Peak triceps surae muscle activity is not specific to knee flexion angles during MVIC

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Peak Triceps Surae Muscle Activity Is Not Specific to Knee Flexion Angles During MVIC

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Abstract
There is limited research on peak activity of the separate triceps surae muscles in select knee flexion (KF) positions during a maximum voluntary isometric contraction (MVIC) used to normalize EMG signals. The aim of this study was to determine how frequent peak activity occurred during an MVIC for soleus (SOL), gastrocnemius medialis (GM), and gastrocnemius lateralis (GL) in select KF positions, and if these peaks were recorded in similar KF positions. Forty-eight healthy individuals performed unilateral plantar-flexion MVIC in standing with 0°KF and 45°KF, and in sitting with 90°KF. Surface EMG of SOL, GM, and GL were collected and processed in 250 ms epochs to determine peak root-mean-square amplitude. Peak activity was most frequently captured in standing and rarely in sitting, with no position selective to SOL, GM or GL activity. Peak GM and GL activity was more frequent in 0°KF than 45°KF, and more often in similar KF positions than not. Peak SOL activity was just as likely in 45°KF as 0°KF, and more in positions similar to GM, but not GL. The EMG amplitudes were at least 20% greater in positions that captured peak activity over those that did not. The overall findings support performing an MVIC in more than one KF position to normalize triceps surae EMG. It is emphasized that no KF position is selective to SOL, GM, or GL alone.

Keywords
Electromyography, Maximum voluntary isometric contraction, Triceps surae muscles, Normalization, Ankle plantar-flexion, Heel-raise

1. Introduction
Normalization of electromyography (EMG) signals is important for the valid and reliable interpretation of EMG data (De Luca, 1997, Lehman and McGill, 1999, Yang and Winter, 1984). Eliciting a maximum voluntary isometric contraction (MVIC) to normalize EMG is considered standard research practice and the strategy is suggested to increase the accuracy and replication of EMG data in dynamic tasks (Knutson et al., 1994). Furthermore, this normalization process is advocated as reliable for comparing and reducing the between-subject variability of EMG signals, and validated for results interpretation (Burden, 2010). Recently, muscle-specific positions used for normalization to an MVIC have undergone scrutiny which has resulted in concerns and the call for further research on the efficiency and specificity of given positions to solicit peak activity from select muscles (Carvalho et al., 2010, Chopp et al., 2010, Vera-Garcia et al., 2010).

In a recent study of 12 positions used to acquire an MVIC to normalize the EMG signals from shoulder muscles, Chopp et al. (2010) found that some positions captured the peak activity of several muscles simultaneously, whereas only specific muscles were identified in other positions. Vera-Garcia et al. (2010) determined that no one position was optimal for generating peak muscular activity of specific trunk muscles, and recommended that several positions be employed to determine MVIC values for normalization. Utilizing several knee flexion (KF) positions to normalize the EMG signals of the triceps surae (TS) muscles has been described and recommended for many years (Perry et al., 1981) as the TS is comprised of three anatomically and apparently functionally distinct components: soleus (SOL), gastrocnemius medialis (GM), and gastrocnemius lateralis (GL) (McGowan et al., 2009, Neptune et al., 2001, Sasaki and Neptune, 2006). GM and GL are bi-articular, insert above the knee, are more influenced by change in KF than SOL which is mono-articular and inserts below the knee. To acquire peak EMG activity of the separate TS muscles during an ankle plantar-flexion MVIC, researchers traditionally follow the anatomical premise that increased KF places the bi-articular GM and GL at mechanical disadvantage and hence encourages peak activity from the mono-articular SOL (Cresswell et al., 1995, Kennedy and Cresswell, 2001, Signorile et al., 2002). When adhering to classic manual muscle testing procedures (Clarkson, 2000, Heers et al., 2003, Hislop and Montgomery, 2007, Perry et al., 1981), a straight-leg KF position (0°) in standing is used to target GM and GL, whereas a bent-leg KF position in standing
(40–45°) or in sitting, prone, or 4-point-kneeling (90°) is used for SOL. While the inferred TS muscle-selectivity of KF positions has construct validity primarily based on anatomical and physiological bases, with the EMG literature indicating the potential for TS muscle-specificity by varying KF (Arampatzis et al., 2006, Bogey et al., 2003, Carlsson et al., 2001, Cresswell et al., 1995, Heers et al., 2003, Miyamoto and Oda, 2003, Perry et al., 1981, Wakahara et al., 2007), there is only limited research on peak activity of the individual TS muscles during an MVIC performed in select KF positions.

Perry et al. (1981) compared selective TS muscle activity during an MVIC as performed in manual muscle testing and found that a standing heel-raise in 0°KF and 40°KF maximally activated GM and SOL, respectively; but that the KF degree was not selective to either muscle. Carlsson et al. (2001) differed from the classic manual muscle assessment procedures by using isokinetic equipment rather than external manual resistance, and compared selective TS activity in standing with 0°KF, prone with 0°KF, and sitting with 90°KF. Like Perry et al. (1981), the highest GM activity was found in standing with 0°KF, as was SOL’s. These results contrast with the commonly employed EMG normalization and clinical assessment strategies using 90°KF for SOL (Abbiss et al., 2010, Rudolph and Snyder-Mackler, 2004), as well as research that reports preferential facilitation of the muscle with increasing KF (Cresswell et al., 1995, Tamaki et al., 1997), especially at 90°KF (Signorile et al., 2002).

Although the studies by Perry et al., 1981, Carlsson et al., 2001 provide fundamental knowledge on select TS activity at different KF angles during an MVIC, there is a lack of consideration of GL and of within-subject variability with the possibility that select KF positions for one TS muscle could elicit maximum activity in the other muscle as well (Carlsson et al., 2001, Perry et al., 1981). Instead, the amount of activity in select KF positions are traditionally averaged across participants and subsequently compared to determine each positions’ effectiveness to generate the highest activity level. None of the literature to date states how often the various positions actually generate peak SOL, GM, or GL activity in individual participants (Arampatzis et al., 2006, Bogey et al., 2003, Carlsson et al., 2001, Cresswell et al., 1995, Heers et al., 2003, Miyamoto and Oda, 2003, Perry et al., 1981, Wakahara et al., 2007).

There is a dearth of research exploring whether select KF positions consistently capture specific peak SOL, GM, and GL activity during an MVIC; or whether their individualized maxima sometimes occur in other KF positions, too.

The purpose of this study was to determine whether an MVIC of the TS muscles acquired by a maximal unilateral heel-raise performed in: (1) standing 0°KF, (2) standing 45°KF and (3) sitting 90°KF; was selective to a TS muscle and significantly different between positions, and if the specific peak EMG amplitudes of each muscle were captured in similar positions. Specifically, this required the study to: establish the KF position that most frequently elicited peak SOL, GM, and GL amplitudes during an MVIC; calculate the difference between the EMG amplitudes captured in the KF positions eliciting peak activity to those that did not; and verify if positions that elicited the peaks for one TS muscle was more likely to elicit the peaks from the other two. It was hypothesized that during plantar-flexion MVIC testing performed against similar loads (1) peak GM and GL activity would be acquired more often in 0°KF than 45°KF, and rarely in 90°KF; (2) peak SOL activity would be more frequent in 45°KF and 90°KF than 0°KF; and (3) peak GM and GL activity would be captured in similar KF positions, whereas SOL would not. Secondary aims were to estimate if the number of MVIC trials performed influenced which trial evoked maximal muscle activity, if the presence of cross-talk between the TS muscles was significant, and if testing order, sex, age group, body-mass index category, and physical activity level influenced which position captured peak TS activity.
2. Methodology

2.1. Experimental design
A repeated-measures design was used and the study required participants to attend one session in a university EMG-biomechanics laboratory at the Centre for Physiotherapy Research. The research was approved by the University of Otago Human Ethics Committee.

2.2. Participants
A total of 48 participants were recruited and their demographics are presented in Table 1. Individuals were included if they reported being in good general health and were excluded if they had a current or previous injury to the Achilles tendon or TS muscles, or a medical condition that could limit their ability to perform and sustain a maximal unilateral heel-raise. Equal numbers of males and females from both a younger (18–25 years) and an older (35–45 years) population were targeted in the study design to consider the effect of sex and age on muscle function. The age ranges were selected to designate groups at relative low and high risk of TS pathology and Achilles tendon disorders, respectively (Baur et al., 2004, Leppilahti and Orava, 1998, Mademli and Arampatzis, 2008, Tumilty, 2007). Volunteers were recruited by word of mouth, selected e-mail distribution lists, and poster-board advertisements placed within the local community. All participants provided written informed consent prior to testing and were familiarized with all experimental procedures.
Table 1. Means (95%CI) and ratios of the demographic characteristics of participants by age-and-sex group. BMI ratios (Under:Normal:Over:Obese) are from the World Health Organization. Activity ratios (High:Moderate:Low) are from the International Physical Activity Questionnaire. Footedness ratios (Left:Right) are from the Dunedin Footedness Inventory. YM: younger males; YF: younger females; OM: older males; OF: older females; and BMI: Body mass index.

<table>
<thead>
<tr>
<th>Participants (n)</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>BMI (ratio)</th>
<th>Activity (ratio)</th>
<th>Footedness (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YM (n = 12)</td>
<td>22.4 (21.2, 23.5)</td>
<td>177.4 (173.9, 181.0)</td>
<td>71.7 (65.2, 78.2)</td>
<td>22.7 (21.4, 24.0)</td>
<td>0:11:1:0</td>
<td>8:4:0</td>
<td>1:11</td>
</tr>
<tr>
<td>YF (n = 12)</td>
<td>22.7 (21.4, 24.0)</td>
<td>165.1 (162.4, 167.7)</td>
<td>61.1 (54.3, 67.9)</td>
<td>22.4 (20.2, 24.6)</td>
<td>1:7:4:0</td>
<td>4:8:0</td>
<td>0:12</td>
</tr>
<tr>
<td>OM (n = 12)</td>
<td>41.1 (39.1, 43.0)</td>
<td>177.7 (173.5, 181.8)</td>
<td>81.7 (72.2, 91.2)</td>
<td>25.9 (23.0, 28.8)</td>
<td>0:5:4:3</td>
<td>6:5:1</td>
<td>2:10</td>
</tr>
<tr>
<td>OF (n = 12)</td>
<td>41.5 (39.4, 43.7)</td>
<td>166.5 (161.3, 171.7)</td>
<td>66.6 (60.1, 73.2)</td>
<td>24.1 (21.8, 26.4)</td>
<td>0:7:5:0</td>
<td>5:7:0</td>
<td>1:11</td>
</tr>
<tr>
<td>All (n = 48)</td>
<td>31.9 (29.1, 34.8)</td>
<td>171.7 (169.2, 174.1)</td>
<td>70.3 (66.3, 74.3)</td>
<td>23.8 (22.7, 24.8)</td>
<td>1:30:14:3</td>
<td>23:60:1</td>
<td>4:44</td>
</tr>
</tbody>
</table>
The EMG signal of the lower-limb muscles has been shown to be influenced by footedness (Valderrabano et al., 2007), level of physical activity (Behm and St-Pierre, 1998, Larsson et al., 2006), and body-mass composition (Caruso et al., 2005, Lowery et al., 2002, Nordander et al., 2003). Therefore, the Dunedin Footedness Inventory was used to determine if participants were left or right footed (Schneiders et al., 2010); the self-administered short-form International Physical Activity Questionnaire to classify levels of physical activity as low, moderate, or high (Craig et al., 2003, Sjöström et al., 2005); and the body-mass index (BMI) categories recommended by the World Health Organization to categorize participants’ as under, weight of normal weight over-weight, or obese (WHO, 2000, WHO, 2004).

2.3. Maximum voluntary isometric contraction

2.3.1. Positions

The activity of the TS muscles during MVIC trials were collected from the dominant lower-limb during a unilateral heel-raise at maximal height. Since TS muscle activity during dynamic tasks may be influenced by foot placement (Aruin, 2006, Ribeiro et al., 2007, Riemann et al., 2011) and type (Murley et al., 2009), participants served as their own controls and performed all MVIC trials using their habitual and most comfortable weight-bearing foot position. Trials were captured at end-range of plantar-flexion to standardize ankle range of motion between subjects and trials. Three MVIC trials were performed at end of active plantar-flexion range in the three following positions:

(1) Standing 0°KF
(2) Standing 45°KF
(3) Sitting 90°KF

The selected positions were chosen based on common manual muscle assessment procedures used in musculoskeletal clinical practice (Clarkson, 2000, Hébert-Losier et al., 2009, Hislop and Montgomery, 2007), advocated strength training protocols (Alfredson et al., 1998, Signorile et al., 2002), and methods used in research to acquire EMG normalization values from the individual TS muscles during an MVIC (Abbiss et al., 2010, Heers et al., 2003, Perry et al., 1981). The order of KF position testing was block randomized within sex-by-age groups before participant recruitment and was allocated to participants as they were tested. The process accounted for the possible effects of testing order.

A long-arm goniometer (Fred Sammons Inc., Bissell Healthcare Corporation, IL, USA) was used by the examiner (KH-L) to position the participants’ knee following standard goniometry guidelines (Clarkson, 2000; Reese et al., 2010), where the parallel alignment of the femur and tibia determined 0°KF. An electromechanical goniometer (Noraxon USA Inc., Scottsdale, AZ) was also fixed to the lateral aspect of participants’ bare limb using double-sided tape with the fulcrum aligned with the knee-joint center, the proximal arm with the greater trochanter, and the distal arm with the lateral malleolus. Angular readings were displayed in real-time on a computer screen to provide a visual feedback to participants and to allow the examiner to monitor KF position during an MVIC trial.

2.3.2. Protocol

Each individual completed three trials per KF position for a total of nine MVIC trials. Each MVIC lasted approximately 7 s and included 2 s of ramped-effort, 3 s of stable maximum isometric-hold, and 1–2 s of gradual decreased-effort; this was followed by a 2 min rest period. An MVIC trial was repeated if the select KF position was not maintained within a ±2.5° range. All participants were given individualized and standardized training, instructions, and practice trials until individuals were comfortable and the examiner determined that MVIC performance was acceptable according to MVIC standards (Merletti et al., 1999). Prior to testing, an appropriate
rest period was allocated to eliminate any muscle fatigue which may have occurred during the familiarization process.

2.3.3. Testing apparatus
To optimize participant safety and obtain a near-maximal loading of the anti-gravitational TS muscles during the MVIC trials, a Smith machine (Multipower M053 Technogym®; Gambettola, Italy) with a counter-balanced floating barbell system was used. The machine allowed the vertical displacement of free weights on a barbell, which was secured within two lateral steel uprights. A series of short horizontal posts extending from the lateral uprights allowed fixation of the barbell at various heights and also permitted the placement of security blocks at lower levels to prevent the weighted-barbell from falling below a predetermined height. All participants were familiarized with the security features and the safe-use of the equipment prior to use.

In the two standing positions (0°KF and 45°KF), participants rested the barbell of the Smith machine over the upper scapular and trapezius regions of their shoulders; and in sitting (90°KF), over the distal end of their femurs (Abbiss et al., 2010, Heers et al., 2003, Kendall et al., 2005). Extra padding was added if the bar pressure caused discomfort to participants. The amount of weight added to the barbell was adjusted to equate to the maximal load which an individual could lift and sustain in each position.

2.3.4. Instructions
For each MVIC, participants were instructed to “push the barbell upwards using both legs”, to “lift their heels as high as possible”, to “transfer their body-weight to their dominant leg”, to “adjust their knee flexion position” according to the test position, to “maintain the knee position and the heel at maximum height”, and to “push/resist as hard as they could” while the examiner applied an additional downwards resistance through the barbell. The instructions were standard for all individuals as was the verbal encouragement to reach maximum contraction levels during each MVIC trial.

2.4. Instrumentation
2.4.1. Electromyography
The EMG signals of SOL, GM, and GL were recorded by surface electrodes. Skin preparation and electrode positioning followed the SENIAM (Hermens et al., 1999) and ISEK (Merletti and Torino, 1999) recommendations. Participants’ skin was shaven, lightly abraded, and cleaned with alcohol swabs to reduce tissue impedance and electrode-to-skin artefacts (Ball and Scurr, 2010, Winter, 1991). To correctly position the electrodes, all muscle bellies were first identified by palpation while under contraction. Paired Ag–AgCl gelled-electrodes (Ambu® Blue Sensor SP) were then positioned on the relaxed muscle bellies of: (1) SOL, on the lower medial third of the posterior leg, approximately two-thirds of the distance between the medial femoral condyle and medial malleolus; (2) GM, just distal to the knee on the upper-third of the posterior leg and over the maximal medial girth; and (3) GL, similar to GM, but on the maximal lateral girth. All paired-electrodes were trimmed to reduce the inter-electrode distance to <20 mm and were oriented parallel to the underlying muscle fibers. A single ground electrode was positioned over the tibial tuberosity. To measure skin impedance, a multimeter (Fluke 70 series II®, Tequipment.NET™, Long Branch, NJ) was used. The skin preparation process was repeated until an impedance level of <3000 Ω was measured at all electrode sites (Konrad, 2005). Surface EMG signals were collected at a sampling rate of 3000 Hz using a Noraxon TeleMyo 2400 T G2™ and MyoResearch XP Master Edition Software™ package (Noraxon USA Inc., Scottsdale, AZ). The telemetered system applied a band-pass filter of 10–500 Hz; had an input impedance >100 MΩ; a common mode rejection ratio >100 dB; a baseline AC noise <1 μV rms; an input range ±3.5 mV; and a gain of 1000. The data were transmitted and digitized with 16-bit resolution for all analog inputs using a series of 30 data-points for the zero-correction of all acquired signals. Prior to testing, participants performed active plantar-flexion contractions to verify the EMG signals collected. If artefacts were observed, the EMG equipment installation was readjusted before acquiring MVIC data.
2.4.2. Experimental procedures
All participants attended a single session for testing. The examiner recorded the age, height, and weight of participants on the day of testing. Participants then completed the self-administered physical activity questionnaire, were assessed for footedness, and were familiarized with all testing equipment, procedures, and protocols. After MVIC training, the EMG electrodes were positioned and the acquired signals were verified. According to the allocated random testing order of KF positions, the examiner adjusted the weight of the barbell to the established individualized near-maximum load and placed participant's knee according to the requirements of the test position. The standardized MVIC instructions where provided, with the additional instruction to maintain the KF angle displayed on the computer monitor. All three MVIC trials in a select KF position were performed before proceeding to the next KF testing position.

2.5. Data analysis
2.5.1. Data processing
The EMG signals collected during a stable 3 s maximum isometric-hold period of each MVIC trial were processed to determine root-mean-square (rms) amplitudes (μV) in 250 ms epochs from the signal of each TS muscle. The MVIC trials that elicited the greatest rms amplitude from SOL, GM, and GL, respectively, were retained for subsequent analyses.

A cross-correlation processing technique was used to determine whether the EMG signals collected from one TS muscle contained cross-talk from the other two (Lowery et al., 2003, Winter et al., 1994). Cross-talk was assessed over the 3 s isometric-hold of all the trials eliciting the peak EMG amplitude of any muscle; and considered negligible when the absolute values of the mean and peak coefficients were <.30 (De Luca, 1997, Vink et al., 1989, Winter et al., 1994).

2.5.2. Statistical analysis
Means and 95% confidence intervals (95%CI) were used to report results from frequencies and odd ratios computations. Contingency tables with χ²-test were used to determine if the number of times a KF position, and a trial within a position, elicited the peak amplitudes for each TS muscle and was different. The amount of difference in the EMG amplitudes recorded in KF positions that captured peak activity compared to those that did not was also investigated. Population-averaged logistic regressions were employed to determine if the position which elicited the peak amplitude in one TS muscle also elicited the peak amplitude in the other two muscles. The influence of testing order, sex, age group, BMI category and physical activity level on the KF position that generated peak TS muscle activity was analyzed via stepwise multinomial logistic regressions. All analyses considered the repeated-measures design of the study.

The significance level was set at $p \leq .05$ prior to all analyses that were performed using the statistical software package R™ version 2.11.1.

3. Results
3.1. Peak EMG amplitudes of the triceps surae muscles
3.1.1. Frequency of peaks in different KF positions
The number of times each KF position elicited the greatest amplitude for one of the TS muscles during a plantar-flexion MVIC is also detailed (Table 2). The frequency by which the positions generated the greatest amplitudes was significantly different in all three TS muscles ($p < .001$ in Table 2). Positions 0°KF, 45°KF and 90°KF, respectively, provided 75%, 21%, and 4% of the greatest amplitudes recorded for GM; 65%, 27%, and 8% for GL; and 42%, 56%, and 2% for SOL. The sitting 90°KF position only generated 5% of the greatest EMG amplitudes when considering all the three muscles compared to 95% which were from the two positions in standing (0°KF
and 45°KF). Further analyses between the two standing positions demonstrated that while 0°KF compared to 45°KF more frequently provided peak amplitudes for GM (p < .001) and for GL (p = .007), no difference was observed for SOL (p = .307). An additional table comparing the probabilities of 45°KF and 90°KF to elicit peak TS muscle amplitudes compared to 0°KF is provided in the supplementary files (see Supplementary Table 1). The odds of peak GM and GL activity in 0°KF were 3.6 (1.8, 7.3; p < .001) and 2.4 times (1.3, 4.6; p = .007) higher than in 45°KF; with 45°KF 1.4 times (.8, 2.4; p = .307) more likely than 0°KF to capture peak SOL activity, although not reaching significance.

Table 2. Frequency (n) and percent (%) of total participants where each knee position elicited peak triceps surae muscle activity. Frequencies were compared between KF position, with the level of significance (p) reported comparing frequencies to the 0°KF position ($\chi^2$). SOL: soleus; GM: gastrocnemius medialis; GL: gastrocnemius lateralis; and KF: knee flexion.

<table>
<thead>
<tr>
<th>Position</th>
<th>SOL (%(n))</th>
<th>GM (%(n))</th>
<th>GL (%(n))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°KF</td>
<td>20 (42%);--</td>
<td>36 (75%);--</td>
<td>31 (65%);--</td>
</tr>
<tr>
<td>45°KF</td>
<td>27 (56%); p = .307</td>
<td>10 (21%); p &lt; .001</td>
<td>13 (27%); p = .007</td>
</tr>
<tr>
<td>90°KF</td>
<td>1 (2%); p &lt; .001</td>
<td>2 (4%); p &lt; .001</td>
<td>4 (8%); p &lt; .001</td>
</tr>
</tbody>
</table>

3.1.2. Amplitude of EMG activity in KF positions not capturing peak activity

The EMG amplitude recorded in the KF position that elicited peak activity during an MVIC was significantly greater than the amplitudes recorded in the two other positions, for all TS muscles (p < .001 in Table 3). When comparing positions to the one that captured peak activity, EMG amplitudes were approximately 19% and 45% lower in KF positions that recorded the second highest and lowest levels of EMG activity for a given TS muscle, respectively.

Table 3. Percent (95%CI) with level of significance (p) of triceps surae muscle activity in a KF position when compared to the KF position that recorded the peak activity ($\chi^2$). SOL: soleus; GM: gastrocnemius medialis; GL: gastrocnemius lateralis; and KF: knee flexion. Position 1: KF position with peak EMG activity; Position 2: KF position with second highest EMG activity; Position 3: KF position with lowest EMG activity.

<table>
<thead>
<tr>
<th>Position by amplitude</th>
<th>SOL (% of peak)</th>
<th>GM (% of peak)</th>
<th>GL (% of peak)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position 1</td>
<td>100;--</td>
<td>100;--</td>
<td>100;--</td>
</tr>
<tr>
<td>Position 2</td>
<td>80.8 (77.0, 84.7); p &lt; .001</td>
<td>81.5 (77.2, 85.7); p &lt; .001</td>
<td>82.0 (78.5, 85.5); p &lt; .001</td>
</tr>
<tr>
<td>Position 3</td>
<td>48.4 (43.5, 53.2); p &lt; .001</td>
<td>55.5 (50.4, 60.5); p &lt; .001</td>
<td>63.5 (59.2, 67.9); p &lt; .001</td>
</tr>
</tbody>
</table>

3.1.3. Relationship between the peak EMG amplitudes of the TS muscles and KF positions

A population-averaged logistic regression model was used to determine if a position that captured peak amplitude for one TS muscle also captured the peaks of the other two muscles, interaction considered. The interaction between the other two TS muscles had no significant effect and was therefore removed from the model. Subsequent regression analyses demonstrated that if a position recorded peak SOL activity, the position was 3.2 times (1.2, 8.7; p = .02) more likely to record peak GM activity also, but only 1.2 times (.5, 2.8; p = .711) for GL. However, if a position captured peak GM activity, the odds for that position to also record peak GL activity increased 8.6-fold (3.2, 23.1; p < .001).

3.1.4. Influence of confounders, number of trials and cross-talk

Stepwise multinomial regression analyses demonstrated that testing order, sex, age group, BMI category, and physical activity level had no significant influence on which KF position generated peak TS muscle amplitude. Furthermore, peak amplitudes were derived almost equally from the first, second, and third MVIC trial.
performed within a select KF position; with no significant difference between the trials for SOL ($p = .724$), GM ($p = .157$), or GL ($p = .216$) (Table 4). The presence of cross-talk in the EMG data was also determined to be not significant for any of the muscles, and all the average and all the peak absolute cross-correlation coefficients were below the .30 threshold. Details are provided as supplementary data (see Supplementary Table 2).

Table 4. Frequency (n) and percent (%) of total participants where the first, second or third trial within a select position elicited peak triceps surae muscle activity. Frequencies were compared between trials, and no significant differences between trials were determined ($\chi^2; p \geq .05$). The level of significance ($p$) of the frequencies between the second and third trials compared to the first is reported ($\chi^2$). SOL: soleus; GM: gastrocnemius medialis; and GL: gastrocnemius lateralis.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Muscle (% of (n))</th>
<th>SOL</th>
<th>GM</th>
<th>GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>16 (33%);—</td>
<td>22 (46%);—</td>
<td>12 (25%);—</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>18 (38%); $p = .732$</td>
<td>12 (25%); $p = .086$</td>
<td>17 (35%); $p = .353$</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>14 (29%); $p = .715$</td>
<td>14 (29%); $p = .182$</td>
<td>19 (40%); $p = .209$</td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion
The overall findings of this research support the use of different KF positions, in standing, to acquire peak activity of the three TS muscles during a plantar-flexion MVIC; while highlighting that no one position selectively elicits the peak amplitude of any given TS muscle, with sitting being not effective. In accordance with our first and third hypotheses, the peak amplitudes of GM and GL were more frequently generated in 0°KF, rarely in 90°KF, and more likely to be elicited in the same KF position than not. However, our second hypothesis was not accepted in that 90°KF was not effective in capturing the peak amplitudes of SOL, and 0°KF was just as effective as 45°KF for this purpose.

A plantar-flexion MVIC performed in open-chain (e.g., prone, supine, and 4-point-kneeling) is reported to generate lower levels of TS muscle activity than in closed-chain (e.g., standing or sitting) (Carlsson et al., 2001, Carlsson et al., 2003). Like Carlsson’s et al. (2001) results, our data indicate that within a closed-chain configuration, sitting is not as effective as standing for acquiring maximal EMG activity of the TS muscles, even at equivalent external loads. Only 5% of the peak EMG amplitudes of the three TS muscles were recorded in sitting with 90°KF. This position is commonly employed in research to normalize the EMG signal of SOL (Abbiss et al., 2010, Blackburn et al., 2008, Sousa et al., 2007); but standing appears to be a better alternative for all the TS muscles, SOL included.

Employing more than one KF position for normalizing TS muscle EMG to a plantar-flexion MVIC has been described in research methods for at least 30 years (Mizner and Snyder-Mackler, 2005, Perry et al., 1981), and is shown to influence the relative activity of SOL, GM, and GL (Cresswell et al., 1995). Furthermore, using two KF positions is common practice in everyday assessment, rehabilitation, conditioning, and strengthening of the distinct TS muscles (del Porto et al., 2010, Magnussen et al., 2009). Despite this underlying knowledge and widespread clinical use, many studies still employ only one KF position for the simultaneous acquisition of EMG normalization values for all three TS muscles, which range from 0°KF to 120°KF (Ball and Scurr, 2010, Ochala et al., 2005, Simoneau et al., 2009). Our results indicate that employing at least two KF angles in standing is more effective and accurate than just using one in standing to determine peak EMG amplitudes of the TS. Although peak GM and GL activity was more frequent in 0°KF, more than 20% was collected in 45°KF; whereas the greatest SOL amplitude was elicited almost equally in 0°KF (42%) and 45°KF (56%).

Considering that this study investigated only two specific KF positions in a standing, we consulted published works that had also used standing heel-raise to acquire an MVIC of the TS muscles in an attempt to determine
an optimal set of KF angles. This provided insight on TS muscle activity in standing with 0°KF (Ball and Scurr, 2010, Heers et al., 2003, Kasahara et al., 2007, Perry et al., 1981), 20°KF (Jönhagen et al., 2009), 40°KF (Perry et al., 1981), and 60°KF (Bogey et al., 2003); however, combining and comparing results across studies was not feasible given the heterogeneity in research aims, procedures, equipment, normalization methods, and/or type of EMG processing techniques. Although we cannot offer a gold standard set of KF angles, our results still recommend performing MVIC trials in at least two KF angles in standing rather than in sitting; one near 0°KF and the other near 45°KF, which appears to optimize the acquisition of peak EMG amplitudes for SOL, GM, and GL. Moreover, most published research that use more than one KF angle during an MVIC assumed that peak SOL activity was only captured in greater KF, peak GM and GL in lesser KF, and did not consider a crossover (Heers et al., 2003, Hsu et al., 2006, Mizner and Snyder-Mackler, 2005, Rudolph and Snyder-Mackler, 2004). Our findings indicate that the peak amplitudes from all three TS muscles can occur in any given KF position during an MVIC trial in standing, and therefore no assumptions should be made in regards to KF specificity towards individualized peak TS muscle activity. We recommend that the EMG of SOL, GM, and GL during an MVIC collected in standing be processed from all KF positions used, with the peak amplitude subsequently employed for their individualized normalization (Winter, 1991). The common utilization and standardization of this systematic approach for normalizing individual TS muscle to an MVIC in EMG research would contribute towards enhancing the general scientific merit of EMG results by increasing cross-study data comparisons; promoting reliability, coherent validity, inferential, and inductive reasoning; as well as facilitating meta-analysis (Borenstein and Hedges, 2009, Holloway and Todres, 2003).

As hypothesized, a position that elicited peak activity in GM was more likely to also elicit peak activity in GL rather than SOL. The bi-articular GM and GL have complementary roles in stabilizing the knee and accelerating the lower-limb during gait (Ferri et al., 2003, Fiebert et al., 2000). The mono-articular SOL has a more pronounced role in postural stability and body-weight support with less dynamic influence at the knee. The higher probability for a KF position to elicit peak activity in GM and GL over SOL reflects the muscles’ respective anatomical configurations and functional roles (Sasaki and Neptune, 2006). Rationalizing why peak SOL activity was more likely to be in the same KF position than GM but not GL is more complicated: However, studies on the effect of load (Seynnes et al., 2008), percent of MVIC (Giordano and Segal, 2006, Kinugasa and Akima, 2005), cycling biomechanics (Wakeling, 2009), and gait parameters (Gefen, 2002) on TS muscle function also report that SOL and GM EMG amplitudes and recruitment patterns are similar, whereas those from GL tend to differ. Further investigations are required to verify the underlying mechanisms linked to the observed associations between KF positions and specific peak activity of SOL, GM and GL. We however emphasize that association does not equate causation and caution against extending the interpretation of processed EMG data collected during an MVIC from one TS muscle to another muscle.

Our secondary analyses found that peak TS activity was derived almost equally from the first, second and third MVIC trial performed within a select KF position. This highlights the importance of completing the three recommended trials advocated by the SENIAM and ISEK organizations during MVIC data collection, as the three have equal chances of capturing peak muscle activity. Equal numbers of males and females designated to age groups at low (18–25 years) and high (35–45 years) risk of Achilles tendon and TS injuries were recruited and tested as sex, age and Achilles tendon degeneration could have confounded results. However, testing sequence, sex, age group, and BMI and physical activity categories, had no significant influence on the position found to generate peak activity in the separate TS muscles. This suggests that the MVIC procedures used in this study can be extended to all healthy individuals from 18 to 45 years of age, including those at a potentially low or high risk of Achilles tendon and TS injuries based on age. The fact that the signals contained no significant amount of cross-talk indicates that the procedures, techniques and equipment used adequately captured the individual activity of the TS muscles during an MVIC, thus strengthening the results.
As in all studies, there are both strengths and limitations. Although results can be generalized to a healthy population of 18 to 45 years of age, the MVIC methods used in this study may not be appropriate in pediatrics and/or geriatrics and/or individuals with musculoskeletal disorders or other medical conditions. Results and interpretation of manual muscle testing procedures are perceived to be limited as they depend on the examiner (Mulroy et al., 1997, Schmitt and Cuthbert, 2008, Smidt and Rogers, 1982). However, the set-up used in this study allowed for weights to be adjusted until participants were exerting a maximum plantar-flexion effort in all select KF positions, and were not able to sustain the heel-raise position with further load. The examiner was an experienced physical therapist, skilled in the application of manual muscle testing procedures, and followed a standardized protocol to increase the reliability of testing (Schmitt and Cuthbert, 2008, Smidt and Rogers, 1982). In addition, when compared to research of a similar design (Carvalho et al., 2010, Cresswell et al., 1995, Vera-Garcia et al., 2010), this study examined a relatively large sample size.

5. Conclusion
As recently suggested for upper-limb muscles (Chopp et al., 2010), we recommend the systematic use of more than one position for normalizing EMG to an MVIC of the TS muscles, since no one position is specific or selective to peak SOL, GM, or GL activity. MVIC normalization methods should be performed in standing in at least two KF positions, as sitting was not effective for capturing peak activity of the TS muscles; even for SOL. This knowledge will assist in future EMG research on TS muscle activity and help to refine the physical therapeutic evaluation, rehabilitation, training, and development of injury prevention strategies specific to the TS muscles. Greater scientific merit can be attained in EMG research by utilizing common and valid normalization procedures to capture peak individual TS muscle activity.

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