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# PERIPHERAL ARTERY DISEASE AND ACTIVITY-INDUCED SHIFTS IN QUADRICEPS MEDIAN FREQUENCY DURING TREADMILL WALKING: A PRELIMINARY STUDY

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<sup>A</sup> Study Design; <sup>B</sup> Data Collection; <sup>C</sup> Statistical Analysis; <sup>D</sup> Manuscript Preparation

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**Abstract.** Peripheral artery disease (PAD) is associated with altered gait biomechanics. No previous research study has investigated the effect of activity on muscle activation in individuals with PAD. The purpose of this study was to investigate the effect of PAD on muscle activation in response to a ten-minute walking task. **METHODS:** Ten healthy young adults, ten healthy older adults and ten individuals with PAD performed a ten-minute treadmill walking trial at a self-selected velocity. Surface EMG was recorded from the vastus lateralis and medial gastrocnemius during five steps in the first and tenth minutes of the walking trial. EMG signals were rectified and smoothed using the root mean squared (RMS) with a 20 ms smoothing window. Peak RMS EMG and median frequencies (Mdf) were calculated. Mixed-model ANOVAs with Tukey's post-hoc was used to determine effects of group and activity on peak RMS EMG and Mdf. **RESULTS:** PAD was associated with significantly greater reductions in Mdf of the vastus lateralis compared to healthy young and healthy older adults. No significant differences were observed in peak RMS EMG. **DISCUSSION:** PAD is associated with exaggerated rates of fatigue in the quadriceps but not the gastrocnemius. Efficacy of evidence-based therapeutic interventions should be further investigated.

**Key words:** peripheral artery disease, fatigue, median frequency, gait, EMG, aging

## Introduction

Peripheral artery disease (PAD) is an atherosclerotic disease that affects between 20% and 30% of older adults (Hirsch et al. 2001; McDermott et al. 2001) and up to 12 million individuals in the United States (Becker et al. 2002; Nehler et al. 2003). PAD is associated with reduced blood flow, known as intermittent claudication (IC), and most commonly affects the muscles of the thigh and pelvis. The PAD-induced reductions in blood flow often result in ischemic muscle pain and altered gait mechanics in response to physical activity (McDermott et al. 2001; Scott-Pandorf et al. 2007; Celis et al. 2009; Myers et al. 2009; Koutakis et al. 2010a). Previous research has demonstrated that IC associated with PAD is associated with fatigue, reductions in daily physical activity (McDermott et al. 2000), diminished muscle strength (Scott-Okafor et al. 2001) and postural instability (Gardner and Montgomery 2001). These reductions in functional capacity lead to exaggerated risks of falling, limited capacity to complete activities of daily living (ADLs) and a diminished quality of life (Feinglass et al. 1996).

The reduced functional capacity observed in individuals with PAD is a manifestation of changes in the underlying movement patterns. Several research studies have demonstrated that individuals with PAD exhibit abnormal gait biomechanics (Chen et al. 2008; Celis et al. 2009; Koutakis et al. 2010a; Koutakis et al. 2010b; Myers et al. 2010). Though PAD has been suggested to most commonly affect the muscles of the pelvis and thigh, recent research has revealed that joint mechanics are altered throughout the entirety of the lower extremity including the ankle, knee and hip joints (Chen et al. 2008; Celis et al. 2009). Specifically, research has demonstrated that individuals with PAD have reduced ankle plantarflexion excursion and sagittal plane ranges of motion compared to healthy controls (Celis et al. 2009). Conversely, another study suggested that individuals with PAD may adopt an ankle-based strategy, exhibiting reduced hip flexion and increased ankle plantarflexion excursions in early stance as well as increased ankle dorsiflexion during late stance (Chen et al. 2008). In addition to augmented lower extremity kinematics, research has also shown that individuals with PAD also exhibit altered lower extremity kinetics. Previously published data have demonstrated that individuals with PAD produce significantly reduced hip and knee extensor moments in early stance, diminished hip flexor and knee extensor moments in mid-stance and reduced ankle plantar flexor moments in late stance (Chen et al. 2008; Koutakis et al. 2010a). Further, it has been demonstrated that unilateral claudication in PAD results in bilateral multi-joint biomechanical adaptations during gait (Koutakis et al. 2010b) and that these aberrant gait biomechanics are independent of the onset of claudication pain (Chen et al. 2008; Crowther et al. 2008a; Crowther et al. 2008b; Koutakis et al. 2010b). These research findings clearly reveal that gait mechanics are altered as a result of PAD.

Recent evidence suggests that aberrant gait mechanics associated with PAD are not significantly improved in response to improved peripheral blood flow with medication therapy (Huisinga et al. 2010a; Huisinga et al. 2010b). Further, it has been suggested that the unique gait mechanics associated with PAD are not solely due to reductions in blood flow to muscular tissues, but may also be the manifestation of changes in the health of the peripheral nervous system as a result of a chronic ischemic environment (Koopman et al. 1996; Pipinos et al. 2007; Celis et al. 2009). Nonlinear measures of complexity and regularity have been used previously to assess the stability of the motor program in healthy and diseased populations (Stergiou and Decker 2011). Recent research has revealed that individuals with PAD have greater moment-to-moment variability during treadmill walking (Myers et al. 2010). In a study that investigated linear and nonlinear measures of lower extremity variability during gait, it was revealed that individuals with PAD had significantly greater standard deviations, coefficient of variation and largest Lyapunov

exponent values than healthy, age-matched controls (Myers et al. 2010). In addition to representing the variability of a movement pattern, nonlinear measures of variability have been suggested to be reflective of the stability of the self-organizing neuromuscular system (Pincus 1991; Stergiou and Decker 2011). In the study by Myers et al. (2010) individuals with PAD were free of claudication pain during testing which suggests that the increased variability observed in individuals with PAD can be attributed to the chronic ischemic environment associated with PAD. These data suggest that the neuromuscular program of individuals with PAD exhibits increased noise and reduced stability compared to healthy, age-matched controls (Myers et al. 2009).

Fatigue has been identified as a potential mechanism underlying the observed changes in kinematic and kinetic patterns associated with PAD (Gasparini et al. 2012). The phenomenon of fatigue, however, has not been well defined and has been attributed to a variety of heterogeneous mechanisms underlying reduced performance (Eldadah 2010). Several sites have been suggested to underlie fatigue-related reductions in muscle performance including peripheral and central structures. Typically, peripheral fatigue has been attributed to changes within the environment of the muscle (Eldadah 2010) while central fatigue has been associated with changes in the nervous system resulting in reduced descending neural drive or exaggerated inhibition (Allman and Rice 2002). Though peripheral and central fatigue have unique mechanisms, both result in reduced muscle performance and can be assessed by changes in muscle activation. Median frequency has been identified as a valid and reliable measure of fatigue (Watanabe and Akima 2010). Specifically, median frequency is a composite measure of the number and firing rate of activated motor units (Watanabe and Akima 2010), and shifts in median frequency have been suggested to correspond with reductions in either the number of activated motor units or motor unit firing rate, both of which are associated with fatigue. In submaximal fatiguing contractions, previous research has revealed increases in EMG amplitude and has demonstrated that these increases in amplitude are the result of increased numbers of activated motor units and greater motor unit firing rates (Allman and Rice 2002).

While previously reported nonlinear measures of variability have suggested that the neuromuscular system of individuals with PAD is less stable than healthy controls, no previous study has directly investigated the differences in muscle activation patterns in healthy aging and PAD. Further, the diminished oxygen supply associated with PAD would result in reduced muscular performance and muscular fatigue. While previous research studies have examined gait kinematics and variability during a treadmill walking task (Chen et al. 2008; Celis et al. 2009; Myers et al. 2009; Koutakis et al. 2010a; Koutakis et al. 2010b), no previous study has addressed the effect of fatigue on lower extremity mechanics or muscle activation patterns. Therefore, the purpose of this study was to quantify the effects of PAD on muscle activation patterns during treadmill walking. As reduced blood flow due to PAD can result in substantial muscular fatigue, this study also aimed to investigate the effects of fatigue associated with a prolonged walking task on muscle activation patterns. It was hypothesized that individuals with PAD would exhibit disproportionately greater reductions in muscle activation intensity and median frequency than healthy young and healthy older adult controls.

## Methods

### Participants

Ten healthy young adults (YAC), ten healthy older adults (OAC) and ten individuals with Peripheral Artery Disease (PAD) were recruited from the local north-central West Virginia (USA) region and the greater Fairmont State University community to participate in the study. Ages and anthropometric measurements are presented in

Table 1. Young adults were between the ages of 18 and 25 years while healthy older adults and individuals with PAD were between 50 and 85 years of age. All participants completed a verbal medical history and individuals with PAD had diagnoses confirmed by their physician. Any participant was excluded if they had a history of major musculoskeletal injury or a known neurological disease that would limit lower extremity function or gait. Participants with PAD were excluded if they could not walk without assistance, and/or were categorized as 'high risk' according to American College of Sports Medicine Guidelines (Thompson 2010). The experimental protocol was approved by the Fairmont State University Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained prior to the participation of each participant in the current study.

**Table 1.** Anthropometric characteristics of healthy young adults (YAC), healthy older adults (OAC) and individuals with peripheral artery disease (PAD)

Group	Age (yrs)	Height (m)	Mass (kg)	Treadmill Speed (m/s)
YAC	20.8 ±2.9	1.64 ±0.06	61.7 ±5.8	1.21 ±0.31
OAC	55.2 ±3.4 <sup>a</sup>	1.70 ±0.07	86.0 ±24.9	1.34 ±0.22
PAD	70.8 ±12.3 <sup>a,b</sup>	1.66 ±0.06	78.7 ±8.0	1.03 ±0.36

<sup>a</sup> denotes significant difference compared to the YAC group, <sup>b</sup> denotes significant difference compared to the OAC group.

## Laboratory Testing

Each participant had anthropometric measures including age, height and mass taken prior to testing. Surface electromyography (EMG) was recorded from the left vastus lateralis (VL) and medial gastrocnemius (MG) using a BIOPAC MP35 (1000 Hz, BIOPAC, Inc., Goleta, CA, USA). The vastus lateralis and medial gastrocnemius muscles were selected due to previously reported reductions in functional performance during the gait cycle in individuals with PAD (Celis et al. 2009; Koutakis et al. 2010; Koutakis et al. 2010). The skin over the VL and MG were shaved, alcohol washed and abraded to minimize electrical resistance. Surface electrodes were placed according to the recommendations from existing literature (Perotto et al. 2005) and were confirmed using manual muscle testing. Elastic wraps were placed over the thigh and shank to minimize movement artifact and cross-talk.

## Experimental Procedure

Prior to completing the submaximal treadmill exercise, participants performed three maximal voluntary isometric contractions (MVICs) of the knee extensors and ankle plantarflexors. Knee extensor MVICs were characterized by the participant sitting on a table with legs suspended above the floor. A leather strap with a chain connected to the wall was placed around the distal tibia to inhibit knee extension. With the knee at 90° of flexion, the participant performed three maximal knee extension contractions while surface EMG was collected. Ankle plantarflexor MVICs were characterized by the participant performing a standing plantarflexion task (heel raise) while investigators provided exaggerated resistance such that the participant could not raise their heels off of the ground. MVICs were recorded for use in normalization of EMG values. A 2-minute rest period was taken between MVIC trials.

Following the recording of MVICs, each participant was asked to determine a treadmill speed at which they could walk for 10 continuous minutes. Participants then had several minutes of rest and only began experimental

testing upon verbal confirmation that they were not fatigued and had no claudication pain (Myers et al. 2010). Each participant then performed level treadmill walking at their self-selected speed while surface EMG of the VL and MG were recorded. Testing ended after ten minutes of treadmill walking or when claudication pain prevented protocol completion (Table 1). After completion of the level walking task, participants walked for an additional three minutes at decreasing speeds as a cool down. If individuals with PAD terminated testing due to claudication, the cool down was completed after cessation of the claudication pain.

### Data Analyses

VL and MG signals were analyzed from five consecutive strides during the first (pre-activity) and last minutes (post-activity) of the ten-minute walking trial. Median frequency (MdF) was calculated using custom software (MatLab 2010, Mathworks, Natick, MA, USA). The raw EMG signal was converted to the frequency domain using a Fast Fourier Transform and the median value of the frequency domain was calculated using previously described methods (Watanabe and Akima 2010). Shifts in median frequency associated with activity were calculated as the difference between pre- and post-activity median frequencies (Watanabe and Akima 2010).

To calculate muscle activation amplitudes, raw EMG signals were amplified ( $\times 10$  k) and band-pass filtered (50 Hz–400 Hz) using custom software (MatLab 2010, Mathworks, Natick, MA, USA). Filtered EMG signals were rectified and smoothed using the root mean square (RMS) with a 20 millisecond smoothing window. Peak values of the RMS curves for the VL and MG were determined for each stride and normalized to peak RMS values recorded during the pre-trial MVIC. Normalized EMG values are presented as a percent of MVIC.

### Statistical Analyses

Two mixed model repeated measures analysis of variance (ANOVA) with Tukey's post-hoc were used to determine the significant effects of group and activity on peak EMG and median frequency values, respectively. A univariate ANOVA was used to determine the effect of group on activity-induced shifts in median frequency. In the presence of a significant main effect of group on median frequency shifts, follow up t-tests were used to determine the simple effects of group. Three Student's t-tests were used to determine significant effects of group on treadmill velocity. All tests were two-sided and significance was set at  $p < 0.05$ . SPSS 18.0 was used to conduct all statistical tests (IBM, Armonk, NY, USA 10504).

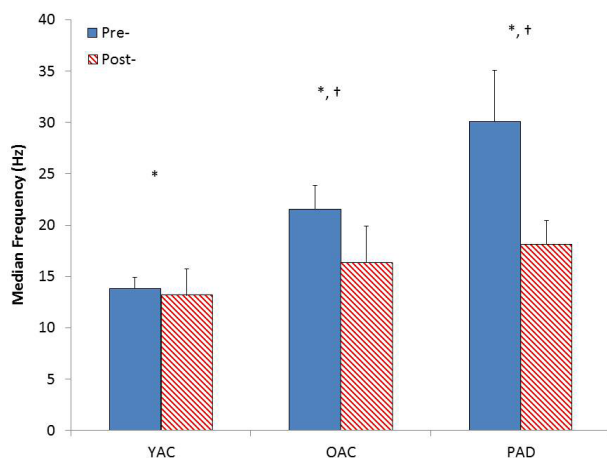
### Results

Participant characteristics are presented in Table 1. The YAC group was significantly younger than either the OAC ( $p < 0.001$ ) or PAD groups ( $p < 0.001$ ). Further, the OAC group was significantly younger than the PAD group ( $p = 0.020$ ). No differences in height were observed between the YAC and the OAC ( $p = 0.091$ ) or PAD groups ( $p = 0.246$ ) as well as between the OAC and PAD groups ( $p = 0.232$ ). The YAC group weighed significantly less than either the OAC ( $p = 0.047$ ) or PAD groups ( $p = 0.004$ ). No significant differences in weight were observed between the OAC and PAD groups ( $p = 0.297$ ).

Treadmill velocities were similar between the groups. Specifically, no significant differences were observed between the YAC and either the OAC ( $p = 0.207$ ) or individuals with PAD ( $p = 0.289$ ). Further, no differences in treadmill velocity were observed between OAC and individuals with PAD ( $p = 0.095$ ).

## Median Frequency

Figure 1 presents the average median frequencies (M<sub>d</sub>F) of the vastus lateralis in the YAC, OAC and PAD groups during the first and last minute of the treadmill walking trial. The mixed model analysis of variance revealed significant effects of group ( $p = 0.003$ ) and activity ( $p = 0.034$ ). The post-hoc analysis indicated that individuals with PAD had significantly greater vastus lateralis M<sub>d</sub>F than the YAC group ( $p = 0.002$ ) with conditions collapsed; however, M<sub>d</sub>F in the PAD group were not significantly different from the OAC group ( $p = 0.134$ ) with conditions collapsed. Vastus lateralis M<sub>d</sub>F were not significantly different between the YAC and OAC groups ( $p = 0.093$ ) with conditions collapsed. The YAC group had significantly smaller shifts in M<sub>d</sub>F between pre- and post- activity than either the OAC ( $p = 0.040$ ) or PAD groups ( $p = 0.001$ ). No significant differences in M<sub>d</sub>F shifts were observed in either the OAC or PAD groups ( $p = 0.061$ ).

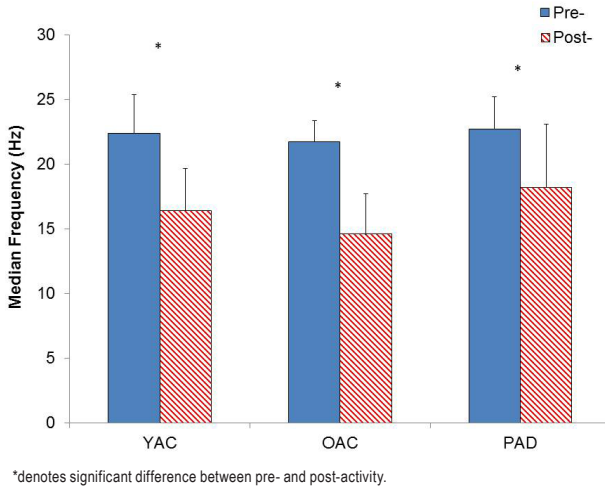


\* denotes significant difference between pre- and post-activity. † denotes significantly greater shift in M<sub>d</sub>F compared to YAC.

**Figure 1.** A comparison of vastus lateralis median frequency values of the healthy young adults (YAC), healthy older adults (OAC) and individuals with peripheral artery disease (PAD) in the first (solid, blue) and last minutes (diagonal, red) of a ten-minute treadmill walking trial.

In the medial gastrocnemius (Figure 2), M<sub>d</sub>F were significantly reduced in response to activity ( $p = 0.039$ ). Post-hoc analyses did not reveal significant simple effects of activity. Specifically, M<sub>d</sub>F of the gastrocnemius in the YAC group were not significantly different than those in the OAC ( $p = 0.927$ ) or PAD groups ( $p = 0.944$ ) with conditions collapsed. Further, the gastrocnemius M<sub>d</sub>F in OAC and PAD groups were not significantly different with conditions collapsed ( $p=0.769$ ). The shifts in M<sub>d</sub>F (pre- to post-activity) in the medial gastrocnemius were not significantly different between the YAC and OAC ( $p = 0.304$ ) or PAD groups ( $p = 0.442$ ). Further, the OAC and PAD groups exhibited no significant differences in M<sub>d</sub>F shifts of the medial gastrocnemius ( $p = 0.304$ ).

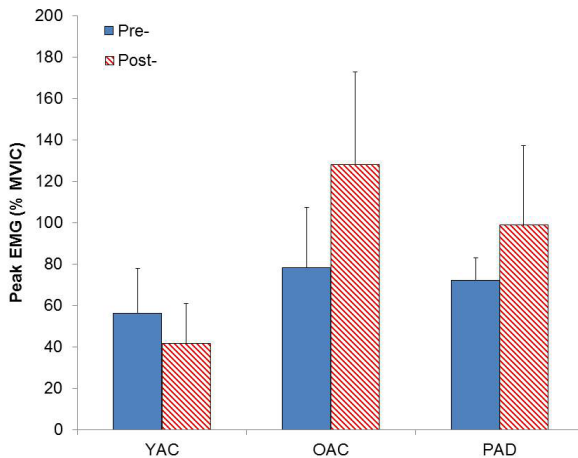




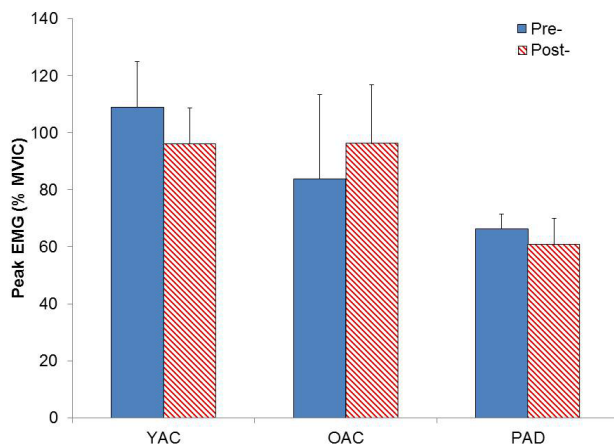
**Figure 2.** A comparison of gastrocnemius median frequency (Mdf) values of the healthy young adults (YAC), healthy older adults (OAC) and individuals with peripheral artery disease (PAD) in the first (solid, blue) and last minutes (diagonal, red) of a ten-minute treadmill walking trial

### Peak RMS EMG

Peak normalized quadriceps EMG values are presented in Figure 3. No significant main effects of group ( $p = 0.228$ ) or activity ( $p = 0.405$ ) were observed. Figure 4 presents peak normalized gastrocnemius EMG values. No significant main effects of group ( $p = 0.069$ ) or activity ( $p = 0.315$ ).



**Figure 3.** A comparison of peak normalized vastus lateralis EMG values in healthy young adults (YAC), healthy older adults (OAC) and individuals with PAD during the first (blue, solid) and last (diagonal, red) minutes of a ten-minute treadmill walking trial



**Figure 4.** A comparison of normalized peak gastrocnemius EMG values in healthy young adults (YAC), healthy older adults (OAC) and individuals with PAD during the first (blue, solid) and last (diagonal, red) minutes of a ten-minute treadmill walking trial

## Discussion

Gait abnormalities associated with PAD have been investigated using a variety of techniques including step length, step width, three-dimensional gait analysis and non-linear measures of regularity. However, no previous study has addressed changes in muscle activation in response to activity. The current study presents novel findings pertaining to changes in Mdf and muscle activation intensity in healthy aging and PAD.

The findings of the present study demonstrate disproportionately greater shifts in Mdf of the vastus lateralis in individuals with PAD compared to healthy young adults. Mdf is a composite measure of the number and firing rate of the activated motor units beneath the surface electrode. Shifts in Mdf have been commonly used in scientific literature as a measure of fatigue (Watanabe and Akima 2010), though the source of fatigue (central vs. peripheral) cannot be determined. In the present study, participants performed a ten-minute walking task at a self-selected velocity. Though statistically insignificant, individuals with PAD walked at a slower velocity than healthy young adults. Reduced walking velocity is associated with lower absolute mechanical demand; however, in response to lower absolute mechanical demand, individuals with PAD exhibited significantly greater shifts in median frequency, indicating disproportionately greater levels of fatigue. The atherosclerotic nature of PAD results in reduced blood flow to and oxygenation of lower extremity musculature in response to activity (Koopman et al. 1996; Hirsch et al. 2001; Becker et al. 2002). Several possible adaptations may occur in response to reduced oxygenation of the lower extremity musculature. One possible adaptation includes the activation of a greater number of type II muscle fibers due to type I motor unit burn out in a hypoxic environment (Gasparini et al. 2012). While this mechanism of muscle fatigue may underlie the findings of some studies of simulated arterial occlusion (Myers et al. 2010), individuals with PAD do not suffer from acute periods of ischemia, but from chronic ischemic conditions. Therefore, it is much more likely that long-term adaptation has occurred in individuals with PAD. It has been suggested that these chronic hypoxic conditions result in changes in muscle function and phenotype in individuals with PAD (Brass et al. 2004; Pipinos et al. 2008a; Pipinos et al. 2008b; Gasparini et al. 2012). Specifically, in response to the chronic reduction

in oxygenation of type I muscle fibers, the number of available type I muscle fibers is diminished and muscle fiber type distribution is shifted toward a predominance of fatigable type II muscle fibers (Brass et al. 2004, Pipinos et al. 2008b). The current data demonstrate that while shifts in MdF were significantly greater in the PAD compared to healthy young adults, peak EMG did not change suggesting that motor unit firing rates were reduced in response to the hypoxic environment associated with PAD.

While in the current study PAD resulted in significant changes in the frequency components of neuromuscular activation in the vastus lateralis, these data suggest that PAD did not significantly affect frequency component of the medial gastrocnemius activation. Previous research has suggested that in individual patients with PAD, some muscles may be more affected than others. PAD is most notable in the muscles of the thigh and pelvis suggesting that the proximal muscles may be more directly affected by reduced blood flow than distal musculature. For example Chen et al. (Chen et al. 2008) and Koutakis et al (Koutakis et al. 2010a; Koutakis et al. 2010b) observed significant reductions in hip and knee contributions to gait. Conversely, Celis et al. (Celis et al. 2009) observed significant differences in ankle kinematics in individuals with PAD compared to healthy controls, but did not observe any differences at the knee or hip joints. However, previous research has suggested that while PAD is classified as an atherosclerotic disease, secondary dysfunction may occur in the nervous system due to chronic ischemia (Chen et al. 2008; Celis et al. 2009). Individuals with PAD in this study may not have experienced claudication of the medial gastrocnemius. It is likely that in more advanced PAD a greater level of nervous dysfunction may result in greater lower extremity dysfunction including reduced hip, knee and ankle joint moments and powers.

An interesting finding of the current study was that no differences in muscle activation intensity or MdF were noted between the healthy young and healthy older adults. It has been previously demonstrated that advancing age is associated with significant changes in lower extremity kinematics and kinetics (Judge et al. 1996; DeVita and Hortobagyi 2000) and neuromuscular activation patterns (Hortobagyi and DeVita 2000; Hortobagyi et al. 2003; Powell et al. 2008; Bice et al. 2011) during activities of daily living. Specifically, it has been shown that healthy older adults perform activities of daily living at a greater relative effort and with greater normalized EMG values (Hortobagyi et al. 2003). Further, it has also been demonstrated that older adults adopt a hip-based gait strategy and that advancing age is associated with a distal to proximal shift in lower extremity joint torques during gait (Judge et al. 1996; DeVita and Hortobagyi 2000). The current data suggest that the neuromuscular activation strategy (i.e. activation intensity of the quadriceps and gastrocnemius) was not significantly altered in healthy aging. The lack of differences in muscle activation intensity in combination with a reduction in the volume of lean mass associated with advancing age may underlie the previously reported reductions in the contributions of distal musculature to the gait cycle (DeVita and Hortobagyi 2000).

Though the findings of this study are novel and present unique data pertaining to the changes in neuromuscular activation patterns in response to healthy aging and PAD, the authors acknowledge several limitations of the study. The small sample size used in the current study limits the statistical power and may result in not finding statistically significant differences between the groups. Further, the mean age of individuals with PAD was significantly greater than the healthy older adults limiting the interpretation of these findings as the observed differences between the healthy older adults and individuals with PAD may not be solely attributable to PAD, but also to advancing age.

## Conclusions

In conclusion, individuals with PAD exhibit altered lower extremity gait mechanics. The findings of the current study suggest these unique lower extremity kinematics and kinetics may be the result of changes in either the number of motor units activated or the motor unit firing rate of driving musculature. Changes to motor unit firing rate could be a manifestation of reduced health of the peripheral nervous system as a result of the chronic ischemic environment of PAD. Future research may seek to address the nature of the mechanisms underlying these altered neuromuscular strategies.

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