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Adjustments with running speed reveal neuromuscular adaptations during landing associated with high mileage running training

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Abstract

It remains to be determined whether running training influences the amplitude of lower limb muscle activations prior to and during the first half of stance, and whether such changes are associated with joint stiffness regulation and usage of stored energy from tendons. Therefore, the aim of this study was to investigate neuromuscular and movement adaptations before and during landing in response to running training across a range of speeds. Two groups of high mileage (HM; >45 km/wk, n=13) and low mileage (LM; <15 km/wk, n=13) runners ran at four speeds (2.5-5.5 m·s^{-1}) while lower limb mechanics and electromyography of the thigh muscles were collected. There were few differences in pre-landing activation levels, but HM runners displayed lower activations of the Rectus Femoris, Vastus Medialis and Semitendinosus muscles post-landing, and these differences increased with running speed. HM runners also demonstrated higher initial knee stiffness during the impact phase compared to LM runners which was associated with an earlier peak knee flexion velocity, and both were relatively unchanged by running speed. In contrast, LM runners had higher knee stiffness during the slightly later weight acceptance phase and the disparity was amplified with increases in speed. It was concluded that initial knee joint stiffness might predominantly be governed by tendon stiffness rather than muscular activations before landing. Estimated elastic work about the ankle was found to be higher in the HM runners which might play a role in reducing weight acceptance phase muscle activation levels and improve muscle activation efficiency with running training.

Key words

Knee stiffness, Electromyography, Lower limb kinematics, Co-activation, Energy return

New and noteworthy

Although neuromuscular factors play a key role during running, the influence of high mileage training on neuromuscular function has been poorly studied, especially in relation to running speed. This study is the first to demonstrate changes in neuromuscular conditioning with high mileage training, mainly characterised by lower

thigh muscle activation after touch-down, higher initial knee stiffness and greater estimates of energy return, with adaptations being increasingly evident at faster running speeds.

1 **Introduction**

2 Although it is established that neuromuscular factors, such as stretch-shortening behaviour of lower limb

3 muscles and tendons, play a vital role in the effectiveness of absorption and generation of force during running

4 (14–16, 47, 63), very few studies have examined the influence of running training on neuromuscular function

5 during running, particularly across a range of running speeds (6, 12). Previous studies that have examined

6 neuromuscular adaptations to higher endurance training volumes in cycling have found evidence to suggest that

7 the movements become more skilled, and this adaptation is characterised by reductions in muscle activation

8 amplitudes and durations (17, 19, 20). Comparisons between high and low mileage runners (assessed for a single

9 running speed) have shown that running training was associated with slight decreases in pre-landing muscle

10 activation levels and reduced activation levels of extensor muscles during the first half of stance (7, 18).

11 However, most investigations of training effects on neuromuscular function have not involved distance running

12 and only consider short-term training effects (19). It is likely that the influence of training on neuromuscular

13 function during running may be more evident when comparing distance runners with a large difference in

14 training mileage level, and assessing adaptations across a range of running speeds.

15 A general concept of neuromuscular efficiency has been proposed, where running training is likely to influence

16 the amplitude of lower limb muscle activation prior to and during the initial phase of ground contact which

17 augment lower extremity stiffness and the use of stored elastic energy (6, 38). This concept has developed from

18 the seminal work of Cavagna, Saibene and Margaria (16), who highlighted the important contribution of elastic

19 recoil energy from stretched tendons and muscles during running. They also highlighted that this mechanism

20 becomes more useful with increases in running speed (15, 16). In addition, estimates of an increased lower limb

21 joint work during stance and the storage and release of elastic energy from the lower limb tendons have been

22 linked to increased physiological efficiency (reduced rate of oxygen consumption or improved economy at a

23 given speed) during running (65, 78). This association is further supported by improvements in running economy

24 found after a period of running and strength training in which stiffness (and energy recoil ability) of the Achilles

25 tendon was increased (2, 26), something that has also been confirmed in inveterate trained endurance runners (4).

26 Increased pre-landing muscle activation and joint stiffness regulation with training have been found during drop

27 landing and hopping activities (37, 38). These findings lead to speculation that a similar response is likely to

28 occur over time with prolonged running training, but the influence of training on pre-landing muscle activation

29 levels remains to be established. It has also been proposed that simultaneous activation of several muscles acting

30 about a joint provides joint stability during ground contact and plays an important role in stiffness regulation (9,

31 38), although such a direct association between joint stiffness and muscle co-activation levels about that joint has

32 not been widely verified yet.

33 The rates of knee flexion during ground contact have been shown to be directly associated with running speed (3,

34 51, 56). In addition, lower limb joint stiffness, in particular stiffness of the knee, has been found to increase

35 when running faster in order to reduce joint deformations during landing (3, 31, 41). Moreover, lower limb

36 muscle activation and co-activation levels have both been reported to increase with running speed before initial

37 ground contact (44, 75), as well as during landing (42, 59, 73). Despite this research attention, the associations

38 between lower limb muscle pre-landing and post-landing activations, co-activations and joint stiffness setting

39 across a range of running speeds have yet to be explored from a running training perspective.

40 Given the importance of the knee joint in running, it was hoped that by examining knee joint stiffness and

41 muscle activation levels (those muscles that act about the knee joint) across a range of running speeds, the

42 adaptations of neuromuscular factors due to running training could be closely explored for the first time. The

43 modulation of thigh muscle pre-activation, co-activation and knee joint stiffness with running speed would also

44 provide fundamental information on neuromuscular control mechanisms during running. Therefore, this study

45 investigated muscle (co-)activation before and during landing, as well as knee stiffness and estimated elastic

46 work during landing between two groups of runners with substantially different levels of running training, over a

47 range of running speeds. It was hypothesised that runners with a high training volume would 1) show greater

48 pre-activation of knee joint muscles before touch-down; 2) have associated greater knee stiffness during early

49 stance; 3) display lower muscle (co-)activation post-landing; 4) demonstrate larger amounts of elastic work

50 about the ankle and 5) that neuromuscular differences between the high and low mileage running groups would

51 be greater at higher running speeds.

52

53 **Methods**

54 *Subjects and protocol*

55 Thirty-seven runners (29 males and 8 females) were recruited to participate in this study. Subjects were healthy

56 at the time of testing and had no previous history of major injuries of the lower limbs within the last six months.

57 All subjects had been running for at least six months. Of the 37 runners, 26 were assigned to either a low mileage

58 (LM; <15 km/wk, n=13), or a high mileage (HM; >45 km/wk, n=13) group, with clear disparity between the two

59 groups (Fig. 1). The cut-off used for the high mileage group was the same as employed in a previous

60   investigation (7). In accordance with the Liverpool John Moores University ethics regulations, all subjects

61   completed an informed consent form prior to data collection.

62   Prior to debrief of the experimental protocol, each subject did a warm-up of approximately five minutes of

63   overground running at a self-selected easy pace. Subjects then performed five overground running trials at each

64   of four running speeds (2.5 m·s^{-1}, 3.5 m·s^{-1}, 4.5 m·s^{-1} and 5.5 m·s^{-1}) and one maximal sprint along a 70 metre

65   indoor runway with a large force platform mounted midway. They started at a self-selected distance from the

66   force platform which was sufficient (15-20 metres) to get up to the required speed. Runners were asked to

67   maintain the required speed from 5 m before to 5 m after the force platform. Running speeds were performed in

68   a different mixed order for every subject, based on a Latin Square design. During the trials, kinematic, kinetic

69   and EMG data of the right leg were recorded. All subjects wore their regular running shoes, to avoid adaptations

70   to unfamiliar footwear.

71   Three-dimensional (3D) kinematic and kinetic data were synchronously collected with an eight camera motion

72   analysis system (Qqus 300+, Qualisys Inc., Gothenburg, Sweden), in combination with a ground embedded force

73   platform (90 x 60 cm, 9287B, Kistler Holding AG, Winterthur, Switzerland). Motion and force data were

74   sampled at 500 Hz and 1500 Hz, respectively. Data were then filtered with a digital dual low-pass Butterworth

75   filter at 15 Hz for motion, and 60 Hz for force. Running speed during trials was measured and controlled with

76   timing gates (Brower Timing Systems, Draper, UT, USA) which were placed 3 m apart before and after the force

77   platform. Only trials within a ± 10% range of the target speed were accepted. Braking and propulsive impulses

78   from the force data were visually screened to ensure that subjects were not speeding up or slowing down during

79   the contact period with the force platform.

80   A retro- reflective marker set was attached to each subject's right lower extremity and pelvis according to a

81   previously published convention (55). A static calibration was collected of subjects standing with their feet

82   approximately shoulder width apart and knees fully extended. This static trial determined local coordinate

83   systems, the location of joint centres and the foot, shank, thigh and pelvis segment lengths of each subject. All

84   marker positional data was tracked using Qualisys Track Manager Software (Qualisys Inc., Gothenberg,

85   Sweden) and exported to Visual3D (C-motion, Germantown, MD, USA) for further processing and analysis.

86   Lower extremity 3D joint angles and angular velocities and accelerations were calculated using an X-Y-Z Euler

87   angle rotation sequence. Euler sequence represented flexion/extension, abduction/adduction and axial rotation.

88   All joint kinematics were decomposed about a joint coordinate system with the distal relative to the proximal

89   segments (30). Segments inertial properties were based on Dempster data (22) and represented as geometric

90   volumes (34).

91   *Muscle activation and co-activation*

92   Surface EMG of the Rectus Femoris, Vastus Medialis, Biceps Femoris and Semitendinosus was recorded at 1500

93   Hz using a wireless Noraxon system (TeleMyo DTS Telemetry system, Noraxon USA Inc., Scottsdale, AZ,

94   USA). Other authors have found these muscles to play essential roles during landing in running (25, 33, 69, 82).

95   The EMG system was synchronised with motion and force data. In accordance with SENIAM recommendations,

96   bipolar Ag/AgCl alloy dual surface electrodes (Noraxon Dual EMG electrode, USA) with a spacing of 2 cm,

97   were placed on the main bulk of the muscles, parallel to the muscle fibres (35). To reduce skin impedance, each

98   subject's skin was shaved, abraded with sandpaper and cleaned with an alcohol swab. Proper placement of the

99   electrodes was confirmed prior to testing by observing EMG signals as the subjects performed knee extension

100   (Rectus Femoris, Vastus Medialis) and knee flexion exercises (Biceps Femoris, Semitendinosus).

101   Raw EMG data were filtered using a digital high- and low-pass Butterworth filters at cut-off frequencies of 20

102   and 500 Hz respectively, and full-wave rectified. Signals were then smoothed with a moving root mean square

103   (RMS) window of 50ms, yielding the linear envelope for each muscle. Finally, EMG of the five trials for each

104   running speed were averaged. All EMG running trial data were normalized to each suject's peak EMG amplitude

105   value during a maximal sprint. This normalization procedure was adopted based on previous recommendations,

106   that showed EMG data normalization to a dynamic sprint was a more reliable and repeatable method compared

107   to the more commonly used static maximal voluntary contraction (1, 81).

108   To quantify knee extensor and flexor co-activation (Fig. 2), a co-activation ratio was derived based on the

109   relative simultaneous activation of quadriceps (Quads = Σ (Rectus Femoris + Vastus Medialis)) and hamstrings

110   (Hams = Σ (Biceps Femoris + Semitendinosus)), and was calculated as following:

111
$$Co-activation\ ratio = \frac{Quads + Hams}{2} \cdot \left(\begin{array}{l} \dfrac{Hams}{Quads}\ if\ Hams < Quads \\[2ex] \dfrac{Quads}{Hams}\ if\ Hams > Quads \end{array} \right)$$

112   The derived co-activation ratio was based on a combination of various co-activation methods used in previous

113   literature (9, 40, 66, 70, 80), and calculated for each time point between 60ms before landing to PKF. For muscle

114   co-activation ratios that only describe the simultaneous activation of multiple muscle groups, equal activation of

115   these muscle groups can be achieved at different magnitudes of activity. When both muscle groups are active for

116   20% of their maximum for instance, this will result in the same co-activation ratio as when both muscles are

117   maximally active. The advantage of including the first part of the equation in the co-activation ratio used in this

118   study compared to other co-activation calculations, is that it takes into account the magnitude of the combined

119   muscle activations, as well as the relative activation of quadriceps and hamstrings. The first part of the equation

120   accounts for the magnitude of total muscular activation. The second part of the equation represents the relative

121   activation of the two muscle groups, and was calculated by dividing the muscle group with the lowest

122   normalized activation by the muscle group with the highest activation. Hamstrings were taken as the divisor if its

123   value was greater than the quads (Fig. 2a, grey areas), and vice versa (Fig. 2a, white area). As such, the co-

124   activation ratio always had a value between 0 and 1, with 1 being both equal and maximal activation of

125   quadriceps and hamstrings. Co-activation ratio, as well as muscle activation of the individual muscles, was

126   calculated for each trial and then averaged over the five trials for each running speed.

127   A time window from 60ms before landing to peak knee flexion (PKF) was subdivided into three separate phases.

128   Since muscle pre-activation has been found to play an important role for landing (66, 68, 82), a pre-landing

129   phase was defined before initial contact (IC). As described in previous literature, the thigh muscles primarily

130   turn on around 30-80 ms before landing (54, 60). To include preparatory muscle activity before touch down, as

131   well as making the pre-landing phase comparable to the other phases during landing, the pre-landing phase was

132   defined from 60ms before landing to IC. The first half of stance (i.e. IC to PKF) was split up into an initial

133   impact and weight acceptance phase (Fig. 3) (9, 24, 45, 80). The initial impact phase, during which the first

134   impact force peak occurs (Fig. 3a), was defined from IC to the timing of PKAV. This phase (which typically

135   lasts 20-40 ms) is often deemed a 'passive' phase since there is little or no opportunity to actively control the

136   rotations of the body segments other than adjusting the initial conditions of landing and allowing the passive

137   structures to control the body motion (11). The weight acceptance phase, during which both the vertical ground

138   reaction force and the knee angular velocity show an obvious change in shape and slope (Fig. 3b), was defined

139   from the timing of PKAV to PKF at mid-stance. These two distinct phases were used to describe knee joint

140   stiffness in more detail than the typical measure of stiffness used in the literature (based on the relationship

141   between of the knee moment and angular displacement), which assumes the landing to be a single phase and

142   might therefore be an over simplistic approach (Fig. 3c). In addition to stiffness, mean values of thigh muscle

143   activation and co-activation were calculated for each phase to quantify (co-)activation and compare muscle

144   activity during the different phases of landing.

145 *Knee kinematic and spatiotemporal characteristics*

146 Kinematic and spatiotemporal knee variables were determined that have previously been associated with running

147 training (37, 58) and speed (32, 41, 43). Investigated variables included the time the knee started to flex before

148 IC, knee angle at IC and PKF, knee range of motion between IC and PKF, time to PKF, knee angular velocity at

149 IC and at PKAV, time from IC to PKAV, knee stiffness, stance time, and knee and ankle joint work. Knee

150 flexion was defined as positive from the anatomical position.

151 Knee joint stiffness was defined for the initial impact phase from IC to PKAV ($K_{knee}1$), and the weight

152 acceptance phase from PKAV to PKF ($K_{knee}2$). For both phases a knee stiffness was calculated according to a

153 method similar to what was used by Dutto and Braun (24) and Li, Heiderscheit, Caldwell and Hamill (48):

154
$$K_{knee}i = \frac{I \cdot \frac{\Delta(\omega_i^2)}{\Delta(\theta_i^2)}}{ROM_i} \qquad (\text{in } \frac{kg \cdot m^2 \cdot \frac{(rad^2 \cdot s^{-2})}{rad^2}}{deg} = \text{kg} \cdot \text{m}^2 \cdot \text{s}^{-2} \cdot \text{deg}^{-1} = \text{Nm} \cdot \text{deg}^{-1})$$

155 where I is the mass of the subject times the thigh length squared ($m \cdot l_{thigh}^2$), ω the knee angular velocity in rad·s^{-1},

156 θ the knee angle in radians, and ROM the knee range of motion in degrees, for the two phases of landing (i=1,2).

157 For ω and θ the knee angle was defined as the angle between thigh and shank, with knee flexion being negative.

158 (Note: working out the units for this formula gives kg·m^2·s^{-2}·deg^{-1}. Since kg·m^2·s^{-2} is dimensionally equivalent to

159 Nm according to the SI unit system, joint stiffness is expressed in Nm·deg^{-1}). Fig. 4 illustrates stiffness for the

160 initial impact and weight acceptance phase for a typical LM and HM subject trial. With unique stiffness

161 corresponding to the two different phases during the first half of ground contact (48), the approach used in this

162 study provides more detailed information regarding knee joint function during running compared to the typical

163 stiffness measure used in the literature (Fig. 3c). As such, an objective and sensitive individual assessment of

164 knee joint function can be obtained (49, 77) that permits changes associated with running training to be

165 identified.

166 *Knee and ankle joint work*

167 Knee and ankle net joint mechanical power in the sagittal plane were calculated using a Newton-Euler inverse

168 dynamics approach similar to previous studies (65, 71, 79). The joint powers were normalized to body mass

169 (W·kg^{-1}) and integrated over the duration of stance to calculate the joint work done (J·kg^{-1}). All the positive and

170 negative work was summed independently to determine the amount of total positive (W_a^+) and negative work

171 (W_a^-) at the knee and ankle joints. In a previously described detailed model (65), an estimate of elastic work at

172 the ankle joint was calculated over a stride. In summary, this calculation assumed that all of the energy absorbed

173   at the ankle during the stance phase occurred elastically and thus allowed us to examine the potential

174   contribution of elastic energy storage and release. The estimation of elastic work about the ankle joint involved

175   integrating the ankle power curve of the negative phase (energy absorption) followed by the positive phase

176   (energy generation). Note the integration was made only on these portions of the joint power curves. It was

177   assumed that all the negative work about the ankle was equal to the elastic energy storage. The integration of the

178   ankle joint power curves over the absorption and generation phases allowed an estimation of positive mass-

179   specific muscular work at the ankle joint that could not be provided by elastic work. Therefore the following

180   equation was used to estimate elastic work about the ankle joint (W_{el}^+) (65):

$$W_{el}^+ = 2 \cdot \left(\int_{t_{st1}}^{t_{st2}} P_a^+ \, dt - W_{mus,st}^+ \right)$$

182   where $W_{mus,st}^+$ is the positive mass specific muscle work during stance at the ankle, $P_a^+ dt$ is the integrated

183   positive power over the stance phase.

184   *Statistical analysis*

185   Two-way ANOVAs were performed to determine whether there was a significant main effect for either group or

186   speed, or an interaction (group·speed) for all knee angle and angular velocity parameters, knee stiffness, knee

187   and ankle joint work, and elastic work about the ankle. Muscle activation and co-activation ratio were analysed

188   using a three-way ANOVA (group·speed·phase). If there were significant main effects ($P < 0.05$) found, a

189   pairwise comparison with Bonferroni correction post-hoc analyses were performed. If there were significant

190   group interactions, a series of independent t-tests with Bonferroni correction were used for post-hoc multiple

191   comparisons (53). Also, partial Eta squared (η_p^2) values were calculated and Cohen's (21) rules of thumb were

192   applied to determine effect sizes (i.e. 0.01 = small, 0.06 = medium, 0.14 = large). Throughout all analyses, the

193   significance level was set at $\alpha = 0.05$ with a 95% confidence interval. All statistical analysis procedures were

194   performed using SPSS (v23, IBM, SPSS Inc., Chicago, IL, USA).

195

196   **Results**

197   *Group characteristics*

198   Group mean (± SD) characteristics described in Table 1 showed that the HM group had a significantly higher

199   maximal sprint speed ($P < 0.05$), faster 5 km personal best times ($P < 0.001$), and higher weekly running mileage

200   ($P < 0.001$) than the LM runners. However, there were no differences for age, weight, height, thigh length, or

201 running experience. Furthermore, no differences were observed in running speed between groups across all the

202 four speeds.

Muscle activation and co-activation

204 Three-way interactions between muscle (co-)activation, running speed and phase of landing showed LM runners

205 had significantly more Rectus Femoris, Vastus Medialis, and Semitendinosus ($P = 0.001$) activity than HM

206 runners (Fig. 5). In addition, activation of the Rectus Femoris, Vastus Medialis, Biceps Femoris and

207 Semitendinosus, and co-activation ratio all significantly increased with speed across groups, as well as for both

208 groups of runners separately (Fig. 6; $P < 0.001$), with large effect sizes ($\eta_p^2 > 0.14$). Moreover, muscular activity

209 of all muscles and co-activation significantly differed between the three phases of landing across both groups of

210 runners, as well as for the separate LM and HM groups ($P < 0.001$).

211 A significant interaction between running mileage and speed was found for Semitendinosus activity (Fig. 7a; $P <$

212 0.05). The between mileage group difference increased with increasing running speed, and at 5.5 m·s^{-1} the LM

213 runners had significantly ($P < 0.01$) higher Semitendinosus activity than HM runners. Similarly, Rectus Femoris

214 recruitment and co-activation strategies showed substantial differences between the LM and HM group at the

215 higher speeds (Fig. 7b-c). Rectus Femoris activation increased more in LM runners than HM runners as running

216 speed increased (Fig. 7b), with a significant difference between groups at 5.5 m·s^{-1} ($P < 0.05$). Moreover, where

217 thigh muscle co-activation was similar for both groups at lower speeds, LM runners dramatically increased their

218 co-activation by 44% between 4.5 and 5.5 m·s^{-1} (Fig. 5, 6 and 7c).

219 The interaction between mileage group and activation strategies during the different phases of landing was

220 similar for both quadriceps muscles (Fig. 5 and 8). Rectus Femoris (Fig. 8a; $P = 0.08$) and Vastus Medialis (Fig.

221 8b; $P < 0.05$) were both more active in LM than HM runners during the landing phases, but not before IC.

222 There was a significant interaction between running speed and landing phase for Vastus Medialis activity (Fig. 9;

223 $P = 0.002$, $\eta_p^2 = 0.07$), with a significant increase in activation between all four speeds during the pre-landing (P

224 < 0.001) and initial impact phase ($P < 0.05$). Especially during the initial impact phase of landing a dramatic

225 increase of 40% Vastus Medialis activity was found between 2.5 and 5.5 m·s^{-1}. Unlike initial impact, Vastus

226 Medialis activity only increased ($P < 0.001$) by 13% between 2.5 m·s^{-1} and 4.5 m·s^{-1} during the weight

227 acceptance phase.

228   *Knee kinematics and spatiotemporal characteristics*

229   A significant interaction between mileage group and running speed was found for stance time (Fig. 10c; $P <$

230   0.001), knee range of motion (Fig. 10d; $P = 0.007$) and knee stiffness $K_{knee}2$ (Fig. 10f; $P < 0.001$). Stance time

231   significantly decreased with increasing running speed ($P < 0.001$), and was shorter for the HM compared to LM

232   runners across all four speeds (Fig. 10c). Although there was no significant main effect of training mileage on

233   stance time (Table 2; $P = 0.056$), a large effect size was found ($\eta_p^2 = 0.14$). Moreover, stance time was

234   significantly shorter in HM compared to LM runners at 2.5 m·s^{-1} (249±25 vs 277±20 ms; $P = 0.005$) and 3.5 m·s^{-1}

235   ^{1} (214±22 vs 231±16 ms; $P < 0.05$).

236   Similarly, knee range of motion over the first half of stance was found to be significantly smaller at 2.5 m·s^{-1}

237   (24±4 vs 27±4 deg; $P = 0.02$) and 3.5 m·s^{-1} (25±3 vs 27±3 deg; $P < 0.05$) in the HM runners (Fig. 10d), despite

238   there being no significant main group effect. Nevertheless, a significant main effect of running speed was found

239   ($P < 0.001$) with a significantly decrease in knee range of motion between 4.5 and 5.5 m·s^{-1} (Table 2).

240   The HM group flexed their knee significantly earlier before IC (Fig. 10a and 11; $P = 0.03$, $=_p^2 = 0.19$), and

241   reached PKAV significantly sooner during stance (Fig. 10b and 11; $P = 0.005$, $=_p^2 = 0.28$) compared to the LM

242   group (Table 2).

243   During the initial impact phase, HM runners adopted a significantly higher knee stiffness than LM runners for all

244   speeds (Fig. 10e; $P < 0.05$, $\eta_p^2 = 0.15$). On average, $K_{knee}1$ was found to be 58% higher in better trained runners

245   (Table 2). In contrast, knee stiffness $K_{knee}2$ was significantly lower in HM runners for all four speeds ($P < 0.05$,

246    $<_p^2 = 0.37$). The difference between both groups significantly increased with running speed (Table 2; $P < 0.001$).

247   At 5.5 m·s^{-1} the less-trained runners had a weight acceptance stiffness that was 130% higher (153±60 vs 67±32

248   Nm·deg^{-1}), compared to HM runners.

249   In both groups of runners the time the knee started to flex before IC ($P = 0.03$), as well as the time from IC to

250   PKF ($P < 0.001$) became significantly shorter with increasing running speed (Table 2, Fig. 10a and 11).

251   Moreover, as runners ran faster they significantly increased knee flexion angle at IC, PKF angle, knee angular

252   velocity at IC, and PKAV (Table 2, Fig. 11; $P < 0.001$). However, the time from IC to PKAV and $K_{knee}1$ were

253   unaffected by running speed regardless training levels.

254   *Knee and ankle joint work*

255   There were no significant interactions between mileage group and running speed for knee and ankle joint work,

256   or elastic work. Also no significant differences between mileage groups were found for knee joint work and

257 positive ankle work during the stance phase (Table 2). However, negative ankle work (Fig. 10g; $P = 0.01$, $=_p^2 =$

258 0.24) and elastic work about the ankle joint (Fig. 10h; $P = 0.005$, $=_p^2 = 0.29$) were both significantly higher in

259 HM runners than in LM runners (Table 2). Also, there were significant increases with running speed ($P < 0.001$)

260 of positive and negative knee joint work, positive and negative ankle joint work (Fig. 10g), and elastic work

261 about the ankle joint (Fig. 10h) for both groups of runners.

262

263 **Discussion**

264 The primary purpose of this study was to investigate neuromuscular and movement adaptations that occur with

265 high running training mileage across a range of steady state speeds. It was anticipated that adjustments with

266 increasing running speed would more clearly discern the influence of running training. This novel investigation

267 found 1) no differences between the training mileage groups in pre-activation of muscles around the knee joint

268 prior to IC; 2) HM runners had an increased knee stiffness during the initial impact phase ($K_{knee}1$) across all

269 speeds, but displayed lower knee stiffness during the weight acceptance phase ($K_{knee}2$) with increasing mileage

270 group differences as running speed increased; 3) the HM group demonstrated lower amplitudes of muscle

271 activation post landing (initial impact and weight acceptance phases) compared to LM runners and these

272 relatively lower levels were amplified at faster running speeds; 4) after touch-down the HM runners had greater

273 ankle negative work and estimated elastic work about the ankle across all running speeds. Collectively, these

274 differences in the HM training group reflect changes in neuromuscular conditioning that were increasingly

275 evident at faster running speeds.

276 While the present results (Fig. 5 & 6) support previous findings of greater muscle activation magnitudes around

277 the thigh with increases in running speed (23, 29, 42, 44, 73), to the authors knowledge this is the first study that

278 has investigated neuromuscular adaptations between runners of different training mileage background across a

279 broad range of speeds. The first hypothesis that high training volume would be associated with lower muscle

280 activation levels of the quadriceps and hamstrings can be partly accepted because three of the four muscles

281 examined (Rectus Femoris, Vastus Medialis and Semitendinosus) displayed significantly reduced magnitudes in

282 the HM group for the fastest running speeds (4.5 and 5.5 m·s^{-1}). The reductions were mainly associated with the

283 post landing phases (particularly in the quadriceps muscles) with no significant differences in activation levels

284 found in the pre-landing (or pre-activation) phase. We found two previous studies (5, 27) which examined

285 neuromuscular activity of shank muscles (i.e. Gastrocnemius and Tibialis Anterior), between runners of different

286   training mileage background at a single running speed (3.3 and 4.0 $m \cdot s^{-1}$, respectively). Both studies reported

287   reduced activation levels post landing in the HM runners and, similar to our findings, found only small

288   differences in muscle pre-activation magnitudes prior to landing between mileage groups. However, the present

289   authors have not found any studies that investigated the influence of training mileage on thigh muscle activation

290   levels during running.

291   A key finding of this study was that for the transition from 4.5 $m \cdot s^{-1}$ to 5.5 $m \cdot s^{-1}$ (the fastest speed examined), the

292   Rectus Femoris and Semitendinosus, muscle activation levels of the LM grouped runners were substantially

293   higher than the HM runners when compared to the slower speeds (Fig. 5 & 7). In addition, we also observed a

294   large 44% increase in muscle co-activation in the LM group from 4.5 to 5.5 $m \cdot s^{-1}$ (Fig. 6e & 7c). It is plausible

295   that the greater muscle activation levels found in the LM group relative to the HM group are partly due to the

296   novelty of the task, as this group of runners might have been less familiar with running at high speeds. Since

297   muscular activity is gradually decreased and modulated with learning over time due to continued practice,

298   unfamiliarity of a task could lead to greater muscle activity and associated co-activations levels (61, 76).

299   Furthermore, for some tasks, large increases in muscle co-activation might be related to the need to generate high

300   joint impedance (the combined influence of stiffness, viscosity and inertia aspects) control (57). Previous studies,

301   that examined the control of lower limb joint stability during unfamiliar tasks, have suggested that the central

302   nervous system increases joint stiffness along with muscle co-activation in the direction of the instability (13,

303   27). The observed sudden increases in both muscle co-activation and knee stiffness during the weight acceptance

304   phase ($K_{knee}2$) in the LM group during running at 5.5 $m \cdot s^{-1}$, may be a protective mechanism to stabilise an

305   unstable knee joint, via impedance control (27) and may thus be metabolically more costly in muscle activation

306   terms (28). However, the LM runners in this study were very likely not in the situation where joint instability

307   was perceived and therefore there may not have been sufficient stimulus for such a protective response strategy

308   (even at the fastest running speed of 5.5 $m \cdot s^{-1}$). The muscle co-activation levels closely mimicked the speed-

309   related changes in joint stiffness across all running speeds, which is most clearly demonstrated by the sudden

310   increase of both co-activation levels and joint stiffness $K_{knee}2$ in the transition from 4.5 to 5.5 $m \cdot s^{-1}$ for the LM

311   group (Fig. 6, 7c & 10f). These very similar changes with running speed in knee joint stiffness during weight

312   acceptance and muscle co-activation about the knee provide some clear evidence to suggest that these two

313   factors are very closely associated (a link that is not that well established or accepted in the literature).

314   Although we did not directly measure elastic work of muscle-tendon units (MTUs) about the ankle and work in

315   the in-series muscles, another plausible explanation for the relatively decreased activation levels of the Rectus

316 Femoris,Vastus Medialis and Semitendinosus muscles during the weight acceptance phase for the HM group

317 could be due to a greater storage and release of elastic strain energy about the ankle. Biewener (10) in his

318 comparative observations across several animal species (including humans), proposed a working proximo-distal

319 gradient theory which suggests that the longer fibered, proximal segment muscles act as a modulator of limb

320 work, while the short-fibered distal limb muscles with longer tendons are designed for efficient force generation

321 and facilitate the recovery of elastic tendon energy. A typical observation in this study, that could be aligned to

322 this theory, was that the proximal leg segment (i.e. the thigh) orientation remains relatively unchanged during the

323 landing phases of stance, while the majority of the rapid knee joint motion is the result of changes in orientation

324 of the more distal segments of the lower limb (i.e. the shank) and angular motion about the ankle. The observed

325 rapid motion occurring at the ankle (as shown in Fig. 3d) during the impact phase of landing may be associated

326 with significant tendon stretch, particularly the Achilles tendon. Another finding in this study was that the

327 duration of the initial impact phase was not altered with running speed within a specific group of runners (Fig.

328 10b & 11). This might indicate a predominantly structural basis for the duration of that phase (e.g. stiffness of

329 the Achilles tendon) which should be explored in future work. Elastic recoil of the tendon could possibly modify

330 the time course of muscle activation in the proximal segments during the slightly later weight acceptance phase

331 in HM runners and perhaps reduce muscle activation levels during that phase (as indicated above). Based on

332 dynamic muscle-tendon studies on animals, Roberts (63) indicated that rapid joint flexion immediately after

333 landing during locomotion is associated with little change in muscle fibre length but is facilitated rather by

334 tendon stretch. Other studies that have examined limb muscle-tendon behaviour with *in vivo* measurements of

335 medial Gastrocnemius and Soleus muscles during the first half of stance for human running have shown that

336 tendon stretch accounts for the majority of the MTU length change (46, 50). It has been shown that despite

337 increases in length of the Soleus MTU with running speed, the length of the Soleus muscle fascicles changed

338 very little (46).

339   The present results showed that the HM group had substantially higher ankle negative work (Fig. 10g) along

340 with greater elastic work about the ankle across all running speeds. These findings are similar to previous studies

341 that have examined joint work with increasing running speeds (71, 72). There were no differences in knee joint

342 work and ankle positive work, but we observed greater negative ankle work and elastic work about the ankle in

343 the HM runners. In the early work of Cavagna (14), he proposed that with increasing running speed the work

344 done by the contractile component decreases progressively due to i) a relatively larger length change taken up by

345 the tendon during the stretch and ii) a decrease in force with the velocity of shortening. Interestingly, other

346   modelling studies found with increasing running speed a greater contribution of positive work was provided by

347   the tendon rather than the contractile machinery of the Soleus and Gastrocnemius (47, 78). Our results indeed

348   clearly demonstrate that the contribution of elastic work about the ankle is greater in response to HM endurance

349   training. As pointed out above, this enhancement in utilisation of elastic recoil energy about the ankle may be

350   related to lower muscle activation levels of the HM runners, since the demand to use muscle contractile

351   characteristics for positive work during stance was likely reduced. Despite the fact that increased muscle work

352   has been found to be correlated to greater EMG activation (muscle shortening) during increased incline running

353   (64), the proxy of using muscle activation for muscle work is equivocal when considering isometric contractile

354   muscle behaviour. Given that isometric muscle contraction performs no work (36) and due to the inability to

355   measure proximal muscle forces directly (10), future research is warranted to explore and understand the *in vivo*

356   relationship between joint work and the in-series muscles of the distal segments, and the contribution of muscle

357   activations and work about the thigh segment.

358   The present study found knee angular displacements and velocities at touch-down, and peak values during stance

359   that resembled those in the literature (56). The knee was flexing at the instant of landing for both groups but it

360   began earlier prior to landing in the HM group across all speeds. A similar, early preparatory knee flexion prior

361   to landing was also reported by Horita, Komi, Nicol and Kyröläinen (38) who showed that good drop jump

362   performances had this movement pattern but not the low performance jumps. Moreover, these authors explained

363   that this early knee preparatory flexion prior to ground contact was associated with high initial knee stiffness

364   after landing. In accordance, the present results also reported increased initial knee stiffness ($K_{knee}1$) in the HM

365   runners. Furthermore, this increase in initial knee stiffness along with knee flexion timing before IC was not

366   influenced by speed. Although the knee angle at IC was similar between mileage groups, with the HM runners

367   flexing the knee earlier prior to IC, joint dynamics (flexion angular velocity and flexion angular acceleration) at

368   IC tended to be increased with the HM group during running at the two fastest running speeds. The curves

369   illustrated in Fig. 11 clearly show that the HM group displayed rapid changes in knee angular acceleration (or

370   increased magnitude of angular jerk) just after IC which is similar across all running speeds. Knee stiffness

371   during the impact phase ($K_{knee}1$) was also minimally influenced by running speed and it is plausible that this

372   impact phase transient knee motion in the HM group is associated with elevated knee (and perhaps Achilles

373   tendon) stiffness. It remains to be determined whether these increases in IC joint dynamics and knee angular jerk

374   just after landing facilitate increased storage of strain energy in the Achilles tendon. To the authors' knowledge,

375   the knee movement adaptations as a result of HM training found in this study have not been reported elsewhere.

376 This may be due to the relatively high quality of the kinematic data collected for this investigation. With the high

377 sampling rate (500 Hz) of the segment trajectories, more accurate joint angular velocities and accelerations

378 during the early stance phase of running were obtained, together with greater temporal resolution for the timings

379 of key variables such as knee flexion onset prior to landing and peak knee flexion velocity just after landing.

380 Also, segment tracking markers were placed in the middle of the shank and thigh segments rather than at the

381 knee joint where errors due to soft tissue movement relative to the underlying bone can be excessive (8).

382 Of the two hamstring muscles investigated, the Semitendinosus appeared to be more sensitive than Biceps

383 Femoris to the influence of running training. There was a tendency for Semitendinosus activation levels to be

384 higher during the pre-activation and weight acceptance phases in the LM group whereas few group differences

385 can be observed with the Biceps Femoris (see Fig. 5 and 6). These Semitendinosus-Biceps Femoris differences

386 between training groups became larger with increasing running speed, but it is unclear why the Biceps Femoris

387 activation levels seem to be unaffected by training status. With increasing running speed you would expect the

388 rate of stretch of the hamstrings to be increased, and Schache, Dorn, Wrigley, Brown and Pandy (73) predicted

389 using a musculoskeletal model that the eccentric activation and rate of stretch of the Semitendinosus during the

390 late swing phase of running (just prior to ground contact) was greater than for the Biceps Femoris lateral

391 hamstring. Other recent work (39), has also provided evidence that the Semitendinosus has increased loading of

392 its muscle fibres and tendinous tissue compared to the Biceps Femoris during rapid contractions with a flexed

393 knee. However, these indications of likely differing loading profiles between the two hamstrings muscles,

394 investigated in this study, remain to be explored in the context of training effects.

395 A limitation of this study is the cross sectional design. The authors acknowledge that the neuromuscular

396 adaptations observed in the HM group may not be solely in response to the weekly running mileage but may

397 include other factors related to their training background (such as training speeds, resistance training, flexibility

398 and stretching). For example, with previous studies reporting the benefits of resistance and/or plyometric training

399 to the elastic work of the lower limbs during running (2, 62, 67, 74), it is possible that the HM runners previously

400 or currently performed other forms of training that contributed to the neuromuscular adaptations observed in the

401 present study. Given this limitation and lack of knowledge in the area of neuromuscular adaptations to running

402 training volume, a possible future direction would be to conduct a longitudinal training intervention study that

403 examined responses following a controlled, well documented programme of running training. Another limitation

404 of this study is that we did not directly measure *in vivo* the dynamic function of the lower limb muscle-tendons,

405 but used an estimation of elastic work based on inverse dynamics of ankle joint work. Although the methods

406 used in this study provide an overall approximation of the contribution of the elastic work during running,

407 possible future avenues would be to use advanced imaging techniques such as shear wave imaging that are being

408 introduced to quantify how the lower extremity tendons behave dynamically during locomotion (52).

409

410 **Conclusion**

411 The changes in knee joint motion and neuromuscular behaviour with both running speed and training status

412 revealed some clear and fundamental associations between knee joint stiffness and muscle co-activation levels

413 about that joint. We found that high mileage training was associated with changes in neuromuscular conditioning

414 which was mainly characterised by lower activation of muscles surrounding the knee joint during the weight

415 acceptance phase, higher initial knee stiffness and greater estimates of elastic energy return about the ankle.

416 Overall, these neuromuscular adaptations were increasingly evident at the faster running speeds.

417

418 **Acknowledgement**

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590

591

Figure captions

Figure 1. Weekly running mileage of all runners was used to assign them to either a low mileage (<15 km/wk,

n=13) or a high mileage (>45 km/wk, n=13) group. Each bar represents each individual subject's weekly running

mileage.

Figure 2. (A) Activation patterns of quadriceps (Rectus Femoris and Vastus Medialis) and hamstrings (Biceps

Femoris and Semitendinosus) from 60 ms before landing to peak knee flexion, for a typical trial of a

representative subject. Where hamstring activation decreases in preparation for landing, quadriceps become

more active and have their maximal activation during stance. **(B)** Co-activation of quadriceps and hamstrings

was defined by the magnitude of muscle activation, as well as the relative simultaneous activation. The co-

activation ratio was highest when all muscles had simultaneous high activation levels.

Figure 3. (A-B) A typical vertical ground reaction force (GRF) pattern (A) and knee angular velocity trace from

60 ms before initial contact (IC) to toe-off for an exemplar subject running at 5.5 m·s^{-1} (B). The relationship

between the vertical GRF and knee angular velocity is characterised by the clear change in angular velocity after

the impact force peak. The initial impact phase (which typically lasts 20-40 ms) was defined from IC to peak

knee angular velocity (PKAV). The weight acceptance phase was defined from PKAV to peak knee flexion

(PKF). **(C)** The slope of the straight line over the first half of stance in the knee moment-angle curve is typically

used as a constant joint stiffness measure. Note that the distinct characteristics of landing are not identified by

this typically adopted knee stiffness calculation method, which assumes the landing to be a single phase and

neglects the passive and active phases characterised by the changes in knee angular velocity. **(D)** A schematic

stick figure illustrates the changes in knee and ankle angle between IC and PKF . Note that the orientation of the

thigh segment (angle relative to the vertical) does not change during landing, and changes in knee angle are

predominately related to the rotation of the shank. The time between two subsequent sticks is 4 ms.

Figure 4. Knee joint stiffness calculation for a typical trial of a representative LM (black dashed line) and HM

(grey solid line) runner. Stiffness was defined as the slope of the squared knee angular position (θ) – velocity (ω)

620   curve. The slopes of the lines from initial contact (IC) to peak knee angular velocity (PKAV) and from PKAV to

621   peak knee flexion (PKF) represent the stiffness of the knee for the initial impact phase ($K_{knee}1$) and the weight

622   acceptance phase ($K_{knee}2$) respectively.

623

624   **Figure 5.** Muscle activation levels and muscle co-activation for the LM (black dashed line) and HM (grey solid

625   line) groups with standard deviations at four running speeds, from 60 ms before initial contact (IC; vertical

626   dashed line) to peak knee flexion. Activation levels for each muscle were normalized to the peak EMG

627   amplitude of a maximal sprint. Note that differences between groups mainly appeared after IC and increased

628   with speed, in particular for Rectus Femoris, Vastus Medialis, Semitendinosus and muscle co-activation from 4.5

629      $m \cdot s^{-1}$ to 5.5 $m \cdot s^{-1}$.

630

631   **Figure 6.** Mean (±SD) normalized muscle activation for the Rectus Femoris (A), Vastus Medialis (B), Biceps

632   Femoris (C), Semitendiosus (D) and co-activation ratio (E) for the LM (black) and HM (grey) group, during the

633   pre landing, initial impact and weight acceptance phases of landing across running speeds. Especially during the

634   initial impact and weight acceptance phases the muscle activation levels showed more increase with speed in LM

635   runners compared to the HM group. Note the sharp increase of the co-activation ratio in the LM group during in

636   the initial impact phase from 4.5 $m \cdot s^{-1}$ to 5.5 $m \cdot s^{-1}$.

637

638   **Figure 7.** Mean normalized muscle activations of the Semitendinosus (A) and Rectus Femoris (B), and co-

639   activation ratio (C), at four speeds for LM and HM runners. Semitendinosus and Rectus Femoris muscle

640   activations progressively increased with running speed in both groups, however, the LM grouped runners

641   displayed a progressively steeper increase in muscle activation with running speed as compared to the HM

642   group. A significantly greater magnitude of muscle activation in Semitendinosus and Rectus Femoris was found

643   between the groups at 5.5 $m \cdot s^{-1}$. Similarly, the co-activation ratio showed a substantial (but not significant)

644   increase of 44% in the LM group from 4.5 $m \cdot s^{-1}$ to 5.5 $m \cdot s^{-1}$. *Significant difference between groups, $P < 0.05$.

645

646   **Figure 8.** Mean muscle activations for the Rectus Femoris (A) and Vastus Medialis (B) between the LM and

647   HM group for the three phases of landing. Activation levels were averaged across speeds for each separate phase

648 per group of runners. Quadriceps muscles did not show any differences between groups in activation levels prior

649 to landing, but LM runners had greater quadriceps activation during the initial impact and weight acceptance

650 phase.

651

652 **Figure 9.** Mean Vastus Medialis activation during the three phases of landing for each running speed. Vastus

653 Medialis activation was averaged across LM and HM runners. Particularly during the initial impact phase Vastus

654 Medialis activation showed large increases with running speed. *Significant difference between all running

655 speeds, $P < 0.001$. †Significant difference between 2.5 $m \cdot s^{-1}$, 3.5 and 4.5 $m \cdot s^{-1}$, $P < 0.05$.

656

657 **Figure 10.** Knee kinematic and kinetic characteristics for the low mileage (black) and high mileage (grey)

658 groups for four running speeds. The time from the start of knee flexion (KF) to initial contact (IC) (A), the time

659 from IC to peak knee angular velocity (PKAV) (B), stance time (C), knee range of motion (D), initial impact

660 knee stiffness $K_{knee}1$ (E), weight acceptance knee stiffness $K_{knee}2$ (F), ankle negative work (G) and elastic work

661 (H), was averaged for each group of runners per running speed. *Significant difference between groups,

662 †significantly different from 2.5 $m \cdot s^{-1}$, ‡ significantly different from 3.5 $m \cdot s^{-1}$, §significantly different from 4.5

663 $m \cdot s^{-1}$, $P < 0.05$.

664

665 **Figure 11.** Knee angle, angular velocity and angular acceleration curves for the LM (black dashed line) and HM

666 (grey solid line) across the four running speeds, from 60 ms before initial contact (IC; vertical dashed line) to

667 peak knee flexion (PKF). Knee flexion before IC and peak knee angular velocity after IC occurred earlier in the

668 HM runners compared to the LM group. Note that despite the reduced time to PKF with increasing running

669 speed (as expected) the time to PKAV remained constant across speeds. HM runners showed slightly higher

670 angular velocities at IC, and rapid changes in angular acceleration after IC (increased magnitude of angular jerk)

671 across all running speeds.