**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, mainlycharacterised 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**.**

# Introduction

Although it is established that neuromuscular factors, such as stretch-shortening behaviour of lower limb muscles and tendons, play a vital role in the effectiveness of absorption and generation of force during running (14–16, 47, 63), very few studies have examined the influence of running training on neuromuscular function during running, particularly across a range of running speeds (6, 12). Previous studies that have examined neuromuscular adaptations to higher endurance training volumes in cycling have found evidence to suggest that the movements become more skilled, and this adaptation is characterised by reductions in muscle activation amplitudes and durations (17, 19, 20). Comparisons between high and low mileage runners (assessed for a single running speed) have shown that running training was associated with slight decreases in pre-landing muscle activation levels and reduced activation levels of extensor muscles during the first half of stance (7, 18). However, most investigations of training effects on neuromuscular function have not involved distance running and only consider short-term training effects (19). It is likely that the influence of training on neuromuscular function during running may be more evident when comparing distance runners with a large difference in training mileage level, and assessing adaptations across a range of running speeds.

A general concept of neuromuscular efficiency has been proposed, where running training is likely to influence the amplitude of lower limb muscle activation prior to and during the initial phase of ground contact which augment lower extremity stiffness and the use of stored elastic energy (6, 38).This concept has developed from the seminal work of Cavagna, Saibene and Margaria (16), who highlighted the important contribution of elastic recoil energy from stretched tendons and muscles during running. They also highlighted that this mechanism becomes more useful with increases in running speed (15, 16). In addition, estimates of an increased lower limb joint work during stance and the storage and release of elastic energy from the lower limb tendons have been linked to increased physiological efficiency (reduced rate of oxygen consumption or improved economy at a given speed) during running (65, 78). This association is further supported by improvements in running economy found after a period of running and strength training in which stiffness (and energy recoil ability) of the Achilles tendon was increased (2, 26), something that has also been confirmed in inveterate trained endurance runners (4).

Increased pre-landing muscle activation and joint stiffness regulation with training have been found during drop landing and hopping activities (37, 38). These findings lead to speculation that a similar response is likely to occur over time with prolonged running training, but the influence of training on pre-landing muscle activation levels remains to be established. It has also been proposed that simultaneous activation of several muscles acting about a joint provides joint stability during ground contact and plays an important role in stiffness regulation (9, 38), although such a direct association between joint stiffness and muscle co-activation levels about that joint has not been widely verified yet.

The rates of knee flexion during ground contact have been shown to be directly associated with running speed (3, 51, 56). In addition, lower limb joint stiffness, in particular stiffness of the knee, has been found to increase when running faster in order to reduce joint deformations during landing (3, 31, 41). Moreover, lower limb muscle activation and co-activation levels have both been reported to increase with running speed before initial ground contact (44, 75), as well as during landing (42, 59, 73). Despite this research attention, the associations between lower limb muscle pre-landing and post-landing activations, co-activations and joint stiffness setting across a range of running speeds have yet to be explored from a running training perspective.

Given the importance of the knee joint in running, it was hoped that by examining knee joint stiffness and muscle activation levels (those muscles that act about the knee joint) across a range of running speeds, the adaptations of neuromuscular factors due to running training could be closely explored for the first time. The modulation of thigh muscle pre-activation, co-activation and knee joint stiffness with running speed would also provide fundamental information on neuromuscular control mechanisms during running. Therefore, this study investigated muscle (co-)activation before and during landing, as well as knee stiffness and estimated elastic work during landing between two groups of runners with substantially different levels of running training, over a range of running speeds. It was hypothesised that runners with a high training volume would 1) show greater pre-activation of knee joint muscles before touch-down; 2) have associated greater knee stiffness during early stance; 3) display lower muscle (co-)activation post-landing; 4) demonstrate larger amounts of elastic work about the ankle and 5) that neuromuscular differences between the high and low mileage running groups would be greater at higher running speeds.

# Methods

## Subjects and protocol

Thirty-seven runners (29 males and 8 females) were recruited to participate in this study. Subjects were healthy at the time of testing and had no previous history of major injuries of the lower limbs within the last six months. All subjects had been running for at least six months. Of the 37 runners, 26 were assigned to either a low mileage (LM; <15 km/wk, n=13), or a high mileage (HM; >45 km/wk, n=13) group, with clear disparity between the two groups (Fig. 1). The cut-off used for the high mileage group was the same as employed in a previous investigation (7). In accordance with the Liverpool John Moores University ethics regulations, all subjects completed an informed consent form prior to data collection.

Prior to debrief of the experimental protocol, each subject did a warm-up of approximately five minutes of overground running at a self-selected easy pace. Subjects then performed five overground running trials at each 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 indoor runway with a large force platform mounted midway. They started at a self-selected distance from the force platform which was sufficient (15-20 metres) to get up to the required speed. Runners were asked to maintain the required speed from 5 m before to 5 m after the force platform. Running speeds were performed in a different mixed order for every subject, based on a Latin Square design. During the trials, kinematic, kinetic and EMG data of the right leg were recorded. All subjects wore their regular running shoes, to avoid adaptations to unfamiliar footwear.

Three-dimensional (3D) kinematic and kinetic data were synchronously collected with an eight camera motion analysis system (Qqus 300+, Qualisys Inc., Gothenburg, Sweden), in combination with a ground embedded force platform (90 x 60 cm, 9287B, Kistler Holding AG, Winterthur, Switzerland). Motion and force data were sampled at 500 Hz and 1500 Hz, respectively. Data were then filtered with a digital dual low-pass Butterworth filter at 15 Hz for motion, and 60 Hz for force. Running speed during trials was measured and controlled with timing gates (Brower Timing Systems, Draper, UT, USA) which were placed 3 m apart before and after the force platform. Only trials within a ± 10% range of the target speed were accepted. Braking and propulsive impulses from the force data were visually screened to ensure that subjects were not speeding up or slowing down during the contact period with the force platform.

A retro- reflective marker set was attached to each subject’s right lower extremity and pelvis according to a previously published convention (55). A static calibration was collected of subjects standing with their feet approximately shoulder width apart and knees fully extended. This static trial determined local coordinate systems, the location of joint centres and the foot, shank, thigh and pelvis segment lengths of each subject. All marker positional data was tracked using Qualisys Track Manager Software (Qualisys Inc., Gothenberg, Sweden) and exported to Visual3D (C-motion, Germantown, MD, USA) for further processing and analysis. Lower extremity 3D joint angles and angular velocities and accelerations were calculated using an X-Y-Z Euler angle rotation sequence. Euler sequence represented flexion/extension, abduction/adduction and axial rotation. All joint kinematics were decomposed about a joint coordinate system with the distal relative to the proximal segments (30). Segments inertial properties were based on Dempster data (22) and represented as geometric volumes (34).

## Muscle activation and co-activation

Surface EMG of the Rectus Femoris, Vastus Medialis, Biceps Femoris and Semitendinosus was recorded at 1500 Hz using a wireless Noraxon system (TeleMyo DTS Telemetry system, Noraxon USA Inc., Scottsdale, AZ, USA). Other authors have found these muscles to play essential roles during landing in running (25, 33, 69, 82). The EMG system was synchronised with motion and force data. In accordance with SENIAM recommendations, bipolar Ag/AgCl alloy dual surface electrodes (Noraxon Dual EMG electrode, USA) with a spacing of 2 cm, were placed on the main bulk of the muscles, parallel to the muscle fibres (35). To reduce skin impedance, each subject’s skin was shaved, abraded with sandpaper and cleaned with an alcohol swab. Proper placement of the electrodes was confirmed prior to testing by observing EMG signals as the subjects performed knee extension (Rectus Femoris, Vastus Medialis) and knee flexion exercises (Biceps Femoris, Semitendinosus).

Raw EMG data were filtered using a digital high- and low-pass Butterworth filters at cut-off frequencies of 20 and 500 Hz respectively, and full-wave rectified. Signals were then smoothed with a moving root mean square (RMS) window of 50ms, yielding the linear envelope for each muscle. Finally, EMG of the five trials for each running speed were averaged. All EMG running trial data were normalized to each suject’s peak EMG amplitude value during a maximal sprint. This normalization procedure was adopted based on previous recommendations, that showed EMG data normalization to a dynamic sprint was a more reliable and repeatable method compared to the more commonly used static maximal voluntary contraction (1, 81).

To quantify knee extensor and flexor co-activation (Fig. 2), a co-activation ratio was derived based on the relative simultaneous activation of quadriceps (Quads = Σ (Rectus Femoris + Vastus Medialis)) and hamstrings (Hams = Σ (Biceps Femoris + Semitendinosus)), and was calculated as following:

The derived co-activation ratio was based on a combination of various co-activation methods used in previous literature (9, 40, 66, 70, 80), and calculated for each time point between 60ms before landing to PKF. For muscle co-activation ratios that only describe the simultaneous activation of multiple muscle groups, equal activation of these muscle groups can be achieved at different magnitudes of activity. When both muscle groups are active for 20% of their maximum for instance, this will result in the same co-activation ratio as when both muscles are maximally active. The advantage of including the first part of the equation in the co-activation ratio used in this study compared to other co-activation calculations, is that it takes into account the magnitude of the combined muscle activations, as well as the relative activation of quadriceps and hamstrings. The first part of the equation accounts for the magnitude of total muscular activation. The second part of the equation represents the relative activation of the two muscle groups, and was calculated by dividing the muscle group with the lowest normalized activation by the muscle group with the highest activation. Hamstrings were taken as the divisor if its value was greater than the quads (Fig. 2a, grey areas), and vice versa (Fig. 2a, white area). As such, the co-activation ratio always had a value between 0 and 1, with 1 being both equal and maximal activation of quadriceps and hamstrings. Co-activation ratio, as well as muscle activation of the individual muscles, was calculated for each trial and then averaged over the five trials for each running speed.

A time window from 60ms before landing to peak knee flexion (PKF) was subdivided into three separate phases. Since muscle pre-activation has been found to play an important role for landing (66, 68, 82), a pre-landing phase was defined before initial contact (IC). As described in previous literature, the thigh muscles primarily turn on around 30-80 ms before landing (54, 60). To include preparatory muscle activity before touch down, as well as making the pre-landing phase comparable to the other phases during landing, the pre-landing phase was defined from 60ms before landing to IC. The first half of stance (i.e. IC to PKF) was split up into an initial impact and weight acceptance phase (Fig. 3) (9, 24, 45, 80). The initial impact phase, during which the first impact force peak occurs (Fig. 3a), was defined from IC to the timing of PKAV. This phase (which typically lasts 20-40 ms) is often deemed a ‘passive’ phase since there is little or no opportunity to actively control the rotations of the body segments other than adjusting the initial conditions of landing and allowing the passive structures to control the body motion (11). The weight acceptance phase, during which both the vertical ground reaction force and the knee angular velocity show an obvious change in shape and slope (Fig. 3b), was defined from the timing of PKAV to PKF at mid-stance. These two distinct phases were used to describe knee joint stiffness in more detail than the typical measure of stiffness used in the literature (based on the relationship between of the knee moment and angular displacement), which assumes the landing to be a single phase and might therefore be an over simplistic approach (Fig. 3c). In addition to stiffness, mean values of thigh muscle activation and co-activation were calculated for each phase to quantify (co-)activation and compare muscle activity during the different phases of landing.

## Knee kinematic and spatiotemporal characteristics

Kinematic and spatiotemporal knee variables were determined that have previously been associated with running training (37, 58) and speed (32, 41, 43). Investigated variables included the time the knee started to flex before IC, knee angle at IC and PKF, knee range of motion between IC and PKF, time to PKF, knee angular velocity at IC and at PKAV, time from IC to PKAV, knee stiffness, stance time, and knee and ankle joint work. Knee flexion was defined as positive from the anatomical position.

Knee joint stiffness was defined for the initial impact phase from IC to PKAV (Kknee1), and the weight acceptance phase from PKAV to PKF (Kknee2). For both phases a knee stiffness was calculated according to a method similar to what was used by Dutto and Braun (24) and Li, Heiderscheit, Caldwell and Hamill (48):

 (in = kg∙m2∙s-2∙deg-1 = Nm∙deg-1)

where *I* is the mass of the subject times the thigh length squared (m∙lthigh2), *ω* the knee angular velocity in rad∙s-1, *θ* the knee angle in radians, and ROM the knee range of motion in degrees, for the two phases of landing (i=1,2). For *ω* and *θ* the knee angle was defined as the angle between thigh and shank, with knee flexion being negative. (Note: working out the units for this formula gives kg∙m2∙s-2∙deg-1. Since kg∙m2∙s-2 is dimensionally equivalent to Nm according to the SI unit system, joint stiffness is expressed in Nm∙deg-1). Fig. 4 illustrates stiffness for the initial impact and weight acceptance phase for a typical LM and HM subject trial. With unique stiffness corresponding to the two different phases during the first half of ground contact (48), the approach used in this study provides more detailed information regarding knee joint function during running compared to the typical stiffness measure used in the literature (Fig. 3c). As such, an objective and sensitive individual assessment of knee joint function can be obtained (49, 77) that permits changes associated with running training to be identified.

## Knee and ankle joint work

Knee and ankle net joint mechanical power in the sagittal plane were calculated using a Newton-Euler inverse dynamics approach similar to previous studies (65, 71, 79). The joint powers were normalized to body mass (W∙kg-1) and integrated over the duration of stance to calculate the joint work done (J∙kg-1). All the positive and negative work was summed independently to determine the amount of total positive () and negative work () at the knee and ankle joints. In a previously described detailed model (65), an estimate of elastic work at the ankle joint was calculated over a stride. In summary, this calculation assumed that all of the energy absorbed at the ankle during the stance phase occurred elastically and thus allowed us to examine the potential contribution of elastic energy storage and release. The estimation of elastic work about the ankle joint involved integrating the ankle power curve of the negative phase (energy absorption) followed by the positive phase (energy generation). Note the integration was made only on these portions of the joint power curves. It was assumed that all the negative work about the ankle was equal to the elastic energy storage. The integration of the ankle joint power curves over the absorption and generation phases allowed an estimation of positive mass-specific muscular work at the ankle joint that could not be provided by elastic work. Therefore the following equation was used to estimate elastic work about the ankle joint ( (65):

where is the positive mass specific muscle work during stance at the ankle, is the integrated positive power over the stance phase.

## Statistical analysis

Two-way ANOVAs were performed to determine whether there was a significant main effect for either group or speed, or an interaction (group∙speed) for all knee angle and angular velocity parameters, knee stiffness, knee and ankle joint work, and elastic work about the ankle. Muscle activation and co-activation ratio were analysed using a three-way ANOVA (group∙speed∙phase). If there were significant main effects (*P* < 0.05) found, a pairwise comparison with Bonferroni correction post-hoc analyses were performed. If there were significant group interactions, a series of independent t-tests with Bonferroni correction were used for post-hoc multiple comparisons (53). Also, partial Eta squared (ηp2) values were calculated and Cohen’s (21) rules of thumb were applied to determine effect sizes (i.e. 0.01 = small, 0.06 = medium, 0.14 = large). Throughout all analyses, the significance level was set at α = 0.05 with a 95% confidence interval. All statistical analysis procedures were performed using SPSS (v23, IBM, SPSS Inc., Chicago, IL, USA).

# Results

## Group characteristics

Group mean (± SD) characteristics described in Table 1 showed that the HM group had a significantly higher maximal sprint speed (*P* < 0.05), faster 5 km personal best times (*P* < 0.001), and higher weekly running mileage (*P* < 0.001) than the LM runners. However, there were no differences for age, weight, height, thigh length, or running experience. Furthermore, no differences were observed in running speed between groups across all the four speeds.

## Muscle activation and co-activation

Three-way interactions between muscle (co-)activation, running speed and phase of landing showed LM runners had significantly more Rectus Femoris, Vastus Medialis, and Semitendinosus (*P* = 0.001) activity than HM runners (Fig. 5). In addition, activation of the Rectus Femoris, Vastus Medialis, Biceps Femoris and Semitendinosus, and co-activation ratio all significantly increased with speed across groups, as well as for both groups of runners separately (Fig. 6; *P* < 0.001), with large effect sizes (ηp2 > 0.14). Moreover, muscular activity of all muscles and co-activation significantly differed between the three phases of landing across both groups of runners, as well as for the separate LM and HM groups (*P* < 0.001).

A significant interaction between running mileage and speed was found for Semitendinosus activity (Fig. 7a; *P* < 0.05). The between mileage group difference increased with increasing running speed, and at 5.5 m∙s-1 the LM runners had significantly (*P* < 0.01) higher Semitendinosus activity than HM runners. Similarly, Rectus Femoris recruitment and co-activation strategies showed substantial differences between the LM and HM group at the higher speeds (Fig. 7b-c). Rectus Femoris activation increased more in LM runners than HM runners as running speed increased (Fig. 7b), with a significant difference between groups at 5.5 m∙s-1 (*P* < 0.05). Moreover, where thigh muscle co-activation was similar for both groups at lower speeds, LM runners dramatically increased their co-activation by 44% between 4.5 and 5.5 m∙s-1 (Fig. 5, 6 and 7c).

The interaction between mileage group and activation strategies during the different phases of landing was similar for both quadriceps muscles (Fig. 5 and 8). Rectus Femoris (Fig. 8a; *P* = 0.08) and Vastus Medialis (Fig. 8b; *P* < 0.05) were both more active in LM than HM runners during the landing phases, but not before IC.

There was a significant interaction between running speed and landing phase for Vastus Medialis activity (Fig. 9; *P* = 0.002, ηp2 = 0.07), with a significant increase in activation between all four speeds during the pre-landing (*P* < 0.001) and initial impact phase (*P* < 0.05). Especially during the initial impact phase of landing a dramatic increase of 40% Vastus Medialis activity was found between 2.5 and 5.5 m∙s-1. Unlike initial impact, Vastus Medialis activity only increased (*P* < 0.001) by 13% between 2.5 m∙s-1 and 4.5 m∙s-1 during the weight acceptance phase.

## Knee kinematics and spatiotemporal characteristics

A significant interaction between mileage group and running speed was found for stance time (Fig. 10c; *P* < 0.001), knee range of motion (Fig. 10d; *P* = 0.007) and knee stiffness Kknee2 (Fig. 10f; *P* < 0.001). Stance time significantly decreased with increasing running speed (*P* < 0.001), and was shorter for the HM compared to LM runners across all four speeds (Fig. 10c). Although there was no significant main effect of training mileage on stance time (Table 2; *P* = 0.056), a large effect size was found (ηp2 = 0.14). Moreover, stance time was 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 (214±22 vs 231±16 ms; *P* < 0.05).

Similarly, knee range of motion over the first half of stance was found to be significantly smaller at 2.5 m∙s-1 (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 there being no significant main group effect. Nevertheless, a significant main effect of running speed was found (*P* < 0.001) with a significantly decrease in knee range of motion between 4.5 and 5.5 m∙s-1 (Table 2).

The HM group flexed their knee significantly earlier before IC (Fig. 10a and 11; *P* = 0.03, =p2 = 0.19), and reached PKAV significantly sooner during stance (Fig. 10b and 11; *P* = 0.005, =p2 = 0.28) compared to the LM group (Table 2).

During the initial impact phase, HM runners adopted a significantly higher knee stiffness than LM runners for all speeds (Fig. 10e; *P* < 0.05, ηp2 = 0.15). On average, Kknee1 was found to be 58% higher in better trained runners (Table 2). In contrast, knee stiffness Kknee2 was significantly lower in HM runners for all four speeds (*P* < 0.05, <p2 = 0.37). The difference between both groups significantly increased with running speed (Table 2; *P* < 0.001). At 5.5 m∙s-1 the less-trained runners had a weight acceptance stiffness that was 130% higher (153±60 vs 67±32 Nm∙deg-1), compared to HM runners.

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 PKF (*P* < 0.001) became significantly shorter with increasing running speed (Table 2, Fig. 10a and 11). Moreover, as runners ran faster they significantly increased knee flexion angle at IC, PKF angle, knee angular velocity at IC, and PKAV (Table 2, Fig. 11; *P* < 0.001). However, the time from IC to PKAV and Kknee1 were unaffected by running speed regardless training levels.

## Knee and ankle joint work

There were no significant interactions between mileage group and running speed for knee and ankle joint work, or elastic work. Also no significant differences between mileage groups were found for knee joint work and positive ankle work during the stance phase (Table 2). However, negative ankle work (Fig. 10g; *P* = 0.01, =p2 = 0.24) and elastic work about the ankle joint (Fig. 10h; *P* = 0.005, =p2 = 0.29) were both significantly higher in HM runners than in LM runners (Table 2). Also, there were significant increases with running speed (*P* < 0.001) of positive and negative knee joint work, positive and negative ankle joint work (Fig. 10g), and elastic work about the ankle joint (Fig. 10h) for both groups of runners.

# Discussion

The primary purpose of this study was to investigate neuromuscular and movement adaptations that occur with high running training mileage across a range of steady state speeds. It was anticipated that adjustments with increasing running speed would more clearly discern the influence of running training. This novel investigation found 1) no differences between the training mileage groups in pre-activation of muscles around the knee joint prior to IC; 2) HM runners had an increased knee stiffness during the initial impact phase (Kknee1) across all speeds, but displayed lower knee stiffness during the weight acceptance phase (Kknee2)with increasing mileage group differences as running speed increased; 3) the HM group demonstrated lower amplitudes of muscle activation post landing (initial impact and weight acceptance phases) compared to LM runners and these relatively lower levels were amplified at faster running speeds; 4) after touch-down the HM runners had greater ankle negative work and estimated elastic work about the ankle across all running speeds. Collectively, these differences in the HM training group reflect changes in neuromuscular conditioning that were increasingly evident at faster running speeds.

While the present results (Fig. 5 & 6) support previous findings of greater muscle activation magnitudes around the thigh with increases in running speed (23, 29, 42, 44, 73), to the authors knowledge this is the first study that has investigated neuromuscular adaptations between runners of different training mileage background across a broad range of speeds. The first hypothesis that high training volume would be associated with lower muscle activation levels of the quadriceps and hamstrings can be partly accepted because three of the four muscles examined (Rectus Femoris, Vastus Medialis and Semitendinosus) displayed significantly reduced magnitudes in the HM group for the fastest running speeds (4.5 and 5.5 m∙s-1).The reductions were mainly associated with the post landing phases (particularly in the quadriceps muscles) with no significant differences in activation levels found in the pre-landing (or pre-activation) phase. We found two previous studies (5, 27) which examined neuromuscular activity of shank muscles (i.e. Gastrocnemius and Tibialis Anterior), between runners of different training mileage background at a single running speed (3.3 and 4.0 m∙s-1, respectively). Both studies reported reduced activation levels post landing in the HM runners and, similar to our findings, found only small differences in muscle pre-activation magnitudes prior to landing between mileage groups. However, the present authors have not found any studies that investigated the influence of training mileage on thigh muscle activation levels during running.

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 Rectus Femoris and Semitendinosus, muscle activation levels of the LM grouped runners were substantially higher than the HM runners when compared to the slower speeds (Fig. 5 & 7). In addition, we also observed a 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 that the greater muscle activation levels found in the LM group relative to the HM group are partly due to the novelty of the task, as this group of runners might have been less familiar with running at high speeds. Since muscular activity is gradually decreased and modulated with learning over time due to continued practice, unfamiliarity of a task could lead to greater muscle activity and associated co-activations levels (61, 76). Furthermore, for some tasks, large increases in muscle co-activation might be related to the need to generate high joint impedance (the combined influence of stiffness, viscosity and inertia aspects) control (57). Previous studies, that examined the control of lower limb joint stability during unfamiliar tasks, have suggested that the central nervous system increases joint stiffness along with muscle co-activation in the direction of the instability (13, 27). The observed sudden increases in both muscle co-activation and knee stiffness during the weight acceptance phase (Kknee2) in the LM group during running at 5.5 m∙s-1, may be a protective mechanism to stabilise an unstable knee joint, via impedance control (27) and may thus be metabolically more costly in muscle activation terms (28). However, the LM runners in this study were very likely not in the situation where joint instability was perceived and therefore there may not have been sufficient stimulus for such a protective response strategy (even at the fastest running speed of 5.5 m∙s-1). The muscle co-activation levels closely mimicked the speed-related changes in joint stiffness across all running speeds, which is most clearly demonstrated by the sudden increase of both co-activation levels and joint stiffness Kknee2 in the transition from 4.5 to 5.5 m∙s-1 for the LM group (Fig. 6, 7c & 10f). These very similar changes with running speed in knee joint stiffness during weight acceptance and muscle co-activation about the knee provide some clear evidence to suggest that these two factors are very closely associated (a link that is not that well established or accepted in the literature).

Although we did not directly measure elastic work of muscle-tendon units (MTUs) about the ankle and work in the in-series muscles, another plausible explanation for the relatively decreased activation levels of the Rectus Femoris,Vastus Medialis and Semitendinosus muscles during the weight acceptance phase for the HM group could be due to a greater storage and release of elastic strain energy about the ankle. Biewener (10) in his comparative observations across several animal species (including humans), proposed a working proximo-distal gradient theory which suggests that the longer fibered, proximal segment muscles act as a modulator of limb work, while the short-fibered distal limb muscles with longer tendons are designed for efficient force generation and facilitate the recovery of elastic tendon energy. A typical observation in this study, that could be aligned to this theory, was that the proximal leg segment (i.e. the thigh) orientation remains relatively unchanged during the landing phases of stance, while the majority of the rapid knee joint motion is the result of changes in orientation of the more distal segments of the lower limb (i.e. the shank) and angular motion about the ankle. The observed rapid motion occurring at the ankle (as shown in Fig. 3d) during the impact phase of landing may be associated with significant tendon stretch, particularly the Achilles tendon. Another finding in this study was that the duration of the initial impact phase was not altered with running speed within a specific group of runners (Fig. 10b & 11). This might indicate a predominantly structural basis for the duration of that phase (e.g. stiffness of the Achilles tendon) which should be explored in future work. Elastic recoil of the tendon could possibly modify the time course of muscle activation in the proximal segments during the slightly later weight acceptance phase in HM runners and perhaps reduce muscle activation levels during that phase (as indicated above). Based on dynamic muscle-tendon studies on animals, Roberts (63) indicated that rapid joint flexion immediately after landing during locomotion is associated with little change in muscle fibre length but is facilitated rather by tendon stretch. Other studies that have examined limb muscle-tendon behaviour with *in vivo* measurements of medial Gastrocnemius and Soleus muscles during the first half of stance for human running have shown that tendon stretch accounts for the majority of the MTU length change (46, 50). It has been shown that despite increases in length of the Soleus MTU with running speed, the length of the Soleus muscle fascicles changed very little (46).

 The present results showed that the HM group had substantially higher ankle negative work (Fig. 10g) along with greater elastic work about the ankle across all running speeds. These findings are similar to previous studies that have examined joint work with increasing running speeds (71, 72). There were no differences in knee joint work and ankle positive work, but we observed greater negative ankle work and elastic work about the ankle in the HM runners. In the early work of Cavagna (14), he proposed that with increasing running speed the work done by the contractile component decreases progressively due to i) a relatively larger length change taken up by the tendon during the stretch and ii) a decrease in force with the velocity of shortening. Interestingly, other modelling studies found with increasing running speed a greater contribution of positive work was provided by the tendon rather than the contractile machinery of the Soleus and Gastrocnemius (47, 78). Our results indeed clearly demonstrate that the contribution of elastic work about the ankle is greater in response to HM endurance training. As pointed out above, this enhancement in utilisation of elastic recoil energy about the ankle may be related to lower muscle activation levels of the HM runners, since the demand to use muscle contractile characteristics for positive work during stance was likely reduced. Despite the fact that increased muscle work has been found to be correlated to greater EMG activation (muscle shortening) during increased incline running (64), the proxy of using muscle activation for muscle work is equivocal when considering isometric contractile muscle behaviour. Given that isometric muscle contraction performs no work (36) and due to the inability to measure proximal muscle forces directly (10), future research is warranted to explore and understand the *in vivo* relationship between joint work and the in-series muscles of the distal segments, and the contribution of muscle activations and work about the thigh segment.

The present study found knee angular displacements and velocities at touch-down, and peak values during stance that resembled those in the literature (56). The knee was flexing at the instant of landing for both groups but it began earlier prior to landing in the HM group across all speeds. A similar, early preparatory knee flexion prior to landing was also reported by Horita, Komi, Nicol and Kyröläinen (38) who showed that good drop jump performances had this movement pattern but not the low performance jumps. Moreover, these authors explained that this early knee preparatory flexion prior to ground contact was associated with high initial knee stiffness after landing. In accordance, the present results also reported increased initial knee stiffness (Kknee1) in the HM runners. Furthermore, this increase in initial knee stiffness along with knee flexion timing before IC was not influenced by speed. Although the knee angle at IC was similar between mileage groups, with the HM runners flexing the knee earlier prior to IC, joint dynamics (flexion angular velocity and flexion angular acceleration) at IC tended to be increased with the HM group during running at the two fastest running speeds. The curves illustrated in Fig. 11 clearly show that the HM group displayed rapid changes in knee angular acceleration (or increased magnitude of angular jerk) just after IC which is similar across all running speeds. Knee stiffness during the impact phase (Kknee1) was also minimally influenced by running speed and it is plausible that this impact phase transient knee motion in the HM group is associated with elevated knee (and perhaps Achilles tendon) stiffness. It remains to be determined whether these increases in IC joint dynamics and knee angular jerk just after landing facilitate increased storage of strain energy in the Achilles tendon. To the authors’ knowledge, the knee movement adaptations as a result of HM training found in this study have not been reported elsewhere. This may be due to the relatively high quality of the kinematic data collected for this investigation. With the high sampling rate (500 Hz) of the segment trajectories, more accurate joint angular velocities and accelerations during the early stance phase of running were obtained, together with greater temporal resolution for the timings of key variables such as knee flexion onset prior to landing and peak knee flexion velocity just after landing. Also, segment tracking markers were placed in the middle of the shank and thigh segments rather than at the knee joint where errors due to soft tissue movement relative to the underlying bone can be excessive (8).

Of the two hamstring muscles investigated, the Semitendinosus appeared to be more sensitive than Biceps Femoris to the influence of running training. There was a tendency for Semitendinosus activation levels to be higher during the pre-activation and weight acceptance phases in the LM group whereas few group differences can be observed with the Biceps Femoris (see Fig. 5 and 6). These Semitendinosus-Biceps Femoris differences between training groups became larger with increasing running speed, but it is unclear why the Biceps Femoris activation levels seem to be unaffected by training status. With increasing running speed you would expect the rate of stretch of the hamstrings to be increased, and Schache, Dorn, Wrigley, Brown and Pandy (73) predicted using a musculoskeletal model that the eccentric activation and rate of stretch of the Semitendinosus during the late swing phase of running (just prior to ground contact) was greater than for the Biceps Femoris lateral hamstring. Other recent work (39), has also provided evidence that the Semitendinosus has increased loading of its muscle fibres and tendinous tissue compared to the Biceps Femoris during rapid contractions with a flexed knee. However, these indications of likely differing loading profiles between the two hamstrings muscles, investigated in this study, remain to be explored in the context of training effects.

A limitation of this study is the cross sectional design. The authors acknowledge that the neuromuscular adaptations observed in the HM group may not be solely in response to the weekly running mileage but may include other factors related to their training background (such as training speeds, resistance training, flexibility and stretching). For example, with previous studies reporting the benefits of resistance and/or plyometric training to the elastic work of the lower limbs during running (2, 62, 67, 74)**,** it is possible that the HM runners previously or currently performed other forms of training that contributed to the neuromuscular adaptations observed in the present study. Given this limitation and lack of knowledge in the area of neuromuscular adaptations to running training volume, a possible future direction would be to conduct a longitudinal training intervention study that examined responses following a controlled, well documented programme of running training. Another limitation of this study is that we did not directly measure *in vivo* the dynamic function of the lower limb muscle-tendons, but used an estimation of elastic work based on inverse dynamics of ankle joint work. Although the methods used in this study provide an overall approximation of the contribution of the elastic work during running, possible future avenues would be to use advanced imaging techniques such as shear wave imaging that are being introduced to quantify how the lower extremity tendons behave dynamically during locomotion (52).

# Conclusion

The changes in knee joint motion and neuromuscular behaviour with both running speed and training status revealed some clear and fundamental associations between knee joint stiffness and muscle co-activation levels about that joint. We found that high mileage training was associated with changes in neuromuscular conditioning which was mainly characterised by lower activation of muscles surrounding the knee joint during the weight acceptance phase, higher initial knee stiffness and greater estimates of elastic energy return about the ankle. Overall, these neuromuscular adaptations were increasingly evident at the faster running speeds.

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# 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 at5.5 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) curve. The slopes of the lines from initial contact (IC) to peak knee angular velocity (PKAV) and from PKAV to peak knee flexion (PKF) represent the stiffness of the knee for the initial impact phase (Kknee1) and the weight acceptance phase (Kknee2) respectively.

**Figure 5.** Muscle activation levels and muscle co-activation for the LM (black dashed line) and HM (grey solid line) groups with standard deviations at four running speeds, from 60 ms before initial contact (IC; vertical dashed line) to peak knee flexion. Activation levels for each muscle were normalized to the peak EMG amplitude of a maximal sprint. Note that differences between groups mainly appeared after IC and increased with speed, in particular for Rectus Femoris, Vastus Medialis, Semitendinosus and muscle co-activation from 4.5 m·s‑1 to 5.5 m·s‑1.

**Figure 6.** Mean (±SD) normalized muscle activation for the Rectus Femoris (A), Vastus Medialis (B), Biceps Femoris (C), Semitendiosus (D) and co-activation ratio (E) for the LM (black) and HM (grey) group, during the pre landing, initial impact and weight acceptance phases of landing across running speeds. Especially during the initial impact and weight acceptance phases the muscle activation levels showed more increase with speed in LM runners compared to the HM group. Note the sharp increase of the co-activation ratio in the LM group during in the initial impact phase from 4.5 m·s‑1 to 5.5 m·s‑1.

**Figure 7.** Mean normalized muscle activations of the Semitendinosus (A) and Rectus Femoris (B), and co-activation ratio (C), at four speeds for LM and HM runners. Semitendinosus and Rectus Femoris muscle activations progressively increased with running speed in both groups, however, the LM grouped runners displayed a progressively steeper increase in muscle activation with running speed as compared to the HM group. A significantly greater magnitude of muscle activation in Semitendinosus and Rectus Femoris was found between the groups at 5.5 m·s‑1. Similarly, the co-activation ratio showed a substantial (but not significant) increase of 44% in the LM group from 4.5 m·s‑1 to 5.5 m·s‑1. \*Significant difference between groups, *P* < 0.05.

**Figure 8.** Mean muscle activations for the Rectus Femoris (A) and Vastus Medialis (B) between the LM and HM group for the three phases of landing. Activation levels were averaged across speeds for each separate phase per group of runners. Quadriceps muscles did not show any differences between groups in activation levels prior to landing, but LM runners had greater quadriceps activation during the initial impact and weight acceptance phase.

**Figure 9.** Mean Vastus Medialis activation during the three phases of landing for each running speed. Vastus Medialis activation was averaged across LM and HM runners. Particularly during the initial impact phase Vastus Medialis activation showed large increases with running speed. \*Significant difference between all running speeds, *P* < 0.001. †Significant difference between 2.5 m·s‑1, 3.5 and 4.5 m·s‑1, *P* < 0.05.

**Figure 10.** Knee kinematic and kinetic characteristics for the low mileage (black) and high mileage (grey) groups for four running speeds. The time from the start of knee flexion (KF) to initial contact (IC) (A), the time from IC to peak knee angular velocity (PKAV) (B), stance time (C), knee range of motion (D), initial impact knee stiffness Kknee1 (E), weight acceptance knee stiffness Kknee2 (F), ankle negative work (G) and elastic work (H), was averaged for each group of runners per running speed. \*Significant difference between groups, †significantly different from 2.5 m·s‑1, ‡ significantly different from 3.5 m·s‑1, §significantly different from 4.5 m·s‑1, *P* < 0.05.

**Figure 11.** Knee angle, angular velocity and angular acceleration curves for the LM (black dashed line) and HM (grey solid line) across the four running speeds, from 60 ms before initial contact (IC; vertical dashed line) to peak knee flexion (PKF). Knee flexion before IC and peak knee angular velocity after IC occurred earlier in the HM runners compared to the LM group. Note that despite the reduced time to PKF with increasing running speed (as expected) the time to PKAV remained constant across speeds. HM runners showed slightly higher angular velocities at IC, and rapid changes in angular acceleration after IC (increased magnitude of angular jerk) across all running speeds.