

1 Adaptations in equine axial movement and muscle activity occur during induced fore- and hindlimb
2 lameness: a kinematic and electromyographic evaluation during in-hand trot.

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20 **ABSTRACT**

21 **Background:** The inter-relationship between equine thoracolumbar motion and muscle activation
22 during normal locomotion and lameness is poorly understood.

23 **Objectives:** To compare thoracolumbar and pelvic kinematics and longissimus dorsi (longissimus)
24 activity of trotting horses between baseline and induced forelimb (iFL) and hindlimb (iHL) lameness.

25 **Study design:** Controlled experimental cross-over study.

26 **Methods:** Three-dimensional kinematic data from the thoracolumbar vertebrae and pelvis, and
27 bilateral surface electromyography (sEMG) data from longissimus at T14 and L1, were collected
28 synchronously from clinically non-lame horses (n = 8) trotting overground during a baseline
29 evaluation, and during iFL and iHL conditions (2-3/5 AAEP), induced on separate days using a
30 lameness model (modified horseshoe). Motion asymmetry parameters, maximal thoracolumbar
31 flexion/extension and lateral bending angles, and pelvis range of motion (ROM) were calculated from
32 kinematic data. Normalised average rectified value (ARV) and muscle activation onset, offset and
33 activity duration were calculated from sEMG signals. Mixed model analysis and statistical parametric
34 mapping compared discrete and continuous variables between conditions ($\alpha=0.05$).

35 **Results:** Asymmetry parameters reflected the degree of iFL and iHL. Maximal thoracolumbar flexion
36 and pelvis pitch ROM increased significantly following iFL and iHL. During iHL, peak lateral bending
37 increased towards the non-lame side (NLS) and decreased towards the lame side (LS). Longissimus
38 ARV significantly increased bilaterally at T14 and L1 for iHL, but only at LS L1 for iFL. Longissimus
39 activation was significantly delayed on the NLS and precipitated on the LS during iHL, but these clear
40 phasic shifts were not observed in iFL.

41 **Main limitations:** Findings should be confirmed in clinical cases.

42 **Conclusions:** Distinctive, significant adaptations in thoracolumbar and pelvic motion and underlying
43 longissimus activity occur during iFL and iHL and are detectable using combined motion capture and
44 sEMG. For iFL, these adaptations occur primarily in a cranio-caudal direction, whereas for iHL, lateral
45 bending and axial rotation are also involved.

46 1. INTRODUCTION

47 Lameness and back pain are common clinical issues in horses that are often interrelated; lame
48 horses can exhibit adaptive thoracolumbar movement and horses with back pain can show clinical
49 signs of forelimb (FL) and/or hindlimb (HL) lameness.¹ Lameness is one of the main reasons for
50 veterinary consultation,² and the prevalence of back problems has been reported to be as high as
51 94% in ridden horses.³ Despite this, the aetiology and clinical manifestation of equine back pain and
52 the inter-relationship with FL and/or HL lameness, remain poorly understood, creating a diagnostic
53 challenge.^{4,5}

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55 Quantitative equine gait analysis has been applied to measure axial motion in non-lame horses⁶⁻⁹
56 and to quantify adaptive changes in axial motion in horses with induced lameness or back pain¹⁰⁻¹³
57 during treadmill locomotion. Increased thoracolumbar range of motion (ROM) was observed in horses
58 with induced unilateral back pain¹² and FL lameness,¹⁰ but not during induced unilateral HL
59 lameness.¹¹ These studies have advanced our understanding of adaptive axial movement associated
60 with pain avoidance during treadmill locomotion, but clinical observations during overground
61 locomotion indicate decreased thoracolumbar ROM during FL and/or HL lameness, which contradicts
62 published findings.^{11,12} Furthermore, the underlying neuromuscular mechanisms that ultimately
63 facilitate these movement adaptations are poorly understood and have not been quantified during
64 standardised lameness conditions.

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66 Surface electromyography (sEMG) offers a solution to this shortcoming by quantifying isolated muscle
67 activation through recordings of summated motor unit action potentials from electrodes placed on the
68 skin over superficial muscles.¹⁴ Zaneb et al.¹⁵ used sEMG to quantify back muscle activity during
69 treadmill trot and detected significantly lower amplitude ratios bilaterally from longissimus dorsi
70 (longissimus) in a group of horses with chronic, unilateral HL lameness. They interpreted this finding
71 as a “more distinct resting phase” between active contractions of longissimus.¹⁵ Unfortunately, axial
72 movement was not quantified to corroborate this interpretation and comparisons were drawn from
73 horses with subjectively assessed and non-standardised lameness. In recognition of this, we have
74 therefore initiated research to directly compare appendicular (St. George et al. under review) and

75 axial movement and muscle activity between non-lame and standardised lameness conditions during
76 overground locomotion.

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78 This study aimed to quantify and compare thoracolumbar and pelvic kinematics and longissimus
79 activity in horses' thoracic and lumbar regions during overground trot in non-lame and induced
80 forelimb (iFL) and hindlimb (iHL) lameness conditions. Based on previously reported findings and
81 clinical observations, we hypothesised that there will be different adaptations during iFL and iHL, with
82 the changes in ROM and longissimus activity being more localised to the thoracic and lumbar regions,
83 respectively.

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86 2. MATERIALS AND METHODS

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88 Ethical approval for this study was obtained from Utrecht University (CCD: AVD108002015307) and
89 the University of Central Lancashire (RE/17/08a_b).

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91 2.1 Horses

92 Eight horses (Mean \pm SD age: 9.2 \pm 3.9 years, height: 161.3 \pm 3.4 cm, body mass: 582.1 \pm 39.4 kg, 7
93 mares, 1 stallion) were used. Horses were in regular ridden exercise, were accustomed to being
94 walked and trotted in hand, and were deemed clinically non-lame (<1/5 AAEP Lameness Scale)
95 through visual assessments by two equine veterinarians (T.S., F.S.B).

96

97 2.2 Kinematic instrumentation

98 Three-dimensional (3D) kinematic data were collected using an optical motion capture (OMC) system
99 of 18 high-speed infrared cameras^a. The OMC system was hardware synchronised to the sEMG
100 system and recorded time series for both data types in one file for further processing. The calibrated
101 volume for data collection was 56 m long and 10 m wide. Super-spherical, retro-reflective markers^b
102 (19 mm diameter) were attached over anatomical landmarks, as presented in Figure S1a. Individual
103 markers and a marker cluster on the head were attached using double-sided adhesive tape, with an
104 additional drop of cyanoacrylate glue used for the hoof and limb markers.

105

106 2.3 sEMG Instrumentation

107 sEMG data were collected bilaterally from longissimus using wireless sEMG sensors^c with a fixed
108 inter-electrode distance of 10 mm. Ultrasonography was used for the detection of the desired
109 locations over longissimus, at the T14 and L1 vertebrae, 6 cm lateral to midline.¹⁶ Once identified,
110 each skin location was clipped of hair, then thoroughly cleaned using isopropyl alcohol. A small
111 amount of electrolytic solution (0.9% saline) was applied to each electrode before attaching sensors
112 to the prepared skin locations using double-sided adhesive interface strips^d, with the electrodes
113 oriented perpendicular to the underlying muscle fibre direction.^{17,18} Additional adhesion included a
114 drop of cyanoacrylate glue on the double-sided tape, attached to the top and bottom of the sensor,
115 above each electrode pair (Figure S1b).

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117 2.4 Data collection

118 To simulate a real-world lameness examination, sEMG (2000 Hz) and 3D kinematic (200 Hz) data
119 were synchronously collected from in-hand trot trials, conducted on a straight, hard, indoor runway
120 during control and induced lameness (iFL, iHL) conditions. Four trials (passes down the runway) were
121 conducted per condition. Data were initially collected from the control condition to determine the
122 baseline gait pattern of each horse (baseline 1). Then, mild iFL (2-3/5 AAEP Lameness Scale) was
123 temporarily induced by mechanical screw pressure applied to the sole of the hoof using a modified
124 horseshoe.¹⁹ Lameness induction was applied, graded, and monitored by veterinarians (T.S., F.S.B.).
125 Horses were randomly divided into two groups (n=4) for right and (n=4) left iFL, in a cross-over
126 design. Following iFL, trot trials were repeated. After a washout period of at least 24 hours, the same
127 data collection process was repeated for baseline 2 and iHL conditions, where iHL was again
128 randomised to the right (n=4) or left (n=4) HL.

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130 2.5 Data Analysis

131 2.5.1 Kinematic processing and analysis

132 Stride segmentation was based on the detection of gait events using kinematic data as described by
133 Roepstorff et al.²⁰ Upper body vertical displacement of poll, withers and pelvis were high-pass filtered
134 (Butterworth 4th order) with the cut-off frequency adjusted to the stride frequency of each
135 measurement.²¹ Kinematic variables were calculated as previously described for upper body
136 asymmetry²² and for thoracolumbar and pelvic motion⁶ and are described in detail in Supplementary
137 Item 1. Briefly, the thoracolumbar angle was calculated using cranial and caudal segments, defined
138 using markers located on the T6 and T13 vertebrae, and on the T13 vertebra and the tuber sacrale,
139 respectively. Thoracolumbar flexion/extension angle was defined in the sagittal plane with flexion as
140 positive and extension as negative, and lateral bending angle was defined in the transverse plane,
141 with bending to the LS (lame side) as positive and NLS (non-lame side) as negative.⁶ For the pelvic
142 segment, pitch and yaw were defined relative to a line between the withers and tuber sacrale
143 markers, with roll defined relative to the horizontal.⁶ Pelvis pitching rotations were defined as negative
144 during flexion and positive for extension and pelvis roll and yaw rotations were defined as downward
145 (ventral) and forward (cranial) movements of the tuber coxae on the LS and NLS, respectively.⁶

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In order to progress to further data analysis, the measured motion asymmetry differences between an individual horse's baseline and lameness induction had to exceed previously described reference values for upper body motion asymmetry of 13 mm for head movement (MinDiff Poll or MaxDiff Poll) and 5 mm for hindquarter (pelvic) motion (MinDiff Pelvis and/or MaxDiff Pelvis) and with standard deviations less than their respective means.²³

2.5.2 sEMG data processing and analysis

Raw sEMG signals were DC-offset removed, high-pass filtered (Butterworth 4th order, 40 Hz cut-off),²⁴ and full-wave rectified. Discrete sEMG variables were calculated for each stride and included the average rectified value (ARV) and timings of sEMG activity onset, offset, and resultant activity duration for each muscle location.

ARV was calculated from full-wave rectified signals using stride duration as the temporal domain. As NLS and LS of longissimus were analysed separately, contralateral HL impact events were employed for stride segmentation for sEMG variables. Outliers in ARV data, defined as 2 standard deviations outside the mean ARV values within each horse, muscle location, and condition, were excluded from further analysis. To ensure that the same strides were analysed within the LS and NLS for each condition and muscle location, detected outlier strides were excluded for both muscle locations (T14 and L1) within that stride. To reduce inter-subject variability, within-horse ARV data were normalised to a reference voluntary contraction (RVC) defined as the maximum value observed for each muscle location relative to the corresponding baseline condition.²⁵ This permitted examination of the proportional change in muscle activity between baselines and the corresponding iFL/iHL conditions.

Muscle activity onset and offset events were calculated across strides, in accordance with the double threshold method.²⁶ Events were calculated from enveloped signals (Butterworth 4th order, low-pass filter, 10 Hz cut-off), with an amplitude threshold defined as 20% of the peak amplitude value of each individual sEMG signal and the timing threshold defined as 5% of the average gait cycle duration from the control condition across all horses.²⁶ Given the variation in baseline activity amplitude for longissimus signals and in accordance with St George et al.²⁶, the amplitude threshold was increased

176 or reduced by 5% to improve accuracy for certain horse/muscle combinations. Onset, offset, and
177 resultant activity duration for each muscle were normalised to percentage stride duration.

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179 To complement the discrete variables, continuous sEMG data, in the form of time and amplitude-
180 normalised sEMG signals across all strides/conditions were prepared for analysis.²⁷ Within-horse,
181 enveloped sEMG signals (Butterworth 4th order, low-pass filter, 25 Hz cut-off) were normalised to an
182 RVC: the peak amplitude value of enveloped signals, observed for each muscle location across all
183 strides (excluding detected outlier strides) from the corresponding baseline condition. As the RVC
184 represents a submaximal contraction, it was possible for both normalised ARV and continuous data
185 from the iFL/iHL conditions to exceed 100% of the RVC.

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187 2.6 Statistical Analyses

188 To increase statistical power, asymmetry parameters from right iFL and iHL were multiplied by -1 to
189 mirror the indices and thus categorise all data as if they were derived from left limb inductions only.
190 For the remaining variables, including sEMG variables, data from right iFL and iHL, were also
191 mirrored. Therefore, all results are reported as results of the lame side (LS) and the non-lame side
192 (NLS). The original kinematic values, without the mirroring procedure applied, are presented in Table
193 S1.

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195 Linear mixed models were used to estimate the effect of lameness induction. iFL and iHL were
196 modelled separately. Stride level data for discrete kinematic and sEMG variables were entered into
197 the model for the baseline condition and the corresponding induced lameness conditions (baseline 1
198 and iFL, baseline 2 and iHL) from each horse. Models were calculated in open-source R-studio
199 (version 3.6.3) using the package lme4 (version 1.1-15), with horse ID as a random effect and
200 condition as fixed effect. Additionally, separate models were conducted to evaluate the impact of
201 speed on results, using speed as a random slope to correct for this variable. Model fit was assessed
202 using q-q plots and boxplots of the residuals. For each model, results are presented as estimated
203 marginal means, standard error (SE) and 95% lower and upper confidence intervals calculated using
204 the software package emmeans (version 1.7.1). Significance values were corrected for multiple
205 comparisons using the false discovery rate method.

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Statistical parametric mapping (SPM), a technique increasingly used to investigate differences in ambulatory behavior, was employed to analyse continuous kinematic and sEMG data, (i.e., complete time series of the normalised signals from one stride).^{27,28,29} Time and amplitude normalised stride values for sEMG data and angle-time curves for kinematic data were assembled into 1*101*1 vector fields (median stride, 101 datapoints per stride and one dimension per data point) for each signal, condition, and horse. The open source spm1d package (version M.0.4.1) was used to conduct SPM analysis in Matlab (version 2020b).²⁸ For both sEMG and kinematic data, separate analyses were performed to compare signals between baseline and the corresponding iFL/iHL conditions. For group-level kinematic and sEMG data, paired samples t-tests were performed. For individual sEMG data, Hotelling's T2 tests were performed on T14 and L1 locations together, but separately for the LS and NLS. If significant results were found in a Hotelling's T2 test, paired samples t-tests were performed as post-hoc analyses. The two-tailed significance level was set at $\alpha = 0.05$ and p values were adjusted for multiple comparisons using the Bonferroni correction.

222 3. RESULTS

223

224 3.1 General Descriptive Findings

225 Thoracolumbar movement and longissimus activation patterns during trot are presented in a
226 supplementary video (SV1), containing the moving 3D model and associated kinematic and sEMG
227 signals from a representative horse during the baseline 1 condition. A total of 647 strides were used
228 for kinematic analysis (163: baseline 1, 132: baseline 2, 189: iFL and 163: iHL). A total of 508 and 504
229 strides were employed for the separate sEMG analysis of the LS (138: baseline 1 and iFL, 116:
230 baseline 2 and iHL) and NLS (136: baseline 1 and iFL, 116: baseline 2 and iHL), respectively. Across
231 all horses, muscle locations and conditions, a biphasic activation pattern was observed for
232 longissimus, with activation bursts consistently occurring between $33.1 \pm 4.8\%$ to $51.8 \pm 4.7\%$ and
233 $84.3 \pm 4.5\%$ to $100.9 \pm 4.6\%$ of stride duration. Additional bursts or elongation of the bi-phasic pattern
234 were observed, albeit less consistently, at $13.5 \pm 4.0\%$ to $24.6 \pm 4.8\%$ and $64.6 \pm 3.9\%$ to $75.5 \pm 3.7\%$
235 of stride duration. Linear mixed model results for iFL and iHL are presented in Tables 1 and 2, with
236 sEMG activation timings presented separately in Table S2. To allow for comparison of the effect of
237 speed on results, the following sections include data from both models, with (Table 1, S2) and without
238 (Table 2, S2) statistical correction for speed. Unless otherwise stated, this section describes results
239 from the speed-corrected model. Statistical correction for speed has not been applied to the
240 continuous time-series data presented in Figures 1 – 6.

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242 3.2 Effect of Forelimb Lameness Induction

243 3.2.1 Kinematic parameters

244 An increase in most asymmetry variables was found for iFL (Tables 1, 2, and S1), mainly Poll MinDiff
245 (53.73 mm, $p < 0.001$) and Withers MinDiff (13.14 mm, $p < 0.001$). Changes in thoracolumbar motion for
246 iFL were characterised by a significant decrease in peak flexion angle ($p < 0.05$), and slight, but non-
247 significant decreases in peak extension and peak left and right lateral bending angles (Table 1,
248 Figures 1 and 2). Changes in pelvic motion were characterised by a significant increase in pitch
249 ($p < 0.0001$) and non-significant decreases in pelvis yaw and roll (Table 1, Figure 3). Non-speed
250 corrected findings (Table 2) were similar except for pelvis yaw ROM, which increased significantly
251 ($p < 0.05$) without speed-correction. SPM results for kinematic data from the thoracolumbar and pelvic
252 segments for the group of horses are presented in Figures 2 (a, b) and 3 (a– c), respectively, and
253 showed no significant differences between conditions.

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256 3.2.2 sEMG parameters

257 Significant increases ($p < 0.0001$) and decreases ($p < 0.05$) in ARV were respectively observed at the
258 LS and NLS L1 sites during iFL, but changes in ARV at T14 locations were non-significant when
259 compared to baseline (Table 1). Activity duration of longissimus significantly increased ($p < 0.0001$) at
260 the LS, T14 site, but was not significantly altered at the other locations. In general, onset/offset
261 timings were not significantly influenced by iFL (Figure 1, Table S2) and any significant alterations in
262 timings were not restricted to specific activation bursts, occurring both earlier and later across sensor
263 sites (Table S2). Contrasting sEMG results were observed between models with- and without
264 statistical correction for speed (Table 2, Table S2). For example, significant increases in ARV from the
265 T14 site on the NLS and LS were observed during iFL ($p > 0.0001$) when speed was not corrected for
266 (Table S2). Significant differences in activity onset/offset timings were also observed more frequently
267 in the non-speed corrected model (Table S2).

268

269 sEMG waveforms from individual horses showed significant differences between conditions when
270 analysed using SPM, as illustrated by “Horse 4” in Figure S2. SPM post-hoc analysis of LS sEMG
271 data revealed that significant differences between conditions are primarily influenced by significant
272 increases in amplitude at the L1 location (Figure S2). However, when sEMG data were grouped
273 across all horses, SPM results revealed that such differences were not significant (Figure 4).

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275 3.3 Effect of Hindlimb Lameness Induction

276 3.3.1 Kinematic Parameters

277 An increase in most asymmetry variables was found for iHL (Tables 1, 2 and S1), mainly pelvis
278 MinDiff (22.25mm, $p < 0.001$), pelvis MaxDiff (27.87mm, $p < 0.001$) and Hip Hike Swing (61.73mm,
279 $p < 0.001$). Changes in thoracolumbar motion were characterised by a significantly larger peak
280 extension angle and significantly smaller peak flexion angle ($p < 0.0001$) (Figure 5, Table 1). Peak
281 lateral bending angle significantly decreased ($p < 0.001$) and increased ($p < 0.0001$) on the LS and NLS,
282 respectively (Figure 5, Table 1). Changes in pelvic motion were characterised by a significant
283 increase in pitch and yaw ($p < 0.0001$), and non-significant changes in roll ($p > 0.05$) (Figures 3d– f,
284 Table 1). Results from the non-speed corrected model (Table 2), were congruent with results from the
285 speed corrected model (Table 1) except for pelvis yaw ROM, which was non-significant when speed
286 was not corrected for. SPM results showed no significant differences between conditions for
287 thoracolumbar motion (Figures 2c, d), but significant differences were observed for pelvis pitch and
288 roll during the lame diagonal stance (Figures 3d, e) ($p < 0.05$).

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290 3.3.2. *sEMG Parameters*

291 Significant increases in ARV were observed bilaterally at T14 and L1 longissimus sites ($p < 0.0001$)
292 (Table 1). At both T14 and L1, activation onset/offset events were generally detected significantly
293 earlier in the stride cycle on the LS, and later on the NLS ($p < 0.05$) (Figures 5 and 6, Table S2). On
294 the LS, longissimus activity duration significantly increased at T14 ($p < 0.0001$) and decreased at L1
295 ($p < 0.0001$) (Table S2). ARV and sEMG activation timing results from the non-speed corrected model
296 (Table 2, S2), were congruent with results from the speed corrected model (Table 1, S2), except for
297 two activation events, which showed significant differences between conditions ($p < 0.05$) when speed
298 was not corrected for (Table S2).

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300 sEMG waveforms from individual horses showed significant differences between conditions when
301 analysed using SPM, as illustrated in "Horse 6" (Figure S3), but when sEMG data were grouped
302 across all horses, SPM results revealed that such differences were not significant (Figure 6).

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306 **4. DISCUSSION**
307

308 This study combined motion capture and sEMG technology to quantify and compare thoracolumbar
309 and pelvic kinematics and longissimus activity, between baseline and standardised iFL and iHL
310 conditions. Kinematic asymmetry indices provided quantitative evidence for the successful induction
311 of iFL and iHL across all horses, which resulted in different, significant changes in thoracolumbar and
312 pelvic ROM, and longissimus muscle activity. iFL was characterised by significant decreases in peak
313 thoracolumbar flexion and increases in pelvis pitching ROM (Figure 1). These adaptations were also
314 observed during iHL, plus significant increases on LS and decreases on NLS in peak thoracolumbar
315 lateral bending angle and increases in peak thoracolumbar extension angle and pelvis yaw ROM
316 (Figure 5). Clear adaptations in longissimus activation patterns were observed during iHL, with
317 significant bilateral increases in amplitude across T14 and L1 and distinct phasic shifts reflecting
318 precipitated (LS) and delayed (NLS) muscle activation onset/offset within the stride cycle. In
319 comparison, adaptations in longissimus activation patterns did not generally change during iFL, with
320 no distinct phasic shifts in activation observed, but with significant changes in amplitude only
321 observed at the L1 locations. Therefore, findings from this study support the hypothesis that iFL and
322 iHL cause different adaptations in thoracolumbar and pelvic ROM and longissimus activity, but do not
323 support the hypothesis that these changes are localised to the thoracic and lumbar areas during iFL
324 and iHL, respectively.

325

326 **4.1 Kinematic adaptations of thoracolumbar and pelvic motion during iFL and iHL**

327 The overall finding that mild, reversible iFL and iHL result in different measurable adaptations in
328 thoracolumbar and pelvic motion agrees with previous studies that reported increases in overall
329 thoracolumbar flexion/extension ROM during iFL,¹⁰ but no significant differences during iHL.¹¹ In
330 contrast, horses in this study adapted to iFL by significantly decreasing peak thoracolumbar flexion
331 during LS stance phase (Figures 1 and 2a), without significantly altering peak extension or lateral
332 bending angles, and to iHL by significantly decreasing peak thoracolumbar flexion and increasing
333 extension (Figures 2c and 5). Comparisons between studies are limited by methodological differences
334 in data processing and analysis and the fact that horses were evaluated during treadmill locomotion,
335 in which thoracolumbar motion differs from overground locomotion.^{10,11,31,32} However, our findings are
336 congruent with clinical observations of increased stiffness/decreased flexibility of the thoracolumbar
337 region in horses presenting FL and HL lameness. Further, our findings for iHL (Figure 2c) agree with
338 a descriptive study that reported decreased extension during LS stance and increased extension

339 during NLS stance in a single clinical hindlimb lameness case (right tarsal osteoarthritis) compared to
340 a non-lame horse during overground trot.³⁰

341

342 Gómez-Álvarez et al.¹⁰ related compensatory “head nod” during iFL and its concurrent effects on
343 thoracic flexion/extension to significant increases in extension angles of individual thoracic and lumbar
344 vertebrae during lame diagonal stance. Indeed, an examination of group-averaged iFL time-angle
345 curves in Figure 2a reveals a general, albeit non-significant, trend for increased extension and
346 decreased flexion during lame diagonal stance. Thus, asymmetrical head and neck movement during
347 iFL appears to affect the subtle, but largely non-significant, asymmetries observed in group-averaged
348 thoracolumbar flexion/extension. Discrete data revealed that peak thoracolumbar flexion was
349 significantly decreased during iFL and based on Figure 2a, this was attributed to the flexion peak
350 bridging at the end of lame and non-lame diagonal stance phases (Figure 1). Significant increases in
351 thoracic flexion, as observed by Gómez-Álvarez et al.¹⁰ during non-lame diagonal stance, were not
352 found in this study for group-averaged data, although individual kinematic data reveals that certain
353 horses exhibited this movement pattern, particularly the two horses with the highest MinDiff Poll
354 values (i.e., the highest degree of iFL) (Table S1). Significant increases in T10 and T13 lateral
355 bending angles towards the LS during lame diagonal stance have been observed and interpreted as
356 an attempt to shift the centre of mass towards the NLS.¹⁰ Again, group-averaged lateral bending data
357 from our study does not support this finding, but individual horses exhibited increased lateral bending
358 towards the LS. Thus, in accordance with known inter-horse variance in back motion during non-lame
359 locomotion^{6,8,33}, findings from this study suggest that individual horses adopt different adaptation
360 strategies, most notably during iFL.

361

362 4.2 Electromyographic activity of the longissimus and adaptations during iFL and iHL

363

364 Longissimus is the largest equine epaxial muscle. Based on its anatomical location and attachments,
365 it is thought to extend the spine when activated bilaterally in a concentric contraction, whereas
366 unilateral concentric activation results in lateral bending and/or axial rotation.¹⁶ Here, longissimus had
367 a bilateral, biphasic activation pattern in each stride cycle, with each burst corresponding to the
368 second half of HL stance, where thoracolumbar flexion occurs (Figures 1 and 5). This biphasic pattern
369 is well-documented in sEMG studies of quadrupedal trot on a treadmill^{16, 34-37}, with longissimus

370 function generally attributed to eccentric activity that stabilises the thoracolumbar spine during passive
371 flexion.³⁶⁻⁴¹ Across these studies, there are both inter-individual variation in activation timing^{36,39,42} and
372 variations in the number of activation bursts.^{40,42,43} Our findings support inter-individual variation of
373 longissimus activation, with some horses showing additional activation events in the first half of HL
374 stance, producing additional bursts or elongation of the bi-phasic pattern. Von Scheven⁴³ explicitly
375 described these additional bursts of longissimus activity in some horses during treadmill trot and, in
376 the current study, they preceded peak thoracolumbar extension at approximately HL mid-stance
377 (Figures 1 and 5). This is the first known study to acquire sEMG data from longissimus during
378 overground quadrupedal trot on a hard surface, which is an important consideration given the known
379 effect of both treadmill and surface type on locomotion, loading patterns, and workload.^{31,32,44} Indeed,
380 loading experiments to alter locomotor forces acting on the trunk and hindlimbs of dogs, have noted
381 adaptations in longissimus activation.^{40,41} Therefore, overground locomotion on a hard-surfaced
382 runway, as studied here, may yield different longissimus activation patterns. However, further
383 research comparing muscle function during overground vs. treadmill locomotion and examination of
384 antagonist muscles (e.g., rectus abdominus) is required to confirm this.

385

386 Bilateral, significant increases in ARV observed at T14 and L1 during iHL support the theory posed by
387 Barrey et al.⁴⁵ that bilateral adaptations in longissimus activity represent a stabilising function against
388 compensatory sagittal plane forces during iHL, namely reduced vertical acceleration and
389 displacement of the centre of mass during LS stance and vice versa during NLS stance.¹³ Supporting
390 this, observational analysis of Figures 5 and 6 depicts increases in sEMG amplitude during iHL that
391 are most pronounced in longissimus activation bursts during the first half of HL stance, where
392 significant adaptations in thoracolumbar extension occurred, albeit to varying degrees between
393 horses, likely because of documented adaptations in vertical forces acting on the trunk.¹³ These
394 findings contrast with a study¹⁵ reporting significantly lower bilateral longissimus amplitude in horses
395 with chronic, unilateral HL lameness, which was interpreted as a “more distinct resting phase”
396 between muscular activation bursts. Contrasting differences in longissimus activity could be related to
397 chronicity of existing HL lameness compared to the acute, induced lameness evaluated in our study,
398 but further comparative research is required to confirm this.¹⁵ Interestingly, non-significant changes in
399 sEMG amplitude were also reported by Fischer et al.⁴⁶ for the LS and NLS of longissimus activity at

400 L3/L4 sites in dogs with unilateral iHL during treadmill trot. Again, methodological differences make
401 direct comparisons between studies difficult, particularly in relation to the type of locomotion (treadmill
402 vs. overground), sEMG processing and analysis methods,^{15,47} and lameness studied (acute/induced
403 vs. chronic cases).¹⁵

404

405 Longissimus activation is affected by vertical and horizontal components of HL pro-retractor
406 muscles.⁴¹ Temporal adaptations in HL pro-retraction have been described during iHL⁴⁷, and in
407 accordance with these changes, significantly delayed NLS longissimus activation timings were
408 observed in our study and in Fischer et al.,⁴⁶ who also reported a non-significant trend for earlier
409 activation on the LS, which was largely significant in our study. Trunk rotation towards the NLS has
410 been described during iHL^{11,13} as a means to unload the LS HL.¹³ Significant changes in discrete
411 lateral bending angles and continuous pelvic ROM data (Figures 3d, e), indicate that this
412 compensatory mechanism was also observed in the current study. Lateral bending toward the NLS
413 and pelvis roll and yaw rotations towards the LS were also found in this study, with significant
414 differences in the SPM results for pelvic roll during LS stance (Figure 3d). It has been suggested that
415 compensatory longitudinal rotations of the back and pelvis during iHL are driven by increased activity
416 of NLS epaxial, as well as HL protractor muscles.⁴⁶ The significant increases in NLS longissimus
417 amplitude observed in this study, as well as NLS superficial gluteal, biceps femoris and
418 semitendinosus observed in St. George et al. (under review) support the realisation of increased
419 lateral bending of the back towards the NLS and of the pelvis towards the LS. Taken together, these
420 findings are the first to support postulated muscular adaptations for known compensatory
421 weightbearing and movement patterns of the limbs, back, and pelvis during hindlimb lameness.

422

423 To our knowledge, this is the first study to examine equine muscle function during forelimb lameness.
424 In contrast to iHL, longissimus amplitude and activation patterns at the sites evaluated remained
425 largely unaltered during iFL, except for recordings at the LS L1 site, which significantly increased in
426 amplitude (Figure 1). This finding appears to support the suggestion by Gómez-Álvarez et al.¹⁰: that
427 increased activation of longissimus lumborum occurs during lame diagonal stance to produce lateral
428 bending towards the LS in an attempt to shift the centre of mass towards the NLS in the horizontal
429 plane.⁴⁸ It is possible that the pronounced increases in LS L1 activity reflect an active contraction to

430 aid lateral bending towards the LS. However, inter-individual differences in L1 activation were
431 apparent in this study, further supporting the finding that horses adapt to iFL using individual
432 compensatory movement patterns. Interestingly, significant changes were not observed in the
433 thoracic recording sites, which were hypothesised to exhibit the greatest change during iFL, due to
434 their closer proximity to the well-described compensatory “head-nod”.¹³ It is possible that the “head
435 nod” produces subtle changes in thoracolumbar flexion/extension, but not enough to necessitate
436 increased muscle activation of the longissimus at this region.

437

438 4.3 Clinical relevance and further considerations

439 The lameness induction model was considered ideal for this preliminary research, as it produces a
440 highly reliable and standardised condition for study, but indeed lameness encountered clinically is
441 variable and often chronic in nature. Further, inter-individual variation in the dataset from a small
442 sample could be considered a limiting factor, but we argue that this finding reflects challenges within
443 the clinical world, as well as previous research.^{6,8,33} Prior to this study, only clinical perceptions about
444 adaptations in epaxial muscle activation during equine lameness existed. Although findings from this
445 study offer the first objective data on underlying muscular adaptations in the equine back during
446 lameness, clinical extrapolation of preliminary data is challenging. Thus, further studies employing a
447 larger sample of clinical lameness cases are required. Nevertheless, it is clear from our results that
448 adaptation mechanisms to lameness are complex and single limb lameness can affect kinematic and
449 muscle activation of the back in an acute lameness model.⁴⁹

450

451 The known effect of speed on kinematic³⁶ and sEMG variables⁵⁰, was addressed in this study by
452 presenting results from models with- and without a statistical correction for speed. This is especially
453 relevant, as significant changes in stride velocity during equine lameness are known.⁴⁷ Therefore, it is
454 only the adaptations in speed-corrected variables (Table 1, S2) that can be considered clinically
455 relevant, as they are not confounded by the effects of speed and are thus the result of induced
456 lameness. Finally, fewer group-averaged variables were found to be significantly altered during
457 lameness conditions when analysed using SPM compared to linear mixed models. This discrepancy
458 between the analysis of discrete and time series variables agrees with previous studies of equine
459 biomechanics data.^{26,27,29} As alluded to in previous studies, this is likely because alpha is more tightly

460 controlled when using SPM and the known variation in equine biomechanics data affects the level of
461 significance using SPM.^{27,29} Based on this, Smit et al.²⁷ and Hobbs et al.²⁹ suggest that reaching
462 significance may not be as important when using SPM to evaluate clinical implications and that data
463 from individual horses should be assessed to ensure that subtle changes are not overlooked when
464 considering group-level data. Our findings agree with this, as the clusters of data points that reached
465 significance following SPM post-hoc analysis of within-horse sEMG data (Figures S2 and S3), were
466 often in accordance with the significant increases in discrete ARV and activation onset/offset variables
467 (Table 1 and 2), suggesting that time series data from individual horses should be evaluated when
468 clinically assessing the effects of equine lameness.

469

470

471 **5. CONCLUSION**

472
473 Distinctive differences in thoracolumbar and pelvic motion and underlying longissimus activity occur
474 during iFL and iHL and have been measured here for the first time using combined motion capture
475 and sEMG. iFL was characterised by significant decreases in peak thoracolumbar flexion angle,
476 significant increases in pelvis pitching ROM, and significant changes in sEMG amplitude at L1 sites.
477 In contrast, iHL was characterised by several significant adaptations including increases in
478 thoracolumbar lateral bending towards the NLS and decreases towards the LS, decreased peak
479 thoracolumbar flexion and increased peak extension angles, and increased pelvis yaw and pitching
480 ROM. These kinematic changes during iHL occurred alongside significant bilateral increases in
481 longissimus activity and clear phasic shifts in activation timings. These findings suggest that, during
482 iFL, thoracolumbar and pelvic movement adaptations occur primarily in the cranio-caudal direction,
483 but this seemingly does not necessitate significant adaptations in longissimus activation at the
484 thoracic regions studied here. Instead, significant changes in longissimus activation at the lumbar
485 regions were observed during iFL, but this was largely horse-specific and may reflect another
486 compensatory mechanism of increasing LS lateral bending to horizontally shift the centre of mass
487 away from the affected limb. Whereas findings suggest that compensation for iHL primarily involves
488 lateral bending and axial rotation to shift the centre of mass horizontally, and that these adaptations
489 are facilitated by significant phasic shifts and increases in longissimus activation at both of the
490 thoracic and lumbar regions studied here. The subtle and often horse-specific nature of these
491 adaptations drives home the importance of future research to determine whether the significant
492 changes observed here constitute clinically meaningful changes and to develop further objective
493 clinical evaluation techniques for the equine back. These future studies are particularly important
494 because many of the kinematic adaptations, and certainly the underlying neuromuscular adaptations,
495 to lameness, as observed here, are undetectable through human observation alone.

496 **MANUFACTURERS' ADDRESSES**

497

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502

503 Table 1. Speed corrected data as estimated marginal means (EM Mean) and standard error (SE) for
504 discrete variables from baseline and iFL and iHL lameness conditions and estimated mean marginal
505 differences (EM Mean Difference, EM Mean % Difference) between corresponding baseline and
506 induced lameness conditions and associated p-values.

Variable	Induction	Baseline		Induction		EM Mean Difference	EM Mean % Difference	p-value
		EM Mean	SE	EM Mean	SE			
Stride Duration (s)	FL	0.77	0.01	0.75	0.01	-0.01	1.30	<0.001
	HL	0.73	0.01	0.71	0.01	-0.02	2.74	<0.001
Asymmetry Variables (mm)								
MinDiff Poll	FL	-3.36	5.30	-57.09	5.22	-53.73	n/a	<0.001
	HL	-5.72	5.17	-13.85	5.10	-8.13	n/a	<0.001
MaxDiff Poll	FL	-7.18	5.38	-29.47	5.72	-22.29	n/a	<0.001
	HL	-2.87	3.04	-11.95	2.92	-9.08	n/a	<0.001
MinDiff Withers	FL	-2.36	1.64	-15.51	1.75	-13.14	n/a	<0.001
	HL	-2.07	1.70	10.96	1.72	13.04	n/a	<0.001
MinDiff Pelvis	FL	1.03	1.14	3.25	1.29	2.22	n/a	<0.001
	HL	0.34	2.68	-21.91	2.67	-22.25	n/a	<0.001
MaxDiff Pelvis	FL	0.68	3.27	6.29	3.28	5.61	n/a	<0.001
	HL	4.78	1.37	-23.08	1.39	-27.87	n/a	<0.001
Hip Hike Swing	FL	0.81	4.22	13.98	4.23	13.17	n/a	<0.001
	HL	2.89	6.31	-58.84	6.33	-61.73	n/a	<0.001
Maximum Thoracolumbar Angle (degrees)								
Left/ LS Lateral Bending	FL	4.41	0.71	4.39	0.73	-0.02	0.45	0.9
	HL	3.54	1.00	2.91	1.00	-0.63	17.80	<0.001
Right/ NLS Lateral Bending	FL	-2.14	0.74	-2.04	0.77	0.11	5.14	0.7
	HL	-3.37	1.00	-4.26	1.00	-0.89	26.41	<0.001
Extension	FL	-23.75	1.13	-23.67	1.08	0.07	0.29	0.6
	HL	-21.03	1.10	-21.64	1.11	-0.61	2.90	<0.001
Flexion	FL	-16.36	0.85	-16.56	0.85	-0.20	1.22	0.03
	HL	-15.97	0.91	-16.34	0.91	-0.36	2.25	<0.001
Pelvic ROM (degrees)								
Pitch	FL	7.88	0.57	8.39	0.57	0.50	6.35	<0.001
	HL	8.49	0.66	9.27	0.66	0.77	9.07	<0.001
Roll	FL	7.53	0.78	7.40	0.77	-0.13	1.73	0.4
	HL	7.26	0.68	7.10	0.67	-0.16	2.20	0.4
Yaw	FL	3.20	0.33	3.10	0.33	-0.10	3.12	0.07
	HL	3.32	0.21	3.45	0.21	0.12	3.61	<0.001
Longissimus sEMG ARV (%)								
T14, NLS	FL	97.33	4.53	93.89	4.92	-3.44	3.5	0.09
	HL	82.29	6.83	108.23	6.78	25.94	31.52	<0.001
T14, LS	FL	91.19	1.78	88.78	1.96	-2.41	2.6	0.1
	HL	79.95	5.43	117.52	5.25	37.58	47	<0.001
L1, NLS	FL	93.05	1.53	89.35	1.76	-3.70	4.0	0.03

507
508

	HL	89.99	5.53	111.17	5.59	21.18	23.54	<0.001
L1, LS	FL	116.57	22.05	193.35	24.75	76.78	65.9	<0.001
	HL	84.73	4.35	97.23	4.31	12.50	14.75	<0.001

509 Table 2. Non-speed corrected data as estimated marginal means (EM Mean) and standard error (SE)
 510 for discrete variables from baseline and iFL and iHL lameness conditions and estimated mean
 511 marginal differences (EM Mean Difference, EM Mean % Difference) between corresponding baseline
 512 and induced lameness conditions and associated p-values.

Variable	Induction	Baseline		Induction		EM Mean Difference	EM Mean % Difference	p-value
		EM Mean	SE	EM Mean	SE			
Stride Speed (m/s)	FL	3.13	0.10	2.87	0.10	-0.26	8.31	<0.001
	HL	3.09	0.12	3.03	0.12	-0.06	1.94	0.02
Stride Duration (s)	FL	0.74	0.02	0.78	0.02	0.04	5.41	<0.001
	HL	0.75	0.01	0.74	0.01	-0.01	1.33	<0.001
Asymmetry Variables (mm)								
MinDiff Poll	FL	-3.99	4.42	-57.35	4.41	-53.36	n/a	<0.001
	HL	-3.04	3.19	-14.28	3.09	-11.25	n/a	<0.001
MaxDiff Poll	FL	-4.62	6.26	-24.27	6.25	-19.65	n/a	<0.001
	HL	-3.40	2.14	-13.07	2.04	-9.68	n/a	<0.001
MinDiff Withers	FL	-1.97	2.06	-14.13	2.06	-12.16	n/a	<0.001
	HL	-2.61	1.75	11.23	1.73	13.84	n/a	<0.001
MinDiff Pelvis	FL	-0.65	1.75	0.79	1.75	1.44	n/a	0.05
	HL	1.40	2.13	-21.24	2.11	-22.64	n/a	<0.001
MaxDiff Pelvis	FL	3.46	1.34	9.31	1.32	5.85	n/a	<0.001
	HL	5.60	2.55	-25.74	2.52	-31.34	n/a	<0.001
Hip Hike Swing	FL	3.92	2.33	16.72	2.32	12.80	n/a	<0.001
	HL	7.51	5.03	-56.32	4.98	-63.83	n/a	<0.001
Maximum Thoracolumbar Angle (degrees)								
Left/ LS Lateral Bending	FL	4.53	0.65	4.72	0.65	0.19	4.19	0.4
	HL	4.20	0.80	3.26	0.80	-0.94	22.38	<0.001
Right/ NLS Lateral Bending	FL	-2.10	0.70	-2.27	0.70	-0.17	8.10	0.4
	HL	-2.84	0.93	-3.93	0.93	-1.09	38.38	<0.001
Extension	FL	-22.54	0.96	-22.72	0.96	-0.18	0.80	0.2
	HL	-21.82	1.05	-22.50	1.05	-0.68	3.12	<0.001
Flexion	FL	-16.62	0.83	-16.91	0.83	-0.29	1.74	<0.001
	HL	-16.00	0.80	-16.33	0.80	-0.32	2.00	<0.001
Pelvis ROM (degrees)								
Pitch	FL	8.39	0.48	9.13	0.48	0.73	8.70	<0.001
	HL	8.63	0.42	9.68	0.42	1.05	12.17	<0.001
Roll	FL	7.12	0.66	7.25	0.66	0.12	1.69	0.4
	HL	7.25	0.63	7.21	0.63	-0.04	0.55	0.8
Yaw	FL	3.02	0.32	3.30	0.32	0.28	9.27	0.02
	HL	3.16	0.23	3.23	0.23	0.07	2.22	0.2
Longissimus sEMG ARV (%)								
T14, NLS	FL	86.73	6.09	80.30	6.09	-6.43	7.41	<0.001
	HL	85.08	4.83	109.81	4.81	24.73	29.07	<0.001
T14, LS	FL	88.01	3.31	82.65	3.31	-5.36	6.09	<0.001

	HL	86.66	5.73	118.28	5.72	31.62	36.49	<0.001
L1, NLS	FL	86.76	3.75	78.91	3.75	-7.85	9.05	<0.001
	HL	83.12	4.00	101.76	3.98	18.64	22.43	<0.001
L1, LS	FL	92.48	30.70	166.55	30.68	74.07	80.09	<0.001
	HL	86.28	2.77	95.08	2.77	8.81	10.21	<0.001

513

514

515 **LIST OF FIGURE LEGENDS**

516

517 Figure 1: Graphs show mean (solid line) and standard deviation (shaded area) of amplitude-
518 normalised, linear-enveloped sEMG signals from LS and NLS longissimus (L1 location) and time-
519 angle curves for thoracolumbar flexion/extension and lateral bending from representative “horse 4”
520 during baseline 1 (blue) and iFL (red) conditions. Within the sEMG graphs, upward and downward
521 arrows demarcate sEMG activity onset and offset, respectively, for baseline 1 (blue arrows) and iFL
522 (red arrows). Data are time-normalised between LS hindlimb impact events. Line drawings show the
523 outline of the horse at different stages of the stride cycle, as illustrated by horizontal bars showing
524 mean stance phase for each limb (baseline 1: blue bars, iFL: red bars). Within the line drawings, red
525 arrows illustrate significant (solid arrows) and non-significant (outline arrows) decreases in
526 thoracolumbar flexion/extension (vertical arrows) and lateral bending (horizontal arrows) following iFL.
527 Significant increases in pelvis pitching are illustrated as curved, green arrows around the transverse
528 axis.

529

530 Figure 2: SPM results for time-normalised thoracolumbar kinematic data across the group of horses
531 for flexion/extension (a, c) and lateral bending (b, d) during baseline 1 and iFL (a, b) and baseline 2
532 and iHL (c, d). Upper graphs illustrate median (solid line) and standard deviation (shaded area)
533 kinematic data for baseline (blue) and induced lameness (red) conditions. Lower graphs illustrate the
534 paired samples t-test SPM result (black solid line) and the critical thresholds for significance (red
535 dashed line). Data are time-normalised between impacts of the LS hindlimb.

536

537 Figure 3: SPM results for time-normalised pelvis segment kinematics across the group of horses for
538 pitch (a, d), roll (b, e), yaw (c, f) during baseline 1 and iFL (a, c), and baseline 2 and iHL (d, f). Within
539 each subpanel, upper graphs illustrate median (solid line) and standard deviation (shaded area)
540 kinematic data for baseline (blue) and induced lameness (red) conditions. Lower graphs illustrate the
541 paired samples t-test SPM result (black solid line) and the critical thresholds for significance (red
542 dashed line). Data are time-normalised between impacts of the LS hindlimb. Grey shaded areas
543 indicate regions with statistically significant differences between conditions.

544

545 Figure 4: SPM results for time and amplitude-normalised longissimus sEMG data across the group of
546 horses during baseline 1 (blue) and iFL (red) conditions for T14 (a, b) and L1 (c, d) locations on the
547 LS (a, c) and NLS (b, d). Within each subpanel, upper graphs illustrate median (solid line) and
548 standard deviation (shaded area) sEMG data and lower graphs illustrate the paired samples t-test
549 SPM result (black solid lines) and the critical thresholds for significance (red dashed line). Data are
550 time normalised between ipsilateral hindlimb impact events.

551

552 Figure 5: Graphs show mean (solid line) and standard deviation (shaded area) of amplitude-
553 normalised, linear enveloped sEMG signals from LS and NLS longissimus (L1 location) and time-
554 angle curves for thoracolumbar flexion/extension and lateral bending from representative “horse 2”
555 during baseline 2 (blue) and iHL (red) conditions. Within the sEMG graphs, upward and downward
556 arrows demarcate sEMG activity onset and offset, respectively, for baseline 2 (blue arrows) and iHL
557 (red arrows). Data are time-normalised between LS hindlimb impact events. Line drawings show the
558 outline of the horse at different stages of the stride cycle, as illustrated by horizontal bars showing
559 mean stance phase for each limb (baseline 2: blue bars, iHL: red bars). Within the line drawings,
560 arrows illustrate significant (solid arrows) and non-significant (outline arrows) increases (green arrow)
561 and decreases (red arrow) in thoracolumbar flexion/extension (vertical arrows) and lateral bending
562 (horizontal arrows) following iHL. Significant increases in pelvis pitch and yaw are illustrated as
563 curved, green arrows around the transverse and vertical axes, respectively.

564

565 Figure 6: Results of SPM of time and amplitude normalised sEMG data from longissimus across the
566 group of horses during baseline 2 (blue solid line/shaded area) and iHL (red solid line/shaded area)
567 for T14 (a, b) and L1 (c, d) locations on the LS (a, c) and NLS (b, d). Within each subpanel, upper
568 graphs illustrate median (solid line) and standard deviation (shaded area) sEMG data and lower
569 graphs illustrate the paired samples t-test SPM result (black solid lines) and the critical thresholds for
570 significance (red dashed line). Data are time normalised between ipsilateral limb impact events.

571

572

573 **LIST OF LEGENDS FOR SUPPLEMENTARY ITEMS**

574

575 Supplementary Video (SV1): Video showing axial movement and muscle activity from one
576 representative horse (Horse 4) and stride at trot during the baseline 1 condition. In the first clip,
577 thoracolumbar flexion/extension angle and sEMG signals from the left and right longissimus dorsi at
578 T14 and L1 locations are presented as separate graphs in the right panel, alongside the moving
579 three-dimensional model in the middle panel, to illustrate muscle activation in relation to movement
580 during one trot stride. In a second clip, thoracolumbar lateral bending angle is presented alongside
581 the sEMG signals and the moving three-dimensional model from the same representative horse and
582 trot stride. Video clips were exported and adapted from Visual3D (C-motion Inc.) software.

583

584 Supplementary Item 1: detailed description of calculations for discrete kinematic variables are a
585 separate document within the folder.

586

587 Figure S1: Retro-reflective markers and surface electromyography (sEMG) sensors attached to one
588 subject (a) at the following anatomical locations: 1. Marker cluster attached to the head, 2. Poll, 3.
589 Thoracic (T) 6, 4. T10, 5. T13, 6. Lumbar (L) 1, 7. L3, 8. L5, 9. Between the tuber sacrale, 10. Sacral
590 vertebra (S) 3, 11. S5, and bilaterally over: 12. Proximal end spina scapula, 13. Greater tubercle of
591 the humerus, 14. Lateral tuberosity radius, 15. Marker cluster attached to distal radius, 16. Marker
592 cluster attached to mid 3rd metacarpus bone (MCIII), 17. Centre of rotation metacarpophalangeal joint
593 (MCPJ), 18. Lateral hoof wall (at the centre of rotation of the distal interphalangeal joint (DIPJ), 19.
594 Tuber coxae, 20. Greater trochanter femur, 21. Lateral tibia plateau, 22. Marker cluster attached to
595 distal tibia, 23. Proximal end 4th metatarsal bone (MCIV), 24. Marker cluster attached to mid 3rd
596 metatarsal bone (MTIII), 25. Centre of rotation metatarsophalangeal joint (MTPJ), 26. Lateral hoof
597 wall. Bilateral sEMG sensor sites for Longissimus at T14 (27) and L1 (28). Inset (b): showing
598 prepared skin sites and adhesion technique for markers and sensors at L1 (28).

599

600 Figure S2: Individual SPM results for horse 4 with induced forelimb lameness, showing the stride and
601 amplitude normalised sEMG signal for T14, L1, LS and NLS. Results are compared between baseline
602 1 (BAS) (blue/ with shaded area) and iFL (red/ with shaded area). Graphs within the top two rows

603 show the median stride (solid lines) and their standard deviations (shaded areas). Graphs within the
604 middle row show individual SPM results for hotelling T2 (black solid lines) with the respective critical
605 thresholds (red dashed lines). Graphs within the bottom two rows show post-hoc analysis for each
606 site separately (T14, L1) at LS and NLS, with the SPM t-statistic (black solid lines) and the critical
607 threshold (red dashed lines). Statistically significant areas are indicated with the grey shaded areas,
608 where $p < 0.05$. Data are time normalised between ipsilateral hindlimb impact events.

609
610 Figure S3. Individual SPM results for horse 6 with induced hindlimb lameness, showing the stride and
611 amplitude normalised sEMG signal for T14, L1, LS and NLS. Results are compared between baseline
612 1 (BAS) (blue/ with shaded area) and iFL (red/ with shaded area). The top two panels show the
613 median stride (solid lines) and their standard deviations (shaded areas). The middle graphs show
614 individual SPM results for hotelling T2 (black solid lines) with the respective critical thresholds (red
615 dashed lines). The bottom two rows show post-hoc analysis for each site separately (T14, L1) at LS
616 and NLS, with the SPM t-statistic (black solid lines) and the critical threshold (red dashed lines).
617 Statistically significant areas are indicated with the grey shaded areas, where $p < 0.05$. Data are time
618 normalised between ipsilateral hindlimb impact events.

619
620 Table S1. Resume of kinematic variables of all horses for baseline 1,2 and after iFL and iHL – note
621 that data is not mirrored and presented data is the original induced limb: MinDiff (difference between
622 the two minima of the movement) and MaxDiff (difference between the two maxima of the movement)
623 Hip Hike Swing (the difference between the upward movement of the left and right tuber coxae during
624 swing phase) and Hip Hike Stance (difference between the upward movement of the left and right
625 tuber coxae during stance).

626
627 Table S2: Estimated marginal means (EM Mean) and standard error (SE) for baseline and lameness
628 induction conditions, and estimated differences (EM Mean Difference) between corresponding
629 baseline and induction conditions and associated p-values for discrete sEMG activation onset, offset
630 and activity duration variables (% stride) for longissimus dorsi. Data for iFL and iHL lameness
631 conditions are presented from the models with- (speed corrected data) and without (non-speed
632 corrected data) a speed*condition fixed effect. Bilateral sEMG data are presented for each muscle
633 site (L1 and T14) from the NLS and LS, based on the side of induced lameness.

634

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