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1 The between-day reliability of peroneus longus EMG during walking

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1 Abstract

2 The peroneus longus (PL) is a rearfoot evtor, important in frontal plane foot motion. Studying
3 PL function has been limited by previous electromyography (EMG) studies reporting poor
4 between-day reliability. Due to its close proximity to adjacent muscles, EMG measures of PL
5 may be susceptible to crosstalk, thus correct electrode placement is vital. The aim of this study
6 was to use ultrasound to aid placement of small surface EMG electrodes and determine the
7 between-day reliability of PL EMG in healthy participants' walking. Ten participants walked
8 barefoot and shod at a controlled, self-selected speed. Six trials per condition, per session, were
9 recorded over two days (mean (SD): 5 (3) days apart). The muscle belly was located using
10 ultrasound. EMG was recorded with surface electrodes (Trigno™ Mini, Delsys, Inc.) at 2000
11 Hz. Amplitude was normalized to the peak per gait cycle and time normalized to the gait cycle.
12 Reliability of discrete variables were primarily assessed with the standard error of measurement
13 (SEM), plus the coefficient of multiple correlation (CMC), the coefficient of variation (CV) and
14 the intra-class correlation coefficient (ICC). The pattern of the EMG profile was consistent.
15 The SEM of peak amplitude was 4% (3-8%) and 3% (2-5%) for barefoot and shod respectively.
16 For timing of the peak the SEM was 2% (1-3%) and 1% (1-2%) for barefoot and shod
17 respectively. Low SEM of discrete variables suggests good reliability of PL EMG during
18 walking supporting the future use of this protocol. Therefore activation of PL can be
19 confidently studied in repeated-measures study designs.

20

1 Background

2 The peroneus longus (PL) is a rearfoot evtor and electromyography (EMG) studies have
3 investigated its function. However, reliability is among the most important factors regarding
4 measurement accuracy (Hopkins, 2000) and poor between-day reliability of PL has been
5 reported during walking (Barn et al., 2012; Murley et al., 2010). In patients with rheumatoid
6 arthritis (RA), for example, peak PL amplitude in the combined midstance/propulsion phase
7 had an ICC of 0.03-0.19 and standard error of measurement (SEM) of 17-18% (Barn et al.,
8 2012). If between-day reliability of PL EMG is indeed poor, then our ability to study normal
9 and abnormal activation of PL, and intervention effects, will be limited.

10 Due to being close to adjacent muscles, EMG measures of PL are susceptible to crosstalk
11 (Campanini et al., 2007). Shifting electrode position can change peak PL activation relative to
12 the central location by up to 29% (SD=13%) (Campanini et al., 2007). Crosstalk from tibialis
13 anterior (TA) particularly, could explain poor between-day reliability of PL surface EMG in
14 the study by Barn and colleagues (2012) if electrodes were inadvertently placed closer to the
15 TA during one session than another. With a small sample size (n=5), the mean difference
16 between sessions would be particularly susceptible to extreme errors in electrode placement.
17 Identifying the borders of the PL with prior ultrasound scanning may facilitate sensor
18 placement. Additionally with small EMG sensors muscle borders can be more easily avoided.
19 The small head (25 mm x 12 mm x 7 mm) of the Delsys Trigno™ Mini sensor (Fig. 1., Delsys,
20 Inc., Boston, USA) makes it suitable for recording from muscles that are difficult to isolate and
21 susceptible to crosstalk (Delsys). The presence of RA may also have added variability by
22 influencing muscle function in the study by Barn et al (2012). The reliability of surface PL
23 EMG in a healthy population is unknown.

24 Using fine-wire EMG (Murley et al., 2010) eliminates crosstalk, however intramuscular wire
25 electrodes are potentially sensitive to their position relative to active motor units (Kadaba et
26 al., 1985). Possible variation between sessions does not appear to be overcome by normalising
27 to maximum voluntary contractions (MVCs) or dynamic normalisation to a self-selected fast
28 walking speed (Murley et al., 2010). Performing an MVC possibly introduces error,
29 particularly if participants are unfamiliar with performing maximal movements in the frontal
30 plane. Participants may also not truly produce maximum effort if a forceful contraction causes
31 discomfort with fine-wire in the muscle. Furthermore good reliability of PL EMG using surface

1 electrodes would be advantageous because fine-wire EMG is invasive and requires specialist
2 skills and equipment.

3 Therefore the aim of this study was to use ultrasound to aid placement of small surface EMG
4 electrodes and determine the between-day reliability of PL EMG in healthy participants'
5 walking.

6 **Methods**

7 Ten healthy participants (two male, eight female), mean (SD) age of 28 (4) years, body height
8 of 1.69 (0.07) m, body mass of 67.13 (9.22) kg walked barefoot and shod in standardized
9 shoes at a self-selected walking speed. Six good trials were recorded per condition in a session,
10 and two sessions were performed over two days, a mean (SD) of 5 (3) days apart. Three-
11 dimensional kinematics and kinetics were recorded simultaneously with EMG through
12 Qualysis Track Manager Software (QTM, version 2.13, Qualysis, Gothenburg, Sweden) in
13 order to normalise EMG to the gait cycle. Reflective markers were placed on anatomical
14 landmarks to track three dimensional movement using 15 infra-red cameras (Oqus 400,
15 Qualysis, Gothenburg, Sweden). Ground reaction force data was recorded using two AMTI
16 force plates (OR6, USA, Type: BP400600, dimensions: 600mm x 400mm). The study
17 conformed to the Declaration of Helsinki and was granted approval by the University of Salford
18 ethics panel and informed consent was obtained per subject.

19 **EMG sensor placement**

20 Firstly approximate sensor location was found on the right leg using the Surface
21 Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) guidelines
22 (Hermens et al., 2000). Specifically, participants laid supine and the lateral malleolus and the
23 fibula head were marked. A third mark was made at 25% of the distance from the fibula head
24 to the lateral malleolus. Secondly the muscle belly of PL was scanned in the transverse plane
25 level with the middle mark using ultrasound at 5-8 MHz (Linear 60 mm probe, Echo Blaster
26 128 CEXT, Telemed Medical Systems, Milan, Italy). The ultrasound probe was coated in water
27 soluble gel. A blunt metal stick was placed under the probe to gently apply pressure to the skin,
28 to visualise deformation in the ultrasound image level with the two borders of the muscle in
29 turn (Fig. 2.). The probe was removed and a mark was made by the stick with a surgical marker.
30 The marks were typically within ~1 cm of the SENIAM placement in the longitudinal direction,
31 but often more anterior/posterior of the line between the fibula head and malleolus depending
32 on the individual. Marks from session one were not visible in session two. Skin was cleaned of

1 gel and wiped with an alcohol wipe. The sensing head of a Trigno™ Mini was then affixed
2 between the two marks orientated in the longitudinal direction of the shank and PL muscle
3 fibres. The main sensor of the Trigno™ Mini was affixed over the tibia. Tape was fixed over
4 the sensing head and cable to minimise potential movement artefact. The EMG signal was
5 tested by everting while standing on the left leg. If unsatisfactory the sensor was removed, the
6 location wiped and the sensor reapplied.

7 Analysis

8 Marker data were collected, identified and labelled with QTM software (V.2.13, Qualysis AB,
9 Sweden) and then further processed and analysed in Visual 3D (V.6, C-Motion, Inc., USA).
10 Walking speed was controlled in processing using mean stride time. Trials with stride time
11 outside mean \pm 5% were excluded.

12 A 75 ms window was used to calculate the root mean squared (RMS) EMG from each trial
13 and averaged across all included gait cycles. The RMS signals were exported to ASCII files
14 after normalisation to the gait cycle for further analysis in MATLAB (R2016b). Amplitude was
15 normalized to the peak of each gait cycle and then averaged across trials for each condition.
16 Our previous work found peak normalisation superior to normalising to MVCs in shank
17 muscles for reducing variability (Onmanee, 2016).

18 Statistics

19 The SEM is advocated for reliability studies because it is easy to interpret as it is expressed in
20 the units of measurement itself and unaffected by the range of the measurement (Atkinson and
21 Nevill, 1998; Baker and McGinley, 2013; Deschamps et al., 2011; McGinley et al., 2009).
22 Although the intra-class correlation coefficient (ICC) depends on sample heterogeneity
23 (Atkinson and Nevill, 1998; Baker and McGinley, 2013; Hopkins, 2000) and the coefficient of
24 multiple correlation (CMC) depends on the range of the data (McGinley et al., 2009), both are
25 commonly used (Barn et al., 2012; McGinley et al., 2009; Murley et al., 2010). The ICC (3,1)
26 and CMC were calculated in order to compare to other reliability studies and because reporting
27 multiple reliability statistics is recommended (Atkinson and Nevill, 1998; Luiz and Szklo,
28 2005).

29 The CMC was calculated to compare the average PL EMG profile between sessions using
30 Microsoft Excel. The ICC (3,1) and SEM ($SD\Delta/\sqrt{2}$) for the peak and timing of the peak were
31 calculated using a freely downloadable Microsoft Excel spreadsheet for consecutive pairwise

1 analysis (Hopkins, 2015). The CV was calculated as: $(SEM/Grand\ mean)*100$ (Batterham and
2 George, 2003; Hopkins, 2000).

3 Results

4 Mean (SD) of PL peak amplitude (% of peak per gait cycle) was 90 (4) on day 1 and 92 (3) on
5 day 2 when barefoot and 87 (7) on day 1 and 91 (3) on day 2 when shod. The timing of initial
6 PL activation (% of the gait cycle) was 44 (2) on both days when barefoot and 45 (3) on day 1
7 and 44 (2) on day 2 when shod.

8 The EMG profiles of PL were repeatable in both barefoot and shod (Fig. 3.), with very good
9 CMCs (0.91 and 0.88 respectively). Reliability of discrete variables was moderate to very good
10 for PL for all statistics except the ICC for peak amplitude. For barefoot, reliability was
11 generally moderate for the peak (SEM: 3% (2-5), ICC: 0.23 (-0.33-0.67), CV: 3%) and very
12 good for the timing of the peak (SEM: 1% (1-2) of gait cycle, ICC: 0.85 (0.52-0.96), CV: 2%).
13 Similarly for shod, reliability was moderate for the peak (SEM: 4% (3-8), ICC: 0.44 (0-0.82),
14 CV: 5%) and very good for the timing of the peak (SEM: 2% (1-3) of gait cycle, ICC: 0.72
15 (0.20-0.92), CV: 3%).

16 Discussion

17 The aim of this study was to determine the reliability of PL EMG in walking. The PL EMG
18 profile and discrete variables were repeatable, supporting the future use of this protocol.

19 The between-day reliability of discrete EMG variables in PL was more reliable in our study
20 than in the study by Barn et al. (2012). In the previous study the SEM (95% CI) for peak
21 amplitude in early stance was 19% (-53, 51) and 12% (-27, 40) for barefoot and shod
22 respectively. Low reliability might be expected in early stance if crosstalk with TA was present
23 because there is high levels of TA activity in this phase. Considering the peak in the combined
24 midstance/propulsion phase, when PL is most active, the SEM for peak amplitude was 17%
25 and 18% for barefoot and shod respectively in the previous study, but only 3% and 4% in our
26 study. The SEM of the time of peak in the combined midstance/propulsion phase was 8% and
27 9% in the previous study and 1% and 2% in the present study for barefoot and shod
28 respectively.

29 The greater between-day reliability in the combined midstance/propulsion phase in the present
30 study versus the earlier study may be due to differences in power, study populations, and/or
31 normalisation. Barn et al. (2012) acknowledged the limitation of a sample size of five, due to

1 the challenge of recruiting RA patients. They also suggested that RA may have contributed to
2 the variability in both raw and normalised EMG signals. Fluctuations in inflammatory
3 cytokines can influence muscle function and subsequent EMG recordings and variability in
4 MVCs may have been an issue as differences in joint tenderness between days may affect the
5 capacity to perform an MVC (Barn et al., 2012). A limitation of our study is that only healthy
6 participants were tested, thus reliability of this approach with patients is unknown.

7 The ICC (3,1) for peak PL amplitude in barefoot walking in our study 0.23 (-0.33-0.67) was
8 worse than the ICC (2,1) of peak PL amplitude in the midstance/propulsion phase during
9 barefoot walking in the earlier study using fine-wire: 0.53 (0.17–0.77) and 0.52 (0.15–0.76)
10 for MVC and sub-maximum normalisation respectively (Murley et al., 2010). However in the
11 study by Murley et al. (2010) a systematic bias (% mean difference) of 7.7% existed between
12 sessions for peak amplitude in the combined midstance/propulsion phase using sub-maximum
13 normalisation. The difference in mean of peak amplitude in the present study while barefoot
14 was only 2% and the SEM 3% (2-5) and CV (3%) were low despite poor reliability according
15 to the ICC. The ICC (3,1) of 0.85 (0.52-0.96) in our study for time of peak amplitude barefoot
16 was superior to time of peak in midstance in the earlier study ICC (2,1): 0.58 (0.27–0.78).
17 Differences between the studies may be due to differences between fine-wire and surface EMG.
18 Repeatability is similar in soleus, TA and medial gastrocnemius for surface and fine-wire EMG
19 (Bogey et al., 2000; Onmanee, 2016). However it is unknown if these results apply to PL
20 because motor units could be distributed differently in PL and if a different number of active
21 motor units are recorded from between sessions then this could affect between-day reliability
22 (Onmanee, 2016). Nonetheless the present protocol is less invasive than fine-wire EMG.

23 In conclusion, the PL EMG profile was repeatable between sessions during barefoot and shod
24 walking. Good reliability was also demonstrated for the majority of statistical measures of
25 reliability for peak PL amplitude and time of peak amplitude. Therefore activation of PL can
26 be confidently studied in repeated-measures study designs like interventions using this
27 protocol.

28 Acknowledgements

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30 or not-for-profit sectors.

1 Declaration of conflicting of interests

2 The manuscript was prepared by J.R. The preparation of the manuscript was primarily
3 supervised by C.N. All authors were involved in the drafting and approving of the manuscript.

4 C.N. owns equity in a company that manufactures foot orthoses. Other authors have no
5 conflicts of interest to declare.

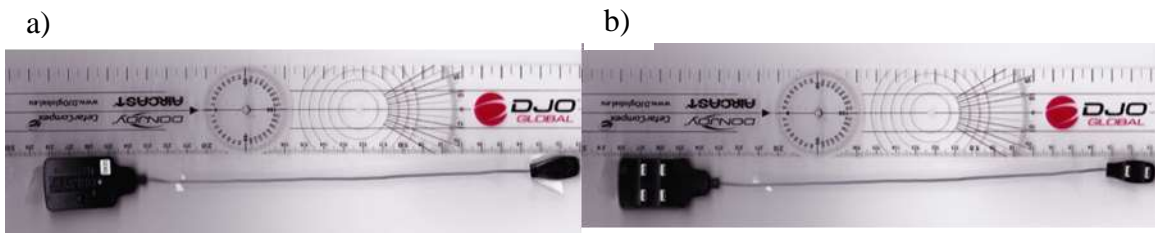
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Fig. 1. Delsys Trigno™ Mini sensor to scale, shown a) from above and b) the electrodes on the underside

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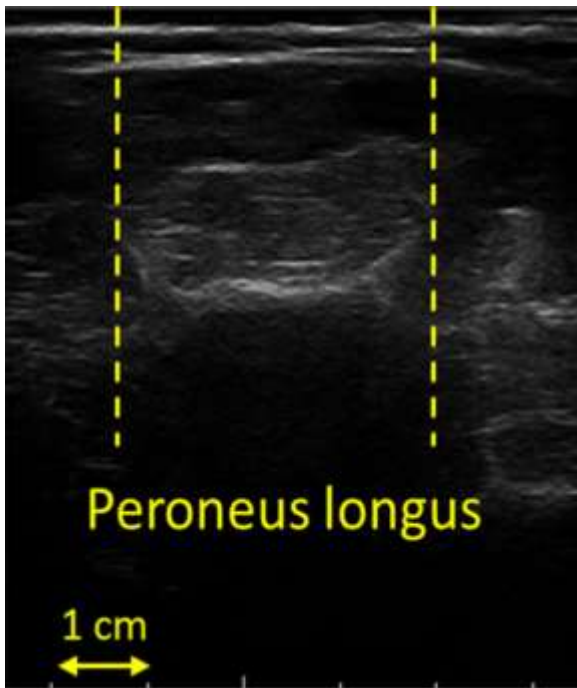


Fig. 2. Ultrasound image of the peroneus longus in the transverse plane, dashed lines indicate the borders of the PL muscle

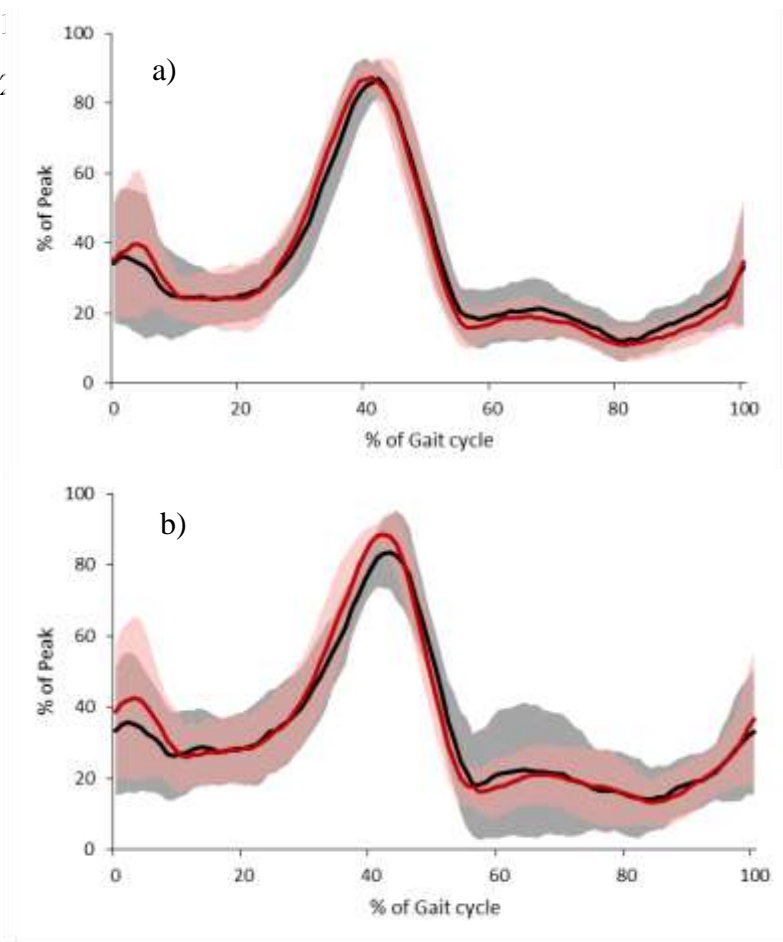


Fig. 3. Peroneus longus EMG average profiles \pm SD expressed as a percentage of the gait cycle in a) barefoot and b) shoes. Day 1: black lines and Day 2: red lines