1 Comparison of limb kinematics between collected and lengthened (medium/extended) trot in 2 two groups of dressage horses on two different surfaces. 3

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29 Authorship: Walker, Tranquille, Dyson & Murray contributed to study design, data analysis 30 and interpretation and preparation of the manuscript. Walker, Tranquille, Brandham and 31 Northrop acquired the data. Northrop carried out surface analysis. Newton performed statistical 32 analysis and contributed to the manuscript. All authors approved the final version of the 33 manuscript. 34

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37 SUMMARY

38 Background: Dressage horses are often asked to work in lengthened paces during training and 39 competition, but to date there is limited information about the biomechanics of dressage-40 specific paces. Preliminary work has shown increased fetlock extension in extended compared 41 with collected paces, but further investigation of the kinematic differences between collected, 42 medium and extended trot in dressage horses is warranted. Objectives: Investigation of the 43 effect of collected versus medium/extended trot on limb kinematics of dressage horses. Study 44 design: Prospective kinematic evaluation. Methods: Twenty clinically sound horses in active 45 dressage training were used: Group 1) ten young horses (≤ 6 years) were assessed at collected 46 and medium trot; Group 2) ten mature horses (≥9 years) were assessed at collected and 47 extended trot. All horses were evaluated on two different surfaces. High-speed motion-capture 48 (240Hz) was used to determine kinematic variables. Forelimb and hindlimb angles were 49 measured at midstance. Descriptive statistics and mixed-effect multilevel-regression analyses 50 were performed. Results: Speed and stride length were reduced and stride duration increased 51 at collected compared with medium/extended trot. Lengthened trot (medium/extended trot) 52 was associated with increased fetlock extension in both the forelimbs and hindlimbs in both 53 groups of horses. Changes were greater in Group 2 compared with Group 1. Shoulder and 54 carpus angles were associated with forelimb fetlock angle. Hock angle was not significantly 55 influenced by pace. Surface had no effect on fetlock or hock angles. Main limitations: Only 2D 56 motion analysis was carried out. Results may have been different in horses with more extreme 57 gait characteristics. Conclusions: Medium/extended trot increases extension of the forelimb 58 and the hindlimb fetlock joints compared with collected trot in both young and mature dressage 59 horses, respectively.

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62 INTRODUCTION

63 Dressage horses are often asked to work in lengthened paces during training and competition, 64 but to date there is limited information about the biomechanics of dressage-specific paces [1-65 7]. The current literature highlights the high prevalence of injuries of the suspensory apparatus 66 and the metacarpophalangeal or metatarsophalangeal (fetlock) joints in dressage horses [8-11]. 67 Dressage-specific movements may be implicated in causation or sub-clinical injuries may be 68 exacerbated by the highly repetitive nature of dressage training [12]. However to determine 69 this we need to first understand the biomechanics of dressage-specific paces, therefore 70 investigation of the kinematic differences between collected, medium and extended trot in 71 dressage horses is warranted.

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73 During the stance phase the limbs are progressively loaded until peak load at midstance. In the 74 forelimbs this results in shoulder and elbow flexion and carpus and fetlock extension; in the 75 hindlimbs there is hip, stifle, and hock flexion and fetlock extension [13,14]. In all limbs, the 76 role of the suspensory apparatus is to limit fetlock extension; consequently any variable which 77 increases fetlock extension is likely to increase load on the joint and the suspensory apparatus, 78 [15,16] and therefore may increase injury risk to these structures. Increased speed and stride 79 length and reduced stride duration in medium and extended trots compared with collected trot 80 have been described [3]. More recently it was shown that changes in temporal variables can 81 influence extension in trot [17-18]. These findings were supported by a pilot study of four 82 mature advanced dressage horses in which greater fetlock extension and hock flexion were 83 found in extended trot compared with collected trot [7].

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85 Epidemiological data has highlighted surface as a risk factor for injury in dressage horses [8,86 9]. Surface properties have been found to influence limb kinematics in horses competing in

other disciplines such as racing [19-20] and trotting [21-24], but there has been minimal
investigation on the effect of surface in collected or extended trot. Greater fetlock extension at
extended trot has been reported in dressage horses on a synthetic surface compared with dirt
[21], which suggests that surface may influence kinematics at this pace.

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92 The study aimed to investigate forelimb and hindlimb kinematics in: 1) young dressage horses 93 at collected and medium trot and 2) mature dressage horses at collected and extended trot. It 94 was hypothesised that 1) increased forelimb and hindlimb fetlock extension and hock flexion 95 would be seen at medium/extended trot compared with collected trot; 2) medium/extended trot 96 would have greater speed, stride length and reduced stride duration compared with collected 97 trot; 3) speed, stride length and stride duration and forelimb joint angles would be correlated 98 with forelimb fetlock extension and speed, stride length and stride duration and hindlimb joint 99 angles would be correlated with hock angle and hindlimb fetlock extension; 4) hindlimb fetlock 100 extension and hock flexion would be related to maximal hindlimb protraction and retraction 101 angles; 5) forelimb and hindlimb fetlock extension and hock flexion would be affected by 102 surface.

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105 MATERIALS AND METHODS

106 Horses

107 A power calculation indicated that a sample size of 19 horses was required to detect a difference

108 at a significance level P<0.05 for distal metatarsal coronary band vertical ratio (MTCR) which

109 represents hind fetlock extension, and hock angles based on pilot data [7].

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111 Twenty clinically sound horses, with no history of suspensory ligament injury, in active 112 dressage training were used: Group 1) ten young (≤ 6 years) horses working at novice to 113 elementary level dressage [25]; Group 2) ten mature (≥9 years) horses working at Prix St 114 Georges and above [25]. Horses were conventionally shod or barefoot. Horses did not wear 115 boots or bandages. All horses were assessed on two different outdoor surfaces (Surfaces A and 116 B, Table 1). Surface composition was analysed by taking a sample from each arena and carrying out simple material tests to quantify percentage moisture, sand, fibre and wax as 117 118 described in previous work [26]. The arena conditions were chosen because they simulated 119 surface composition and preparation routinely used for training and competing dressage horses. 120

121 All horse were evaluated by an experienced veterinarian (RM-Diplomate of the American 122 College of Veterinary Surgeons) in-hand at walk and trot in straight lines and in-hand at walk 123 in 5m diameter circles on a firm surface to ensure that they were free from lameness or graded 124 <1/8 lame [27]. Domed 30mm markers were placed at predetermined anatomical sites (Figure 125 1A) on the left and right sides by a single experienced technician (blinded for review), verified 126 by a veterinarian, according to palpable surface landmarks [28]. Marker placement 127 repeatability has been previously validated [7]. Horses were warmed-up by their normal rider, 128 as they would be at a competition, for up to 30 minutes before testing.

Testing took place at a single venue, on both surfaces consecutively in a randomised order, using a cross-over design. When the horses moved from the first surface to the second, 10 minutes were available for acclimatisation (duration used was rider-determined). Each horse was ridden at collected trot sitting (the degree of collection depended upon the stage of training) and at medium (Group 1) or extended (Group 2) trot sitting in a straight line marked out with cones (Figure 1B).

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136 Data Collection

137 High-speed motion-capture (240Hz, 1280 x 720 pixels) was used to assess each horse from the 138 left side. The camera (Casio EX-FH250¹) was placed 6m from the middle of the trot pathway 139 and the field of view was 5m wide and 3m high (Figure 1A). The camera was calibrated using 140 a known object in the field of view and also using a known measurement on the horse. These 141 were both compared to ensure that the calibration was accurate to 0.5 mm. A minimum of four 142 strides for each type of trot on each surface were collected. Strides were recorded when the 143 horse passed the camera. A single complete stride was selected per pass, because the field of 144 view prohibited recording of consecutive strides. Recordings were retained for analysis if the 145 stride was correct according to the Fédération Equestre International Rules for Dressage [29], 146 contained the entire stance phase, and was in the centre third of the field of view (directly in 147 front of the camera) to reduce the camera/marker angle in order to maximise accuracy. This 148 was judged by 3 authors (RM, VW, JB). Speed was calculated from the time it took for each 149 horse to get from the cone at the start of the runway to the cone at the end of the runway and 150 was verified from normal-speed video camera footage.

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154 Images were analysed by an experienced analyst (blinded for review) using previously 155 validated techniques [7]. Data was tracked through the entire stance phase and a low-pass 156 Butterworth filter with a cut off of 15Hz was used. Shoulder, elbow, carpal, forelimb fetlock, 157 hip, stifle and tarsal angles (Figure 2) were determined at midstance, when the fetlock joint was 158 maximally extended and mid swing when the carpus/hock joint was maximally 159 extended/flexed. Fetlock extension angle throughout the stance phase was plotted graphically 160 and the frame of peak fetlock extension was determined. Repeatability of this frame selection 161 was carried out 5 times for 5 horses (Coefficient of variation < 3%). Hindlimb fetlock extension 162 was measured as MTCR at midstance, which was defined as the distance between the fetlock 163 and the coronary band marker. This was calculated as the difference between the vertical location of markers 12 and 13 (Figure 3) on the Y axis at maximal extension of the MTPJ, as 164 165 previously described [7]. The MTCR measurement was used to determine the presence of 166 fetlock hyperextension (defined as marker 12 located below marker 13 at midstance). Using 167 this technique, it was less labour intensive to compare the degree of hyperextension/extension 168 among horses and between groups than measuring static fetlock angles and then making a 169 comparison with midstance angles. This was only performed in the hindlimbs because it is 170 commonly accepted that forelimb fetlock extension occurs during normal locomotion [30,31]. 171 We aimed to determine metatarsophalangeal joint extension compared with the coronary band, 172 using a method which has been successfully applied previously [7]. The measurements of \geq 173 1mm were accurate. Data from the left side only were analysed.

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175 The angle of the dorsal coronary band (marker 11) to a vertical line drawn from the tuber ischii 176 (marker 19) was used to represent hindlimb retraction (Figure 4A). Relative protraction of the 177 hindlimb was calculated as the angle between the dorsal coronary band marker relative to the 178 vertical line drawn from the proximal end of the tuber coxae (marker 15) (Figure 4B). Forelimb 179 protraction was defined as the angle between a vertical line drawn from the cranial eminence 180 of the greater tubercle of the humerus (marker 4) and the dorsal hoof wall marker (marker 10), 181 when the forelimb was in its foremost position just before hoof impact (Figure 4C). Forelimb 182 retraction was defined as the angle between a vertical line drawn from the cranial eminence of 183 the greater tubercle of the humerus (marker 4) and the dorsal hoof wall marker (marker 10) 184 when the forelimb was maximally retracted, but with the toe still in contact with the ground 185 (Figure 4D). These markers were chosen in preference to the spinous process of the 6th thoracic 186 vertebra (marker 24) and the tubera sacrale (marker 22) because they were easier to see and 187 also to minimise any effect of trunk rotation on the measurements.

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189 Statistical Analysis

190 Descriptive statistics and mixed effect multilevel regression analyses were performed using 191 Stata[™] 12.0 software² with statistical significance taken at P≤0.05. All continuous data were 192 considered normally distributed after evaluation graphically using kernel density and normal 193 quantile plots. Outcome variables examined in separate analyses were i) midstance forelimb 194 fetlock angle (°), ii) midstance hock angle (°) and iii) MTCR (cm), which were each considered 195 continuous variables. Kinematic predictor variables of the forelimbs and hindlimbs (Table 2), 196 along with trot pace (collected, medium and extended) and surface (A and B), were assessed. 197 Following preliminary univariable linear regression analyses to examine the relationship 198 between outcome variables and each predictor variable separately, multivariable linear 199 regression was then used to investigate the relationship between outcomes and simultaneous 200 multiple predictor variables. Each capture was one observation (fetlock angle and MTCR 201 n=308, hock angle n=320), because data comprised repeated measures with 16 separate 202 observations made on each of 20 individual horses. Mixed effect multiple linear regression 203 models were developed to evaluate continuous and categorical fixed effects variables as

multiple simultaneous predictors of midstance fetlock and hock joint angles and MTCR, each separately, with horse set as a random effect (intercept) variable in all three models. Model building was by forward stepwise selection of variables, with the final model retaining variables that were significantly associated with the outcome and/or that significantly improved the overall fit of the model, based on likelihood ratio testing. The distribution and outlier values of the standardised residuals (difference between the model predicted and actual outcome values) from each model were also assessed.

211

212 RESULTS

For Group 1 mean age was 5.5±0.7 years and mean height was 167±7 cm. For Group 2 mean
age was 12.3±2.3 years and mean height was 169±6cm. Warm-up duration ranged from 12-29
minutes (mean 18 minutes). Means and standard deviations for all kinematic variables are
shown in Table 2.

217

218 Table 3 summarises final models from mixed effect multiple linear regression analyses with 219 only statistically significant variables retained for predicting i) midstance forelimb fetlock 220 angle, ii) midstance hock angle and iii) MTCR with horse included as a statistically significant 221 (P<0.0001) random effect variable in each model. Results can be considered as representing 222 biologically plausible statistical models to predict values of each of the three continuous 223 outcome measures. Outcome values are derived as the sum of a baseline (intercept) value with 224 addition (positive regression coefficient values) or subtraction (negative regression coefficient 225 values) of estimated parameter values, comprising the product of each predictor variable 226 measurement and its corresponding regression coefficient. Surface was not retained as a 227 statistically significant predictor variable in any of the final models.

Speed and stride length were significantly increased and stride duration was significantly
decreased at medium trot compared with collected trot in Group 1 and at extended trot
compared with collected trot in Group 2 (P<0.0001 for all).

232

233 Forelimb fetlock angle

234 The final model predicted that forelimb fetlock extension angle was significantly increased 235 (positive regression coefficient values) at medium and extended trots compared with collected 236 trot (P<0.001 for both). It also predicted that forelimb fetlock extension angle was significantly 237 decreased (negative regression coefficient value, indicating reduced fetlock extension) when 238 stride length was increased (P=0.05). The final model predicted that forelimb fetlock extension 239 angle significantly decreased (negative regression coefficient values) when shoulder angle was 240 increased (indicating decreased shoulder flexion) (P=0.042). Forelimb fetlock extension angle 241 significantly increased (positive regression coefficient values) when carpus angle was 242 increased (P<0.001).

243

244 Hock angle

Hock angle was not affected by pace. Hock angle significantly decreased, indicating greater
hock flexion, when stride duration (P<0.001) and speed (P=0.002) were increased. Hock angle
significantly increased when hip angle increased (P=0.005). Hock angle significantly
decreased, indicating greater hock flexion, when hindlimb protraction and retraction angles
were increased (P<0.001 for both).

- 250
- 251 MTCR

MTCR significantly decreased, indicating greater hindlimb fetlock extension, at medium andextended trots, both compared with collected trot (P<0.001 for both). MTCR significantly

increased, indicating reduced fetlock extension, when speed increased (P=0.001). The final
model predicted that MTCR significantly decreased, indicating greater hindlimb fetlock
extension, when hindlimb retraction angle was increased (P=0.032).

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258 DISCUSSION

259 This study successfully investigated forelimb and hindlimb kinematics in young and mature 260 dressage horses at collected and lengthened trots. In both groups, lengthened (medium and 261 extended) trot was associated with forelimb and hindlimb fetlock extension, supporting our 262 first hypothesis, and had greater speed, stride length and reduced stride duration compared with 263 collected trot, supporting our second hypothesis. However, hock angle was not affected by 264 pace. The third hypothesis that speed, stride length, stride duration and forelimb joint angles 265 would be correlated with forelimb fetlock extension was partially supported. Forelimb fetlock 266 extension angle was positively correlated with carpus angle, and negatively correlated with 267 shoulder angle and stride length. No correlations between forelimb fetlock angle and speed, 268 stride duration or elbow angle were detected. For the hindlimb, our hypothesis that speed, stride 269 length, stride duration and hindlimb joint angles would be correlated with hock angle and 270 hindlimb fetlock extension was also partially proven. Hock angle significantly decreased when 271 stride duration and speed were increased, and was positively correlated with hip angle but not 272 stifle, MTCR or stride length, MTCR increased with speed (i.e. hindlimb fetlock extension was 273 reduced), but was not related to any of the measured hindlimb joint angles, stride length or 274 duration. The fourth hypothesis that hindlimb fetlock extension and hock flexion would be 275 related to maximal hindlimb protraction and retraction angles was supported by our findings. 276 The fifth hypothesis was unproven; no effect of surface on any outcome variables was detected.

278 Similar findings in both groups indicate that lengthening of the trot stride increases extension 279 of the fetlock joints at midstance compared with collected trot. The suspensory apparatus 280 moderates the extension of the fetlock [15,16, 32-34] and our findings suggests that lengthened 281 paces may increase the strain placed both on the suspensory apparatus and the fetlock in 282 forelimbs and hindlimbs. The magnitude of extension is greater in movements such as 283 cantering and jumping [35], but currently there is no evidence to specify the magnitude or 284 frequency of hyperextension necessary to increase risk of injury. A 6 to 8 degree increase in 285 fetlock joint overextension has been observed due to fatigue in trotting horses [36]. The authors 286 proposed this could increase strain on the suspensory ligament and the supporting structures of 287 the fetlock joint. In this study we did not work the horses to fatigue but, based on the findings 288 on trotters [33,34,36] and show jumpers [35], fatigue may affect the degree of fetlock extension 289 seen in either pace. It should be a consideration when teaching horses collected or lengthened 290 paces because they are likely to fatigue more rapidly when learning new movements. This 291 study aimed to further our knowledge of how collected and extended trot affect fetlock 292 extension so we can begin to understand the factors that are likely to provide an influence. It is 293 expected that the degree of fetlock extension in medium and extended trots, and its potential 294 risk of injury depends on many factors such as musculoskeletal strength and coordination, 295 conformation (static and dynamic), training intensity, training frequency and training volume 296 (potentially including how frequently and for how long the horse is asked to demonstrate 297 lengthened paces), previous injury, and genetics [37]. Further work is warranted to understand 298 the effect of these factors in horses performing different types of trot. No difference in hock 299 flexion angle between collected and lengthened trot was observed in either group. This is 300 contrary to previous results [7], which may be due to differences in sample size, horses' gait 301 patterns, training levels and/or level of collection/extension used in this and the previous study. 302

303 The degree of change in fetlock extension for collected to lengthened trot was greater in Group 304 2 (mature) horses performing extended trot than the Group 1 (young) horses performing 305 medium trot as seen by the greater regression coefficient value for Group 2 compared with 306 Group 1. We aimed to test the types of pace that were considered acceptable for the horses' 307 ages and levels of training. Young horses and those in the lower competitive levels are asked 308 to show lengthened or medium gaits in competition [37], so medium trot was selected for 309 Group 1, while Group 2 were trained to achieve greater collection and extension so could be 310 tested with more exaggerated pace types.

311

312 Greater speed, stride length and reduced stride duration at medium trot compared with collected 313 trot in Group 1 and at extended trot compared with collected trot in Group 2 were observed, as 314 hypothesised. This is consistent with previous findings [1-3,5,17,18], although we observed 315 slower collected, medium and extended trots with a shorter stride length than those observed 316 in national level dressage horses [3] and with slower and shorter strides in the extended trot 317 than recorded in Olympic competitors [5]. This may reflect differences in training level and 318 athletic ability compared with the current study, in which horses were of mixed levels (e.g., 319 Group 2 ranged from Advanced medium to Grand Prix). The type of dressage horse has also 320 changed considerably over the last 20-25 years, so the populations are not directly comparable.

321

Fetlock angle has previously been linked to speed [13,38] so it could be suggested that an increase in fetlock extension could simply be due to the increase in speed at medium and extended trot compared with collected trot. Our findings suggest that temporal variables (speed and stride length) have an influence on fetlock angle, but because they are inherent components of pace it is hard to identify their pure effects in isolation. Pace is also made up of other components, such as duty factor and muscle activation, not all of which were measured in this 328 study, but which would also be accounted for through inclusion of pace. Pace (type of trot) had 329 the principle effect on MTCR and as such would also have accounted for some of these inherent 330 component effects. However, as speed was retained in the MTCR model along with pace, this 331 indicated that there was clearly a still statistically significant residual effect of speed, beyond 332 that already accounted for by pace. Pace is quite a crude variable due to its complexity, but the 333 model suggested that it was the best predictor for MTCR angle. This means that the effect of 334 pace, through all its inherent components is to reduce MTCR in medium or extended trot 335 (increases fetlock extension), but speed also has a slightly positive residual effect (reduces 336 fetlock extension), which further improved the prediction of the model. Increased speed results 337 in slightly reduced fetlock extension, but overall when also accounting for pace there is a net 338 greater extension in medium and extended trot compared with collected trot. This means that 339 medium and extended trot reduce MTCR compared with collected trot, but that reduction is 340 slightly less if the horse is going faster.

341 In the forelimb fetlock extension model, we observed that at medium/extended trot forelimb 342 fetlock extension was increased compared with the collected trot, but the increase in fetlock 343 extension was slightly reduced when the stride length was greater. These findings could relate 344 to the faster speed or increased stride length of the medium and extended trots compared with 345 collected trot, potentially influencing stance duration and therefore loading time. It suggests 346 that although speed and stride length are part of the change in pace, the influence of pace is 347 made up of lots of different constituents, including stance duration and duty factor, all of which 348 need to be thoroughly investigated to understand the mechanism, the impact and potential 349 practical implications of these findings.

350

There were different associations between fetlock angle and other limb joint angles in theforelimbs and hindlimbs. As previously documented [14-16], the forelimbs and hindlimbs are

kinematically different at midstance, which potentially affects the way they moderate forces at midstance. As hypothesised, the forelimb shoulder and carpus angles were associated with forelimb fetlock angle at midstance, although no association with elbow angle at midstance was observed. This suggests that the forelimb as a unit is influenced by trot type and therefore the kinematic and kinetic changes influence many of the structures of the forelimb, not just the suspensory apparatus and fetlock.

359

360 In the hindlimb the reciprocal apparatus provides a connection between the stifle and hock, and 361 also has a connection to the fetlock via the deep digital flexor tendon [39]. However, we 362 observed no association between hindlimb fetlock extension and the angle of any of the 363 hindlimb joints at midstance. In the hindlimb, coxofemoral joint (hip) angle was positively 364 associated with hock angle, which may have implications for loading of the hip and hock. With 365 increased speed and/or greater hindlimb protraction and retraction the hock is more flexed at 366 midstance [13]. The results of the current study indicate that this flexion may be moderated by 367 the action of the hip.

368

369 It was previously suggested that an explanation for increased fetlock extension and increased 370 hock flexion during lengthened paces might be an alteration in protraction and retraction of the 371 hindlimbs between extended and collected trot [7]. In the current study hindlimb protraction 372 and retraction were associated with hock angle, with an increase in protraction/retraction 373 resulting in a decrease in hock flexion angle at midstance. However, hindlimb protraction and 374 retraction angles were not affected by different trot types. Thus the mechanism which causes 375 increased fetlock extension in lengthened, compared with collected trot remains unclear and 376 merits further investigation.

378 Increased hindlimb fetlock extension at medium and extended trots supports previous findings 379 [7]. There is an association between static or dynamic hindlimb fetlock overextension and 380 injury of the hindlimb suspensory apparatus [12, 37]. The current findings suggest that although 381 hindlimb fetlock extension occurred in both groups of horses, the mean value for each trot type 382 did not indicate dynamic hyperextension at the trot, previously defined as the fetlock marker 383 being distal to the coronary band marker at peak fetlock extension [7]. Horses in the current 384 study were subjectively considered to be well-conformed. Horses with a small dorsal fetlock 385 angle may be more at risk of hyperextension compared with better-conformed horses, which 386 may increase risk of injury to the suspensory apparatus [12, 40]. Our findings are only relevant 387 to the trot and other gaits and movements (e.g., canter pirouette) may have different results.

388

389 Surfaces A and B were selected because it was hypothesised that their functional properties 390 would be different. However, no effect of arena surface type was observed in the final models 391 for either collected or extended trot. Lower GRF and decreased maximal fetlock extension were 392 observed on deep, wet sand compared with firm, wet sand [23], suggesting that these two 393 surfaces were functionally dissimilar. Surface material is known to behave differently 394 according to composition, preparation and maintenance [20,41,42]. It is possible that despite 395 differences in surface type and moisture content, the overall make-up and maintenance of 396 surfaces A and B meant functional properties were comparable. Increased fetlock extension at 397 midstance was reported on one surface when it was harrowed versus rolled, however data 398 grouped all gaits together (walk, trot and canter) [41]. Canter would be expected to produce 399 greater fetlock extension [35], which might explain these findings compared with the present 400 study, conducted only in trot. The sample population included horses with a variety of 401 conformations; static or dynamic conformation and the surfaces on which the horses normally 402 train may influence preference of surface type. Fetlock extension was highly variable and 403 heterogeneity within the sample population may explain why no significant difference was404 found between surfaces.

405

406 The study had some limitations. All motion capture was in two dimensions. Three-dimensional 407 analysis would be useful to evaluate other movement planes which may influence strain on the 408 suspensory apparatus and loading of the fetlocks. All testing was carried out on an artificial 409 surface which can influence the measurements acquired and the definition of impact. Rotation 410 of the hoof into the surface may have influenced the accuracy of the MTCR calculation. It also 411 made it difficult to accurately measure stance duration and therefore duty factor. All recruited 412 horses were Warmblood dressage horses which were grouped according to age and training 413 level, however there are likely to be considerable differences in natural athletic ability of the 414 horses, which may influence the findings. Extrapolation of these findings must therefore be 415 done with care, because they may not apply to different breed populations or to horses with 416 different gait characteristics e.g., Andalusian, Lusitano or Lipizzaner. Rider skill may also 417 have had some influence on the gaits of the horse [43]. All horses underwent a subjective 418 conformation assessment, but an objective assessment was not performed and would have been 419 preferable. Each horse and rider combination was evaluated on a single day on both surfaces, 420 and a cross-over design was used for both pace and surface in order to minimise order effect. 421 However in the second session there may have been an influence of previous warm up/mobility 422 and/or fatigue. Testing over multiple days could have reduced these effects; however we aimed 423 to keep the environmental conditions as similar as possible for each horse, because this can 424 influence surface functional properties [44]. Horse performance can also vary from day to day 425 for a variety of reasons. Comparisons between medium and extended trot, or between working 426 and medium trot were not performed due to time constraints. Further work is warranted to

427 assess the difference between working, collected, medium and extended paces and specific

428 movements, such as pirouettes.

429

430 CONCLUSIONS

- 431 Medium or extended trot increase extension of the forelimb shoulder, carpal and fetlock joints
- 432 and the hindlimb fetlock joint compared with collected trot in both young and mature dressage
- 433 horses, respectively.
- 434

435 MANUFACTURERS' ADDRESSES

- 436 ¹ Casio Computer Co Ltd, Tokyo, Japan.
- 437 ² Stata SE 12.1, College Station, Texas, USA. Formatted: Italian (Italy)

438 FIGURE LEGENDS

439 Figure 1: A) Marker placement for data collection: 1) rostral aspect of the facial crest 2) wing 440 of atlas 3) proximal aspect of the scapular spine 4) over the cranial eminence of the greater 441 tubercle of the humerus 5) the lateral epicondyle of the humerus over the lateral collateral 442 ligament of the elbow 6) lateral styloid process of the radius 7) proximal aspect of the third 443 metacarpal bone at the junction with the base of the 4th metacarpal bone 8) distal aspect of the 444 third metacarpal bone over the lateral collateral ligament of the metacarpophalangeal joint 445 9)lateral collateral ligament of the distal interphalangeal joint (designated coronary band) 10) 446 dorsal aspect of the coronary band 11) dorsal aspect of the coronary band 12) lateral collateral 447 ligament of the distal interphalangeal joint (designated coronary band) 13) distal aspect of the 448 third metatarsal bone over the collateral ligament of the metatarsophalangeal joint 14) 449 proximal aspect of the third metatarsal bone at the junction with the base of the 4th metatarsal 450 bone 15) mid talus 16) lateral aspect of the tibial crest 17) medial epicondyle of the distal femur 451 18) proximal aspect of the greater trochanter of the femur 19) ischiatic tuberosity 20) top of 452 tail 21) proximal aspect of the tuber coxae 22) tuber sacrale 23) spinous process of the 4th 453 lumbar vertebra 24) spinous process of the 6th thoracic vertebra. B) Arena set up for testing; 454 showing field of view and runway used.

455

Figure 2: Angles measured from high speed motion capture at midstance. In the forelimb:1)
Shoulder angle; calculated from the proximal aspect of the scapular spine, the cranial eminence
of the greater tubercle of the humerus, the lateral epicondyle of the humerus over the lateral
collateral ligament of the elbow. 2) Elbow angle; calculated from the cranial eminence of the
greater tubercle of the humerus, the lateral epicondyle of the humerus over the lateral
ligament of the elbow, the lateral epicondyle of the humerus over the lateral collateral
ligament of the elbow, the lateral styloid process of the radius. 3) Carpus angle; calculated
from, the lateral epicondyle of the humerus over the lateral collateral ligament of the elbow,

463 lateral styloid process of the radius, proximal aspect of the third metacarpal bone at the junction 464 with the base of the 4th metacarpal bone. 4) Forelimb fetlock (metacarpophalangeal) angle; 465 calculated from the proximal aspect of the third metacarpal bone at the junction with the base of the 4th metacarpal bone, distal aspect of the third metacarpal bone over the lateral collateral 466 467 ligament of the metacarpophalangeal joint, lateral collateral ligament of the distal 468 interphalangeal joint (designated coronary band). In the hindlimb: 5) Hip angle; calculated 469 from the proximal aspect of the tuber coxae, proximal aspect of the greater trochanter of the 470 femur, the medial epicondyle of the femur. 6) Stifle angle; calculated from proximal aspect of 471 the greater trochanter, medial epicondyle of the distal femur, lateral aspect of the tibial crest. 472 7) Hock (tarsal) angle; calculated from the lateral aspect of the tibial crest, mid talus, proximal 473 aspect of the third metatarsal bone at the junction with the base of the 4th metatarsal bone. 474 MTCR; metatarsal coronary band ratio is calculated as the difference between marker 12; 475 lateral collateral ligament of the distal interphalangeal joint (designated coronary band) and 476 marker 13: distal aspect of the third metatarsal bone over the collateral ligament of the 477 metatarsophalangeal joint along the Y axis.

478

Figure 3: Metatarsal coronary band ratio (MTCR). This is calculated as the difference between
marker 12; lateral collateral ligament of the distal interphalangeal joint (designated coronary
band) and marker 13: distal aspect of the third metatarsal bone over the collateral
ligament of the metatarsophalangeal joint along the Y axis. This is determined at midstance-

defined as the point of maximal fetlock extension. The image is calibrated to give a value incentimetres.

485

486 Figure 4: Forelimb and hindlimb protraction and retraction angles. Maximal hindlimb487 retraction (A) was defined as the angle of a line between the dorsal aspect of the coronary band

| 488 | marker to the tuber ischii, relative to vertical. This was measured just before the toe left the |
|-----|--|
| 489 | surface. Maximal hindlimb protraction (B) was defined as the angle of a line between the dorsal |
| 490 | aspect of the coronary band to the proximal aspect of the tuber coxae relative to vertical. This |
| 491 | was measured just before hoof/surface impact. Maximal forelimb protraction (C) was defined |
| 492 | as the angle of a line between the dorsal aspect of the coronary band at the toe to the cranial |
| 493 | eminence of the greater tubercle of the humerus relative to vertical. This was measured just |
| 494 | before hoof/surface impact. Maximal forelimb retraction (D) was defined as the angle of a line |
| 495 | between the dorsal aspect of the coronary band marker to the cranial eminence of the greater |
| 496 | tubercle of the humerus, relative to vertical. This was measured just before the toe left the |
| 497 | surface. |
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- 622 <u>http://www.fei.org/fei/about-fei/publications/fei-books</u>
- 623

- 624 Table 1: Surface composition for the 2 arena surfaces (1 and 2) on which 20 dressage horses
- 625 were assessed at collected and medium/ extended trot. Estimate of composition were based on
- 626 a mean (n = 3 samples per surface) [27].

| Component % | Surface A | | Surface B | | |
|--------------|-----------------|---------------|-----------------------------------|--------------|--|
| - | Mean | sd | Mean | sd | |
| Moisture | 11 | 2 | 6 | 2.6 | |
| Sand | 76 | 1.9 | 46 | 5.8 | |
| Fibre/rubber | 11 | 2.5 | 45 | 5.1 | |
| Wax | 2 | 0.1 | 2 | 0.7 | |
| | | | | | |
| | Small strand | fibre <5cm, | Small felt fibre | <5cm length, | |
| Composition | small grain | rubber <1cm | mainly small grain rubber <1cm | | |
| Composition | diameter, son | me large felt | diameter, some large grain rubber | | |
| | fibre up to 12c | em in length. | >1cm diameter | | |

628Table 2: Mean and standard deviation (sd) for all kinematic variables of the forelimbs and629hindlimbs measured in 20 dressage horses: Group 1 (≤ 6 years of age, n=10) and Group 2 (≥ 9 630years of age, n=10), assessed in straight lines in collected and medium trot and collected and631extended trot, respectively. Shading denotes outcome variables and non-shading denotes632predictor variables.

| | Group | 1- | Group | 1- | Group | 2- | Group | 2 – |
|-----------------------------|----------|------|-------|------|----------|------|---------|------|
| | collecto | ed | mediu | n | collecte | d | extende | ed |
| Variable | Mean | sd | Mean | sd | Mean | sd | Mean | Sd |
| Forelimb fetlock angle (°) | 246.2 | 9.3 | 249.3 | 10.5 | 246.1 | 13.9 | 251.1 | 13.4 |
| Hock angle (°) | 151.9 | 5.0 | 150.2 | 5.0 | 149.2 | 6.0 | 146.6 | 6.0 |
| MTCR (cm) | 2.3 | 0.5 | 1.8 | 0.6 | 1.8 | 0.2 | 1.0 | 0.4 |
| Stride Duration (secs) | 0.8 | 0.01 | 0.7 | 0.01 | 0.8 | 0.1 | 0.7 | 0.1 |
| Speed (m/s) | 2.7 | 0.3 | 3.7 | 0.4 | 2.7 | 0.4 | 4.0 | 0.4 |
| Stride Length (m) | 2.1 | 0.2 | 2.7 | 0.3 | 2.3 | 0.4 | 2.9 | 0.5 |
| Mid stance shoulder angle | | | | | | | | |
| (°) | 126.2 | 8.4 | 125.5 | 7.4 | 122 | 8.7 | 121.9 | 8.8 |
| Mid swing shoulder angle | | | | | | | | |
| (°) | 127.3 | 7.9 | 128.8 | 6.8 | 123. | 8.3 | 125. | 8.8 |
| Mid stance elbow angle (°) | 211.5 | 6.0 | 212.1 | 6.2 | 212.6 | 6.2 | 214.5 | 5.8 |
| Mid swing elbow angle (°) | 250.7 | 10.1 | 253.2 | 9.4 | 253.6 | 7.5 | 256.4 | 9.6 |
| | | | | | | | | |
| Mid stance carpus angle (°) | 183.0 | 5.5 | 182.7 | 5.9 | 180.5 | 5.4 | 180.8 | 4.5 |
| Mid swing carpus angle (°) | 128.5 | 8.5 | 123.2 | 8.7 | 129.7 | 8.5 | 122.1 | 8.2 |
| Mid swing fetlock angle (°) | 175.1 | 9.4 | 170.5 | 10.8 | 179.9 | 14.0 | 174.8 | 14.6 |

| Mid stance hin angle (°) | 71.1 | 1.1 | 71.2 | 19 | 70.0 | 12 | 71.2 | 4.0 |
|-----------------------------|-------|------|-------|------|-------|------|-------|------|
| who stance mp angle () | /1.1 | 4.4 | /1.5 | 4.0 | 70.9 | 4.2 | /1.2 | 4.9 |
| Mid swing hip angle (°) | 64.1 | 3.8 | 63.4 | 4.4 | 62.7 | 3.2 | 61.9 | 3.4 |
| Mid stance stifle angle (°) | 101.6 | 7.2 | 101.4 | 8.0 | 99.6 | 6.4 | 99.2 | 7.6 |
| Mid swing stifle angle (°) | 98.8 | 9.3 | 96.9 | 10.4 | 96.8 | 14.9 | 95.8 | 14.7 |
| Mid swing hock angle (°) | 109.8 | 7.2 | 105.9 | 9.1 | 105.2 | 8.1 | 96.9 | 11.7 |
| Mid swing fetlock angle (°) | 150.1 | 13.9 | 149.5 | 14.1 | 150.7 | 12.5 | 149.0 | 12.1 |
| HL Protraction angle (°) | 11.9 | 1.9 | 12.9 | 1.7 | 11.6 | 2.2 | 13.4 | 2.4 |
| HL Retraction angle (°) | 16.1 | 1.9 | 18.0 | 2.4 | 15.1 | 2.2 | 18.5 | 1.8 |

633 Secs = seconds; m/s = metres per second; m = metres; ⁰ = degrees; MTCR = metatarsal

 $\label{eq:coronary band ratio; cm = centimetres; HL = hindlimb.$

635

637 Table 3: Summary of final models from mixed effect multiple linear regression analyses of i) forelimb fetlock angle (°) (n= 308), ii) hock angle

638 (°) (n 320) and iii) metatarsal coronary band ratio (MTCR; cm) (n=308) with different predictor variables and horse (n=20) included as a

639 statistically significant (P<0.0001) random effect variable in each model for two groups of dressage horse, young (group 1) and mature (group 2).

| Outcome measure | | Regression | Standard | 95% confidence interval | P-value |
|-------------------------|---------------------------------------|-------------------|----------|---------------------------|---------|
| Predictor variable | Comparator for interpretation | coefficient | error | of regression coefficient | |
| | | | | | |
| Forelimb fetlock angle | | (<i>unit</i> = % | | | |
| Intercept | Baseline fetlock angle | 172.7 | 22.0 | 129.6 - 215.9 | - |
| Medium trot (Group 1) | Versus collected trot as baseline | +5.70 | 1.59 | 2.58 - 8.82 | < 0.001 |
| Extended trot (Group 2) | Versus collected trot as baseline | +8.59 | 1.75 | 5.16 - 12.03 | < 0.001 |
| Stride length | Per metre increase of stride length | -2.87 | 1.48 | -5.77 - 0.04 | 0.05 |
| Carpus angle | Per degree increase of carpus angle | +0.61 | 0.10 | 0.41 - 0.82 | < 0.001 |
| Shoulder angle | Per degree increase of shoulder angle | -0.17 | 0.08 | -0.330.01 | 0.042 |
| | | | | | |
| Hock angle | | (unit = % | | | |
| Intercept | Baseline hock angle | 171.5 | 7.4 | 156.9 – 186.1 | - |

| Stride duration | Per second increase of stride duration | -20.9 | 4.94 | -30.611.2 | < 0.001 |
|--|--|--|--------------------------------------|---|---|
| Speed | Per metre per second increase of speed | -1.25 | 0.39 | -2.020.48 | 0.002 |
| Hip angle | Per degree increase of hip angle | +0.21 | 0.08 | 0.06 - 0.36 | 0.005 |
| Hindlimb (HL) protraction angle | Per degree increase of HL protraction angle | -0.49 | 0.11 | -0.710.26 | < 0.001 |
| Hindlimb (HL) retraction angle | Per degree increase of HL retraction angle | -0.39 | 0.11 | -0.600.17 | < 0.001 |
| | | | | | |
| | | | | | |
| Metatarsal coronary band ratio | | (unit = cm) | | | |
| Metatarsal coronary band ratio Intercept | Baseline MTCR distance | (unit = cm) 1.43 | 0.61 | 0.24 – 2.62 | - |
| Metatarsal coronary band ratio Intercept Medium trot (Group 1) | <i>Baseline MTCR distance</i> Versus collected trot as baseline | (unit = cm) 1.43 -0.87 | <i>0.61</i> 0.19 | 0.24 - 2.62 -1.240.50 | -<0.001 |
| Metatarsal coronary band ratio Intercept Medium trot (Group 1) Extended trot (Group 2) | Baseline MTCR distance Versus collected trot as baseline Versus collected trot as baseline | (unit = cm) 1.43 -0.87 -1.23 | 0.61 0.19 0.24 | 0.24 - 2.62 -1.240.50 -1.710.76 | - <0.001 <0.001 |
| Metatarsal coronary band ratio Intercept Medium trot (Group 1) Extended trot (Group 2) Speed | Baseline MTCR distance Versus collected trot as baseline Versus collected trot as baseline Per metre per second increase of speed | (<i>unit = cm</i>) 1.43 -0.87 -1.23 +0.51 | 0.61 0.19 0.24 0.15 | 0.24 - 2.62 -1.240.50 -1.710.76 0.22 - 0.81 | - <0.001 <0.001 0.001 |
| Metatarsal coronary band ratioInterceptMedium trot (Group 1)Extended trot (Group 2)SpeedHindlimb (HL) retraction angle | Baseline MTCR distance Versus collected trot as baseline Versus collected trot as baseline Per metre per second increase of speed Per degree increase of HL retraction angle | (<i>unit = cm</i>) 1.43 -0.87 -1.23 +0.51 -0.06 | 0.61 0.19 0.24 0.15 0.03 | 0.24 - 2.62 -1.240.50 -1.710.76 0.22 - 0.81 -0.110.05 | - <0.001 <0.001 0.001 0.032 |