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ORIGINAL ARTICLE

# Sex differences in neuromuscular control of quadriceps

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## Abstract

**Purpose** Patellofemoral pain syndrome (PFPS) is twice as prevalent in females as males, yet a few studies have evaluated differences in quadriceps muscle control between sexes or across force levels. This study investigated sex differences in quadriceps EMG onset times and amplitude at different force levels during isometric knee extension in asymptomatic males and females and in females with PFPS.

**Methods** Thirteen healthy males, 12 healthy females, and 10 females with PFPS performed isometric knee extension ramp contractions at 25%, 50%, and 75% of maximal voluntary contraction (MVC). Surface EMG was recorded from the vastus lateralis (VL), vastus medialis oblique (VMO), vastus medialis (VM), and rectus femoris (RF).

**Results** Healthy females showed delayed VL (222 ± 67 ms, $p = 0.002$), VMO (357 ± 101 ms, $p = 0.001$), and VM (258 ± 62 ms, $p < 0.001$) recruitment in comparison with healthy males. Healthy males activated the VL earlier than the VM (156 ± 51 ms, $p = 0.02$) and RF (379 ± 74 ms, $p < 0.001$), and at a similar time as the VMO; healthy females activated the VL earlier than the VM (192 ± 53 ms, $p = 0.004$) and VMO (239 ± 73 ms, $p = 0.01$). A lower VMO:VL activation ratio was found at 25% MVC ($p < 0.001$) than at higher force levels.

**Conclusions** Delayed activation of the VMO relative to the VL has been proposed as a risk factor for PFPS. This study confirms a delay in VMO onset time in females.

**Keywords** Electromyography (EMG) · Onset time · Patella · Vastus medialis · Vastus lateralis

## Abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| EMG | Electromyography |
| ICC | Intraclass correlation coefficient |
| MVC | Maximal voluntary contraction |
| PFPS | Patellofemoral pain syndrome |
| RF | Rectus femoris |
| RMS | Root mean square |
| VAS | Visual analog scale |
| VL | Vastus lateralis |
| VM | Vastus medialis |
| VMO | Vastus medialis oblique |

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## Introduction

Patellofemoral pain syndrome (PFPS) is the most common diagnosis of knee joint pathology for anterior knee pain (Foss et al. 2014). It is commonly thought to be due to excessive lateral tracking of the patella in the femoral groove (Pal et al. 2012) caused by delayed and lower recruitment of the vastus medialis oblique (VMO) relative to the vastus lateralis (VL) (McConnell 1996; Boling et al. 2006). Females are 2.23 times more likely to develop PFPS than males (Boling et al. 2010). It is possible that in addition to anatomical differences with males, females may have differences in quadriceps neuromuscular control patterns.

Muscle biopsy studies have revealed that females have a higher proportion of slow twitch fibers and a lower proportion of fast-twitch fibers in the VL than males (Simoneau and Bouchard 1989; Miller et al. 1993). Thus, females may recruit more low-threshold motor units in the VL earlier than males. This could create a lateral pull on the patella. Myer et al. (2005) found that females exhibited a lower VMO:VL activation ratio than males during a dynamic-landing task (Myer et al. 2005). Sex differences in recruitment patterns

of lower extremity muscles are present in healthy individuals (Baur et al. 2010; Peng et al. 2018) and in people with knee dysfunction (Bigham et al. 2018). However, no sex differences were observed in the VMO:VL activation ratio during dynamic knee extension, single leg squat, stepping down, or straight leg raise (Herrington et al. 2006; Bowyer et al. 2008), nor in the onset times of the VMO and VL in a dynamic-stepping task (Cowan and Crossley 2009). These discrepant findings may be due to differences in neuromuscular control across force levels between males and females, but quadriceps control across force levels has not yet been investigated systematically between the sexes.

Muscle activation patterns may change with increasing exertion of force based on the function and size of the quadriceps' sub-portions. Although all the quadriceps muscles generate knee extension force collectively, the VMO stabilizes the patella medially against the opposing force from the distal VL (Bennett et al. 1993). The cross-sectional area of the VL muscle belly is larger than that of other quadriceps sub-portions; therefore, the VL possesses higher force generation capacity (Narici et al. 1989). In healthy individuals with sexes pooled, the vasti muscle activation pattern does not appear to be altered across force levels (Rainoldi et al. 2008; Spairani et al. 2012). However, it is possible that sex affects neuromuscular control during varying levels of force generation as a result of dissimilar muscle fiber characteristics between males and females which may lead to the higher incidence of PFPS in females.

Many studies have found that individuals with PFPS exhibit delayed and lower VMO activation relative to the VL (Cowan et al. 2002; Crossley et al. 2004; Boling 2006; Van Tiggelen et al. 2009; Felicio et al. 2011). However, many have also observed no difference in quadriceps activation (Karst and Willett 1995; Powers et al. 1996; Gilleard et al. 1998; Laprade et al. 1998; Morrish and Woledge 1997; Owings and Grabiner 2002; Cavazzuti et al. 2010). The considerable disagreement as to whether or not there is VMO–VL dysfunction in individuals with PFPS may well be a result of different methodologies and population characteristics (Powers 1998; Chester et al. 2008). Moreover, pain becomes aggravated with increasing force production (Powers 2012), which could also affect neuromuscular control. It is unknown whether individuals with PFPS exhibit altered vasti control at different force levels.

In the present study, we examined temporal control and activation amplitude of the superficial vasti muscles across force levels between healthy males and females and in females with PFPS when performing controlled-force ramp-up tracking tasks. Our aims were twofold. The first was to examine sex differences in onset times and activation levels of the VL, rectus femoris (RF), vastus medialis (VM), and VMO in healthy individuals during isometric knee extension. The second was to assess differences in EMG onset times and amplitude of the quadriceps in females with and without PFPS. We hypothesized that: (1) healthy females would show delayed EMG onset of the VMO relative to the VL, and their VMO onset would also be delayed compared to the VMO onset in healthy males; (2) healthy females would have lower activation levels of the VMO relative to the VL, and their VMO activation amplitude would be also lower compared to the VMO amplitude in healthy males; (3) VMO:VL activation ratio would be smaller in healthy females than in healthy males at higher force levels; (4) females with PFPS would show delayed recruitment of the VMO relative to the VL, and the VMO recruitment would be delayed compared to healthy females, and (5) females with PFPS would show lower activation levels of the VMO relative to the VL, and the VMO amplitudes would be lower compared to the VMO amplitudes in asymptomatic females.

## Methods

### Participants and ethical approval

Participants ($N = 35$) included 25 healthy individuals: 13 males [mean age 26.1 (SD 3.7) years] and 12 females [mean age 27.1 (SD 3.9) years] without any current lower extremity injury or pain, previous leg surgery, immobilization, or arthritis in the dominant leg. Additional 10 females [mean age 25.9 (SD 6.0) years] who had ongoing retro-patellar or peri-patellar pain were recruited for the PFPS group through referrals from local physical therapists or with recruitment flyers. These participants were included if their PFPS symptoms had persisted for at least 1 month and could be induced or aggravated by at least two of the following activities: ascending/descending stairs, hopping/jogging, prolonged sitting, kneeling, or squatting (Boling et al. 2010). All participants completed a questionnaire on their health history and exercise habits. The PFPS group also completed the Anterior Knee Pain Scale (Kujala et al. 1993), which measures PFPS pain severity and the ability to perform functional activities. There were no significant differences in exercise intensity [$F$ (2, 2.74) = 1.84, $p = 0.18$] or exercise time [$F$ (2, 15,492.51) = 0.27, $p = 0.77$] across the healthy male, healthy female, and PFPS female groups (Table 1). All participants signed a written informed consent document, and all experimental procedures were approved by the University of Texas at Austin Institutional Review Board.

### Experimental protocol

All data were collected in the Neuromuscular Physiology Laboratory at the University of Texas at Austin. Participants were directed to avoid any intense physical activity for at least 48 h prior to the experimental procedures. Before data

**Table 1** Average exercise habits for the three participant groups

| | Average exercise intensity* | Average exercise time (min/week) |
|---|---|---|
| Healthy males | 2.7 ± 1.4 | 279.8 ± 305.8 |
| Healthy females | 1.9 ± 1.1 | 208.7 ± 140.0 |
| PFPS females | 2.8 ± 1.2 | 266.1 ± 251.6 |

*PFPS* patellofemoral pain syndrome

*Exercise intensity scores: 1, light; 2, light-to-moderate; 3, moderate; 4, moderate-to-high; 5, high

Values are means ± SD

**Table 2** Pain in the patellofemoral pain syndrome (PFPS) group ($n = 10$)

| | |
|---|---|
| Legs tested | 6 right legs, 4 left legs |
| Symptom duration | 1 month–1 year ($n = 4$)<br>1–3 years ($n = 4$)<br>6 years ($n = 2$) |
| Anterior knee pain scale | 74.9 ± 13.5 (full function score 100) |
| VAS before and after experiment | 0.1 ± 0.3 (range 0–1) |
| VAS during isometric knee extension task | 3.0 ± 2.7 (range 0–8) |

*VAS* visual analog scale

Values are means ± SD

collection, participants were seated for skin preparation and surface EMG electrode application on the tested thigh.

The dominant leg (leg used for kicking a ball) was tested in the healthy group, and the most painful side was tested in the PFPS group. Four pairs of pre-gelled silver/silver chloride surface electrodes (1.92 × 2.86 cm) with 1.91 cm inter-electrode distances were placed over the muscle bellies of the VM, VMO, VL, and RF following the guidelines of Lieb and Perry (1968) and Rainoldi et al. (2008). The electrodes for the VM were placed 12 cm from the center of the patella, oriented 15° medially to the long axis of the femur. The electrodes for the VMO were placed 5 cm from the center of the patella, oriented 50° medially to the long axis of the femur. For the VL, electrodes were placed 10 cm from the center of the patella, oriented 15° laterally to the long axis of the femur. For the RF, electrodes were placed 15 cm from the center of the patella, oriented 10° medially to the long axis of the femur.

Participants were seated in an adjustable chair with the trunk, pelvis, and thighs fixed with straps to ensure that the isometric knee extension task was performed in an upright sitting position with hips and knees flexed at 90°. The ankle on the tested side was secured with a padded restraint attached to a strain gauge (Entran Sensor & Electronics, Fairfield, NJ).

Participants performed 3–5 3-s isometric knee extension maximal voluntary contractions (MVCs) with verbal encouragement from the experimenter. A 1-min rest was given between contractions to avoid muscle fatigue. MVCs were performed until three similar MVC values were obtained. The average value of the three highest MVCs was used to determine the target forces for the submaximal contractions. The intraclass correlation coefficient [ICC (3, 1)] for the MVCs was 0.998, indicating excellent reliability across the MVCs.

Following the MVCs, the participants performed stable ramp contractions up to 25%, 50%, and 75% MVC in random order. The ramp-up task was performed by tracing a line on a computer screen in front of the participant. Feedback of the knee extension force was provided. The speed of the ramp contractions was 7.5% MVC/s up to the three target forces, with 10-s holds at 25% and 50% MVC and 5-s holds at 75% MVC. One successful ramp-up trial with a smooth force generation was collected for each target force. An average of three trials were attempted for each force level to achieve a successful trial. A 1-min rest was given between ramp contractions to prevent muscle fatigue. Requirements for a successful trial were: (1) Starting point: force onset matched the start point of the template, (2) Slope: the force produced during the ramp-up was aligned to the template, (3) Target force: Force reached the target force at the end of the slope, and (4) Holding: Able to maintain the target force.

The PFPS group was asked to report the location and severity of each occurrence of pain using a visual analog scale (VAS) between contraction trials. The descriptive pain data are shown in Table 2. Surface EMG and force data were recorded in Spike2 (version 5.21, Cambridge Electronic Design, Cambridge, UK) with a sampling rate of 1 kHz.

## Surface EMG and force data reduction

Surface EMG data were high-pass filtered at 10 Hz with a fourth-order recursive Butterworth filter in Matlab (version 2017a, Mathworks, Natick, MA). The EMG differential amplifier achieved common mode rejection for 140 dB at 50/60 Hz and contributed noise of approximately 4 μV at 10–1 kHz (Coulbourn Instruments, Allentown, PA).

For each ramp-up trial to 25%, 50%, and 75% MVC, the EMG onset time and average EMG amplitude during the holding phases for each muscle were determined objectively in Matlab. EMG onset was defined as the time at which the EMG RMS amplitude was 3 SD higher than the average-resting RMS for at least 25 ms (Cowan et al. 2002; Aminaka et al. 2011). The average-resting EMG RMS was calculated from the most stable 100-ms window during rest (Konrad 2005). During the experiment, we monitored muscle activation and started the data collection when the participant was fully relaxed with a steady baseline signal. During analysis, we also checked each 100-ms window visually to avoid

any low-level continuous muscle contraction or movement artifacts.

The DC offset was removed from force signals prior to data collection to remove any resting tension after experimental setup. Force onset was calculated as the force RMS reaching 5 SD higher than the average-resting force RMS. We used 5 SD higher than the baseline noise, because the typical resting noise was very small (0.15% MVC). The EMG onset time for each muscle was standardized to the force onset.

EMG amplitude during the holding phase was the average RMS from the most stable 100-ms window after removing the baseline noise (Konrad 2005). The average EMG RMS from the highest 100-ms window during MVCs was calculated after removing the baseline noise (Konrad 2005). The normalized activation amplitude was presented as percentage of the RMS at holding phase divided by RMS at MVC. The VMO:VL activation ratio was calculated as the normalized activation amplitude of the VMO divided by the normalized activation amplitude of the VL.

### Statistical analysis

Statistical analysis of the surface EMG data was performed with SPSS software (version 25.0) (IBM Corp 2017). Univariate repeated-measures ANOVAs with Bonferroni adjustment for multiple comparisons were used to evaluate differences in onset time, activation amplitude, and VMO:VL ratio. The assumption of sphericity was tested, and Huynh–Feldt epsilon correction was used to adjust the degrees of freedom for the averaged tests of significance. The $\alpha$ level of significance was set a priori at $p < 0.05$.

For onset time, a two-way repeated-measures ANOVA was used to examine the between-subjects effect of sex (healthy males, healthy females) and the within-subjects effect of muscle (VM, VMO, VL, RF) in the healthy individuals, with data pooled from the three force levels. This analysis was used to test the Hypothesis 1. Another two-way repeated-measures ANOVA was used to analyze the between-subjects effect of group (healthy females, PFPS females) and the within-subjects effect of muscle (VM, VMO, VL, RF) on onset time in the female participants with data pooled from the three force levels. This analysis was used to test the Hypothesis 4.

For activation amplitude, a three-way repeated-measures ANOVA was used to examine the within-subjects effects of force (25%, 50%, 75% MVC) and muscle (VM, VMO, VL, RF), and the between-subjects effect of sex in the healthy participants. This analysis was used to test the Hypothesis 2. Another three-way repeated-measures ANOVA was used to analyze the effects of force, muscle, and group on activation amplitude in the female participants. This analysis was used to test the Hypothesis 5.

A two-way repeated-measures ANOVA was used to analyze the effect of force and sex on the VMO:VL ratios, which tested the Hypothesis 3. Another two-way repeated-measures ANOVA was applied for the effect of force and group on the VMO:VL ratios. Two one-way ANOVAs with Fisher's least significant difference post hoc tests were used to analyze the differences in exercise intensity and exercise time across the three groups.

## Results

### Onset time

In the healthy participants, there was an interaction effect between muscle and sex [$F$ (2.51, 0.41) = 3.09, $p = 0.037$] on onset time. Bonferroni pairwise comparisons revealed that male participants had an earlier onset time of the VMO ($p = 0.001$), VL ($p = 0.002$), and VM ($p < 0.001$) than females (357 ± 101 ms, 222 ± 67 ms, and 258 ± 62 ms, respectively, Fig. 1). Hypothesis 1 was supported the onset time of the RF was not significantly different between the two sexes ($p = 0.779$).

In male participants, VL onset was 379 ± 74 ms earlier than RF onset ($p < 0.001$) and 156 ± 51 ms earlier than VM onset ($p = 0.02$). However, VL onset time was not significantly different from VMO onset time ($p = 0.874$). RF onset was also 275 ± 97 ms later than the VMO onset ($p = 0.035$) and 223 ± 80 ms slower than the VM onset ($p = 0.039$). In females, VL onset was 239 ± 73 ms earlier than VMO onset ($p = 0.01$) and 192 ± 53 ms earlier than VM onset ($p = 0.004$) (Fig. 1), which also supporting the hypothesis 1.

For healthy and PFPS female participants, there was a main effect of muscle [$F$ (2.82, 0.96) = 3.60, $p = 0.016$] on onset time, with no difference between groups [$F$ (1, 0.26) = 0.57, $p = 0.455$]. There was no difference in muscle onset time between healthy and PFPS females, proving the hypothesis 4 to be incorrect (Fig. 2). To further examine the main effect of muscle, data from the healthy and PFPS females were pooled for Bonferroni pairwise comparisons. There was a trend for the VL onset to be 278 ± 102 ms earlier than VM onset; however, this difference did not reach statistical significance ($p = 0.051$).

### EMG amplitude

There was no significant main effect of sex or group on EMG amplitude. Therefore, the results did not support Hypotheses 2 and 5. We did not observe a lower VMO activation amplitude in females relative to males (Fig. 3) nor a lower VMO activation amplitude in PFPS females relative to healthy females (Fig. 4).

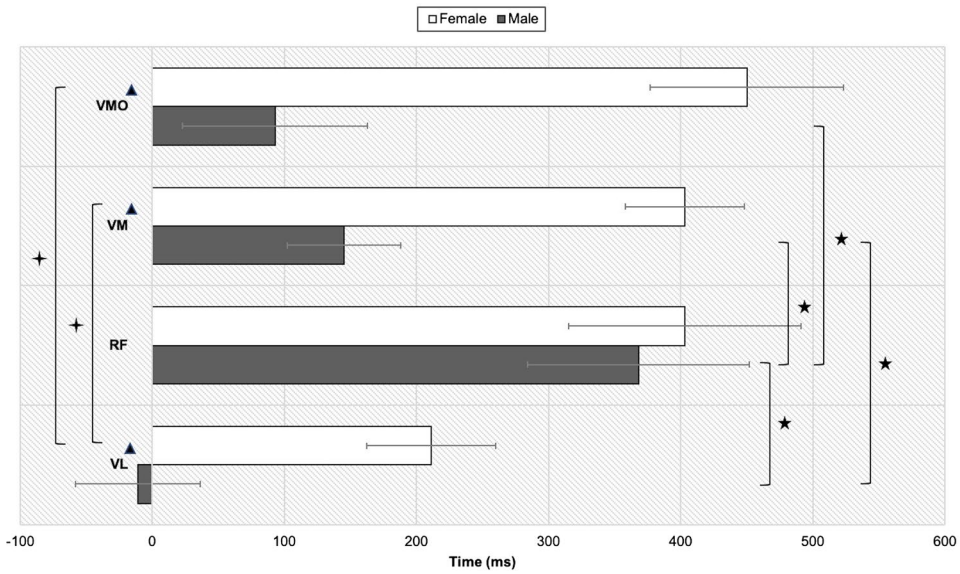


**Fig. 1** EMG onset time of VL, RF, VM, and VMO in healthy males and healthy females. All bars are aligned to the onset of force at zero seconds. Filled triangle indicates a significant difference in EMG onset time between the two sexes. Filled star indicates a significant difference in EMG onset time between two muscles in healthy males. Filled plus indicates a significant difference in EMG onset time between two muscles in healthy females

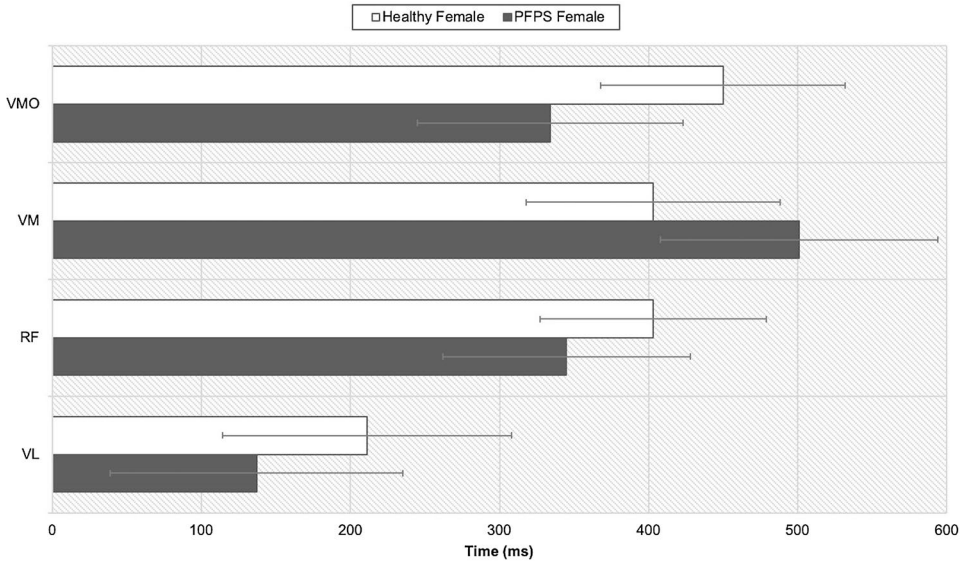


**Fig. 2** EMG onset time of VL, RF, VM, and VMO in healthy and PFPS females. All bars are aligned to the onset of force at 0 s

The data were then pooled from all participants for a two-way repeated-measures ANOVA to assess the possible effect of muscle and force on activation amplitude. There was an interaction effect between force and muscle [$F$ (3.91, 0.05) = 4.33, $p = 0.003$] on normalized EMG amplitude. At the lowest force level (25% MVC), EMG RMS amplitude was 7.0 ± 1.9% EMGmax higher in the VL than in the VMO ($p = 0.004$), 5.6 ± 1.8% EMGmax higher than in the VM ($p = 0.025$), and 8.8 ± 2.2% EMGmax higher than in the RF ($p = 0.001$) (Fig. 5). There was no difference in activation amplitude across the four muscles at 50% and 75% MVC.

## VMO:VL EMG ratio during the holding phases

There was no significant main effect of sex or group on VMO:VL ratios, showing that the results did not support Hypothesis 3. However, there was a main effect of force on the VMO:VL activation ratio during the holding phases [$F$ (1.57, 1.00) = 16.75, $p < 0.001$] with data pooled from all participants. The VMO:VL ratio was greater at higher force levels. The VMO:VL ratio was 0.20 ± 0.04 lower at 25% MVC than at 50% MVC ($p < 0.001$) and 0.29 ± 0.06 lower than at 75% MVC ($p < 0.001$) (Fig. 6). The VMO:VL ratio was not different between 50 and 75% MVC ($p = 0.14$).

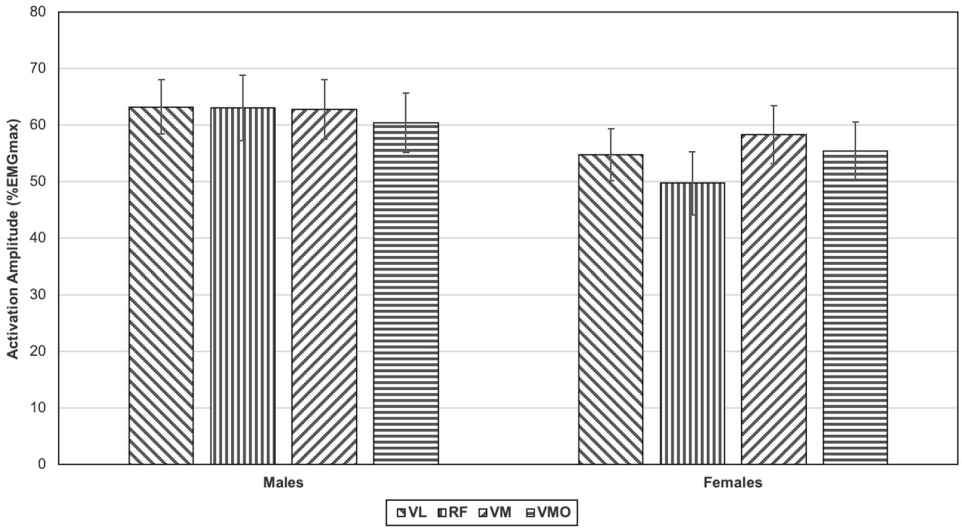


**Fig. 3** Normalized EMG RMS amplitude for the VL, RF, VM, and VMO during the isometric knee extension holding phases for both sexes

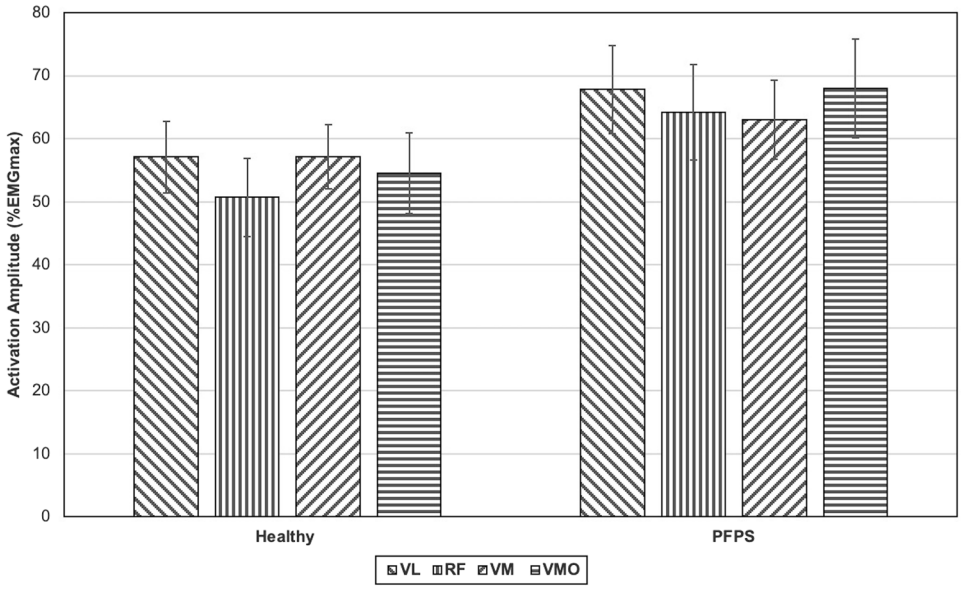


**Fig. 4** Normalized EMG RMS amplitude for the VL, RF, VM, and VMO during the isometric knee extension holding phases for healthy and PFPS groups

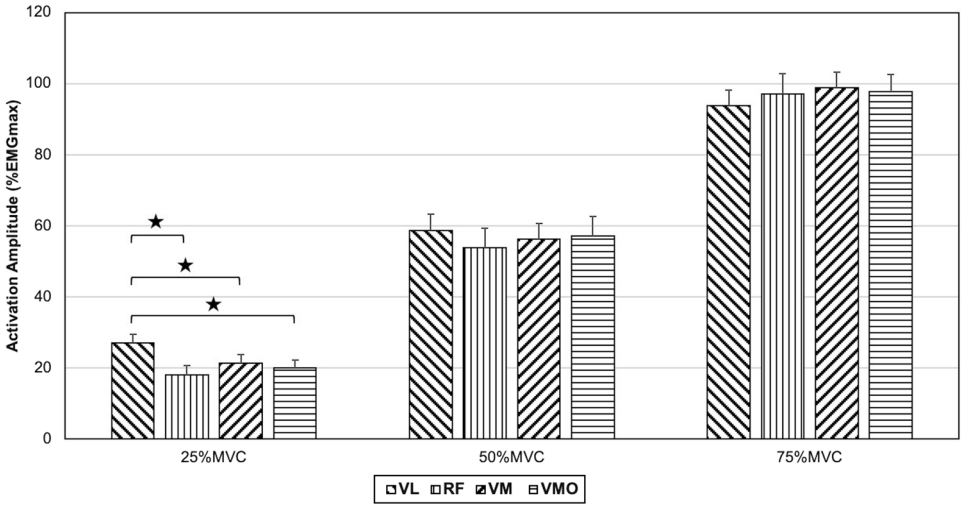


**Fig. 5** Normalized EMG RMS amplitude for the VL, RF, VM, and VMO during the isometric knee extension holding phases of the three force levels with pooled data from all participants. Filled star indicates a significant difference in EMG amplitude between two muscles

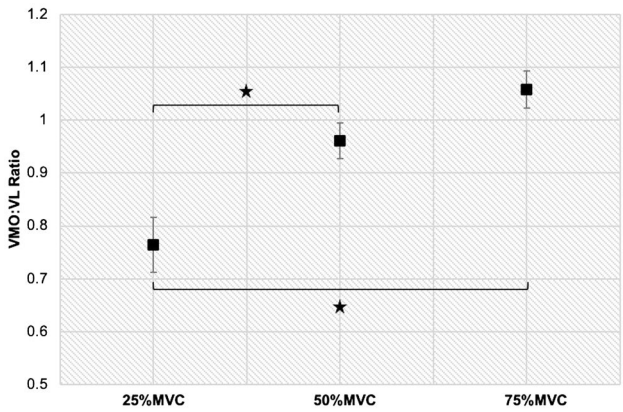


**Fig. 6** VMO:VL normalized activation amplitude ratio during isometric knee extension holding phases at the three force levels with pooled data from all healthy and PFPS participants. Filled star indicates a significant difference in the VMO:VL ratio between two force levels

## Discussion

The primary aim of this study was to evaluate sex differences in recruitment and activation patterns of the superficial quadriceps muscles that could contribute to the higher incidence of PFPS in females. The secondary aim was to evaluate whether the level of force output has an effect on relative quadriceps activation patterns. We found that both asymptomatic and symptomatic females exhibited delayed activation of the vastus medialis complex compared to males. A more equivalent distribution of activation across the vasti muscles occurred at higher force levels.

### Sex differences in quadriceps onset times

In healthy males, VL and VMO onset was earlier than VM and RF onset. Counterbalanced forces pulling on the superior-medial and superior-lateral border of the patella are generated by contraction of the VMO and VL, respectively (Lin et al. 2004). An appropriate activation sequence and comparable activation amplitude of the two muscles ensure patella stability during knee movement (Neptune et al. 2000). Our findings suggest that male participants were able to activate their VMO and VL simultaneously to stabilize the patella while initiating isometric knee extension. However, delayed onset of the VMO and VM relative to the VL was observed in healthy females and in females with PFPS, which indicates an imbalanced muscle control of the patella. Previous examinations of the medial and lateral vasti muscles in asymptomatic knees have reported a similar temporal activation relationship between the VMO and VL (Cowan et al. 2001, 2002; Brindle et al. 2003; Dieter et al. 2014). However, those studies did not evaluate sex differences. The four muscles recorded in female participants were activated after the force onset, which indicates that deeper muscle fibers, such as in the vastus intermedius, contributed to the force initiation.

A few studies have examined sex differences in medial and lateral quadriceps onset times in both healthy individuals and in those with PFPS. Slower medial–lateral quadriceps activation may compromise patellar stability during knee extension initiation and may contribute to the higher incidence of PFPS in females. Sung and Lee (2009) reported that sex differences in muscle onset and activation levels vary with different phases of movement. In comparison with males, females with anterior knee pain exhibited delayed onset of the VM and a lower percentage of VM activation while descending stairs. However, when ascending stairs, females displayed earlier VM onset and equivalent activation levels in comparison with males. Sung and Lee suggested that activation delays observed in the medial quadriceps of females with current knee pain during descending stairs could be a risk factor for knee injuries (Sung and Lee 2009).

Delayed VMO activation is often considered one of the contributing factors to PFPS (McConnell 1996; Boling et al. 2006). However, we did not observe differences in the onset times of the quadriceps muscles in symptomatic versus asymptomatic females. With the symptomatic and asymptomatic females pooled, the VL onset was marginally earlier than VM onset, but the VL onset was not significantly different than the VMO onset. Our results are consistent with the previous studies showing no temporal difference in VMO–VL onset between those with PFPS and asymptomatic individuals (Karst and Willett 1995; Powers et al. 1996; Morrish and Woledge 1997; Gilleard et al. 1998; Laprade et al. 1998; Owings and Grabiner 2002; Cavazzuti et al. 2010). A few studies have evaluated the influence of physical activity level of the participants. Although not statistically significant, the healthy female sample in the present study had a tendency to be more sedentary than the PFPS group (Table 1). Briani et al. (2016) found that females with PFPS who participated in intense physical activity showed a delayed VMO onset when ascending stairs, whereas the females with PFPS who engaged in moderate physical activity showed no delay in VMO onset (Briani et al. 2016).

### Muscle force level and relative quadriceps activity

A more balanced muscle activation pattern among the quadriceps sub-portions was observed during the holding phases at higher force levels in comparison with the lowest force level regardless of sex and group. When isometric knee extension was maintained at the lower force level of 25% MVC, the VL displayed a higher activation amplitude relative to the RF, VM, and VMO. The VL contains the largest cross-sectional area in the quadriceps (Narici et al. 1989), and it is essential to power production during knee

extension (Farahmand et al. 1998). The earlier onset of the VL relative to other quadriceps muscles observed in both healthy and PFPS participants indicates a preferential activation of the VL during isometric knee extension.

With increasing force levels, muscle activation amplitude increased as expected, and the activity was more evenly distributed over the quadriceps muscles. Rainoldi et al. (2008) and Spairani et al. (2012) found that various submaximal contraction levels of isometric knee extension force had no effect on the activation patterns across the VL, VM, and VMO in a healthy population. They reported higher absolute activity of the VMO compared with the VM in isometric knee extension at 10% and 60% MVC (Spairani et al. 2012) and at 60% and 80% MVC (Rainoldi et al. 2008). The differences between these findings and those of the present study may be due to different data reduction methods between absolute and normalized EMG amplitude (Martinez-Valdes et al. 2018). However, during walking, a low-force functional activity, Spairani et al. (2012) found that the VL exhibited a higher activation amplitude relative to the VMO and RF throughout different phases of level walking in individuals with PFPS (Spairani et al. 2012). This is consistent with our findings, suggesting a preferential VL recruitment during low-force contractions.

The VMO:VL ratio was lowest at 25% MVC, in comparison with the higher force levels. With increasing target force, the activation amplitude of the VMO increased. We found that a 50% MVC force level best facilitated an equivalent medial–lateral quadriceps activation. The 75% MVC force level proved to be difficult for participants to maintain for more than 10 s. Thus, moderate levels of force production should be considered for rehabilitation programs for those with PFPS.

## Conclusion

In healthy males, the VL and VMO were activated together prior to the VM and RF during isometric knee extension. Asymptomatic females showed a delayed onset of the VMO relative to the VL, and they also had delayed onsets of the VL, VMO, and VM in comparison with asymptomatic males. This suggests a possible higher risk of knee pain or injury for healthy females than for males. An imbalanced medio-lateral quadriceps muscle activation with a lower VMO:VL ratio was observed at 25% MVC. Isometric knee extensions at 50% MVC are recommended to generate a more evenly distributed quadriceps activation and a higher VMO:VL ratio.

**Author contributions** LG and YP conceived and designed research. YP conducted experiments and analyzed the data. LG and AJ assisted with data interpretation. All authors read and approved the manuscript.

**Funding** None.

**Availability of data and material** Yes.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Code availability** Yes.

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