An exploration of muscle co-activation during different walking speeds and the association with lower limb joint stiffness

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Running title: joint stiffness and muscle co-activation during different walking speeds

Abstract

The aim of this study was to determine the muscle co-activations and joint stiffnesses around the hip, knee, and ankle during different walking speeds and to define the relationships between muscle co-activation and joint stiffness. Twenty-seven healthy subjects (age: 19.6±2.2 years, height: 176.0±6.0 cm, mass: 69.7±8.9 kg) were recruited. Muscle co-activations (CoI) and lower limb joints stiffnesses were investigated during stance phase at different walking speeds using Repeated Measures ANOVA with Sidak post-hoc tests. Correlations between muscle co-activations, joints stiffnesses, and walking speeds were also investigated using Pearson Product Moment correlations. The results indicated that the hip and ankle joints stiffness increased with walking speed ($p<0.001$) during the weight acceptance phase; in addition, a positive correlation between walking speed and Rectus Femoris (RF) and Biceps Femoris (BF) CoI ($p<0.001$), and a negative correlation was between walking speed and tibialis anterior (TA) and lateral gastrocnemius (LG) 17 CoI ($p<0.001$) during the weight acceptance phase, and the RF/BF CoI during pre swing, were observed. These results provide new information on the variations in muscle co-activation around the hip, knee and ankle joints and their association with joint stiffness, and on the responses of stiffness and muscle co-activation to walking speed. The techniques presented could have further application and provides an aid to understanding of the effects of gait retraining and injury mechanisms.

Key words: Muscle activity; Co-contraction; Joint Stiffness; Walking speed.

1. Introduction

Different walking speeds are a requirement in everyday ambulation to adapt to different situations, with greater walking speeds being characterised by higher ground reaction forces (Chiu and Wang, 2007), and an associated increase in the demand of the musculoskeletal system to produce energy to allow forward progression of the body (Peterson et al., 2011). Another aspect of the demand and control of the musculoskeletal system is joint stiffness, which can be considered as the interaction between angular displacement and joint moment which provides information on the control of joint-level mechanics (Frigo et al., 1996). This includes changes in non-uniform dynamic lower limb joint stiffness to manage the range of levels of demand for different activities and mechanical energy exchange which has been associated with variations in the spring-like behaviours of muscles (Santos et al., 2021).

Few studies have looked at all 3 lower limb joints to see if their dynamic joint stiffness responds differently to the demands of fast walking (Akl et al., 2020; Frigo et al., 1996; Jin and Hahn, 2018; Santos et al., 2021). The joint stiffness (K_{joint}) can be expressed as the ratio of the maximum joint moment (ΔM) to the maximum joint flexion angle (Δθ) [$K_{joint}=\Delta M/\Delta\theta$] (Mager et al., 2018), or as the change in moment divided by the change in angle (Hyun and Ryew, 2016). Previous studies indicated that musculotendinous stiffness, which can be passive or active, is associated with joint stiffness (Kelly et al., 2015). It has also been highlighted that passive stiffness is the property of joint structures when there is no muscular activity (Rouse et al., 2013; Zhang and Collins, 2017), whereas muscle activity is taken into account when calculating dynamic stiffness, or quasi-stiffness (Aleixo et al., 2018; Shamaei et al., 2013a).

Several studies have examined the stiffness of the ankle joint during normal walking (Gabriel et al., 2008; Houdijk et al., 2008; Mager et al., 2018; Sanchis-Sales et al., 2016; Shamaei et al., 2013a), and hip joint stiffness (Goldberg and Neptune, 2007). However, only a few studies have examined the stiffness of the hip (Jin and Hahn, 2018), knee (Holt et al., 2003; Jin and Hahn, 2018), and ankle joints (Jin and Hahn, 2018) during different walking speeds. Brughelli and Cronin (2008), Akl et al. (2020), and Kuitunen et al. (2002) showed that knee joint stiffness has a greater impact on controlling leg stiffness than ankle joint stiffness, and Kim and Park (2011) who reported that the moments around the ankle and hip joints are more sensitive to gait speed.

Functional activities require dynamic knee joint stability, and the muscles around the knee must simultaneously contract, or co-activate (Smith et al., 2021). Joint stiffness is hypothesised to promote joint stability through greater antagonist co-activation (Hortobágyi and DeVita, 2000), but to achieve a specific level of net joint work, this also needs more agonist activation (Waanders et al., 2021). In this regard, Akl et al. (2021) and Seidler et al. (1998) demonstrated the significance of alterations in the co-activation of agonist and antagonist muscles during walking. In addition, high hamstrings-to-quadriceps co-activation indices have been reported among individuals with anterior cruciate ligament (ACL) deficiency and after ACL reconstruction (Blackburn et al., 2019; Sherman et al., 2021), and knee osteoarthritis (Mills et al.,

2013) during walking. While traditionally viewed as a beneficial adaptation to preserve stability (Li et al., 1999), excessive co-activation in the absence of injury is poorly understood, and when extrapolated to repetitive movement patterns over time, it may not be advantageous for long-term joint health. For example, lower strength in the hamstrings compared to the quadriceps (i.e., low muscle co-activation) has been associated with a higher risk of lower extremity injury (Knapik et al., 1991).

The study of the factors that influence muscle coactivation and joint stiffness of the lower limb, such as walking speed, can provide indications on the usefulness of this technique, which could be applied to investigations of the effects of interventions in subjects with lower limb impairment and the understanding of the possible mechanisms of injury. Therefore, the purpose of this study was to identify the differences in co-activation of the major lower limb muscles and joint stiffness during different walking speeds, and to explore the associations between muscle co-activation and lower limb joint stiffness. We hypothesized that the stiffness of the lower limb joints would all increase as walking speed increased, and the lower limb muscle co-activations would alter within the gait phases at different walking speeds.

2. Materials and Methods

2.1. Subjects

Twenty-seven volunteers were enrolled in the study, 17 males (age: 19.6±2.2 years, height: 176.0±6.0 cm, mass: 69.7±8.9 kg), and 10 females (age: 19.1±1.9 years, height: 164.0±3.0 cm, mass: 59.6±3.8 kg) from a university student population. Participants were pain- and injury-free and had no prior history of neurological or musculoskeletal injuries to the lower limbs. All participants provided written consent in accordance with the Helsinki Declaration after being informed of the experimental procedures and goals (2013). The hosting institution's Ethical Committee for Human Research approved the project (Ref no. CEFADE 19 2022).

2.2. Experimental Protocol

To define the segment co-ordinate systems, a lower limb marker set with 38 retro reflective markers on anatomical landmarks, and rigid clusters were placed on the foot, ankle, shank, knee, thigh of both legs, as well as on the pelvis (Akl et al., 2020), were used to record 3D kinematics at 200 Hz using an 11 camera Qualisys motion analysis system (Qualisys AB, Gothenburg, Sweden). The anatomical markers were attached using double-sided tape, and clusters of four markers were fixed to the thigh and shank using elastic bandages. In order to facilitate the marker placement and reduce motion artefacts, skin-tight shorts were worn by the subjects. Two 40x60 cm and 2 60x90 cm force platforms were used to record the ground reaction forces at 1000 Hz (Bertec Corporation, OH, USA), which were synchronise with the kinematic data using Qualisys Track Manager Software (Qualisys AB, Gothenburg, Sweden). In addition, surface EMG data of the selected muscles (Rectus Femoris - RF, Biceps Femoris - BF, Tibialis Anterior -TA, and Lateral Gastrocnemius -LG) were recorded using a Trigno EMG Wireless system (Delsys, Boston, MA, USA) sampling at a rate of 2000 Hz. The electrode placement was performed following the Surface

Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) guidelines (Hermens et al., 2000).

Participants were asked to walk at slow, normal, and fast speeds. Prior to the commencement of data collection, each participant was encouraged to walk at their typical, most comfortable speed. They were then asked to practise walking at a slower speed between 80% and 85% of that speed, and then between 115 and 120 percent of that speed (Akl et al., 2020). Data collection began when the subjects said they were confident matching these speeds. During data processing, any slow or fast trials that were outside of the range of these speeds for each subject were disregarded. Every participant completed walking tests over a 12-meter walkway, taking at least 3 steps before and after arriving at the force plates. All variables were averaged for five successful trials, with five gait cycles for each speed for each subject.

2.3. Data Processing

Qualisys Track Manager Software was used to digitise the obtained data (Qualisys, Inc., Gothenburg, Sweden). Marker, force, and EMG data were then exported to Visual3D for analysis (C-Motion, Germantown, MD, USA). To reduce any movement artefacts from the raw EMG data, a high-pass Butterworth filter with a cut-off frequency of 25 Hz was used. The signals were then full rectified and low-pass filtered at 15 Hz to create an enveloped EMG signal (Quittmann et al., 2020). The amplitudes of the enveloped EMG signal were then normalized to the maximum observed signal across all trials at the 3 speeds (Hermens et al., 2000; Oliveira et al., 2017). We assumed symmetry in walking between both legs, so the right leg variables were used for the next processing.

2.4. Muscle Co-Activation

At various walking speeds, the thigh (RF/BF) and calf (TA/LG) muscle co-activations were estimated using the co-activation index (CoI), equation [1]. The CoI was determined independently for the weight acceptance, mid stance, terminal stance, and pre swing gait phases (Di Nardo et al., 2018; Mari et al., 2014). The CoI provides a contribution to the overall activation of the agonist and antagonist muscles during the task, and gives a relative measure of the antagonist muscle (Akl et al., 2021; Oliveira et al., 2017).

$$CoI = \frac{\int_{t_1}^{t_2} EMG_{ant}(t)\, dt}{\int_{t_1}^{t_2} [EMG_{ag} + EMG_{ant}](t)\, dt} \times 100 \qquad [1]$$

where t_1 and t_2 are the start and end of the support phase, EMG_{ant} is the magnitude of EMG from the lower muscle activity, and EMG_{ag} is the magnitude of EMG from the higher muscle activity during normal walking which was used as a reference in the calculation of CoI during the slow and fast walking speeds (Oliveira et al., 2017).

2.5. Joint Stiffness

The stiffness of the hip, knee and ankle joints (Khip, Kknee, Kankle) were represented by displaying the slope of the linear regression of the sagittal plane moments versus sagittal plane angles across the stance phase (Houdijk et al., 2008), and joint stiffnesses were calculated within sections that showed linear characteristics within the different sub-phases of the stance phase of gait (Mager et al, 2018). The stiffnesses of the joints were identified from the slope of the best fit line within the different sub-phases. The stance phase was divided into four sub-phases which were defined according to Gagnat et al. (2020). The weight acceptance phase lasted from the ipsilateral foot strike to the contralateral foot off; the midstance came next, continuing until the ipsilateral knee moment switched from external flexion to extension; and the terminal stance began and persisted until the contralateral foot strike, after which pre-swing continued till the ipsilateral foot off (figure 1).

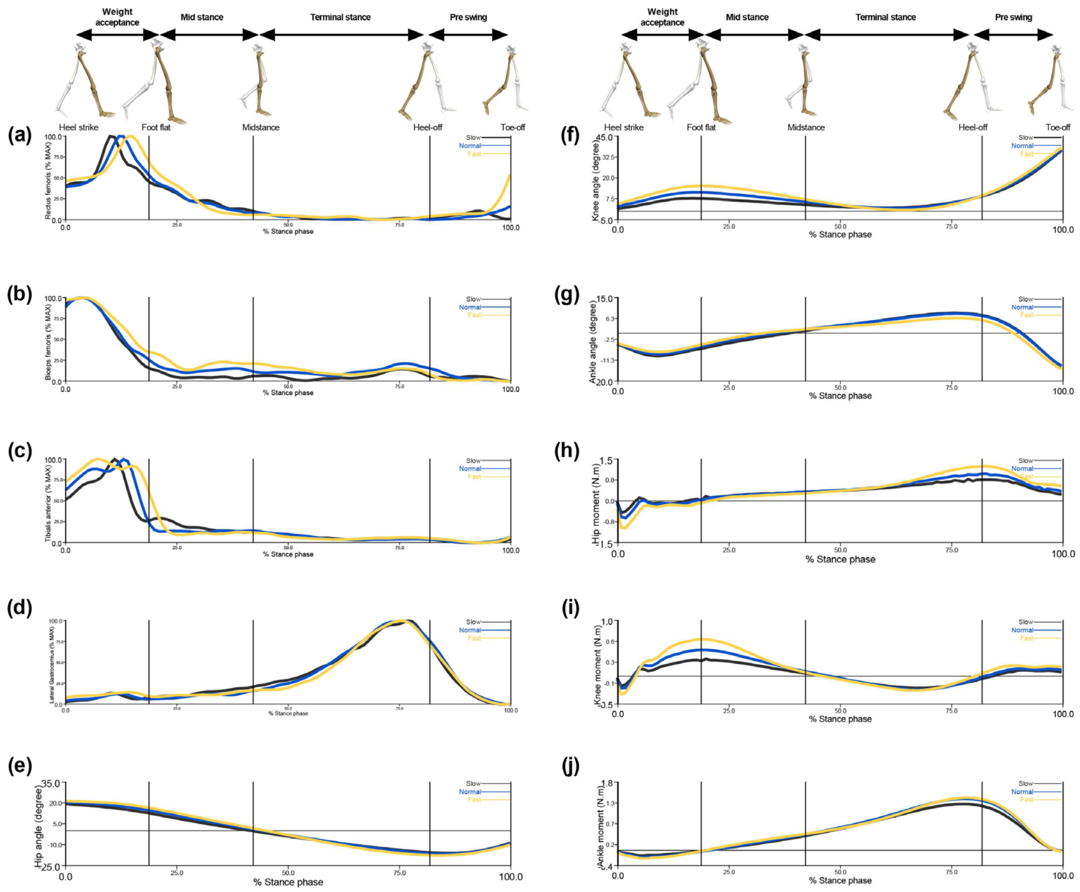

Fig. 1. Means for (a) Rectus femoris (RF), (b) Biceps femoris (BF), (c) Tibialis anterior (TA), (d) lateral gastrocnemius (LG), (e) Hip joint angle, (f) Knee joint angle, (g) Ankle joint angle, (h) Hip joint moment, (i) Knee joint moment, and (j) Ankle joint moment during the sub-phases of stance phase (weight acceptance, mid stance, terminal stance, and pre swing) of the three speeds; Slow (Black), Normal (Blue), Fast (Yellow).

Equation [2] was used to determine joint stiffness, which was computed as the change in joint moment (ΔM) divided by the change in joint angle (ΔΘ) during the stance phase.

$$K_{joint} = \frac{\Delta M}{\Delta \theta} \qquad [2]$$

Where *ΔM* = change in joint moment; *ΔΘ*= change in joint angle (Jin and Hahn, 2018; Mager et al., 2018; Wang et al., 2015).

2.6. Statistical analysis

Shapiro-Wilk tests were used to examine the distribution of the data, and it was determined that all the data was suitable for parametric analysis. Means and 95% confidence intervals were used to report descriptive statistics. To compare the mean joint stiffness variables and muscle co-activation of the lower limb between the 3 walking speeds within the various walking sub-phases, repeated measures analysis of variance (RM-ANOVA) with Sidak post hoc tests were used, each dependent variable was compared across 3 speeds for each phase. Partial eta squared ($\eta^2 p$) was calculated to assess the effect size. In addition, the relationships between muscle co-activations, joint stiffness and walking speeds were also examined using Pearson correlations. IBM SPSS software Statistics v27 was used for all statistical analyses.

3. Results

3.1. Walking Characteristics at Different Speeds

The comparison of gait characteristics at the 3 different walking speeds (slow, normal, and fast) are shown in table 1. the percentages of the differences are reported. With the exception of stride width (p=0.680) all gait variables showed significant main effects between speeds (p<0.001).

Table 1.

The differences in walking characteristics between the three speeds (slow, normal, fast).

Walking Characteristics	**Slow (n=27)**	**Normal (n=27)**	**Fast (n=27)**	**ANOVA P-Value (*η2p*)**	**Different percentages**		
	Mean (SD)	**Mean (SD)**	**Mean (SD)**		**Slow/Normal (%)**	**Slow/Fast (%)**	**Normal/Fast (%)**
Speed (m/s)	0.94 (0.06)	1.12 (0.08)	1.41 (0.10)	<0.001 (0.935)	S < N (16.16)	S < F (33.50)	N < F (20.68)
Cycle Time (s)	1.27 (0.07)	1.11 (0.08)	0.93 (0.06)	<0.001 (0.935)	S > N (14.84)	S > F (37.19)	N > F (19.46)
Stance Time (s)	0.78 (0.06)	0.67 (0.05)	0.55 (0.04)	<0.001 (0.938)	S > N (16.79)	S > F (41.64)	N > F (21.27)
Step Length (m)	0.59 (0.02)	0.62 (0.03)	0.66 (0.04)	<0.001 (0.702)	S < N (4.06)	S < F (10.32)	N < F (6.53)
Step Time (S)	0.63 (0.04)	0.55 (0.04)	0.46 (0.03)	<0.001 (0.925)	S > N (15.69)	S > F (37.83)	N > F (19.13)
Stride Length (m)	1.18 (0.04)	1.23 (0.05)	1.31 (0.06)	<0.001 (0.730)	S < N (4.05)	S < F (9.62)	N < F (5.80)
Swing Time (s)	0.49 (0.02)	0.44 (0.02)	0.38 (0.02)	<0.001 (0.906)	S > N (12.13)	S > F (30.67)	N > F (16.53)
Stride Width (m)	0.13 (0.02)	0.13 (0.02)	0.13 (0.03)	=0.680 (0.067)	S < N (3.79)	S > F (0.79)	N > F (4.76)

η2p= Partial eta squared for effect size.

3.2. Co-activation index and joint stiffness during the different walking phases

3.2.1. Weight acceptance phase

During the weight acceptance phase, the RM-ANOVA demonstrated significant main effects for speed for RF/BF CoI (η^2p= 0.53) (figure 2a), TA/LG CoI (η^2p= 0.21) (figure 2b), K_{hip} (η^2p= 0.79) (figure 2c), and K_{ankle} (η^2p= 0.52) (figure 2e). Additional post hoc comparisons indicated significant increases between slow and normal speeds for: RF/BF CoI (*p<0.05*), K_{hip} and K_{ankle} (*p<0.001*), but not for TA/LG CoI (figure 2b) and K_{knee} (figure 2d). Significant increases were also observed between normal and fast walking speeds for RF/BF CoI, $K_{hip,}$ and K_{ankle} (*p<0.001*), but not for TA/LG CoI and K_{knee}. In addition, significant increases were observed between slow and fast walking speeds for; RF/BF CoI, $K_{hip,}$ and K_{ankle} (*p<0.001*), and significant decreases for TA/LG CoI (*p<0.01*), but not for K_{knee}. In addition, significant positive correlations were seen between walking speed and RF/BF CoI (*r=0.554, p<0.001*), K_{hip} (*r=0.814, p<0.001*), K_{ankle} (*r=0.667, p<0.001*) (figures 2f, 2h, 2j,), and a negative correlation was seen between walking speed and TA/LG CoI (*r=-0.377, p<0.001*) (figure 2g).

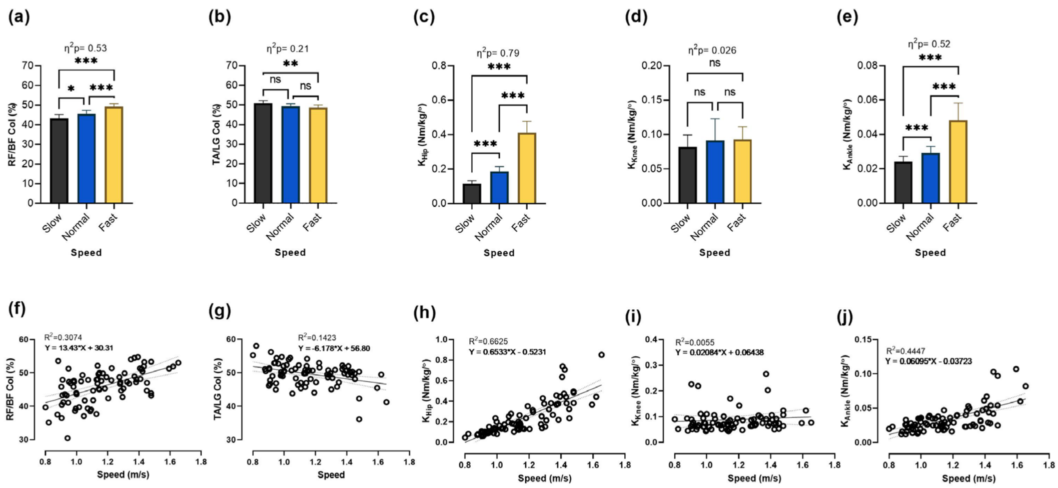

Fig. 2: Weight Acceptance: Pairwise comparisons associated with the significant main effects from the RM-ANOVA with mean and confidence intervals for (a) RF/BF CoI, (b) TA/LG CoI, (c) K_{hip}, (d) K_{knee}, and (e) K_{ankle}. Correlation among walking speed and co-activation index for (f) RF/BF CoI, (g) TA/LG CoI, (h) K_{hip}, (i) K_{knee}, and (j) K_{ankle}. Partial eta squared (η^2p) and asterisk signs represent significant differences between speeds: (***) indicates p < 0.001, (**) indicates p < 0.01, (*) indicates p < 0.05, and (ns) indicates non-significant.

3.2.2. Mid-stance phase

During the mid-stance phase, the RM-ANOVA demonstrated significant main effects for speed for TA/LG CoI (η^2p= 0.24) (figure 3b), K_{hip} (η^2p= 0.30) (figure 3c), and K_{ankle} (η^2p= 0.21) (figure 3e). Additional post hoc comparisons indicated significant differences between slow and normal speeds for TA/LG CoI and K_{hip} (*p<0.01*), but not for RF/BF CoI, K_{knee}, and K_{ankle}, and no significant differences were seen between normal and fast walking speeds. Between slow and fast walking speeds significant differences were seen for TA/LG CoI (*p<0.01*), K_{hip} (*p<0.001*), K_{ankle} (*p<0.05*), but not for RF/BF CoI and K_{knee}. In addition, there was a strong positive association between walking speed and K_{hip} (*r=0.458, p<0.001*), K_{ankle} (*r=0.400, p<0.001*) (figure 3h,3j), and a negative correlation was seen between walking speed and TA/LG CoI (*r=-0.347, p<0.01*) (figure 3g).

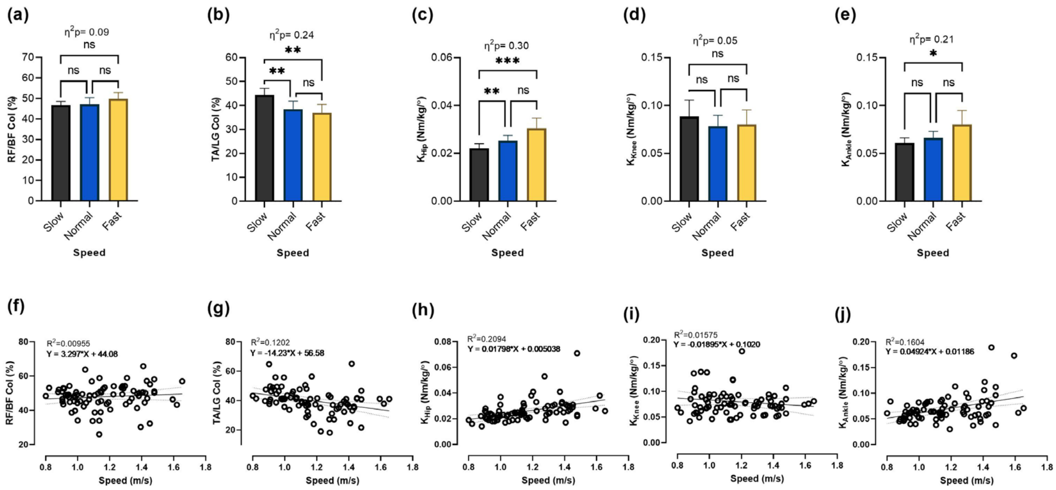

Fig. 3. Mid stance phase: Pairwise comparisons associated with the significant main effects from the RM-ANOVA with mean and confidence intervals for (a) RF/BF CoI, (b) TA/LG CoI, (c) K_{hip}, (d) K_{knee}, and (e) K_{ankle}. Correlation among walking speed and co-activation index for (f) RF/BF CoI, (g) TA/LG CoI, (h) K_{hip}, (i) K_{knee}, and (j) K_{ankle}. Partial eta squared ($\eta^2 p$) and asterisk signs represent significant differences between speeds: (***) indicates p < 0.001, (**) indicates p < 0.01, (*) indicates p < 0.05, and (ns) indicates non-significant.

3.2.3. Terminal stance phase

During terminal stance phase, the RM-ANOVA demonstrated no significant main effects with small effect size ($\eta^2 p$= 0.07) for speed for RF/BF CoI (figure 4a), TA/LG CoI ($\eta^2 p$= 0.05) (figure 4b), K_{hip} ($\eta^2 p$= 0.002) (figure 4c). And significant effects for speed for K_{knee} ($\eta^2 p$= 0.17) (figure 4d), and K_{ankle} ($\eta^2 p$= 0.49) (figure 4e). Additional post hoc comparisons showed differences between slow and normal walking speed for K_{ankle} (*p<0.001*), but not for RF/BF CoI, TA/LG CoI, K_{hip}, and K_{knee}. Between normal and fast walking speeds significant differences were seen between speeds for; K_{knee} (*p<0.05*) and K_{ankle} (*p<0.01*), but not for RF/BF CoI, TA/LG CoI, and K_{hip}. Between slow and fast walking speeds significant differences were seen for K_{knee} and K_{ankle} (*p<0.001*), but not for RF/BF CoI, TA/LG CoI, and K_{hip}. In addition, walking speed and K_{knee} had a significant negative association (*r=-0.262, p<0.05*) (figure 4i), while walking speed and K_{ankle} had a positive correlation (*r=0.546, p<0.001*) (figure 4j).

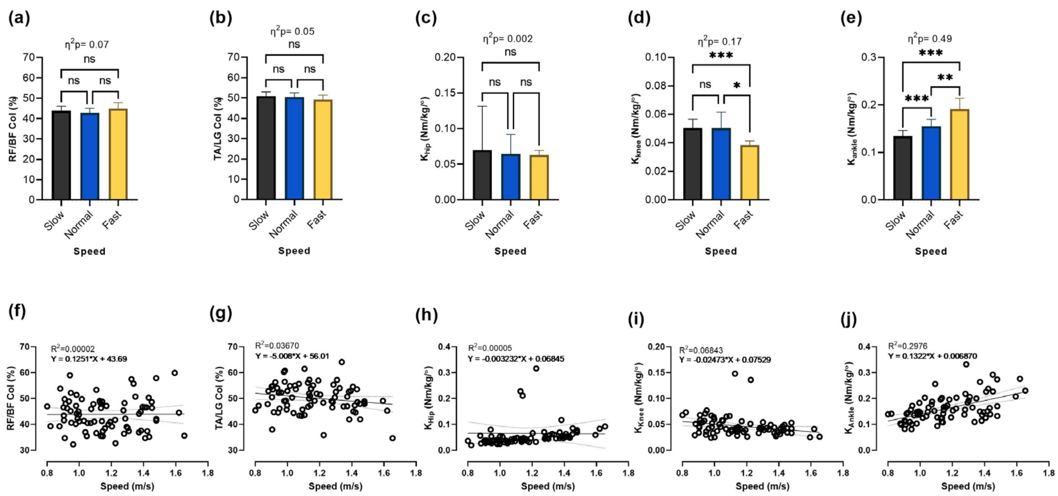

Fig. 4. Terminal stance phase: Pairwise comparisons associated with the significant main effects from the RM-ANOVA with mean and confidence intervals for (a) RF/BF CoI, (b) TA/LG CoI, (c) K_{hip}, (d) K_{knee}, and (e) K_{ankle}. Correlation among walking speed and co-activation index for (f) RF/BF CoI, (g) TA/LG CoI, (h) K_{hip}, (i) K_{knee}, and (j) K_{ankle}. Partial eta squared (η^2p) and asterisk signs represent significant differences between speeds: (***) indicates $p < 0.001$, (**) indicates $p < 0.01$, (*) indicates $p < 0.05$, and (ns) indicates non-significant.

3.2.4. Pre-swing phase

During pre-swing phase, the RM-ANOVA demonstrated significant main effects for speed for RF/BF CoI (η^2p= 0.70) (figure 5a), K_{hip} (η^2p= 0.09) (figure 5c), K_{knee} (η^2p= 0.12) (figure 5d), and K_{ankle} (η^2p= 0.22) (figure 5e). Post hoc comparisons showed significant differences between slow and normal walking speeds for: RF/BF CoI and K_{ankle} (*p<0.01*), but not between TA/LG CoI, K_{hip}, and K_{knee}. Between normal and fast walking speeds significant differences were seen for RF/BF CoI (*p<0.001),* K_{knee} (*p<0.05*), but not for TA/LG CoI, K_{hip}, and K_{ankle}. Significant differences were also seen between slow and fast walking speeds for RF/BF CoI (*p<0.001*), K_{hip} (*p<0.05*), and K_{ankle} (*p<0.01*), but not for TA/LG CoI and K_{knee}. In addition, walking speed and RF/BF CoI had a significant positive association (*r=0.606, p<0.001*) (figure 5f).

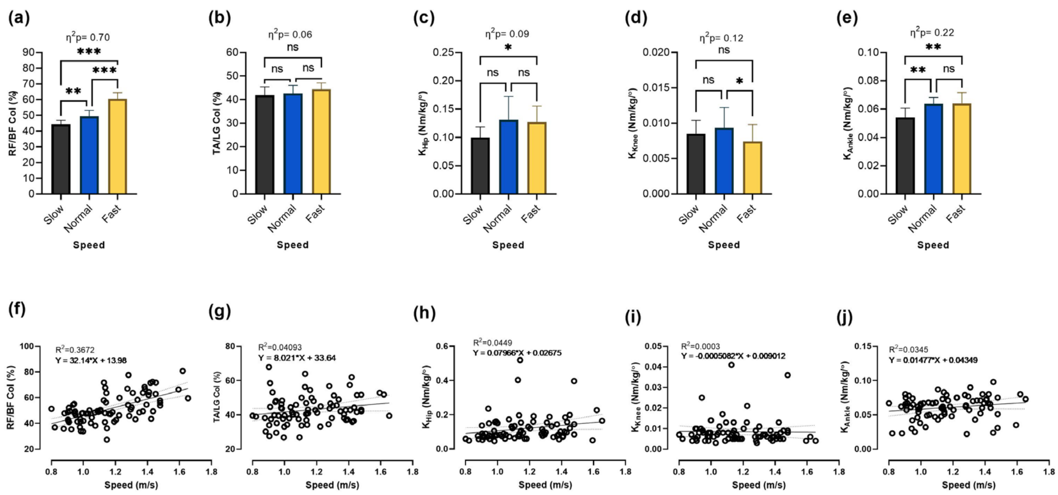

Fig. 5. Pre swing phase: Pairwise comparisons associated with the significant main effects from the RM-ANOVA with mean and confidence intervals for (a) RF/BF CoI, (b) TA/LG CoI, (c) K_{hip}, (d) K_{knee}, and (e) K_{ankle}. Correlation among walking speed and co-activation index for (f) RF/BF CoI, (g) TA/LG CoI, (h) K_{hip}, (i) K_{knee}, and (j) K_{ankle}. Partial eta squared (η^2p) and asterisk signs represent significant differences between speeds: (***) indicates $p < 0.001$, (**) indicates $p < 0.01$, (*) indicates $p < 0.05$, and (ns) indicates non-significant.

4. Discussion

The results of this study showed significant differences in gait characteristics between slow, normal and fast walking speeds, where the average slow walking speed was recorded as 0.94 ± 0.06 m/s, normal walking speed as 1.12 ± 0.08 m/s, and fast walking speed as 1.41 ± 0.10 m/s, which was in agreement with previous findings (Fox and Delp, 2010; Khan et al., 2017). This study also shows the changes in lower limb muscle co-activations and joint stiffnesses of the selected lower limb muscles between the walking speeds.

Further investigations of the muscle co-activations highlighted significant increases in thigh muscle co-activation between slow and normal, slow and fast, and normal and fast walking speeds during the weight acceptance phase. In addition, a significant decrease in calf co-activation between slow and fast walking, although no significant differences in calf co-activation were seen between slow and normal, and normal and fast walking speeds during the weight acceptance phase. These findings show how the knee and hip are controlled when the knee is partially flexed and the hip extends, allowing for weight acceptance and power absorption. Additionally, a combination of distal ankle and proximal hip muscle activation is necessary for control and stability during the mid and terminal stance phases of walking (Tirosh et al., 2013; Winter and Yack, 1987). This is achieved through the thigh muscle co-activation of RF and BF, and calf muscle co-activation of TA and LG to achieve stability during mid stance. However, the results indicated no significant differences in thigh muscle co-activation between the speeds, although significant differences were seen in calf co-activation between slow and normal and slow and fast speeds, with a greater calf co-activation during slow walking during mid stance.

During terminal stance phase when the opposing leg begins to lift off the ground, the support leg's knee and hip continue to extend, and RF, BF, TA, and LG activate synergistically on the knee and ankle joints to control the body progression as more body weight is placed on the support leg (Akl et al., 2021). During the pre-swing phase significant differences of thigh co-activation were detected which increased with speed, whereas there were no significant differences in calf co-activation, this could be because the different duration of terminal stance phase among the walking speeds, especially when moving quickly. The CoI of the thigh and calf muscles varies more between slow and fast speed than between normal and fast speed. Therefore, these findings indicate that controlling walking stability during the 3 walking speeds requires a higher level of antagonist muscle activation.

The K_{hip} showed a significant positive correlation with walking speed during the weight acceptance phase, which is in agreement with Jin and Hahn (2018). Additionally, a strong positive link between K_{hip} and walking speed was seen, with disparities between slow and fast as well as slow and normal walking speed throughout the mid-stance phase. The results of the hip stiffness support previously reported values (Frigo et al., 1996; Huang and Wang, 2016), emphasising the connection between hip moment and hip angle while walking. Due to the nature of this relationship, a stiffer joint is created by a lower angular displacement and a higher joint moment (Dixon et al., 2010; Holt et al., 2003).

In contrast to the hip joint, there were no variations between speeds and knee joint stiffness, which is in agreement with Akl et al (Akl et al., 2020), with the exception of significant differences between slow and fast speeds, and normal and fast speeds during terminal stance phase. This outcome might be the result of a decreased moment relative to knee angular displacement during the double support phase, which increases at terminal stance prior to toe off, and may also indicate a change in the proximal and distal demands placed on RF and BF to control joint stiffness as both are biarticulate muscles crossing the knee and hip joints. The differences during terminal stance in knee stiffness, particularly between slow and fast and normal and fast walking speeds, which indicates a greater stiffness when speed is decreased which has been purported to be a possible risk factor for injury (Apps et al., 2016). According to this interpretation, the knee stiffness rose during the knee flexion phase of the weight acceptance phase but reduced during the knee extension phase, which is in support of the findings by Shamaei et al. (2013b).

Ankle joint stiffness showed a main response to walking speed with a positive significant correlation during weight acceptance, mid stance, and terminal stance of walking stance sub-phases. This result indicates that the ankle joint is generating more mechanical energy than is absorbed and has a greater involvement than the hip and knee joints when walking speeds increase which is in agreement with the results reported by Jin and Hahn (2018).

Significant positive correlations were seen for speed in the K_{hip}, K_{ankle} during the weight acceptance and mid stance phases. In addition, speed was associated with K_{ankle} during terminal stance phase which was in agreement with (Jin and Hahn, 2018). In accordance with earlier investigations, the difference in reaction to hip and knee stiffness also doesn't seem to be related to a higher stiffness adaptability (Frigo et al., 1996; Neptune et al., 2011). The results indicated that the changes to K_{hip} during weight acceptance and mid stance phases as well as the changes to K_{ankle} during weight acceptance, mid stance, and terminal stance phases were associated with a corresponding muscle co-activation. The results suggest an increase in K_{hip} and K_{ankle} with an increase in thigh co-activation and decrease of calf co-activation to provide the necessary stiffness to control the lower limb movement, in agreement with previously reported findings (Wang et al., 2015).

To the authors' knowledge, this is the first study to examine the co-activation of the lower limb muscles along with the stiffness of lower limb joints and the relationships between joints stiffness and co-activation of the thigh and calf muscles with respect to different walking speeds. This provides a greater understanding of the differences and relationships between muscle co-activations, lower limb joint stiffness, and walking speed. The consideration of joint stiffness and muscle co-activation could have further applications when investigating the effects of gait retraining and other interventions in individuals with lower extremity impairment (Arene and Hidler, 2009; De la Fuente et al., 2018) and may help our understanding of possible injury mechanisms (Tam et al., 2017).

This study does have some limitations that require consideration when reviewing the findings. We assumed symmetry in walking between the left and right leg, however individual distributions of joint stiffness and muscle co-activation may differ in cases where asymmetries exist. In addition, we concentrated on exploring differences in walking speeds rather than the effect of sex, which our results suggest could be an important direction for future research. Finally, the EMG data were normalized to the maximum observed signal which is a common method of normalization for dynamic muscle activations, however the use of this technique does complicate comparisons to studies that express activation as a percentage of maximal voluntary isometric contraction (%MVIC). A future area for consideration is the relationship between biarticulate muscles and joint stiffness, which although this study recorded co-activation from 3 biarticulate muscles the relative contributions to the proximal and distal joints of each of the muscles was not considered.

Conclusion

Lower limb joints stiffness is influenced by walking speed, and it is related to thigh and calf co-activation during weight acceptance phase with the exception of knee stiffness. The co-activation of the thigh muscles increased significantly with walking speed during the weight acceptance and pre swing phases. The findings of the study also showed that co-activation of the calf muscles has no differences with walking speed and no relationships with hip, knee, and ankle stiffness during terminal stance and pre swing phases. However, there were positive associations between thigh muscle co-activation and hip stiffness during the weight acceptance and pre swing phases. These results provide more information on the combined responses to walking speed, and show the differences between stiffness of the lower limb joints and co-activation of the lower limb muscles, which could provide greater insights into the effects of gait retraining and injury mechanisms.

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