twenty-nine sport horses with SIJ fixation in extension were randomly assigned to a treatment group (TG) (n=21) or a control group (CG) (n=8). The treatment group received a single osteopathic manipulation targeting the blocked SIJ, while the control group received no intervention. Objective kinematic data, including stride length, and symmetry index, were collected using inertial sensors at four time points: pre-intervention, post-intervention (15 minutes), 3 days, and 15 days. Subjective lameness evaluations and osteopathic examinations were also performed. Generalised linear mixed models were used to analyse the results.

Results: Osteopathic manipulation significantly reduced back muscle tone, back pain, and sacroiliac pain in the treated horses, even at 15 days post-intervention. Biomechanical analysis revealed significant

reduction in protraction ($p <0.05$ before OM, (mean (confidence intervals) CG: 26.59 (23.40-29.77), TG: 30.26 (28.44-32.08). After 15 minutes, CG: 28.38 (25.07-31.69), TG: 29.38 (27.57-31.19). At 3 days, CG: 29.06 (26.02-32.10), TG: 27.98 (26.16-29.81). At 15 days, CG: 29.02 (25.95-32.08), TG: 28.92 (27.10-30.74). An increase in retraction ($p <0.05$) of the hindlimbs, specifically when cantering towards the affected side was also observed, before OM, CG: -36.09 (-40.92 to -31.27), TG: -30.34 (-33.12 to -27.57). After 15 minutes, CG: -34.70 (-39.62 to -29.78), TG: -31.33 (-34.09 to -28.56). At 3 days, CG: -32.68 (-37.40 to -27.96), TG: -31.76 (-34.53 to -28.98). At 15 days, CG: -32.57 (-37.31 to -27.83), TG: -31.47 (-34.24 to -28.70). Additionally, increased head range of motion when cantering towards the unaffected side was observed ($p <0.05$) CG: 200.89 (158.92-242.85), TG: 209.49 (187.29-231.69). After 15 minutes, CG: 200.38 (157.56-243.20), TG: 229.86 (207.22-252.51). At 3 days, CG: 224.95 (182.51-267.38), TG: 215.74 (192.96-238.52). At 15 days, CG: 183.60 (142.73-224.48), TG: 222.83 (200.22-245.44). There were no conclusive results observed at passes in a straight line.

Main Limitations: The main limitation of the study was where there was an intrinsic variability between the subjects.

Conclusion: A single osteopathic manipulation of the blocked SIJ in sport horses resulted in small but significant changes in gait parameters, particularly in hindlimb protraction and retraction when cantering towards the affected side. Further investigation with controlled variables is warranted to validate the impact of equine osteopathic intervention on equine locomotion.

Keywords: equine, osteopathy, sacroiliac joint, biomechanics, inertial sensors, gait analysis

1. Introduction:

The application of osteopathic treatments in veterinary medicine responds to the increasing interest in enhancing equine well-being as well as performance. However, objective assessment of equine locomotion parameters following osteopathic sessions, especially with horses used and trained for competition is lacking.

Vertebral fixations and back pain can lead to compensations and irregularities in equine locomotion [1,2]. Often, the primary cause of these compensations is found in the distal limb. Evidence suggests that osteopathic treatment of a horse's axial skeleton can alter not only spinal biomechanics but also limb biomechanics [3]. Similarly, injuries to the forelimb and hindlimb can affect spinal biomechanics [4,5]. Yet, objective data about how osteopathic manipulations in the distal limbs, pelvis, and neck might influence equine biomechanics are scarce. A high incidence of lumbar and sacroiliac disorders in horses has been reported [6,7], and yet in comparison to humans the neuroanatomical aspects [8] of the equine thoracolumbar region are understudied.

Despite the change in recent years in the evaluation and treatment of horses with back pain [9], it remains an underdiagnosed pathology. Many horses with back pain, especially in the lumbar area, present with other complaints, such as poor performance (76%), behavioural problems (68%), and lameness (50%) [10].

Pain originating from a limb often induces altered limb loading creating abnormal movement patterns and potentially leading to compensation

injuries within the axial skeleton [11]. Sacroiliac dysfunction may create abnormal gait patterns [12,13] in horses without a clearly diagnosed limb lameness, and subtle neurological disorders (e.g., weakness, lack of impulsion), back pain and stiffness, may be present. Back pain can be both a cause and a result of lameness [13], and some authors suggest that 23–32% of horses with limb lameness may have concurrent back pain, and 68–85% of horses with primary back pain may have concurrent limb lameness [14,15]. There is increasing evidence of the clinical importance of compensatory mechanisms and interactions between the axial and appendicular regions with regards to pain and lameness [16].

The optimal function of the (SIJ) is paramount for locomotion but due to its deep position within the hind quarters and the extensive muscle coverage, performing specific tests [17], and obtaining specific diagnostic imaging is challenging [18]. Osteopathic techniques allow for early detection of functional deficits and abnormal motion of this joint [19]. Even if there is currently no universally accepted diagnostic test that serves as the definitive standard for identifying sacroiliac dysfunction and it's difficult to isolate lumbosacral from sacroiliac areas, certain mobilization techniques, fascial palpation, and presence of pain indicate alterations in these areas [20].

The most common presenting complaint of SI dysfunction in horses is stiffness, rider's perception of discomfort, lack of forward impulsion, and poor performance [21–23]. In osteopathic examination by palpation, a SIJ is defined as dysfunctional or fixated when a decrease or

disappearance of movement is felt, there is no rebound of the tissues surrounding the joint and/or there are pain and discomfort on deep palpation, manual mobilization or by observing changes to the response to cutaneous reflexes [20,24]. The benefits of osteopathy may not always be reflected exclusively in biomechanical changes and fields such as neuromuscular function have to be considered [25].

The aim of this study was to examine the effects of a single osteopathic manipulation on a dysfunctional SIJ using an indirect technique manoeuvre with the purpose of decreasing pain and improving mobility. Osteopathic manual examination, visual lameness examination and inertial measuring sensors gathering real-time kinematic data to detect osteopathic-induced locomotor modifications were used for objective gait, and pain analysis. In general, osteopathic clinical practice, numerous joints are routinely manipulated, but in this study only manipulation of the blocked SIJ of the treated group was performed to isolate the biomechanical effects.

The hypothesis was that an objective improvement in the horse's pain and locomotion would be measurable after a single osteopathic vertebral manipulation of the blocked SIJ [26]. The goal was to provide insight on improving equine welfare, rehabilitation and performance.

2. Materials and methods

2.1 Study design

This was a controlled, blinded, randomised clinical trial involving two different horse groups.

2.2 Horses and inclusion/exclusion criteria

Twenty-nine horses (10 mares, 11 geldings, 8 stallions) between 6 and 15 years of age, engaged in showjumping and dressage training and with osteopathic fixation in extension of the SIJ were selected. Sacroiliac fixation was diagnosed by an osteopathic examination [27,28] In many of these subjects, other somatic dysfunctions were detected in other areas, but the SIJ was diagnosed as the main problem.

The horses trained at medium/high level with a regular activity of at least 4-5 days a week. The horses were randomly divided into two groups: a treatment group of 21 horses and control group of 8 horses. All horses presented a SI block in extension but not on the same side, in the treatment group 13 of the 21 cases had a block on the right SIJ; 7 cases on the left and in 1 case, both were affected. In the control group, 4 out of 8 cases showed right SIJ involvement, 3 cases left SIJ, and only one had both affected.

All horses were actively involved in sports training for competition, in continuous regular work, and some even competed during the study period.

Inclusion Criteria:

Sport horses aged 6 to 15 years trained regularly at medium-high level for the past 6–12 months, with a minimum of 4-5 days per week of directed work.

All horses presented at least 3 of the following complaints: discomfort during lateral bending, restriction in hind limb engagement, stiffness in the lumbo-sacral area, rider's complaint of slight discomfort, and lack of forward impulsion. During the osteopathic examination, it was determined that the SIJ was blocked when a decrease or disappearance of movement was present, particularly in lumbosacral extension. Additionally, when the tissues in that joint did not rebound, and when pain or discomfort during deep palpation, manual mobilization, or a painful response to the cutaneous trunci reflex were present the joint blockage was confirmed.

Exclusion Criteria:

A detailed veterinary examination was performed by an experienced veterinarian and horses with defective conformation, lameness of 2/5 or more on the American Association of Equine Practitioners (AAEP) scale, or other obvious musculoskeletal problems were excluded. Also excluded were all horses that had presented lameness or musculoskeletal problems within the last 3 months (e.g., tendinopathy, joint or soft tissue injuries) and those that had received any medical treatment within the last 3 months.

Because these horses had referral veterinarians who performed routine check- ups, horses with a history of radiographic or ultrasonographic pathology of the dorsum, lumbosacral region, or distal extremities were excluded.

2.3 Intervention design

The horses in the treatment group received a single osteopathic treatment using indirect techniques targeting only the SIJ segment, while the ones in the control group received no treatment.

A single osteopathic expert performed the initial evaluation of the horses in both groups and delivered the treatment only to the ones in the treatment group, always utilizing the same indirect high-velocity, low-amplitude thrust technique specific to the SIJ (video 1). The same osteopath also re-evaluated the horses on days 3 and 15 post-treatment. All evaluations and treatments were performed with horses standing quietly on firm, familiar ground to minimize stress and variability.

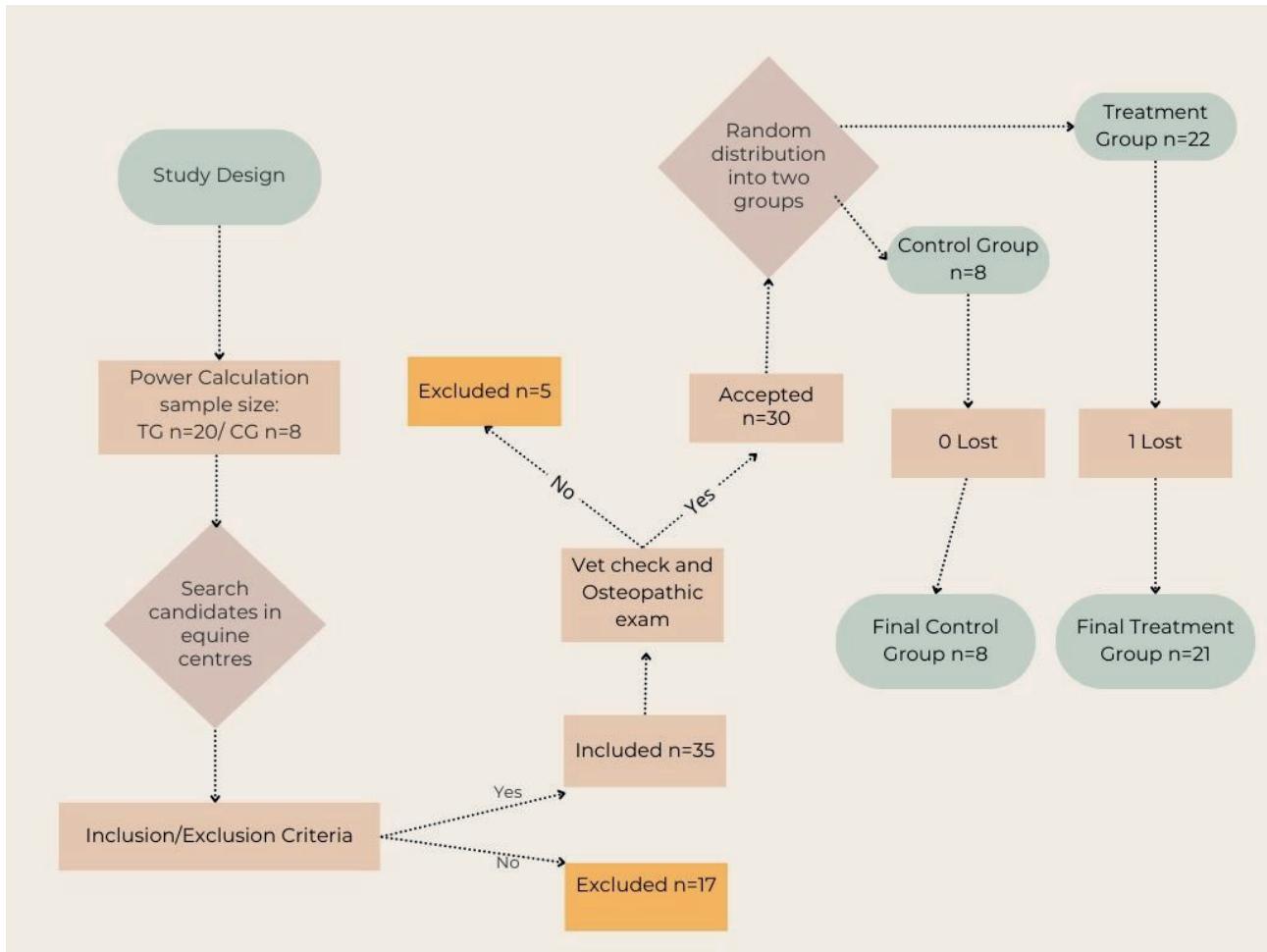


Figure 1. Flowchart horses' selection in a study describing the effect of a single osteopathic manipulation of dysfunctional caudal sacroiliac joint in horses.

2.4 Evaluation

The horses were walked, trotted in a straight line, and lunged at the latter two gaits and at the canter.

Horses underwent three evaluations: a subjective assessment conducted via osteopathic examination, a semiobjective evaluation based on visual lameness examination performed by two veterinarians blinded to the treatment, and an objective examination utilising inertial

sensors at four time points 1: day 1 before, 2: day 1 15 min after manipulation, 3: day 3 and 4: day 15.

2.4.1 Osteopathic physical evaluation and scoring

A physical examination of the horse's neck, back, and sacroiliac area was performed prior to the passes on days 1, 3, and 15 pre movement assessment, by an experienced physiotherapist/osteopath (not blinded).

The osteopathic evaluation prior to every dynamic evaluation was performed by the same osteopath in all cases. It consisted of a physical examination by palpation of the horse's neck, back and sacroiliac area [29] assessing pain or tenderness, muscle tone, joint mobility, and cutaneous reflexes. The evaluation was assessed according to a score of (0-3) for each item created for this study based on other similar scores from various authors (Table 1). The osteopath in this case was not blinded.

Table 1. Scoring of neck and back examination: epaxial muscle tone evaluation; pain assessment; osteopathic assessment of the mobility of the vertebral column and assessment of manual cutaneous reflexes by performing palpation of the thoracolumbar vertebrae and longissimus dorsi muscles (modified from [31–33]).

Clinical Parameter	Scoring			
	0	1	2	3
Muscle tone				
Manual evaluation muscle tone of the muscular groups	Soft musculature with no avoidance	Stiff musculature with no avoidance	Stiff musculature with avoidance characterized by lordosis or movement away from pressure	Stiff musculature with avoidance characterized by kicking or biting
Pain				
5-10 cm off midline, abaxial to spinous process	No obvious avoidance	Avoidance and lordosis of spine or movement away from pressure	Avoidance plus tossing of head and/or tail swish	Violent avoidance characterized by kicking biting or jumping
Vertebral Fixations				
Osteopathic assessment of spine	No fixation	1 segment fixated	2 segments fixated	3 or more segments fixated
Cutaneous reflexes				
Manual reflex evaluation	Normal reflex	Decreased reflex with no clear pain (avoidance)	Clear pain (avoidance) or complete absence of reflex	Violent avoidance characterized by kicking biting or jumping

2.4.2 Lameness semi-objective scoring

All passes were videotaped, and the videos were distributed in random order. They were later evaluated by two double blinded experienced veterinarians: blinded to the treatment and to the order of the passes.

The videos were presented to them individually in a random order with a code number. They were asked to score the degree of lameness according to the AAEP (0-5) of the 4 limbs at the straight trot, walk, and trot and canter in circles. In addition, they were asked to report the quality of the canter according to three items: taking off with correct hind leg, presence or absence of bunny hopping, and switching leads behind, as indicators of poor hind end engagement.

2.4.3 Kinematic data collection

Data were collected at walk and trot in a straight line (40 meters) and at walk, trot and canter in 15-meter circles on a semi-soft surface (competition arena) for minimum 30 strides. All passes were performed by the same operator trying to maintain a constant speed in circles in the different gaits. A common speed was set for each horse in a straight line and maintained for all passes; speed was measured with a Garmin Fenix 5 GPS^a device worn by the operator.

Horses were tested four times 1: before treatment, 2: 15 minutes after manipulation, 3: at 3 days and 4: at 15 days.

Kinematic evaluation of the horse's gait was done through a validated inertial sensor analysis system (EquiMovesTM, Inertia Technology B.V., Enschede, The Netherlands)^b [30,31] using 9 inertial measurement units (IMUs) which were wi-fi connected via an inertial gateway to a computer and processed with the proprietary EquimovesTM software which is a validated system that has been frequently used in previous studies [30]. The EquiMoves system uses a proprietary 2.4 GHz wireless protocol

with a star network topology for the wireless IMU nodes to communicate sensor data to a central gateway device.

The Equimoves® system consists of 9 inertial sensors that can accurately measure low-g acceleration within a range of ± 16 g and high-g acceleration within a range of ± 400 g. A gyroscope can measure angular velocity within a range of ± 2000 degrees per second [30]. The system processes the inertial sensor data by filtering noise with Butterworth filters, handling missing data by retrieving it from local storage on the IMU nodes, performing numerical integration and quaternion operations to calculate displacement, orientation, limb angles, and utilizing cyclic integration methods to determine symmetry parameters from the accelerometer data.

The sensors were placed on the 4 limbs on the distal and lateral side at the 3rd Metatarsal/metacarpal bone, on the top of the neck, on the withers, on the sacral tuber-sacrae and on the dorsal part of the right tuber-coxae and left tuber-coxae (fig 1).

Data was collected from all the passes. The following parameters were analysed: maximal protraction (MP) and maximal retraction (MR) of the limbs (left forelimb (LF), right forelimb (RF), left hindlimb (LH), right hindlimb (RH)), head minimum difference (Mindiff), head maximum difference (Maxdiff), withers Mindiff, withers Maxdiff, sacrum Mindiff, sacrum Maxdiff, Stride Duration, Stance Duration (for all limbs), Head Range of Motion (ROM), Withers ROM, and Sacrum ROM, stride duration, swing/stance phases.

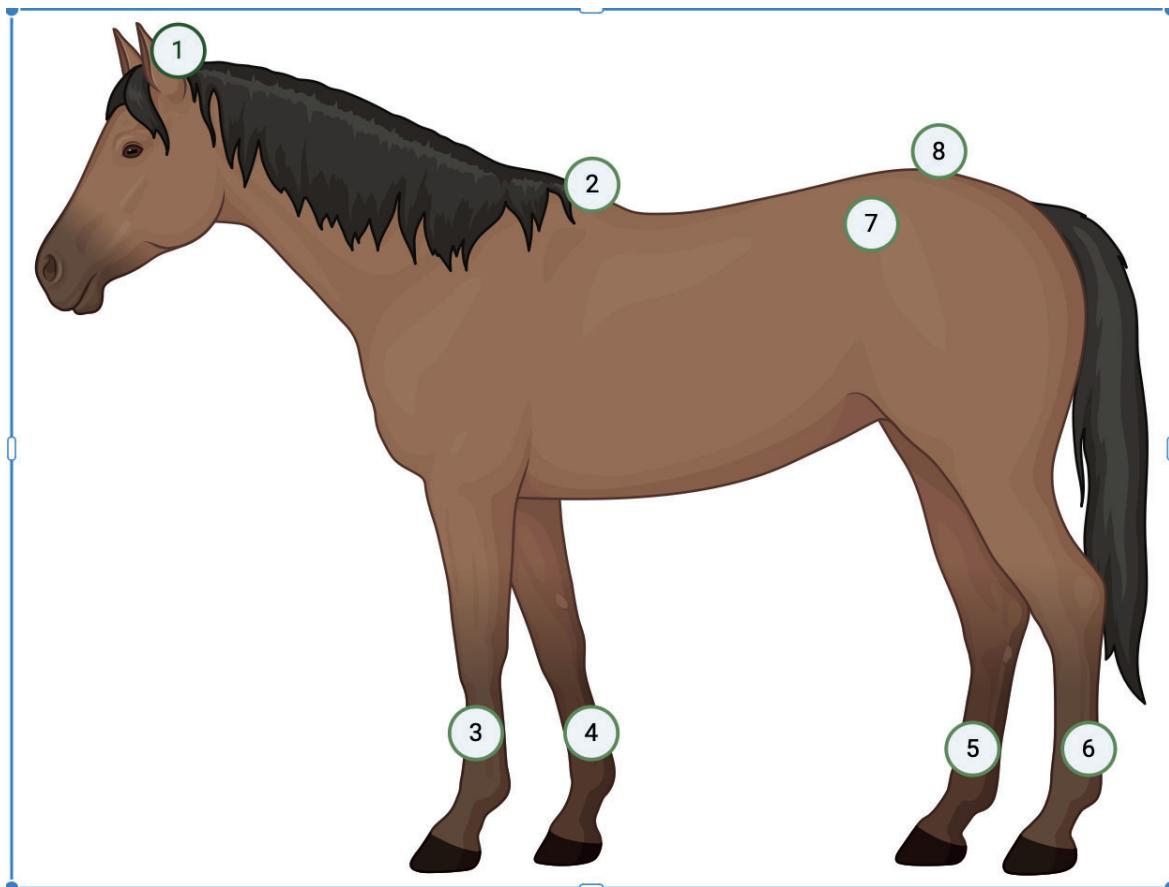


Figure 2. Position of the Imu's sensors: 1. Poll, 2. withers, 3-4 distal and lateral 3rd metacarpal bone, 5-6 distal and lateral 3rd metatarsal bone, 7 Left tuber-coxae, 8 tuber-sacrae, 9 (unseen) Right tuber-coxae.

2.5 Statistical analysis

Data collected from EquiMoves® was exported in .csv format and processed using a custom MATLAB script (MATLAB and Statistics Toolbox Release 2012b, MathWorks, Inc., Natick, Massachusetts, United States)^c to perform the Z-Score method for outlier detection. Outliers were identified based on a threshold set at $Z > 3$ and subsequently removed from the dataset.

Following outlier treatment, the cleaned data was imported into SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp)^d

for further analysis. Normality of the variables was assessed using the Kolmogorov-Smirnov test. Variables failing to meet the normality criterion ($p < 0.05$) were considered non-normally distributed.

To analyse the variance of the results while accommodating both normal and non-normal distributions, a repeated measures General Linear Mixed Models (RM-GLMM) approach was selected. RM-GLMM is a robust statistical model capable of handling mixed effects models and is well-suited for analysing both Gaussian and skewed data. Assumptions of the General Linear Mixed Models (RM-GLMM), including homoscedasticity and multicollinearity were met.

The dataset was binned into variables based on three factors: (i) gait (walk, trot, canter), (ii) natural direction (right, left, straight) and (iii) functional direction (towards the affected side, towards the unaffected side).

Separate RM-GLMM were performed on the kinematic variables provided by Equimoves®, for all the unique combinations of gait*kinematic direction and gait*functional direction. This approach allowed for the examination of specific relationships within these categories. Two fixed effects were included in the model: (i) group (two levels: treatment group, control group and (ii) time (four levels: 1: before treatment, 2:15 minutes after manipulation, 3:at 3 days and 4: at 15 days).

The model was fitted with an identity link and post-hoc comparisons were assessed as deviation contrasts (LSD-corrected), which compared the effects of different levels of the fixed effects.

Both the lameness score and physical evaluation's data matrices were not binned into the previously described logic and a Generalized Linear Model (GLM) was conducted and fitted with the same fixed effects as the previous RM-GLMM model and with Bonferroni-adjusted post-hoc pairwise comparisons.

Significance was set at $p \leq 0.05$ for all analysis.

3 Results

3.1 Osteopathic physical evaluation

Results from the GLM showed a statistically significant decrease in back muscle tone in the treatment group on day 3 and even more on day 15 (Fig. 2) ($p < 0.05$, CI: Table 2), decrease in back pain in the treatment group in two measurements after treatment ($p < 0.05$, CI: Table 3), decreased pain in the sacroiliac area and maintaining the effect on day 15 ($p < 0.05$, CI: Table 4), and reduction of osteopathic fixation in the sacroiliac area ($p < 0.001$, CI: Table 5).

Clinical, but not statistically significant changes were observed in muscle tone in the sacroiliac area ($p = 0.058$, CI: Table 6) cutaneous reflex assessment also improved in most treated horses but was not statistically significantly in relation to the control group ($p > 0.05$, CI: Table 7). (Supplementary (Supp.) Materials- Osteopathic Exam).

Table 2. Mean, standard error (Std Error) and confidence intervals of back muscle tone over the three measures: day 1, day 3, day 15. Control and treatment groups.

Group	Day	Mean	Std. Error	95% Confidence Interval
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				Lower Bound	Upper Bound
Control	1	0.88	0.22	0.42	1.33
	3	1.13	0.21	0.69	1.57
	15	1.00	0.23	0.53	1.47
Treatment	1	0.91	0.14	0.62	1.19
	3	0.67	0.13	0.40	0.93
	15	0.48	0.14	0.19	0.77

Values according to the osteopathic physical examination scoring (table 1).

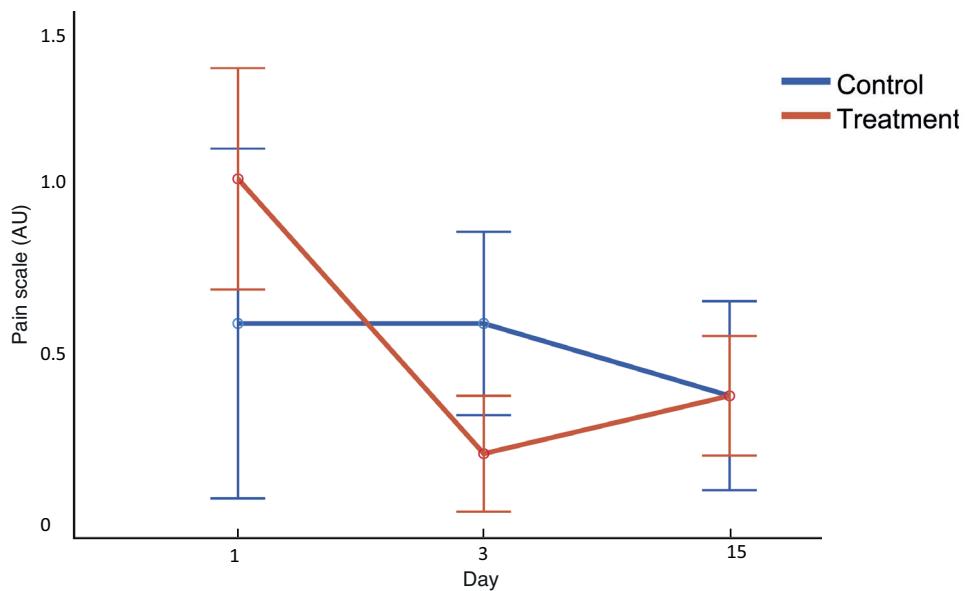


Figure 3. Reported back pain levels of the control group n=8 and the treatment group n=21 according to the osteopathic evaluation using the evaluation scale (Table 1) performed on the three measurement days 1, 3, 15.

Table 3. Mean, standard error (Std Error) and confidence intervals of back pain over the three measures: day 1, day 3, day 15. Control and treatment groups.

Group	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	0.25	0.27	-0.30	0.80
	3	0.88	0.23	0.39	1.36
	15	0.38	0.23	-0.09	0.84
Treatment	1	1.00	0.17	0.66	1.34
	3	0.38	0.14	0.09	0.68

15	0.48	0.14	0.19	0.76
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Values according to the osteopathic physical examination scoring (table 1).

Table 4. Mean, standard error (Std Error) and confidence intervals of pain in the sacroiliac area over the three measures: day 1, day 3, day 15. Control and treatment groups.

Group	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	0.25	0.27	-0.30	0.80
	3	0.88	0.23	0.40	1.36
	15	0.38	0.23	-0.09	0.84
Treatment	1	1.00	0.17	0.66	1.34
	3	0.38	0.14	0.09	0.68
	15	0.48	0.14	0.19	0.76

Values according to the osteopathic physical examination scoring (table 1).

Table 5. Mean, standard error (Std Error) and confidence intervals of osteopathic block in the sacroiliac area over the three measures: day 1, day 3, day 15. Control and treatment groups.

Group	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	1.00	0.00	1.00	1.00
	3	1.00	0.00	1.00	1.00
	15	0.88	0.11	0.65	1.10
Treatment	1	1.00	0.00	1.00	1.00
	3	0.00	0.00	0.00	0.00
	15	0.10	0.07	-0.05	0.24

Values according to the osteopathic physical examination scoring (table 1).

Table 6. Mean, standard error (Std Error) and confidence intervals of muscle tone in the sacroiliac area over the three measures: day 1, day 3, day 15. Control and treatment groups.

Group	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	0.38	0.20	-0.03	0.78
	3	0.50	0.15	0.19	0.81
	15	0.50	0.16	0.16	0.84
Treatment	1	0.33	0.12	0.08	0.59
	3	0.05	0.09	-0.14	0.24
	15	0.10	0.10	-0.11	0.30

Values according to the osteopathic physical examination scoring (table 1).

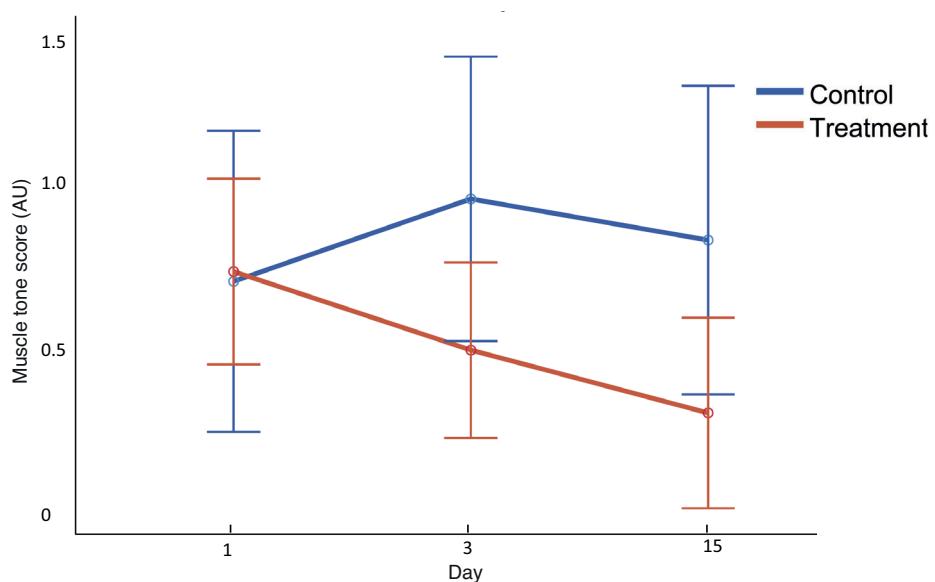


Figure 4. Reported back muscle tone of the control group n=8 and the treatment group n=21 according to the osteopathic evaluation using the evaluation scale (Table 1) performed on the three measurement days 1, 3, 15.

Table 7. Mean, standard error (Std Error) and confidence intervals of cutaneous reflex in the lumbar extension over the three measures: day 1, day 3, day 15. Control and treatment groups.

Group	Day	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	1	0.75	0.31	0.11	1.39
	3	0.50	0.24	0.01	0.99
	15	0.75	0.31	0.11	1.40
Treatment	1	0.85	0.20	0.45	1.25
	3	0.25	0.15	-0.06	0.56
	15	0.55	0.20	0.14	0.96

Values according to the osteopathic physical examination scoring (table 1).

3.2 Lameness evaluation

Upon analysis of the data utilising a GLM, no statistically significant alterations were observed in the lameness evaluation, and any potential deleterious effects of the intervention were conclusively eliminated from consideration. (Supp. Materials-Lameness Exam). These findings were in accordance with initial expectations, given that the baseline lameness score had already been established at a markedly low level prior to the commencement of the study.

3.3 Biomechanical analysis

An RM-GLMM was conducted to assess differences between the treatment and control (untreated) groups over time, in straight line at walk and at trot, and at lunge in three gaits and both directions. The analysis included multiple parameters measured by inertial sensors:

head Mindiff, head Maxdiff, withers Mindiff, Withers max Diff, Sacrum Mindiff, Sacrum Maxdiff, stance duration for all limbs, stride duration, Diagonal advanced placement right and left, maximum and minimum protraction and retraction of all four limbs, and Range of Motion of the head, withers and sacrum. (Supp. Materials-Biomechanical Exam)

Statistical analysis revealed no significant alterations ($p > 0.05$) in any of the kinematic parameters during walk, regardless of movement pattern (straight line or lunging). This lack of significant change was consistent across all time points and between treatment and control groups.

When examining straight-line locomotion, both walk and trot gaits exhibited no statistically significant differences ($p > 0.05$) between the treatment and control groups for any of the measured biomechanical parameters. This absence of significant change persisted throughout the study period, suggesting minimal impact of the intervention on straight-line movement patterns.

In contrast, circular movement at the canter gait yielded statistically significant results ($p < 0.05$) for both the affected and unaffected sides. These significant changes were not observed during circular movement at walk or trot, indicating a gait-specific effect of the intervention. The nature and magnitude of these changes varied depending on whether the horse was moving towards its affected or unaffected side.

Subsequent analysis of the data, categorized according to the direction of movement relative to the affected side, revealed the following outcomes:

3.3.1 Affected side

At canter, significant changes in protraction and retraction were observed in some horses when moving towards their affected side. More specifically, changes in measurements 3 and 4, at 3 days and 15 days, were observed, portraying an increase in retraction of the left hindlimb ($p < 0.05$) (Figure 4, CI: Table 8) and a reduction in protraction of the left hindlimb ($p < 0.05$) (Figure 5, CI: Table 9) of the affected side. No significant changes were observed in other parameters when moving towards the affected side.

Table 8. Mean, standard error (Std Error) and confidence intervals of the left hindlimb retraction of the control and treatment groups over the four measurements: before, 15 min after, day 3 and day 15 at canter, analysed towards the affected side.

Group	Day	Mean	Std. Error	95% Confidence Interval	
				Lower	Upper
Treatment	Before	-30.34	1.37	-33.12	-27.57
	After 15 min	-31.33	1.36	-34.09	-28.56
	Day 3	-31.76	1.37	-34.53	-28.98
	Day 15	-31.47	1.37	-34.24	-28.70
Control	Before	-36.09	2.38	-40.92	-31.27
	After 15 min	-34.70	2.43	-39.62	-29.78
	Day 3	-32.68	2.32	-37.40	-27.96
	Day 15	-32.57	2.33	-37.31	-27.83

All values are expressed in degrees.

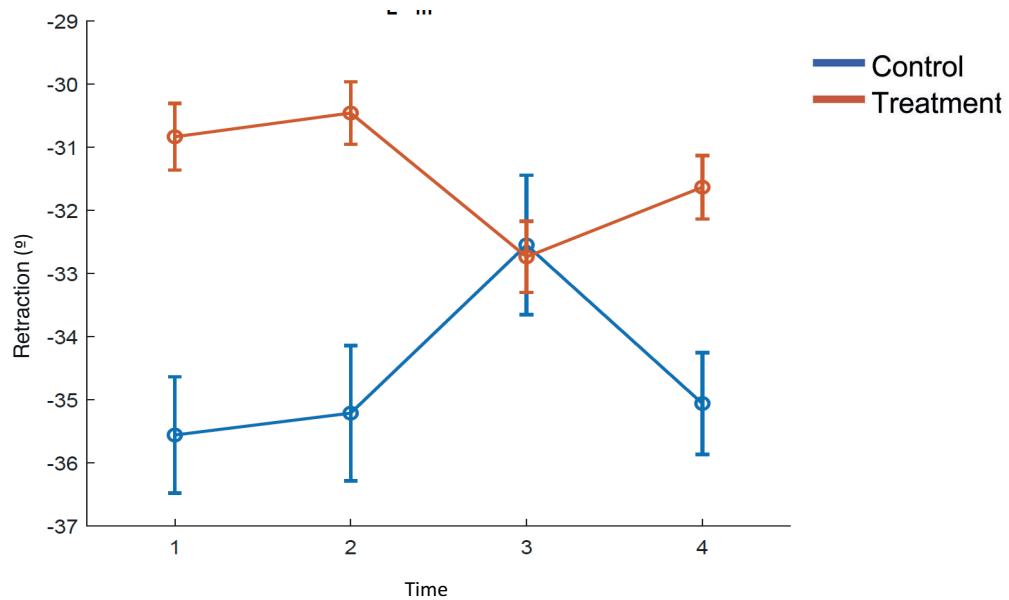


Figure 5. Right forelimb protraction of the control and treatment groups over the four measurements 1: before, 2:15 min after, 3: day 3, 4: day 15 at trot. Analysed with equimoves system® when horses lunging towards the right side.

Table 9. Mean, standard error (Std Error) and confidence intervals of the left hindlimb protraction of the control and treatment groups over the four measurements: before, 15 min after, day 3 and day 15 at canter, analysed towards the affected side.

Group	Day	Mean	Std. Error	95% Confidence Interval	
				Lower	Upper
Treatment	Before	30.26	0.91	28.44	32.08
	After 15 min	29.38	0.90	27.57	31.19
	Day 3	27.98	0.91	26.16	29.81
	Day 15	28.92	0.91	27.10	30.74
Control	Before	26.59	1.59	23.40	29.77
	After 15 min	28.38	1.66	25.07	31.69
	Day 3	29.06	1.51	26.02	32.10
	Day 15	29.02	1.50	25.95	32.08

All values are expressed in degrees.

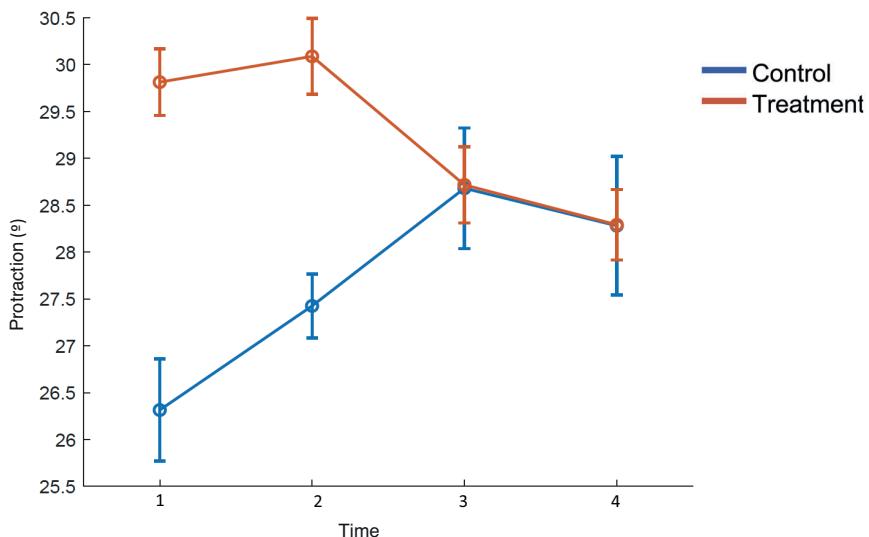


Figure 6. Left hindlimb protraction of the control and treatment groups over the four measurements 1: before, 2:15 min after, 3: day 3, 4: day 15 at canter. Analysed with Equimoves system® when horses were lunging towards the affected side.

3.3.2 Unaffected side

No statistically significant results were found for protraction and retraction when horses cantered towards the unaffected side. However, a significant difference in the increase in the range of movement of the horse's head ROM ($p < 0.05$, CI [207.22 mm – 252.508 mm]) when cantering towards the unaffected side was observed in the treatment group from after the first treatment, which was maintained in measurements 3 and 4 (Figure 6, CI: Table 10) in the treatment group. No significant changes were observed in other parameters when moving towards the unaffected side.

Table 10. Mean, standard error (Std Error) and confidence intervals of the head range of motion of the treatment and control groups over the four measurements: before, 15 min after, day 3 and day 15 at canter, analysed towards the unaffected side.

Group	Day	Mean	Std. Error	95% Confidence Interval	
				Lower	Upper
Treatment	Before	209.49	10.88	187.29	231.69
	After 15 min	229.86	11.14	207.22	252.51
	Day 3	215.74	11.22	192.96	238.52
	Day 15	222.83	11.12	200.22	245.44
Control	Before	200.89	20.69	158.92	242.85
	After 15 min	200.38	21.17	157.56	243.20
	Day 3	224.95	20.96	182.51	267.38
	Day 15	183.60	20.07	142.73	224.48

All values are expressed in mm.

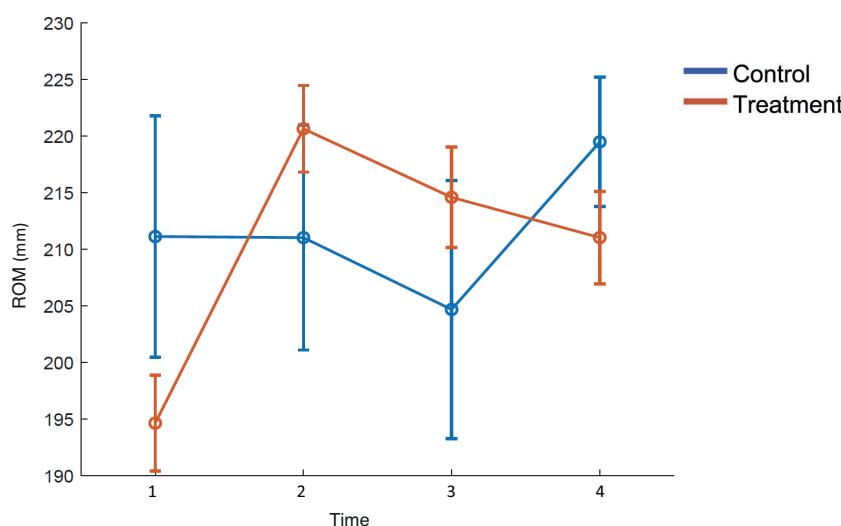


Figure 7. Head range of motion of the treatment and control groups over the four measurements 1: before, 2:15 min after, 3: day 3, 4: day 15 at canter. Analysed with Equimoves system® when horses were lunging towards their unaffected side.

4 Discussion

Despite the increasing number of studies in recent years, SIJ problems remain understudied. The SI is a deep joint receiving multiple shearing forces caused by hind limb loading and despite being sustained by numerous strong ligamentous attachments any prolonged asymmetry renders it vulnerable to dysfunction and injury.

In agreement with previous reports, the clinical and manual osteopathic examination of the horses in this study showed a reduction in muscle tone in the back muscles of the treated horses [32], as well as reduced pain in the back and sacroiliac areas [33,34] both on day 3 and day 15 after the intervention. Previous studies report that positive results may be obtained immediately following a manipulation but occasionally adverse effects too may be observed for a period of several hours after the manipulation [35]. In this study, no improvement was observed 15 minutes after the intervention and no adverse changes were noted at any time point.

No changes were observed in the visual lameness scoring and given that the baseline lameness score had already been established at a low level prior to the inclusion in the study this was not surprising. No increase in lameness, even slight, was observed in any of the limbs after manipulation, which corroborates that osteopathic techniques performed correctly are safe.

No significant changes were identified when horses were assessed in a straight line, either at walk or trot. Similarly, no significant changes were observed when horses were walking and trotting in circles on the lunge. The biomechanical examination yielded statistically significant results

only during canter, which may be attributed to the increased biomechanical demands placed on the sacroiliac joint at this gait.

Objective gait parameter analysis when assessing horses moving towards their affected side, showed significantly increased retraction and decreased protraction of the inside hind limb; on the contrary, changes in the outside hind limb did not show statistical significance. It is possible that the compression of the inside sacroiliac joint when the horse was cantering towards the affected side may be greater, and therefore the consequences in terms of pain, muscle retraction and limited mobility increase as well. Further studies are required to confirm this theory.

According to classical osteopathic principles, horses with SIJ fixation in extension show facilitation of lumbo-sacral flexion and a hindered extension [36]. This leads to a restrained retraction and facilitated protraction, accompanied by a slight latero-flexion of the affected side [37]. It is common for horses with this type of osteopathic fixation to have increased tension in the homolateral lumbar musculature which makes it easier for the lumbar spine to bend laterally to the affected side [38] while also keeping the lumbosacral joint slightly flexed. Psoas tightening and shortening, which may generate slight hip flexion, adduction, and homolateral lumbar latero-flexion. This leads to a reduction in retraction and an increase in hind limb protraction. When reviewing the study results, the treated horses, showed an increase in retraction and less protraction on the affected limb, which would support classical osteopathic biomechanical theories. The results also suggest that manipulating the SIJ fixated in extension may improve

mobility and normalize the horse's tendency to latero-flexion and rebalance protraction and retraction.

It has previously been suggested that sacroiliac pain and dysfunction may be present when signs of reduced protraction and retraction of the hind limbs or lameness, and irregularity of the hind limbs appear [21].

The canter, as a more dynamic and asymmetrical gait compared to walk and trot, places greater stress on the sacroiliac region [39], which may amplify subtle differences in joint function between the treatment and control groups. The sacroiliac joint plays a vital role in transferring forces between the hindlimbs and axial skeleton [40], and this becomes particularly pronounced during this gait. Increased flexion and extension of the lumbosacral region is likely to exacerbate any existing dysfunction. This heightened biomechanical demand may explain why significant changes were only detectable through kinematic analysis at the canter, in accordance with previous research indicating that certain musculoskeletal conditions become more evident at higher gaits [41]. Furthermore, although empirically, riders and veterinarians report problems during cantering in horses with sacroiliac dysfunction.

The absence of significant findings in straight-line movement and at lower-intensity gaits does not necessarily indicate a lack of treatment effect. This indicates that the biomechanical alterations induced by the intervention may be subtle and only measurable under more demanding conditions, such as lunging canter work. These observations emphasise the value of multi-gait analysis in equine biomechanical research, particularly in the context of sacroiliac dysfunction. These findings contribute to the growing body of evidence

that supports the inclusion of assessments at the canter in comprehensive lameness and performance evaluations, thereby highlighting the potential limitations of assessments that are restricted to straight-line or lower-intensity gaits.

The significant inter-subject variability and field conditions have been the primary limitations. It was interesting to work with horses in competition to approach the conditions of clinical osteopaths, which made the results more "real" and applicable to the clinic. However, the laboratory conditions could refine the results. In addition, the horses were evaluated overground and not on the treadmill as in previous studies on equine locomotion.

Typically horses are manipulated on more than one occasion and several dysfunctional areas may be treated in the same session addressing a range of conditions both primary or compensatory. However, it is of scientific interest to study the effects of a single manipulation, administered only once keeping in mind that individuals establish compensatory movement patterns that may confound the effects of a single treatment. The daily variations in the horses' environment and mood may have also had an impact of the gait analysis results.

Although all the horses exhibited SIJ impairment, the location and severity varied. The sampled horses exhibited a range of different secondary somatic dysfunctions. It is well known that assessments of tissue tension and sensitivity strongly depend on the practitioner's interpretation and prior experience. Even when a skilled therapist conducts the assessments, quantifying palpable findings presents

challenges and in addition there is no recognized single reference standard diagnostic test for sacroiliac dysfunction [42]. The same problem exists in human osteopathy [43,44].

The present study focused on the hypomobility of the SIJ and an osteopathic manipulation was applied to increase its mobility. However, there may also be hypermobility problems in this joint [45] that sometimes result in increased muscle tension, especially during the hard training and competition season, to stabilize the area. This can be a confounding factor for the osteopath assessing SIJ mobility and may lead to treating a hypomobility problem that is actually a hypermobility one. This study did not consider possible soft tissue problems that can cause pain, instability, muscle tension and hypomobility in the sacroiliac joint. Although very strict procedures were implemented, the differential diagnosis of the area is not an easy task.

Osteopathic manipulation is a valuable tool for treating early SIJ problems, but it is usually not the only intervention, and it is accompanied by treating any other somatic dysfunctions that may be present, repeating treatments over time and prescribing active exercises after manipulation to improve the mechanics and stability of the joint. The difficulty of obtaining good quality field data from clinical, osteopathic and diagnostic imaging techniques limit the knowledge about its optimal biomechanical behaviour.

5 Conclusion

The key findings of the present study were that a single osteopathic manipulation of the caudal SIJ in sport horses may result in some significant, albeit variable, changes in gait parameters, especially in protraction and retraction of the hindlimbs during canter and more specific when cantering towards the affected side.

This study, even with its limitations, opens the door to further investigating osteopathic manual therapy applied to SIJ restrictions. Semi-objective clinical evaluation techniques, limited follow-up, and the inability to control for all potentially confounding variables have undoubtedly added significant difficulty to the task.

Further research is needed to validate the evidence that osteopathic manipulations improve the performance, welfare and locomotion of sport horses.

Author's declaration of interests

No competing interests have been declared.

Ethical animal research

Research ethics committee oversight not required by this journal:
noninvasive observational study.

Owner informed consent

Owners gave consent for their animals' inclusion in the study.

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Authorship:

The study was designed by Toni Ramon, Dr. Marta Prades, and Dr Constanza B. Gómez Álvarez, who also contributed to the writing, analysis, and interpretation of data. Dr Jorge U Carmona and Marc Elmeua were responsible for the statistical analysis and data analysis. Dr. Marta Prades conducted the initial physical examination and assessment of inclusion/exclusion criteria for the horses. Toni Ramon performed the osteopathic examination and manipulations.

Manufacturers' addresses

a Garmin International Inc. Olathe (Kansas, USA)

b Equimoves System (EquiMoves[®] -www.equimoves.nl) consisting of nine ProMove-mini IMUs (Inertia Technology B.V., Enschede, The Netherlands.

c MATLAB and Statistics Toolbox Release 2012b, MathWorks, Inc., Natick, Massachusetts, United States

d IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY, United States

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General Discussion

To gain a comprehensive understanding of the effects of structural osteopathic manipulations in horses, meticulously designed studies with large sample sizes must be conducted with real-life subjects, whilst minimising variability and potential confounding factors. It is advantageous to perform studies that include horses that are in training in a similar physical form and subject to similar strains.

Horses not only may present with fixations in the same joint, often with differences like side of rotation or side affected, but they also commonly present with other sites of dysfunction. The horses included in these studies presented a fixation of the joint under study and also presented others, which undoubtedly added to the variability and to the difficulty of performing these kinds of studies in a clinical setting.

Horses ridden in training for dressage and showjumping commonly present with signs of increased strain at the lower cervical region and cervical-thoracic junction and in the sacroiliac area [45] For these studies and based on the location of common osteopathic disorders, the joints selected were C6-C7 and the sacroiliac joints. To minimise confounding variables, just the sacroiliac joint (SIJ) was adjusted in one study, whilst only C6-C7 was manipulated in the other. Additionally, only one single intervention was performed, in contrast to other studies [9]. There is growing evidence of the importance of underlying compensatory mechanisms and interactions between the central and peripheral regions in relation to pain and lameness [46] and for this reason, it was deemed crucial to minimize any external influences that may have disrupted the outcomes such as manipulating other joints or treating all the slightly dysfunctional areas that the horses subjected to the study might have presented. The primary objective of this study was to investigate the precise effects of specific osteopathic adjustment on two highly significant and commonly treated joints in sport horses. These joints, which act as hinges, are subject to shear forces and are predisposed to cyclic loads and degenerative processes.

Osteopathic manipulation is an accepted therapeutic tool for the treatment of both the sacroiliac joint and the lower cervical spine, and in humans, manual therapy treatments for sacroiliac joint problems are reported to have high effectiveness [47].

There are numerous systematic reviews that support manual therapy for treating chronic non-specific neck pain, low back pain, and pelvic girdle pain during pregnancy [48–50]. Further studies have demonstrated the efficacy of manual therapy in musculoskeletal pain in different age groups of patients [51–56]

The literature in horses is not as extensive or specific, but there are studies that confirm the effectiveness of osteopathic manipulation on the horse's back. Chiropractic, osteopathy, or manual therapy in general are commonly used in equine practice for the treatment of acute and chronic back pain and lameness, especially mild lameness [5,57]. An extensive analysis of spinal manipulation techniques indicates a significant effectiveness in alleviating thoraco-lumbar discomfort, stiffness, and muscular hypertonicity in horses [58]. Manual therapy has been shown to alleviate pain and dysfunction of the horse's back; however, its effect on lameness and the appendicular skeleton have been hardly studied [26]. In healthy horses treated for back pain, chiropractic has been shown to have minor kinematic effects on limb movements [59].

A number of studies have included experimental horses with induced lameness or back pain [13,15,16,60,61] but what is interesting in the present studies is the inclusion of real clinical cases with not very evident signs of lameness and discomfort, representing the type of horse patient that is found in daily practice and which make the results more relevant. In many instances when considering the goals of osteopathic therapy, the objective is not to treat a specific pathology, but rather to enhance certain qualities, such as symmetry, flexibility, impulsion and improved locomotion.

A comprehensive analysis of various factors, including lameness examination, physical, osteopathic and biomechanical examination,

was deemed essential. The videos of the locomotion of all the horses, collected using the same methodology, were analysed in a randomised fashion and scored by two expert veterinarians blinded to the treatment and they added an important back up for the gait analysis examination.

An osteopathic examination performed by a single examiner is undoubtedly inherently subjective and because of this, a very strict protocol was maintained in the evaluation of pain, muscle tension, mobility and cutaneous reflexes. The information it provided is recorded but should be taken with caution. The decrease in pain in the affected areas and furthermore, the riders' accounts of the effects of treatment (placebo effect included) indicated that the horses may have exhibited improved flexibility following the manipulation.

The selection of the inertial measurement units (IMUs) to measure the effects of the osteopathic manipulations of the cervical and pelvic regions appeared to be very appropriate. Several studies have indicated that IMUs can be employed to effectively measure equine gait parameters in a variety of contexts, including stride variables, upper body kinematics, and flexion-extension movement [30,32]. The IMU's can be used in the same facilities where the horses live which contributes to repeatability of evaluations due to the influence of familiar conditions.

Gait analysis is undoubtedly the most widely used objective tool to evaluate the effects of different therapies on equine locomotion. However, using gait analysis results as the only tool may miss other potentially relevant factors. What the horse perceives, either pain or well-being and the quality of movement and the ease of execution of certain movements amongst other parameters must be considered in the future to evaluate in a more holistic manner.

Multiple studies in humans have proposed theories on the effect of manual therapy on the subject's neurobiology, stating that the manual pressures exerted during manual therapy intervention elicit neurophysiological reactions that can influence the perception of pain [62–64]. Roura et al. explain that these responses occur at three

levels[65]: "1) peripheral, that is, at the tissue level, where the application of MT induces a modulation of inflammatory response after tissue injury [66,67]; 2) spinal: mechanical solicitations activate somatic-autonomic reflexes, which in turn produce indirect neuromuscular responses and trigger intrinsic spinal networks through spinal-spinal loops [68]; and 3) supraspinal, the use of manual contact might regulate brain areas like anterior cingulate cortex, amygdala or periaqueductal grey matter, which are crucial, for example, in pain experience, autonomic responses and hypoalgesia [64,69]. In horses the effects on the autonomic nervous system and in the immune system [70] are described and probably the effects that occur in humans also occur in horses [71]. The relationship between chronic pain and the nervous system is bidirectional in humans, patients with chronic low back pain experience a notable decrease in heart rate variability, with increased sympathetic activity when compared to healthy individuals [65] therefore further studies in this direction are required in horses.

Osteopathic concepts and definitions both in human and animal therapy needs stricter application of the scientific method or evidence-based medicine [72]. In recent years many of these principles have been questioned and are currently being updated always keeping in mind that other mechanisms of action of manual therapy play a role and must be contemplated.

Many of the basic osteopathic biomechanical concepts are being reviewed, starting with Frayette's laws [73], the importance of the direction of manipulation, the consistency of osteopathic assessment between different operators, the validity of the TART [74–76], and even the sacrosanct concept of somatic dysfunction [33].

Interestingly in humans there is evidence that the direction of the manipulation does not change the results [77,78] and no differences have been observed when treatments are applied to whole segments rather than specifically to the theoretically targeted segment [79,80].

A review paper identifying the gaps within the human literature related to the mechanisms of Force-Based Manipulations FBM was published

recently which could be used as a guide for future mechanistic and translational research [81].

Force-based manipulations FBM is a new term for "manual therapy manoeuvre" referring to the passive application of mechanical force to the outside of the body with therapeutic intent, often as part of pain management care (e.g., low-back pain), rehabilitation care, or general wellness and disease prevention. Examples of force-based manipulations include, but are not limited to: light touch, pressure, mobilization, thrust, adjustment, and/or needling [81].

Neural Mechanisms of Force-Based Manipulations are a high priority in the Research Networks in the National Institutes of Health (NIH) and applications that focus on developing resources to refine and test key concepts that will advance and further support study on the neural mechanisms and biomechanics of force-based manipulations are out.??

The relationship between equine spinal pain and their locomotion has been studied [14,82–84] and presence of back pain, its source and consistent assessment is not always straightforward [85].

Pain response to palpation, muscular hypertonicity, thoracolumbar joint stiffness and physical dysfunction are elements often used for physical examination of back pain. Lack of willingness to go forward freely, lack of power, shortened steps, stiffness of the cervical or thoraco-lumbosacral regions are common nonspecific signs of musculoskeletal causes of poor performance in sport horses [86–88] and these signs can be associated with longissimus dorsi spasm on palpation (78.6%), paravertebral muscle stiffness (64.3%), resistance to lateral bending (64.3%), and poor hindlimb impulsion (85.7%) [86].

Even if systematic reviews have shown variable efficacy of osteopathic manipulation in relieving musculoskeletal pain in humans [89,90] , alleviating pain to some degree in a human or a horse can modify behaviour [91], biomechanics [92] and sports performance [91,93] suggesting that pain relief is a key element in improving symptomatology [94].

While it should be noted that bone and soft tissue pain and global assessment are clinically acceptable in the equine back [95], new ways of more objectively assessing pain or mobility (functional and pain assessment scales [96,97]), well-being (facial expressions [98,99]) and others need to be developed, validated and implemented in both research and equine clinical practice.

Lower Cervical

Cervical dysfunction has been observed in horses with mild forelimb gait problems, subtle pain and altered muscle tone. The term "dysfunction" is crucial but often overlooked when discussing poor performance in horses. Symptoms of cervical dysfunction may manifest as either localised or generalised muscle asymmetry, such as atrophy, hypertrophy or hypertonicity. Other signs include stiffness or the inability to move the neck within a normal range of motion, as well as changes in the positioning of the head or neck [100]. Cervical dysfunction can cause altered gait and biomechanics of the forelimb and trunk, resulting in additional dysfunction, pain and lameness. These horses may exhibit behaviours like stopping, refusing to move forward, and even bucking and rolling over backwards if the rider persists in making more strong demands.

Cervical radiculopathy in horses commonly causes localised pain in the lower neck area and lameness in the front limbs. This is due to the involvement of peripheral nerves from the brachial plexus [96]. Another frequently observed clinical indication in horses with cervical discomfort or dysfunction is an inexplicable alteration in behaviour. These horses exhibit unexpected panic or become anxious in familiar environments [101].

Muscle atrophy or hypertrophy, of sudden and rapid onset, especially after trauma may suggest disuse or neurogenic muscle atrophy. Unusual sweating patterns on the side of the neck may also be a symptom of problems with the sympathetic nervous system [6]. Muscle atrophy or underdevelopment often occurs in the lower cervical region (C4-C6), specifically affecting the splenius and semispinalis muscles. Horses with lower cervical pain or stiffness may experience discomfort when the brachiocephalic muscles are manually pressed. This can be detected by applying firm pressure to the muscle, starting from the top and moving towards the lower neck region. Finally, an avoidance response after deep palpation over the cervical transverse processes and articular processes may indicate the presence of bone or joint pain [102].

Certain horses that are affected may exhibit a noticeable weakness or struggle to fully engage the forelimbs at the beginning of stance phase, which can lead to stumbling. If diagnostic analgesia fails to identify the lameness on any other structure of the limb, possibly the problem is located in the neck region. Injection of an analgesic into the articular facets may be used in some circumstances to check whether pain and inflammation in the neck is the cause of lameness [20,30,31] although there is a significant probability that many vertebral levels are impacted and that makes diagnosis difficult and is not free of complications.

There are multiple medical conditions that can be confused with cervical dysfunction and require alternative treatments and approaches. These include cervical vertebral compressive myelopathy (CVCM), equine degenerative myelopathy, complex cervical vertebral malformation syndrome (CCVMS), osteochondral fragmentation, equine protozoal myeloencephalitis, polysaccharide storage myopathies, vitamin E-related myopathies, and myofibrillar myopathies [103,104]. It is also important to exclude any old or recent fractures, which can be challenging to detect due to the difficulty in obtaining quality radiological images of the area, especially in horses with a well-developed neck. It is also important to check all the areas of the brachial plexus from C6 to T2 including the ribs, as well as the mobility and functionality of the thoracic sling. Other common issues include fascia problems, fibrosis, joint capsule synovitis, cervical intervertebral disc disease, and compressive disorders of the spinal cord.

Lymphocytic inflammation has been observed in the dorsal root ganglia or its vicinity in horses diagnosed with "idiopathic hopping-like forelimb lameness" [105]. The clinical significance of dorsal root ganglionitis and its correlation with persistent neck pain in horses has yet to be fully understood.

Approximately 50% of mature horses have been noticed to exhibit degenerative lesions in the articular facets of the final cervical

vertebrae, resulting in unilateral or bilateral changes in the cervical articular margins [106]. These data indicate that it is not possible to diagnose cervical pain or dysfunction only based on radiographic imaging. It is important to exercise caution and avoid overinterpreting osteoarthritis as the main cause of the observed clinical symptoms [102].

Recent studies have found postmortem histological evidence of nerve root injury in horses with unexplained forelimb lameness, when inflammatory mediators associated with osteoarthritis of the cervical articular processes contribute to chemically induced neuritis and the development of neck pain and dysfunction [96].

Pre-assessment of the horses was based on the assessment of tenderness, pain on palpation which has a high inter-operator reliability, muscle tone, mobilisation and segmental mobility restriction, which is clearly less reliable [107,108]. In any case osteopathic manual evaluation is always subjective.

Sacroiliac joint

Sacroiliac joint dysfunction is often a unilateral pathology, and small differences from right to left can be significant, and it should be noted that in healthy horses the symmetry is considerable [109].

Movements of the SIJ are small and coupled only with the flexion of the LSJ [110]. The ligaments surrounding the SIJ have a strong effect on the stabilisation of this joint. Due to the limited amount of movement, its biomechanical study *in vivo* is very difficult [111]. Goff details two types of problems in sacroiliac joint dysfunction the first, presenting as pain and poor performance, is responsive to local analgesia of periarticular structures with poorly defined pathology. The second presents primarily as poor performance with bony pathological changes because of chronic instability. Diagnostic tests based on biomechanics as well as manual provocation for SIJ pain have formed the basis of tests currently used to diagnose SIJ dysfunction in humans [112].

In this study, the focus was mainly on the first of these 2 problems, and hypermobility problems were not treated, as they require specific exercises to improve joint stability and no post rehabilitation exercises were considered in the present study. Nevertheless, and given the difficulty of accurately diagnosing sacroiliac joint disorders [113,114] and since there is no highly specific and sensitive diagnostic test for sacroiliac joint pain [114], it is possible that hypermobility problems were treated as hypomobility problems in some cases [115], despite the experience of both the veterinarian who performed the initial physical examination and the physiotherapist and osteopath who performed the initial examination and treatment.

In fact sacroiliac dysfunction diagnosis and assessment has been based on ruling out other causes of hind limb lameness, along with combinations of ultrasonography, scintigraphy and periarticular anaesthesia of the SIJ [113], but the response to desensitising the sacroiliac joint has been shown to be non-specific [116] and ultrasound-guided injections for equine sacroiliac joints were accurate for peri-articular injection but poor for intra-articular injection, with

injected anaesthetic frequently in contact with interosseous sacroiliac ligaments and neurovascular and synovial structures [117]. Also, some of the horses with sacroiliac joint region pain have concurrent orthopaedic injury or disease [114].

There was a particularly significant finding, namely the increase in retraction of the most affected hind limb with sacroiliac joint fixation. A hindlimb retraction decrease may be one of the main indicators of sacroiliac dysfunction [118] and its increase in the treatment group as compared to the control group may indicate an improvement in SIJD function.

There are several reasons for the lack of observed locomotor changes in the present study and mainly probably since only one single manipulation was performed. In humans, this condition always requires more than one treatment. "A brief treatment involving either mechanical-force, manually-assisted or high-velocity, low-amplitude chiropractic adjustments resulted in a positive impact by decreasing pain and disability in patients diagnosed with sacroiliac joint syndrome" [119] In horses Haussler recommended almost 3 treatments for this kind of condition [9,120]. In the present study the focus was more on the specific mechanisms of a particular osteopathic manipulation than in the benefits of a complete osteopathic treatment.

In view of the above, the purely mechanical approach to osteopathy and the search for results after osteopathic treatment using only classical biomechanical parameters may not be the best option. In the case of pelvic and sacroiliac dysfunction Goff already warned that maybe the IMU's were not the best system to evaluate the horse's pelvis due to skin motion artifact [121].

In SIJD, there are causes other than a purely osteopathic fixation or a mechanical and/or inflammatory or pain-related dysfunction. The relationship between abnormal SIJ motion and altered neuromotor control with clinical disease of the equine SIJ are discussed [112] and involvement of peripheral nerve problems have also been reported: the

sciatic and/or obturator nerves were affected in 26% of cases of horses with SIJD in a post-mortem histological study [122].

There is a large inter-subject variability in SIJ mobility, which requires a large number of cases in both the study and control groups. Recent studies suggest the need to make subgroups according to the type of pelvic mobility of each individual within the same experimental and control groups, which would further increase the need for a much larger number of cases. A recent study carried out on 100 warmblood horses shows that there is substantial individual variation in pelvic roll stride pattern amongst horses[123] and when considering different breeds and sizes and weights the variation may even increase [124].

Research has indicated that it may be required to use breed-specific reference values to differentiate between a normal and pathological gait [124–126]. Enhanced understanding of typical fluctuations in pelvic movement can aid in the detection of abnormalities, such as aberrations in motion patterns linked to mild or bilateral lameness, which are challenging to consistently diagnose using alternative techniques [123].

Limitations

Differences in farrier care and hoof trimming practices between horses and over the study duration may have influenced gait parameters. Rider ability, position, weight and experience were not standardized across horses, allowing for potential placebo or nocebo effects from rider and farrier expectations. Measurement reliability is a concern, as intra- and inter-rater consistency of the orthopedic examination procedures was not established. The musculoskeletal assessment scope was restricted to the neck and back regions, with no comprehensive evaluation of the limbs or other areas beyond the initial screening exam. Furthermore, receiving only a single treatment session limits understanding of potential dose-response relationships, as acute biomechanical changes may require repeated manipulations to manifest.

Conclusions and recommendations for further studies.

The biomechanical studies conducted, employing a single osteopathic manipulation of the sacroiliac and lower cervical joints, have yielded small but significative evidence supporting positive effects on equine locomotor patterns. However, limiting the evaluation of equine osteopathic treatment solely to biomechanical outcomes may fail to fully elucidate the comprehensive benefits of applying osteopathy to horses. Novel, multifaceted systems for assessing the impacts of manual therapies warrant exploration.

Akin to other neuromusculoskeletal disorders in human medicine, numerous factors beyond biomechanics contribute to, modulate, or influence neck and back pain, including anatomical, physical, neurophysiological, genetic, immunological, neuroendocrine, psychological, and social elements [127,128]. For horses, which cohabit with humans in highly specialized environments while routinely performing at elite levels, aspects extending beyond biomechanics should be considered.

In humans afflicted with chronic pain, neuroplastic changes and alterations in brain representations [129] have been documented. Emerging evidence suggests that manual therapy may also influence the autonomic nervous system in horses [130]. The use of facial pain scales [96,97,131] or other objective pain assessment tools should be considered [132,133], as well as bioimpedance recording or dynamic acoustic myography [134], which can detect sustained treatment effects for up to 24-48 hours in muscles such as the trapezius.

The scientific analysis of qualitative parameters, although not entirely objective, should be included in the objectives in the forthcoming years. The arm movements of a dancer and a mechanic, for instance, may exhibit identical ranges of motion, angular velocity, and three-dimensional displacement, yet appear distinctly different to an external human observer, and at some point, science must explain this difference.

Larger sample sizes are necessary to mitigate the effect of the inherent substantial variability in equine movement patterns. Future studies should encompass significantly larger sample sizes for control groups, treatment groups, and subgroups stratified by movement patterns

[123]. The identification of subgroup patterns is valuable for illustrating and enhancing comprehension of variations in pelvic roll patterns, and there is no indication that the lower cervical region would differ in this regard.

Osteopathy must evolve and distance itself from archaic concepts lacking scientific substantiation or validation, such as obsolete biomechanical models or purported mechanisms of action. Within human osteopathy, the mechanistic study model is being abandoned in favor of investigating the neuromuscular changes induced by manual therapy treatments [64,135,136]. The model developed by Bialosky appears to be more scientifically rigorous and meaningful. The study of osteopathy and manual therapy applied to horses cannot lag behind this progress.

List of conclusions

1. Biomechanical analysis of treated horses in both studies shows small but significantly positive effects of a single osteopathic manipulation on gait parameters
2. Osteopathic manipulation of the lower cervical spine in horses with cervical dysfunction results in increased protraction of the inside forelimb when trotting in circles.
3. Osteopathic manipulation of the sacroiliac joint in horses with sacroiliac dysfunction results in increased retraction of the affected limb when cantering to the affected side.
4. Qualitative parameters, such as degree of localized osteopathic dysfunction, degree of pain and muscle tension showed improvement even at 2 weeks.
5. Equine osteopathy research should align with human studies by focusing on neuromuscular changes induced by manual therapy rather than only mechanistic study models.
6. Using additional tools like pain ethograms and pain scales during static and ridden conditions would add to the further documentation of the possible benefits of manual therapy.

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