

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 ($2.5 \text{ m}\cdot\text{s}^{-1}$, $3.5 \text{ m}\cdot\text{s}^{-1}$, $4.5 \text{ m}\cdot\text{s}^{-1}$ and $5.5 \text{ m}\cdot\text{s}^{-1}$) 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 $\pm 10\%$ 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 subject'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:

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

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_{knee1}), and the weight
152 acceptance phase from PKAV to PKF (K_{knee2}). 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
$$K_{knee\ i} = \frac{I \cdot \frac{\Delta(\omega_i^2)}{\Delta(\theta_i^2)}}{ROM_i} \quad \left(\text{in } \frac{kg \cdot m^2 \cdot (rad^2 \cdot s^{-2})}{deg} = kg \cdot m^2 \cdot s^{-2} \cdot deg^{-1} = Nm \cdot deg^{-1} \right)$$

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 $rad \cdot s^{-1}$,
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 $kg \cdot m^2 \cdot s^{-2} \cdot deg^{-1}$. Since $kg \cdot m^2 \cdot s^{-2}$ is dimensionally equivalent to
159 Nm according to the SI unit system, joint stiffness is expressed in $Nm \cdot deg^{-1}$). 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 ($W \cdot kg^{-1}$) and integrated over the duration of stance to calculate the joint work done ($J \cdot kg^{-1}$). 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):

$$181 \quad W_{el}^+ = 2 \cdot \left(\int_{t_{st1}}^{t_{st2}} P_a^+ dt - W_{mus,st}^+ \right)$$

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 (\pm 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.

203 *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 $5.5 \text{ m}\cdot\text{s}^{-1}$ 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 $5.5 \text{ m}\cdot\text{s}^{-1}$ ($P < 0.05$). 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 $5.5 \text{ m}\cdot\text{s}^{-1}$ (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 $5.5 \text{ m}\cdot\text{s}^{-1}$. Unlike initial impact, Vastus
226 Medialis activity only increased ($P < 0.001$) by 13% between $2.5 \text{ m}\cdot\text{s}^{-1}$ and $4.5 \text{ m}\cdot\text{s}^{-1}$ 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 $K_{\text{knee}2}$ (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 $2.5 \text{ m}\cdot\text{s}^{-1}$ (249 ± 25 vs 277 ± 20 ms; $P = 0.005$) and $3.5 \text{ m}\cdot\text{s}^{-1}$
235 (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 $2.5 \text{ m}\cdot\text{s}^{-1}$
237 (24 ± 4 vs 27 ± 4 deg; $P = 0.02$) and $3.5 \text{ m}\cdot\text{s}^{-1}$ (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 $5.5 \text{ m}\cdot\text{s}^{-1}$ (Table 2).

240 The HM group flexed their knee significantly earlier before IC (Fig. 10a and 11; $P = 0.03$, $\eta_p^2 = 0.19$), and
241 reached PKAV significantly sooner during stance (Fig. 10b and 11; $P = 0.005$, $\eta_p^2 = 0.28$) 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, $K_{\text{knee}1}$ was found to be 58% higher in better trained runners
245 (Table 2). In contrast, knee stiffness $K_{\text{knee}2}$ was significantly lower in HM runners for all four speeds ($P < 0.05$,
246 $\eta_p^2 = 0.37$). The difference between both groups significantly increased with running speed (Table 2; $P < 0.001$).
247 At $5.5 \text{ m}\cdot\text{s}^{-1}$ the less-trained runners had a weight acceptance stiffness that was 130% higher (153 ± 60 vs 67 ± 32
248 $\text{Nm}\cdot\text{deg}^{-1}$), 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 $K_{\text{knee}1}$ 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; $P = 0.01$, $\eta_p^2 =$
258 0.24) and elastic work about the ankle joint (Fig. 10h; $P = 0.005$, $\eta_p^2 = 0.29$) 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_{knee1}) across all
269 speeds, but displayed lower knee stiffness during the weight acceptance phase (K_{knee2}) 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 $m \cdot s^{-1}$). 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·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·s⁻¹ to 5.5 m·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·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_{knee2}) in the LM group during running at 5.5 m·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·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_{knee2} in the transition from 4.5 to 5.5 m·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_{knee1}) 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_{knee1}) 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

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- 590
- 591

592 **Figure captions**

593

594 **Figure 1.** Weekly running mileage of all runners was used to assign them to either a low mileage (<15 km/wk,
595 n=13) or a high mileage (>45 km/wk, n=13) group. Each bar represents each individual subject's weekly running
596 mileage.

597

598 **Figure 2. (A)** Activation patterns of quadriceps (Rectus Femoris and Vastus Medialis) and hamstrings (Biceps
599 Femoris and Semitendinosus) from 60 ms before landing to peak knee flexion, for a typical trial of a
600 representative subject. Where hamstring activation decreases in preparation for landing, quadriceps become
601 more active and have their maximal activation during stance. **(B)** Co-activation of quadriceps and hamstrings
602 was defined by the magnitude of muscle activation, as well as the relative simultaneous activation. The co-
603 activation ratio was highest when all muscles had simultaneous high activation levels.

604

605 **Figure 3. (A-B)** A typical vertical ground reaction force (GRF) pattern (A) and knee angular velocity trace from
606 60 ms before initial contact (IC) to toe-off for an exemplar subject running at 5.5 m·s⁻¹ (B). The relationship
607 between the vertical GRF and knee angular velocity is characterised by the clear change in angular velocity after
608 the impact force peak. The initial impact phase (which typically lasts 20-40 ms) was defined from IC to peak
609 knee angular velocity (PKAV). The weight acceptance phase was defined from PKAV to peak knee flexion
610 (PKF). **(C)** The slope of the straight line over the first half of stance in the knee moment-angle curve is typically
611 used as a constant joint stiffness measure. Note that the distinct characteristics of landing are not identified by
612 this typically adopted knee stiffness calculation method, which assumes the landing to be a single phase and
613 neglects the passive and active phases characterised by the changes in knee angular velocity. **(D)** A schematic
614 stick figure illustrates the changes in knee and ankle angle between IC and PKF . Note that the orientation of the
615 thigh segment (angle relative to the vertical) does not change during landing, and changes in knee angle are
616 predominately related to the rotation of the shank. The time between two subsequent sticks is 4 ms.

617

618 **Figure 4.** Knee joint stiffness calculation for a typical trial of a representative LM (black dashed line) and HM
619 (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_{knee1}) and the weight
622 acceptance phase (K_{knee2}) 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 (\pm 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·s⁻¹, 3.5 and 4.5 m·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_{knee1} (E), weight acceptance knee stiffness K_{knee2} (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·s⁻¹, ‡ significantly different from 3.5 m·s⁻¹, §significantly different from 4.5
663 m·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.