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Changes in Head, Withers, and Pelvis Movement Asymmetry in Lame Horses as a Function of Diagnostic Anesthesia Outcome, Surface and Direction



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ABSTRACT

Evaluation of diagnostic anesthesia during equine lameness examination requires comparison of complex movement patterns and can be influenced by expectation bias. There is limited research about how changes in movement asymmetries after successful analgesia are affected by different exercise conditions. Movement asymmetry of head, withers and pelvis was quantified in $N = 31$ horses undergoing forelimb or hindlimb diagnostic anesthesia. Evaluation on a straight line and a circle was performed with subjective diagnostic anesthesia outcome and quantitative changes recorded. Mixed linear models ($P < .05$) analyzed the differences in movement asymmetry before/after diagnostic anesthesia - random factor: horse, fixed factors: surface (soft, hard), direction (straight, inside, outside, inside-outside average), diagnostic anesthesia outcome (negative, partially positive, positive) and two-way interactions. Forelimb diagnostic anesthesia influenced primary movement asymmetry (all head and withers parameters) and compensatory movement asymmetry (two pelvic parameters) either individually ($P \leq .009$) or in interaction with surface ($P \leq .03$). Hindlimb diagnostic anesthesia influenced primary movement asymmetry (all pelvic parameters) and compensatory movement asymmetry (two head and two withers parameters) either individually ($P \leq .04$) or in interaction with surface ($P \leq .01$;) or direction ($P \leq .006$). Direction was also significant individually for two pelvic parameters ($P \leq .04$). Changes in primary movement asymmetries after partially positive or positive outcomes indicated improvement in the blocked limb. Compensatory changes were mostly in agreement with the 'law of sides'. The changes were more pronounced on the hard surface for hindlimb lameness and on the soft surface for forelimb lameness. Withers asymmetry showed distinct patterns for forelimb and hindlimb lameness potentially aiding clinical decision-making.

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1. Introduction

In horses undergoing clinical lameness investigations, diagnostic anesthesia is an essential step in the process of determining

the source(s) of pain causing lameness [1]. As subjective evaluation of the outcome of diagnostic anesthesia has been shown to be affected by expectation bias [2,3], a small number of studies explored kinematic changes in upper body movement asymmetries in order to assess responses to diagnostic anesthesia objectively [4–8]. Positive response to diagnostic anesthesia in horses with forelimb lameness has been linked to a reduction in asymmetry in vertical displacement of head parameters as well as compensatory changes in pelvic movement [4,6]. The compensatory changes associated with forelimb lameness mostly present as 'false lameness' of the contralateral hindlimb [4,6,7] although ipsilateral changes

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have also been observed [6]. Vertical pelvic displacement asymmetry has been shown to decrease in horses with hindlimb lameness after positive response to diagnostic anesthesia [5,8] with a simultaneous reduction in the compensatory head movement patterns resembling ipsilateral forelimb lameness [6,8]. Whether these compensatory strategies in clinically lame horses are consistent across different surfaces and during lunging is yet to be established as these compensatory patterns have so far only been objectively quantified in horses trotting in a straight line on a hard surface and in horses with induced lameness on the circle [9]. Additionally, recent research suggests that withers movement asymmetry should also be considered during lameness evaluation as it might help to distinguish between primary and compensatory lameness [10,11].

When assessing changes in movement asymmetry, correct interpretation of quantitative gait analysis data is paramount. This is particularly the case when comparing repeated measurements, that is, before and after diagnostic anesthesia, as some changes might occur simply due to normal intra-horse variation. For racehorses in training [12] the daily and weekly average variation, measured with an inertial measurement units (IMU) system, was found to be 5 mm–7 mm and 4 mm–6 mm for head and pelvis movement asymmetry respectively. These values are similar to repeatability thresholds of 6 mm for the head and 3 mm for pelvis from another commercially available IMU system [13]. However, the variation for individual horses can exceed these values, with 14 mm–19 mm and 9 mm–13 mm reported for head and pelvis movement asymmetry respectively for intervals containing 90% of the absolute differences over time [12]. In sports horses in regular work, the between-measurement mean variation of movement asymmetry during various exercise conditions, using optical motion capture, was 9 mm–21 mm for head movement asymmetry and as 3 mm–6 mm for pelvis and withers movement asymmetry [14]. The effect of surface should also be considered as a soft surface has been shown to increase variation for head movement symmetry [14] potentially making it harder to visually assess changes in asymmetry reliably. Lunging has also been shown to increase variation for a number of symmetry parameters [14], thereby further highlighting the need to differentiate reliably between clinically significant changes in asymmetry and changes due to natural gait variability between trials and conditions. Additionally, in horses with movement asymmetries on a straight line, circular motion can either reduce or amplify the preexisting asymmetry depending on whether the limb associated with the asymmetry is on the inside or outside of the circle and whether the asymmetry is associated with weight-bearing or propulsion [15,16]. These results underline the importance of discriminating between circle and lameness related asymmetries.

The aim of this study was to quantify differences in movement asymmetry of head, withers and pelvis before and after diagnostic anesthesia based on subjectively judged block efficacy, surface and direction, and their interactions. We hypothesized that:

- 1) Changes in movement asymmetry for diagnostic anesthesia judged subjectively as either 'partially positive' or 'positive' will be significantly different from diagnostic anesthesia judged as 'negative'.
- 2) According to the previously identified compensatory patterns:
 - a) Changes in head movement asymmetry, after positive forelimb diagnostic anesthesia, will be associated with same-side changes in withers movement asymmetry and contralateral changes in pelvis movement asymmetry.
 - b) Changes in pelvis movement asymmetry, after positive hindlimb diagnostic anesthesia, will be associated with contralateral changes in withers movement asymmetry and ipsilateral changes in head movement asymmetry.

We also wanted to explore whether changes after successful diagnostic analgesia would be systematically affected by different surfaces and direction, i.e. movement on a straight line versus lunging.

2. Materials and Methods

2.1. Data Collection

Gait analysis records of horses admitted for lameness investigation at the Royal Veterinary College (RVC) Equine Referral Hospital, London, United Kingdom and at the Pferdeklinik Hochmoor, Germany between January 2019 and June 2020 were evaluated against the following inclusion criteria: (1) Gait analysis data from trot in a straight line and on a circle were obtained before and after at least one diagnostic block, (2) Subjective outcome of diagnostic anesthesia was recorded. If a horse had diagnostic anesthesia administered to more than one limb on the same day, only data from the first blocked limb were analyzed. Multiple blocks administered to the same limb were included in the analysis.

All horses were evaluated on a straight on a hard surface (40 m length) and during circular motion (either lunged or in-hand if necessary) on a soft or a hard surface or both with the circle diameter ranging from 10 m to 20 m. The hard surface was either a nonslip coated tarmac or a paving stone surface while the soft surface was either a sand/fiber mixture or sand-based arena. The horses were trotted up in a bridle by an experienced handler and, typically, the same handler was used throughout the examination. The veterinarians were assessing the horses from any viewpoint they deemed necessary for the given exercise condition.

The quantitative gait analysis was performed with an identical setup consisting of five (or more) inertial measurement units (MTw, second generation, Xsens, Enschede, The Netherlands, tri-axial accelerometer $\pm 16 \times$ gravity, tri-axial gyroscope $\pm 2,000$ deg/S, tri-axial magnetometer ± 1.9 mGauss) attached to the poll, the withers, the sacrum and the left and right tubera coxae. Raw sensor data were transmitted wirelessly at update rates of 60 to 100 Hz to a dedicated Windows (Microsoft, Redmond, WA) laptop computer and processed by a custom graphical user interface written in MATLAB (The Mathworks Inc, Natick, MA) producing PDF readouts of head, withers and pelvic movement symmetry measures (Fig. 1).

2.2. Judgment of Efficacy of Diagnostic Anesthesia

Visual assessment was performed by five experienced veterinary experts (AFJ, RKWS, DMB, MP, and CG) and the efficacy of the blocks was categorized by the clinician in charge of each case. Block outcomes were categorized retrospectively from electronic case records and the subjective outcome was assigned globally to all conditions of a particular block, not individually to each exercise condition. A horse that improved under all conditions would receive a higher score than a horse that only showed improvement under specific exercise condition. The case records included statements about the efficacy of the performed blocks in three subjective categories: 'negative', 'partially positive' or 'positive', or statements about the subjective 'percentage change' after diagnostic anesthesia which were in consultation with the clinicians mapped into the three categories as follows: 'negative' 0%–30%; 'partially positive' >30%–70%; 'positive' >70%–100% [17]. The output of the gait analysis software was available to the clinicians shortly after each evaluation (with a 30–60 seconds delay).

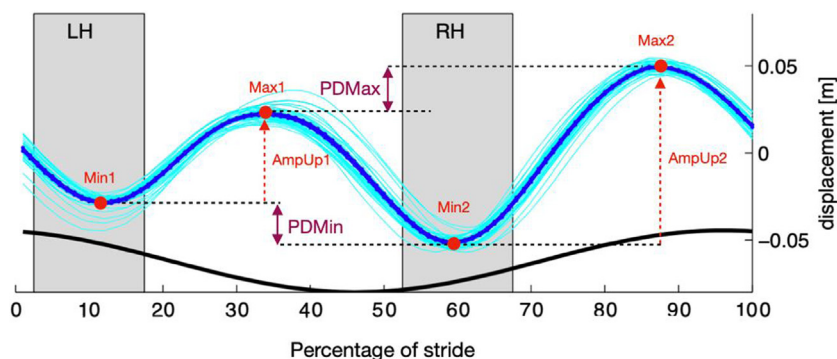


Fig. 1. Example of vertical displacement of the midline of pelvis during a stride and related pelvis movement asymmetry parameters: PDmin: difference between the vertical minima of the midline of pelvis reached during left (Min1) and right (Min2) hindlimb stance, PDmax: difference between the vertical maxima of the midline of pelvis reached after left (Max1) and right (Max2) hindlimb stance, AmpUp1: the upward vertical movement amplitude of midline of pelvis from the lowest point at mid stance to the highest point during the aerial phase reached after left hind stance, AmpUp2: the upward vertical movement amplitude of midline of pelvis from the lowest point at mid stance to the highest point during the aerial phase reached after right hind stance. The gray bars are a visual indicator of which minimum corresponds to the left hind (LH) and to the right hind (RH) stance but are not accurate representations of the duration of the stance phase.

2.3. Movement Asymmetry Parameters

Data were processed by calculating previously described movement asymmetry parameters [18] for each trot stride based on vertical displacement and their median values were tabulated in Microsoft Excel (Microsoft, Redmond, WA) alongside information about the surface (hard, soft) and the movement direction (straight line, left/right circle). In brief, the 10 asymmetry parameters were the difference between the two halves of a stride for the vertical displacement minima, maxima and the upward movement for head (HDmin, HDmax, HDup), withers (WDmin, WDmax, WDup) and pelvis (PDmin, PDmax, PDup). Additionally, hip hike difference (HHD) was calculated, comparing left and right tuber coxae movement amplitudes during contralateral stance. The complete data set was subdivided into two partial datasets, one containing data for diagnostic anesthesia administered to a forelimb, the second for diagnostic anesthesia administered to a hindlimb.

2.4. Data Normalization

In order to be able to combine data from diagnostic anesthesia administered to left and right limbs, differences between asymmetry values before and after diagnostic anesthesia were calculated and these values were 'normalized' to represent diagnostic anesthesia administered to right limbs, that is, difference values of blocks administered to left limbs were inverted (multiplied by negative one). This effectively meant that, for primary changes (i.e., head and withers after forelimb diagnostic anesthesia or pelvis after hindlimb diagnostic anesthesia), negative values indicated a change in asymmetry in the direction which showed improvement on the side of the blocked limb and positive values indicated an increase in the asymmetry assigned to the blocked limb. For compensatory changes, negative values indicated reduction in asymmetry assigned to the ipsilateral limb while positive values indicated reduction in asymmetry assigned to the contralateral limb.

2.5. Classification of Direction

As veterinary textbooks describe that lameness for certain conditions increases with the affected limb on the inside or outside of the circle [19], we wanted to evaluate whether the position of the blocked limb on the circle, that is, to the inside or outside, affected the changes in movement asymmetry. Hence, direction conditions were relabeled as 'inside' and 'outside' rather than 'left' and 'right'. This meant that the direction condition of a horse with a diagnostic anesthesia administered to the right limb circled to the

right was labeled as 'inside' and, similarly, the direction of a horse with a diagnostic anesthesia administered to the left limb circled to the right was labeled as 'outside', that is, the circle direction was described with respect to the 'blocked limb' being on the inside or the outside of the circle. Finally, averages were calculated between 'inside' and 'outside' circle asymmetry values and tabulated as a fourth direction category labeled 'IOavg' (inside-outside average) to evaluate whether 'average-rein asymmetry' could be a useful tool to simplify the evaluation of changes in asymmetry on a circle [16,20].

2.6. Model Building and Data Analysis

All statistical analysis was performed in IBM SPSS (v26, IBM, Armonk, NY). The level of significance was set at $P < .05$. Linear mixed models were implemented with 'case number' (i.e., horse) as random factor and 'direction' (straight, inside, outside, IOAvg), 'surface' (hard, soft) and 'diagnostic anesthesia outcome' (negative, partial, positive) as fixed factors as well as all two-way interactions. The outcome variables were defined as the difference between asymmetry before and after diagnostic anesthesia for each movement asymmetry parameter assessed under the same specific condition (i.e., on the same surface and in the same direction). Separate models were implemented for the subset of forelimb block data and the subset of hindlimb block data. Bonferroni corrections were implemented when investigating pairwise significant differences for the fixed factors. The histograms of the residuals were inspected for normality and the residual values were plotted against fitted values to check for heteroscedasticity.

3. Results

3.1. Horses and Baseline Movement Asymmetry

In total, gait analysis data from 31 horses were included (RVC $N = 25$, Pferdeklunik Hochmoor $N = 6$). Fifteen horses were included only in the forelimb diagnostic anesthesia subset, fifteen horses were included only in the hindlimb diagnostic anesthesia subset and one horse was included in both subsets. This horse was evaluated on two separated days with diagnostic anesthesia administered to a forelimb during the first day and diagnostic anesthesia administered to a hindlimb during the second day.

A summary of the mean baseline movement asymmetry for each parameter, calculated from absolute values of the asymmetry parameters before diagnostic anesthesia was administered, can

be found in Supplementary Item S1. The horses in the forelimb diagnostic anesthesia subset showed larger head movement asymmetries (mean: 10 mm–21 mm, SD = 7 mm–12 mm), compared to the hindlimb diagnostic anesthesia subset (mean: 6 mm–9 mm, SD = 5 mm–10 mm). Conversely, the horses in the hindlimb diagnostic anesthesia subset showed larger pelvis movement asymmetries (mean: 8 mm–15 mm, SD = 5 mm–11 mm) compared to the forelimb diagnostic anesthesia subset (mean: 5 mm–8 mm, SD 3 mm–5 mm). The withers movement asymmetry was of a similar magnitude in both forelimb diagnostic anesthesia subset (mean: 6 mm–12 mm, SD = 4 mm–8 mm) and the hindlimb diagnostic anesthesia subset (mean: 6 mm–13 mm, SD = 4 mm–8 mm).

3.2. Summary of Diagnostic Blocks and Conditions

Before and after measurements were available for 46 forelimb blocks administered to 16 horses (mean 2.9, range 1–8 blocks per horse) and 31 hindlimb blocks were administered to 16 horses (mean 1.9, range 1–6 per horse). Each horse was evaluated in up to six conditions resulting in $N = 220$ data entries related to diagnostic blocks administered to a forelimb (Supplementary Item S2) and $N = 161$ to 168 data entries to diagnostic blocks administered to a hindlimb (Supplementary Item S3). Missing data are related to missing data points from the withers ($N = 4$) and/or left and right tuber coxae sensors ($N = 7$).

The block outcome was subjectively assigned as negative for $N = 129$ (58.6%) data entries in the forelimb diagnostic anesthesia subset and $N = 68$ (40.5%) data entries in the hindlimb diagnostic anesthesia subset, partially positive for $N = 53$ (24.1%) data entries in the forelimb diagnostic anesthesia subset and $N = 49$ (29.2%) data entries in the hindlimb diagnostic anesthesia subset and positive for $N = 38$ (17.3%) for data entries in the forelimb diagnostic anesthesia subset and $N = 51$ (30.4%) data entries in the hindlimb diagnostic anesthesia subset. There were $N = 127$ (57.5%) observations from a hard surface and $N = 93$ (42.3%) observations from a soft surface for the forelimb subset. Similarly, for the hindlimb subset, there were $N = 108$ (64.3%) observations from a hard surface and $N = 60$ (35.7%) observations from a soft surface. For direction, the distribution was similar across all conditions: for straight line there were $N = 49$ (22.3%) observations for the forelimb subset and $N = 37$ (22.0%) observations for the hindlimb subset, inside and outside conditions had each $N = 57$ (25.9%) observations in for the forelimb subset and $N = 44$ (26.2%) observations in the hindlimb subset and for the 'average rein' asymmetry there were $N = 57$ (25.9%) observations in the forelimb subset and $N = 43$ (25.6%) observations in the hindlimb subset.

3.3. Overall Effect of Diagnostic Anesthesia Outcome, Surface, and Direction on Movement Asymmetry Parameters

Forelimb diagnostic anesthesia significantly affected nine parameters either as an individual factor (HDmin, HDmax, HDup, PDup; $P \leq .009$) or during an interaction with surface (WDmin, WDmax, WDup, PDmin, HHD; $P \leq .03$). Hindlimb diagnostic anesthesia affected eight parameters either as an individual factor (HDmax, HDup, PDmin, PDup, HHD; $P \leq .04$) or during an interaction with surface (WDmin, WDup, PDmax; $P \leq .01$;) or direction (WDmin, WDup; $P \leq .006$). Direction was also found to be significantly independent of diagnostic anesthesia outcome for PDup and HHD ($P \leq .04$) for hindlimb diagnostic anesthesia subset. A summary of all models can be found in Supplementary Item S4. Estimated marginal means are only shown for significant individual fixed effects or significant interactions (Tables 1–4).

3.4. Primary Changes After Forelimb Diagnostic Anesthesia

Changes in head and withers movement asymmetry parameters following negative forelimb diagnostic anesthesia were significantly different from changes following partially positive (HDmin, HDup, $P \leq .01$; WDmax and WDup on a soft surface, $P \leq .02$) or positive diagnostic anesthesia (all head parameters, $P \leq .003$; WDmax on a soft, WDup on a hard surface, $P \leq .02$, Tables 1–2). After successful analgesia (positive or partially positive outcome), the estimated marginal means for change in movement asymmetry for head and withers (except WDmin the soft surface positive condition) were negative indicating reduction in asymmetry associated with the blocked limb. Head and withers movement asymmetry changes were not significantly different between positive and partially positive response. The changes in head and withers asymmetry parameters after negative diagnostic anesthesia were not significant.

3.5. Compensatory Changes of the Pelvic Motion After Forelimb Diagnostic Anesthesia

Changes in pelvic movement asymmetry were significantly different between positive and negative forelimb diagnostic anesthesia for PDup ($P = .007$) regardless of surface or direction (Table 1) and for HHD ($P = .046$) on a soft surface (Table 2). Additionally, on the soft surface PDmin and HHD ($P \leq .02$) showed significantly different changes between negative and partially positive diagnostic anesthesia (Table 2). The estimated changes in asymmetry following successful forelimb analgesia (positive and partially positive outcome) were positive indicating a reduction in asymmetry assigned to the hindlimb contralateral to the blocked forelimb. No significant difference was identified between partial and positive response. The changes in the compensatory pelvic asymmetry following negative response were not significant.

3.6. Primary Changes After Hindlimb Diagnostic Anesthesia

Changes in pelvis movement asymmetry following negative response to diagnostic anesthesia were significantly different from changes after successful analgesia (partially positive, $P \leq .03$; positive, $P \leq .03$), regardless of direction or surface, for three pelvis parameters (PDmin, PDup and HHD, Table 1). The pelvis asymmetry changes were also significantly different between negative and positive diagnostic response for PDmax but only on the hard surface ($P \leq .001$, Table 2). For both positive and partially positive outcome, the estimated values for change in movement asymmetry of the pelvis were negative indicating improvement in the asymmetry assigned to the blocked limb. Partially positive response was not significantly different from positive response for any of the pelvis parameters. The changes in asymmetry following negative diagnostic response were not significant.

3.7. Compensatory Changes of Head and Withers Motion After Hindlimb Diagnostic Anesthesia

Compensatory changes in head movement asymmetry were significantly different between negative diagnostic outcome and partially positive (HDmax and HDup, $P \leq .04$) or positive outcome (HDup, $P = .047$) regardless of surface and direction (Table 1). The changes in head movement asymmetry after positive and partially positive response hindlimb diagnostic anesthesia were negative indicating a reduction in head asymmetry ipsilateral to the blocked hindlimb. HDmin parameter was not affected by hindlimb diagnostic anesthesia outcome.

Compensatory changes of withers asymmetry were significantly different between partially positive and negative hindlimb diagnos-

Table 1

Estimated marginal means (mm) with 95% confidence intervals for changes in movement asymmetry for outcome variables with significant 'diagnostic anesthesia outcome' term ($P < .05$) and no significant interactions with 'direction' or 'surface' ($P > .05$).

		Diagnostic Anesthesia Outcome								
		Negative	95% Confidence Interval		Partial	95% Confidence Interval		Positive	95% Confidence Interval	
			Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Forelimb diagnostic anesthesia	HDmin	2.0	-6.1	10.1	-8.4 ^a	-17.6	0.8	-12.2 ^a	-21.8	-2.7
	HDmax	0.8	-1.5	3.2	-2.9	-6.2	0.3	-8.5 ^a	-12.0	-4.9
	HDup	3.3	-4.9	11.4	-12.0 ^a	-21.9	-2.2	-20.1 ^a	-30.5	-9.8
	PDup	-0.1	-2.9	2.7	3.6	0.2	7.0	6.0 ^a	2.4	9.6
Hindlimb diagnostic anesthesia	HDmax	2.5	0.0	5.0	-2.1 ^a	-4.7	0.5	-0.7	-3.5	2.0
	HDup	7.2	1.9	12.4	-2.4 ^a	-8.2	3.4	-2.5 ^a	-8.2	3.4
	PDmin	0.0	-2.4	2.5	-5.0 ^a	-7.9	-2.1	-5.2 ^a	-8.3	-2.2
	PDup	0.5	-3.8	4.9	-9.4 ^a	-14.1	-4.7	-9.1 ^a	-14.1	-4.1
	HHD	2.0	-3.2	7.2	-9.4 ^a	-15.1	-3.7	-12.6 ^a	-18.4	-6.9

^a significantly different from negative response ($P < .05$). Negative values indicate improvement on the side of the blocked limb, positive values indicate improvement on the side contralateral to the blocked limb. Difference between the two halves of a stride for the vertical displacement minima (Dmin), maxima (Dmax) and the upward movement (Dup) for head (H) and pelvis (P). Hip hike difference (HHD) difference between left and right tuber coxae movement amplitudes during contralateral stance. Diagnostic anesthesia outcome based on perceived 'percentage change' in movement asymmetry after diagnostic anesthesia by the clinician in charge of the case: 'negative' 0%–30%; 'partially positive' >30%–70%; 'positive' >70%–100%.

Table 2

Estimated marginal means (mm) with 95% confidence intervals for changes in movement asymmetry for outcome variables with significant 'surface' and 'diagnostic anesthesia outcome' interaction term ($P < .05$).

		Diagnostic Anesthesia Outcome									
		Surface Condition	Negative	95% Confidence Interval		Partial	95% Confidence Interval		Positive	95% Confidence Interval	
				Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Forelimb diagnostic anesthesia	WDmin	Hard	1.3	-2.9	5.5	-2.0	-6.8	2.7	-3.6	-8.8	1.6
		Soft	-0.3	-4.7	4.0	-1.2	-7.1	4.6	2.8	-3.2	8.8
	WDmax	Hard	-1.5	-4.0	1.0	-1.8	-4.7	1.0	-5.7	-8.8	-2.6
		Soft	0.8	-1.7	3.4	-6.2 ^a	-9.7	-2.6	-5.3 ^a	-9.0	-1.7
	WDup	Hard	-0.2	-3.5	3.0	-4.1	-7.9	-0.4	-8.8 ^a	-12.9	-4.6
		Soft	0.3	-3.0	3.6	-7.7 ^a	-12.4	-3.0	-2.9	-7.8	1.9
	PDmin	Hard	0.8	-1.5	3.0	0.2	-2.7	3.2	3.2	-0.3	6.7
		Soft	-1.7	-4.1	0.7	5.9 ^a	1.9	10.0	2.3	-2.0	6.6
	HHD	Hard	1.9	-1.8	5.5	1.7	-2.7	6.0	5.4	0.6	10.3
		Soft	-2.3	-6.1	1.5	8.2 ^a	2.7	13.8	6.9 ^a	1.1	12.7
Hindlimb diagnostic anesthesia	WDmin	Hard	-1.9	-4.2	0.4	1.9 ^a	-0.4	4.2	5.5 ^a	3.4	7.7
		Soft	1.2	-1.3	3.7	2.5	-0.2	5.3	2.8	-0.5	6.3
	WDup	Hard	-1.4	-4.6	1.7	3.7	0.6	6.9	7.6 ^a	4.7	10.6
		Soft	0.8	-2.6	4.2	4.5	0.6	8.4	1.4	-3.4	6.2
	PDmax	Hard	0.1	-2.6	2.9	-3.9	-6.7	-1.0	-7.3 ^a	-10.0	-4.7
		Soft	0.2	-2.7	3.2	-4.7	-8.3	-1.2	-0.3	-4.7	4.1

^a significantly different from negative response ($P < .05$). Negative values indicate improvement on the side of the blocked limb, positive values indicate improvement on the side contralateral to the blocked limb. Difference between the two halves of a stride for the vertical displacement minima (Dmin), maxima (Dmax) and the upward movement (Dup) for withers (W) and pelvis (P). Hip hike difference (HHD) difference between left and right tuber coxae movement amplitudes during contralateral stance. Diagnostic anesthesia outcome based on perceived 'percentage change' in movement asymmetry after diagnostic anesthesia by the clinician in charge of the case: 'negative' 0%–30%; 'partially positive' >30%–70%; 'positive' >70%–100%.

Table 3

Estimated marginal means (mm) with 95% confidence intervals for changes in movement asymmetry for outcome variables with significant 'direction' and 'diagnostic anesthesia outcome' interaction term ($P < .05$).

		Diagnostic Anesthesia Outcome									
		Direction	Negative	95% Confidence Interval		Partial	95% Confidence Interval		Positive	95% Confidence Interval	
				Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Hindlimb diagnostic anesthesia	WDmin	Straight	0.8	-2.3	3.9	1.9	-1.3	5.1	2.6	-0.5	5.7
		Inside	-2.0	-4.8	0.9	2.6	-0.4	5.7	5.5 ^a	2.4	8.5
		Outside	-0.7	-3.6	2.1	2.1	-1.0	5.2	6.4 ^a	3.3	9.4
		In-Out	-1.3	-4.2	1.5	1.6	-1.5	4.7	5.9 ^a	2.9	9.0
	WDup	Straight	2.6	-2.0	7.1	2.8	-1.9	7.6	3.3	-1.3	8.0
		Inside	-3.4	-7.5	0.7	7.2 ^a	2.7	11.7	8.8 ^a	4.3	13.3
		Outside	-0.5	-4.6	3.6	1.8	-2.7	6.3	5.6	1.1	10.1
		In-Out	-1.9	-6.1	2.2	3.9	-0.7	8.5	7.2	2.7	11.7
		Average									

^a significantly different from negative response ($P < .05$). Negative values indicate improvement on the side of the blocked limb, positive values indicate improvement on the side contralateral to the blocked limb. Difference between the two halves of a stride for the vertical displacement minima (Dmin) and the upward movement (Dup) for withers (W). Diagnostic anesthesia outcome based on perceived 'percentage change' in movement asymmetry after diagnostic anesthesia by the clinician in charge of the case: 'negative' 0%–30%; 'partially positive' >30%–70%; 'positive' >70%–100%.

Table 4

Estimated marginal means (mm) with 95% confidence intervals for changes in movement asymmetry for outcome variables with significant 'direction' term ($P < .05$) and no significant interactions with 'diagnostic anesthesia outcome' ($P > .05$).

Hindlimb diagnostic anesthesia	Direction											
	Inside	95% Confidence Interval		Outside	95% Confidence Interval		In-Out Average	95% Confidence Interval		Straight	95% Confidence Interval	
		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
PDup	-11.1 ^a	-16.0	-6.2	-1.3 ^a	-6.2	3.6	-6.0	-10.9	-1.1	-5.1	-10.3	0.1
HHD	-12.2	-18.0	-6.4	-1.8	-7.6	3.9	-7.2	-13.0	-1.3	-4.3	-10.2	1.7

^a significantly different between two direction conditions (i.e., inside vs. outside). Negative values indicate improvement on the side of the blocked limb. Difference between the two halves of a stride for upward movement (Dup) of pelvis (P). Hip hike difference (HHD) difference between of left and right tuber coxae movement amplitudes during contralateral stance.

tic outcome for WDmin on a hard surface ($P \leq .04$, Table 3) and WDup with the blocked hindlimb on the inside ($P \leq .03$, Table 4). For positive and negative diagnostic outcome, the changes were only significantly different for WDmin and WDup on a hard surface ($P \leq .001$), with the blocked hindlimb on the inside of a circle ($P \leq .007$) and, for WDmin only, with the blocked hindlimb on the outside of the circle ($P \leq .01$) and for the 'average' (inside-outside) asymmetry ($P \leq .01$). The withers movement asymmetry changes after successful hindlimb diagnostic anesthesia (partially positive or positive outcome) were positive indicating reduction in withers asymmetry contralateral to the blocked hindlimb.

No significant difference was identified between partial and positive diagnostic anesthesia outcomes for any of the head or withers movement asymmetry parameters. The estimated changes in compensatory asymmetry following negative response were either indicating no change or worsening of the asymmetry.

3.8. Effect of Surface

Surface affected more movement asymmetry parameters in the forelimb diagnostic anesthesia subset (WDmin, Wdmax, WDup, PDmin and HHD) than in the hindlimb diagnostic anesthesia subset (WDmin, WDup, PDmax), Table 2). The head parameters were not affected by surface in any of the models while withers and pelvis were consistently affected by surface. The changes in movement asymmetry were significantly different between positive and negative outcome most consistently for the withers, specifically Wdmax on a soft surface ($P = .02$) and WDup on a hard surface for horses with forelimb lameness ($P = .002$) and WDmin and WDup on a hard surface for horses with hindlimb lameness ($P \leq .001$). Additionally, partially positive changes were significantly different from negative changes for Wdmax and WDup on a soft surface in horses with forelimb lameness ($P \leq .02$). Pelvis parameters were also affected by surface but the changes following negative response were only significantly different from positive outcome for PDmax on a hard surface in horses with hindlimb lameness ($P \leq .001$) and for HHD on a soft surface for horses with forelimb lameness ($P = .046$). In addition, the changes after negative diagnostic anesthesia were significantly different from partially positive response for PDmin and HHD on a soft surface ($P \leq .02$) for horses with forelimb lameness. No significant differences were identified between partially positive and positive response on either surface, and neither was there a significant difference between the positive response on a soft compared to a hard surface.

3.9. Effect of Direction

Direction in interaction with diagnostic anesthesia outcome influenced only withers parameters (WDmin and WDup) in horses undergoing hindlimb diagnostic anesthesia ($P \leq .006$, Supplementary Item S4). The changes in withers movement asymmetry were significantly different between positive and negative outcome for

WDmin for all circle-related direction conditions ($P \leq .01$) and for WDup with the blocked hindlimb on the inside of a circle ($P = .002$, Table 3). For WDup the partially positive outcome was also different from the negative outcome ($P = .02$, Table 3). Regardless of the direction condition, the withers movement asymmetry changes associated with successful analgesia indicated improvement in asymmetry contralateral to the blocked hindlimb. No significant differences were identified between any of the direction conditions (e.g., straight vs. outside after positive outcome). Direction was also found to be significant independently of diagnostic anesthesia outcome for PDup and HHD in horses with hindlimb lameness ($P \leq .03$, Supplementary Item S4) and significant difference was identified between inside and outside direction conditions for PDup only ($P = .02$, Table 4)

4. Discussion

This study aimed to quantify differences in movement asymmetry of head, withers and pelvis based on subjective outcome of diagnostic anesthesia, surface and direction and their interactions. Primary changes in movement asymmetry subjectively evaluated as positive indicated improvement in the blocked limb and the associated compensatory changes followed expected patterns. The only upper body landmark consistently affected by surface in interaction with diagnostic anesthesia outcome was the withers. The pelvis movement asymmetry was also affected by surface, although less consistently, while changes in head movement asymmetry were not influenced by surface. Direction was only significant for horses undergoing hindlimb diagnostic anesthesia – either individually for pelvis movement asymmetry or, in interaction with diagnostic anesthesia outcome for withers movement asymmetry.

4.1. Primary Changes After Forelimb Diagnostic Anesthesia

In the current study, all head parameters were affected by diagnostic anesthesia outcome but not by surface or direction. In agreement with previous studies [4,7], the change in head movement asymmetry following partially positive (EMM: -12.0 mm to -2.9 mm) and positive (EMM: -20.1 mm to -8.5 mm) outcome indicated improvement in asymmetry assigned to the blocked forelimb. Furthermore, in line with our first hypothesis, the changes in head movement asymmetry associated with positive diagnostic anesthesia outcome were significantly different from negative response (EMM: 0.8 mm–3.3 mm) indicating that the degree of head movement asymmetry corresponds to the visual evaluation performed by the veterinarians.

Some changes in movement asymmetry between two trials are expected due to normal inter-run variation but if the changes are higher than the expected variation, the changes are likely due to other factors – in the case of diagnostic anesthesia due to successfully blocking the source of pain resulting in reduced movement asymmetry. This was indeed the case in the current study as the

magnitude of the change in head movement asymmetry for positive outcome was above the test-retest repeatability threshold of 6 mm for vertical head movement asymmetry established for a different IMU system [13] (adjusted to 8 mm–10 mm for the IMU system used in this study using equation from [21]) and the level of variability expected in the same horse measured at different time points with a motion capture system [14].

Interestingly, in a previous study with horses assessed during straight line trot with a similar IMU system [4], the identified thresholds for a positive response to diagnostic anesthesia were of very small magnitudes, -0.19 mm and -4 mm for HDmin and HDmax respectively. Such low values are somewhat surprising, as it has been shown that human eye struggles to identify asymmetries smaller than 25% in a computed generated model of tuber coxae movement [22] although movement asymmetries above 10% could be identified during live assessment [23]. Based on these findings, movement asymmetries below 7 mm–12 mm (i.e., 10% of range of motion of upper body landmarks established in [12,24]) would be hard to identify visually and it could be argued that such low average values may in part be related to expectation bias [3]. In the current study a similar magnitude of change in head movement asymmetry (EMM ≤ 3 mm) was associated with a negative response. However, it is important to note that the veterinarians involved in the current study had access to the gait analysis output which might have influenced the diagnostic anesthesia outcome assigned to the case notes that were consulted retrospectively in this study.

After successful analgesia in a forelimb, withers movement asymmetry changed in concert (same sign) with head movement asymmetry (EMM: -8.8 mm to -2.9 mm) in all conditions except on a soft surface for WDmin (EMM: 2.8 mm). However, the change in withers movement asymmetry was smaller than the changes in head movement asymmetry, up to 62% of the magnitude of change in the head movement asymmetry. These results are in line with our second hypothesis and agree with previous studies in sound horses with head movement asymmetries [11] as well as horses with induced forelimb lameness [10]. While the changes in withers movement asymmetry might be less detectable visually due to the smaller magnitude and due to the fact that the view of the withers might be limited depending on where the observer is standing, the low between-measurement variation of withers vertical movement asymmetry compared to head movement [14] might make withers movement asymmetry a good candidate for the quantification of forelimb lameness or the evaluation of diagnostic anesthesia efficacy.

4.2. Compensatory Changes of the Pelvic Motion After Forelimb Diagnostic Anesthesia

For partially positive or positive forelimb diagnostic anesthesia outcome, all pelvic movement asymmetry parameters apart from PDmax showed improvement in asymmetry contralateral to the blocked forelimb. These results support our second hypothesis about the 'law of sides' and are in agreement with studies of horses with naturally occurring forelimb lameness horses which also showed improvement in asymmetry assigned to the contralateral hindlimb after successful diagnostic analgesia [4,6,7,25]. The evidence for which pelvic movement asymmetry parameters change in response to changes in head movement asymmetry is mixed – one study found that only PDmax was affected [7] while another study identified both PDmin and PDmax compensatory changes, although the magnitude of change in PDmin was much smaller [6]. In another study in horses with induced forelimb lameness, both ipsilateral and contralateral compensatory patterns were identified [9]. In the current study, the compensatory changes after successful forelimb diagnostic analgesia were only associated

with improvement in movement asymmetry assigned to the contralateral hindlimb (EMM: 2.3 mm–6.9 mm) with values similar to the magnitude of expected biological variation [12–14]. The compensatory changes in pelvic movement asymmetry were smaller compared to the changes in head movement asymmetry, up to 32% of the magnitude of changes in the head movement asymmetry. This finding is in line with previous studies which observed compensatory changes in pelvis movement asymmetry that were only 4%–20% of the change in head movement asymmetry [6,9].

4.3. Primary Changes After Hindlimb Diagnostic Anesthesia

Changes in pelvic movement asymmetry were affected by hindlimb diagnostic anesthesia outcome with changes for partially positive (EMM: -9.4mm to -3.9mm) and positive (EMM: -12.6 mm to -5.3 mm) outcome indicating improvement in the movement asymmetry assigned to the blocked hindlimb except for PDmax on a soft surface (EMM: -0.3 mm). This is in agreement with a previous study of horses with hindlimb lameness [8] in which both PDmin and PDmax decreased following diagnostic anesthesia outcomes was subjectively judged as positive. In another study [5], PDmin and HHD were found to be consistently affected by successful diagnostic analgesia showing the largest reduction in movement asymmetry after a positive response while changes in PDmax were less pronounced. Similarly, in the present study all pelvic parameters were affected by diagnostic anesthesia outcome with the largest changes after successful diagnostic analgesia observed for HHD (EMM: -12.6 mm). The largest changes observed for HHD might reflect the strategies of the veterinarians involved in the study as it has been shown that some veterinarians focus more on the upward movement and some on the downward movement of the upper body landmarks [26,27] and, in the case of pelvic movement, some veterinarians might focus more on the movement of the tubera coxae rather than the sacrum. Nevertheless, the changes in all pelvic movement asymmetry parameters associated with positive outcome were, in general, above the test-retest repeatability threshold of 3 mm [13] for pelvic movement asymmetry (adjusted to 5 mm–6 mm for the IMU system used in this study using equation from [21]) and above the magnitude of change in asymmetry expected purely due to biological variation [14]. Hence, it is likely that these changes were indicative of significant improvement in pelvic movement asymmetry and therefore lameness. Furthermore, no significant changes in pelvic movement asymmetry were observed after negative outcome (EMM: 0 mm–2 mm) and, in agreement with our first hypothesis, negative response was significantly different from partially positive or positive response.

4.4. Compensatory Changes of Head and Withers Motion After Hindlimb Diagnostic Anesthesia

Ipsilateral compensatory changes in head asymmetry were previously identified in horses with induced lameness [9] while both contralateral and ipsilateral patterns were observed in lame horses [6]. In the current study, HDmax and HDup were affected by the outcome of hindlimb diagnostic anesthesia while HDmin was not influenced by hindlimb diagnostic anesthesia outcome. This is somewhat surprising as previous studies showed that both HDmin and HDmax change in response to changes in pelvic asymmetry in clinically hindlimb lame horses [6] as well as horses with induced lameness [9]. In the current study, a small number of horses had subsequent diagnostic anesthesia administered to a forelimb, suggesting multilimb lameness, which could explain the lack of change in HDmin after successful hindlimb diagnostic analgesia. The compensatory changes in HDmax after positive or partially response and in HDup after partially positive re-

sponse were showing improvement in head asymmetry ipsilateral to the blocked hindlimb (EMM: -2.5 mm to -2.1 mm) and these changes, albeit small, were significantly different from a negative response (EMM: 2.5 mm to 7.2 mm). Therefore, we can support our second hypothesis that, following successful hindlimb diagnostic analgesia, changes in pelvis movement asymmetry will be associated with changes in ipsilateral head movement asymmetry. The small magnitude of the compensatory changes in head movement asymmetry, both in absolute and relative terms (i.e., in comparison to pelvis movement asymmetry), contradicts previous studies which identified compensatory head movement asymmetry changes of larger magnitudes [6,9]. Additionally, changes in head movement asymmetry of up to 7 mm–20 mm can be expected between two measurements in sound horses [12–14] so differentiation between changes due to improved lameness and changes due to normal inter-run variation might be difficult for small changes in movement asymmetry. On the other hand, lame horses have been shown to have low stride to stride variability [28], hence smaller changes in movement asymmetry after successful diagnostic analgesia could be clinically significant as lame horses might show more consistent movement patterns.

The withers showed significant improvement in movement asymmetry assigned to a forelimb contralateral to the blocked hindlimb for partially positive (EMM: 1.9 mm–4.5 mm) and positive outcome (EMM: 1.4 mm–7.6 mm) – a finding which is in line with our second hypothesis. Similar results were reported for sound horses with upper body vertical movement asymmetries [11] and horses with induced hindlimb lameness [10]. The changes in the withers movement asymmetry had similar magnitude to the changes in primary pelvic movement asymmetry which suggests a strong link between withers and pelvis movement as described in previous studies [10,11]. Due to its low between-measurement variation compared to head movement [14], evaluation of withers movement asymmetry might be beneficial during the process of identification of primary lameness in horses displaying concurrent head and pelvic vertical movement asymmetries.

4.5. Effect of Surface

Veterinary textbooks often describe that lameness for certain orthopedic conditions can be exacerbated on soft or hard surfaces [19] thus amplifying mild asymmetries above the detection threshold of a human eye [22]. In horses with symmetrical movement, the movement symmetry does not seem to be affected by surface [15,29], however, the effect of surface on movement symmetry of clinically lame horses has not been investigated. In the current study, head parameters were not affected by surface which is surprising as a hard surface has been shown to amplify forelimb-related asymmetries [20,29]. On the other hand, withers and pelvic movement asymmetry were affected by surface in interaction with diagnostic anesthesia outcome but posthoc analysis did not reveal any significant differences between the two surfaces for positive response for any of the upper body asymmetry parameters. These results are slightly surprising as veterinary textbooks suggest that specific orthopedic conditions can be exacerbated on different surfaces [19] – for example, horses with foot pain often perform worse on a hard surface while horses with suspensory desmitis or tendonitis tend to be more lame on a soft surface. The lack of significant results with respect to surface type in this study might be due to the heterogeneous group of horses with a variety of orthopedic conditions. Hence, further studies of how specific lesions are affected by different surfaces are warranted.

However, some influence of surface could be identified as more parameters were significantly different between negative and partially positive or positive diagnostic anesthesia outcome on a soft surface when a forelimb was blocked (for WDmax, WDup, PDmin,

HHD) whereas for hindlimb diagnostic anesthesia this was the case for a hard surface (for WDmin, WDup, PDmax). For the soft surface, in some cases the partially positive response was different from negative, but this was not always the case for changes in asymmetry labeled as positive. Some of the contradicting results on a soft surface could be explained by increased variation in movement asymmetries on a soft surface [14]. In addition, most horses in the present study received consecutive diagnostic anesthesia administered on the same day which could help to explain why the change in movement asymmetry after the first diagnostic anesthesia with a partially positive outcome could have been larger in magnitude than the second diagnostic anesthesia with a positive outcome since the veterinarians scored the response to diagnostic anesthesia based on percentage improvement rather than absolute values of change (in mm) in movement asymmetry.

4.6. Effect of Direction

In clinical textbooks, descriptions of increased lameness for certain orthopedic disorders are given when the affected limb is on the outside or on the inside of the circle [19,30]. However, in the current study, the changes in movement asymmetry for positive response were not different between circle and straight-line conditions apart from withers asymmetry parameters in horses undergoing hindlimb diagnostic anesthesia. Previous studies demonstrated that head and pelvis movement asymmetries observed on a straight line can be amplified when the limb to which the asymmetry is attributed is on the inside of the circle [9,16,29]. Additionally, for visual assessment of horses with forelimb foot pain, higher lameness scores were recorded when the horses were lunged with the affected limb on the inside of a circle [31]. In contrast, a different study found that straight-line and circle movement asymmetries were not different for horses with forelimb-related asymmetries but pelvic asymmetry was influenced by circular motion [32]. Surprisingly, the results from the current study suggest that head and pelvic movement asymmetry parameters are not influenced by direction in interaction with diagnostic anesthesia outcome. However, it is important to note that we investigated changes before and after diagnostic anesthesia for the same condition, for example, change in asymmetry with the blocked limb on the inside before and after diagnostic anesthesia, rather than looking at the change between conditions directly. Our focus was not to directly compare the movement asymmetry under different condition but to evaluate the change in asymmetry before and after diagnostic anesthesia which could provide insight into whether specific movement asymmetries are truly 'amplified' (lameness exacerbated) during certain exercise conditions. Previous studies suggested that circle-related asymmetries might be simply 'additive' and that the movement on a circle does not aggravate the lameness as such [9]. This would mean that if the lameness-related movement asymmetry was resolved with diagnostic analgesia, we would see similar change in movement asymmetry on the straight line and on the circle. This has been most consistently demonstrated for PDmin, a pelvic parameter related to impact lameness, with 'worsening' of the asymmetry with the affected limb on the inside and 'reduction' in movement asymmetry with the limb on the outside [9,16,33]. In the current study, PDup and HHD were affected by direction as an individual factor (regardless of the diagnostic anesthesia outcome) but significantly greater changes of movement asymmetry were only identified for PDup with the limb on the inside compared to the outside condition.

For head movement asymmetry, the effect of circular motion is less clear – previous studies concluded that head movement asymmetry observed on a straight-line could be amplified or reduced during lunging depending on the position of the presumably affected limb on a circle (inside or outside) [9,16,20,29]. These re-

sults seem to be in agreement with the clinical textbooks suggesting that, for certain orthopedic disorders, lameness can be exacerbated when the affected limb is on the outside, for example in horses with suspensory desmitis, or on the inside of the circle [19,30]. In contrast, a different study found that the associated 'average-rein' head movement asymmetry values were smaller than condition matched straight-line movement asymmetry values [20] suggesting that some horses with forelimb-related asymmetries might become less asymmetrical on a circle. This could result, on the group level, in the average head movement asymmetry unaltered compared to the straight line measurement. This was indeed the case in the present study as the estimated changes in head movement asymmetry were not affected by direction condition suggesting that similar changes would be observed on a straight line and on a circle or that the changes are not consistent across all the horses. Additionally, since the speed was not strictly controlled for, we cannot rule out that the horses chose to trot at slower speed on the circle which might influence the magnitude of the head movement asymmetries [34]. The results from the current study and previous research [29,35] highlight that horses utilize different adaptations of the vertical head movement when trotting on a circle and further research is needed to establish how specific lesions influence head movement asymmetries on a circle.

While a small number of studies considered the withers movement asymmetry in the context of diagnostic anesthesia [36] or lunging [14,18,37], the compensatory patterns of withers movement asymmetry have only been described in horses trotting in a straight line on a hard surface [10,11]. Interestingly, in the current study the withers were the only upper body landmark influenced by direction in interaction with diagnostic anesthesia outcome in horses with suspected hindlimb lameness. For positive response, the compensatory changes in withers movement asymmetry on a circle (EMM: 6 mm–9 mm) followed the same contralateral pattern as on the straight line (EMM: 3 mm) but, interestingly, the withers movement asymmetry changes were only significantly different between positive and negative response for the circle conditions. Specifically, this was the case for WDmin for all the circle conditions, with the blocked hindlimb on the inside or outside of the circle as well as the average-rein condition, and for WDup with the blocked hindlimb on the inside. Due to its low between-measurement variation both on the straight line and on a circle [14], the withers movement asymmetry might be a useful additional tool when evaluating the outcome of diagnostic anesthesia.

While all the lameness evaluations were carried out by experienced handlers and veterinarians, the speed and circle radius were not strictly controlled and could have influenced the results as these factors may influence the movement asymmetry parameters [34]. Future studies with 'average-rein' parameter where speed and circle radius are closely matched across the two reins could provide further insights into the compensation strategies of horses during lunging. Due to the limited number of horses, it was beyond the scope of this study to divide the horses into groups by a specific orthopedic disorder but further studies with a larger population of horses are warranted to provide further evidence of how movement asymmetries are influenced by specific orthopedic conditions in interaction with movement direction.

4.7. Limitations

When evaluating lameness before and after diagnostic anesthesia, controlling for speed and circle radius may be important as variation in these factors may influence the asymmetry parameters [34]. In the present study, neither speed nor circle radius were strictly controlled, however, all lameness evaluations were carried out by experienced veterinarians and handlers to ensure consistency.

Most horses included in the current study received consecutive diagnostic anesthesia administered on the same day which could have affected the subjective scoring of a diagnostic anesthesia outcome if the veterinarians subconsciously compared the change of the asymmetry to the first time they saw the horse rather than before the most recent block. However, due to similar magnitudes of change in asymmetry after partially positive and positive outcomes, it seems that the veterinarians in the current study based their subjective judgment on the difference observed between two consecutive blocks rather than comparing it to the baseline lameness. Consequently, the change in movement asymmetry after the two consecutive diagnostic anesthesia intervention could have been similar if the lameness improved but not fully resolved after the first 'partial' diagnostic analgesia and the second 'positive' diagnostic analgesia resulted in the lameness being completely abolished.

In the current study, the diagnostic anesthesia outcome was assigned globally and not per condition (i.e., not as separate outcome for straight line and each lunging condition) which could explain why for positive outcomes under some conditions the change in movement asymmetry was only small. If the lameness was exacerbated only under certain exercise conditions, for example during lunging, then the change in movement asymmetry for positive outcome might be small for all the other conditions. The clinicians had access to the gait analysis output (due to processing 30–60 seconds after the evaluations) which might have influenced their subjective judgment but this approach allowed us to explore which parameters or exercise conditions the clinicians considered relevant to their globally assigned diagnostic anesthesia outcome and identify what magnitude of change they considered as 'improvement'. It is unlikely that the clinicians based their judgment of the subjective diagnostic anesthesia outcome solely on the quantitative changes of the movement asymmetry parameters in question due to the fact that the horses were observed under several exercise conditions and overall improvement was then scored globally. Furthermore, while guideline 'thresholds' exists for movement asymmetry on a straight line on a hard surface [13], there are no guideline values for other exercise conditions such as lunging, which means that the veterinarians mostly draw on their experience of what degree of movement asymmetry they consider 'normal' during these conditions. It is also important to note that some horses might improve under certain conditions after successful analgesia of one region while no improvement will be seen for the other exercise conditions due to multiple sources of pain. This might be particularly the case in equine referral hospitals adding another layer of complexity to the decision making in relation to assigning the outcome of diagnostic anesthesia.

Lastly, it is important to note that different technological solutions (specific sensor and/or processing) can influence the values of the measured movement asymmetry [21,28,38]. To ensure consistency, the same IMU system was used in the two equine hospitals included in this study but the interpretation of the changes in movement asymmetry between different systems should be made with caution.

5. Conclusion

The subjectively scored improvement in lameness after diagnostic anesthesia is linked to changes in movement asymmetries of upper body landmarks. For primary movement asymmetries, the changes for partially positive or positive outcomes indicated improvement in the blocked limb while negative outcome was associated with only small changes in asymmetry. The compensatory changes mostly followed expected patterns and were in agreement with the 'law of sides'. Out of the three upper body landmarks evaluated, only withers were consistently affected by surface and

direction in interaction with diagnostic anesthesia outcome. Our results suggest the magnitude of change in the withers movement asymmetry after successful diagnostic analgesia is altered by the exercise conditions with changes being more prominent on a circle and on a hard surface in horses with suspected hindlimb lameness. Pelvis movement asymmetry was affected by surface in interaction with diagnostic anesthesia outcome, although less consistently than withers, while changes in head movement asymmetry were not influenced by surface or direction. Future studies with a larger population of horses are warranted to provide further evidence of how movement asymmetries are influenced by specific orthopedic conditions in interaction with the type of surface or the movement direction.

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Eva Marunova: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Karl Hoenecke:** Data curation, Writing – review & editing. **Andrew Fiske-Jackson:** Investigation, Resources, Writing – review & editing. **Roger K.W. Smith:** Investigation, Resources, Writing – review & editing. **David M. Bolt:** Investigation, Resources, Writing – review & editing. **Melanie Perrier:** Investigation, Resources, Writing – review & editing. **Carolin Gerdes:** Investigation, Resources, Writing – review & editing. **Elin Hernlund:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Marie Rhodin:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Thilo Pfau:** Conceptualization, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing.

Supplementary materials

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