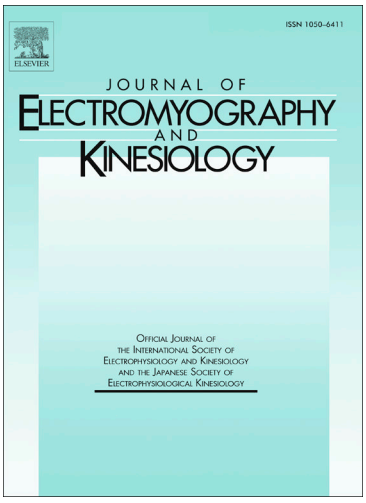

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Comparing Functional Dynamic Normalization Methods to Maximal Voluntary Isometric Contractions for Lower Limb EMG from Walking, Cycling and Running

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Abstract

There is no consensus on the most appropriate method for normalizing an individual's electromyography (EMG) signals from walking, cycling and running in the same data collection. The aim of this study was to compare how the magnitude and repeatability of normalization values differ from three normalization methods and to compare their scaling effect in three moderate intensity activities. Three rounds of maximal voluntary isometric contractions (MVICs), sprint cycling and sprint running were performed to obtain normalization values for each method. EMG from five moderate intensity trials of walking, cycling and running were performed and normalized using each normalization value. Normalization values, coefficients of variation, and peak normalized EMG from the three moderate intensity activities were compared across normalization methods. Sprint running resulted in greater normalization values for 6/9 muscles. MVICs produced the lowest variance in 6/9 muscles. Comparing peak normalized signals of interest across normalization methods, there were significant differences

in 6/9, 7/9 and 8/9 muscles for walking, cycling and running, respectively. When investigating a combination of walking, cycling and/or running EMG data, sprint running is recommended for normalization, over MVICs or sprint cycling, due to its simplicity and its ability to produce a larger normalization value, despite lower repeatability.

1. Introduction

Cycling and running participation (and related research) have increased over the past 30 years in the midst of growing health conscientiousness [Running USA, 2016]. Several studies have reported electromyography (EMG) of lower limb muscles for cycling [Chapman et al., 2008; Dingwell et al., 2008] and running [Bonacci et al., 2010]. EMG is a useful tool in assessing the electrical activity of muscles in biomechanics research [de Luca, 1997], however there is no consensus on the 'best' method to normalize this data for tasks such as cycling and running [Ball & Scurr, 2013; Sousa & Tavares, 2012b].

A common method of processing EMG data is using a linear envelope [Burden & Bartlett, 1999] which entails: removing signal bias, full wave rectification and single- or dual-pass low-pass filtering. Linear enveloping the data is often followed by normalization to a normalization value from a standardized activity. EMG normalization is a key process in the interpretation of EMG signals since it allows for the comparison of the activation of different muscles within an individual and between participants [Lehman & McGill, 1999]. These normalization values are often obtained during a maximal voluntary isometric contraction (MVIC) [Burden, 2010; Merletti & Torino, 1997], though EMG data has also previously been normalized to mean EMG

of a task [Benoit et al., 2003; Tennant et al., 2014], peak EMG of a task [Bonacci et al., 2011; Burden, 2010], submaximal isometric contraction [Ball & Scurr, 2011], or a maximal or submaximal dynamic task [Albertus-Kajee et al., 2010; Albertus-Kajee et al., 2011; Sinclair et al., 2012].

Although MVICs have previously been used for high velocity, ballistic activities such as running [Ball & Scurr, 2010; Kyröläinen et al., 2005] and cycling [Billaut et al., 2006; Hunter et al., 2002], alternate methods have been recommended for such tasks [Ball & Scurr, 2013]. When normalizing EMG data of tasks such as cycling and running to normalization values obtained from MVICs, normalized values have exceeded 100% activation, indicating that the normalization method elicited an activation less than that of the activity being investigated [Ball & Scurr, 2013; Kyröläinen et al., 2017]. MVICs may be inappropriate for normalizing high-velocity tasks due to their inability to reflect the greater neuromuscular drive that is often present in cycling and running [Ball & Scurr, 2013]. In addition, Lewek et al. (2004) found average quadriceps activation during a maximal voluntary exertion to be 93% of that elicited by electrical stimulation. The results also revealed that 25% of healthy participants were unable to voluntarily maximally activate their quadriceps. In the upper limb, Gandevia et al. (1998) found only 23% of voluntary maximal muscular exertions reached the activation recorded during twitch interpolation, specifically for the elbow flexor muscles. MVICs are often used for their relatively simple protocol, but there are some unique factors specific to high-velocity activities (such as impact spikes, neural drive and muscle-skin movement) which can affect the utility of normalizing dynamic tasks to MVICs [Ball & Scurr, 2013].

Further, in obtaining normalization values via MVICs, each muscle group is activated at separate times and it cannot be guaranteed that the intensity level or motivational level between muscles

is exactly the same, which is thought to influence the EMG amplitude of MVICs [Albertus-Kajee et al., 2011]. If the motivational level from one muscle's MVIC trial differs from the motivational level from another muscle's MVIC, then comparing MVIC normalized EMG amplitude between these muscles would be inaccurate, since one muscle could be normalized to an MVIC that was relatively more activated than another when the normalization value was obtained [Sinclair et al., 2015]. A functional task similar to the task under investigation (such as a cycling or running normalization method) would provide the same neural drive and muscular coordination, with all muscles firing at similar relative intensities [Ball & Scurr, 2013]. In cycling and running, it is assumed that, at maximum effort, the lower limb muscles will all activate as much as they can to perform the task, and thus, will all be activated to the same relative intensity.

Previous studies have compared MVICs to dynamic normalization methods in running and cycling [Albertus-Kajee et al., 2010; Albertus-Kajee et al., 2011; Rouffet & Hautier, 2007; Sinclair et al., 2012; Sinclair et al., 2015]. It has been reported that dynamic methods of normalization are more repeatable than or as repeatable as MVIC methods [Sousa & Tavares, 2012b].

Since the purpose of obtaining a normalization value is to implement it in the denominator of the normalization process, it is important to consider how normalization values affect a sample signal of interest. Even if differences are found in normalization values between methods, if there is no difference found to the normalized signals of interest, it could change the interpretation and impact of the findings of the current study.

In the current study, three normalization methods were investigated: MVIC, sprint cycle and sprint run. The sprint cycle and sprint run methods were selected given that it has been

recommended that sprint methods should be performed for normalization when investigating maximal muscle activity in cycling or running, respectively, on a single collection day [Albertus-Kajee et al., 2010; Albertus-Kajee et al., 2011]. To our knowledge, this is the first study to compare MVIC, sprint cycle and sprint running methods of normalization within the same individual.

The purpose of the current study was threefold: 1) to determine which of three normalization methods (MVICs, sprint cycle or sprint run) would produce the greatest normalization value, 2) to determine the repeatability of normalization values from each normalization method across trials and 3) to compare peak normalized 'signals of interest' from moderate intensity walking, cycling and running across the three normalization methods. It was hypothesized that the sprint run would produce the greatest normalization value for all muscles due the greater ballistic motion of the activity. It was also hypothesized that sprint cycle and sprint run would be the more repeatable normalization methods for all muscles compared to MVIC (since it was expected that these activities would feel more natural to the participants, while MVICs have been thought to be unfamiliar to participants [Ball & Scurr, 2013]). Lastly, it was hypothesized that peak normalized EMG signals from moderate intensity trials would be significantly different across the three normalization methods for all muscles.

2. Methods

2.1 Participants

A sample of convenience from the university population consisting of twenty healthy, active young adults (12 male, age: 22.2 ± 3.5 years, height: 1.74 ± 0.13 meters, mass: 69.8 ± 10.5 kg) provided informed consent to participate in this study. Exclusion criteria consisted of any lower

limb injury within the past month, cardiovascular disorder prohibiting safe physical exercise, diagnosed knee osteoarthritis, lower limb injury requiring surgery, or the inability to complete any continuous moderate intensity exercise for 30 minutes such as cycling, running, group or individual exercise, or team sports. Participants also completed an exercise history questionnaire to provide insight into their physical activity experience.

2.2 Instrumentation

Wireless surface electrodes were placed on the participant's right leg (Wave Plus, Cometa srl, Milan, IT; input impedance = 20 MΩ, common mode rejection ratio = 120 dB at 60 Hz) to measure muscle activation. Bipolar Ag/AgCl electrodes (BlueSensor N, Ambu Inc., Glen Burnie, MD, USA) with a center to center inter-electrode distance of 2cm were applied (Figure 1) over the muscle bellies of vastus lateralis (VL), vastus medialis (VM), tibialis anterior (TA), lateral gastrocnemius (LG), medial gastrocnemius (MG), biceps femoris (BF), semitendinosis (ST), gluteus maximus (GM) and tensor fascia latae (TFL), according to SENIAM (Surface EMG for NonInvasive Assessment of Muscles) guidelines [Hermens et al., 2000]. The skin at the electrode sites was shaved, exfoliated and cleaned prior to electrode application. Raw EMG signals were bandpass filtered via the hardware (10–1000 Hz), amplified, and sampled at 2048 Hz.

[Insert Figure 1 near here]

2.3 Experimental Protocol

The experimental protocol is summarized in Figure 2. Three normalization trials were performed for each normalization method in total.

[Insert Figure 2 near here]

Normalization trials were first explained to the participants and, before each MVIC, participants were invited to practice at 50% effort to orient the participant to the protocol. For the sprint cycle task, the cycle ergometer (M3, Keiser Corp, Fresno, CA, USA) was adjusted to the participant [Bini & Hume, 2016] (Figure 3). The participant's feet were strapped onto the pedals via toe-straps and the participant was instructed to not lift their bottom off of the seat for any cycling trial. For the MVIC trials, EMG data was collected for 6s, where the participant ramped up to their maximum intensity over the first 3 seconds, maintained their maximum intensity for 2 seconds and relaxed during the final second. For the sprint cycling trials, EMG data was collected from the start of increasing resistance to when the participant could no longer maintain a cadence of 100 RPM. The sprint cycling trials lasted up to 30 seconds. For the sprint running trials, EMG data was collected from the initiation of their run to the 15 meter mark, where the participant was instructed to reach their top speed. Participants were given 1-3 minutes rest between normalization trials within each normalization trial block [Albertus-kajee et al., 2010; Albertus-Kajee et al., 2011; Rutherford et al., 2011; Sousa et al., 2012a]. A minimum of 1 minute rest was given to the participants, which may have been extended to 3 minutes due to the explanation/reminder of the protocol for the next trial, a position change between equipment or if the participant requested more time.

[Insert Figure 3 near here]

Participants were given up to 5 minutes rest before the start of the moderate intensity trials of interest in order to avoid discomfort and the participants were instructed to identify when they were ready to proceed. Participants were permitted to self-select their walking speed, cycling effort and running pace at a "moderate intensity", or at a 7/10 rating of perceived exertion. A self-selected gait speed was chosen because it has been shown to reduce muscle activity pattern

variability in contrast to a controlled speed [Sousa & Tavares, 2012a]. Each of the five moderate walking trials had a maximum duration of 10s and EMG data was collected for the entirety of the task. For the moderate cycling and moderate running trials, five 30s recordings at the end of every minute were collected.

2.4 Signal Processing

All EMG data processing was completed using Matlab 8.5 (The Mathworks, Release R2015a, Natick, MA): bias removal, full wave rectification and low pass filtering with a dual pass 2nd order Butterworth digital filter with a 6 Hz cutoff frequency [Hubley-Kozey et al., 2006] to produce a linear envelope. Signals with artifacts such as signal dropouts, data spikes and non-biological noise (signal depolarization or wireless interference) were excluded. For normalization trials, an algorithm was coded to flag signals that had a peak less than 0.05V (indicative of signal drop) or a signal over 2V (typically signal spike or depolarization of the signal). Further, after normalization, if a signal was less than 5% (minimum limit of accurate EMG measurement [de Luca, 1997]) or over 100%, the signal was also flagged. All flagged signals were visually inspected and accepted if they did not show signs of artifact.

2.5 Normalization Values

For the 3 trials of each normalization method, the peak EMG magnitudes were extracted for each muscle (1 peak value x 3 trials x 3 methods x 9 muscles). The normalization value for each method was defined as the maximum value of the peaks of the normalization trials for each method, reducing the data to 3 normalization values per muscle (one for each of MVIC, sprint cycle and sprint run). To compare normalization values between methods, mean relative muscle activations were calculated [Rutherford et al., 2011]. This method has been previously used to

compare normalization values between MVICs for the lower limb in participants with osteoarthritis. For each participant, each of the three normalization values was expressed as a percentage of the greatest of the three normalization values. For each muscle, the mean relative muscle activation was the average of these values across participants. Mean values closer to 100% indicate that a method produced a higher normalization value, for more people, for a given muscle. This outcome measure takes into account both the proportion of participants who elicited the greatest normalization value for each method, as well as how the magnitude of the normalization values compare to each other, which makes it a practical criteria for recommendations.

2.6 Peak Normalized EMG

The signals of interest from the moderate trials were normalized using each of the three normalization values, resulting in 45 waveforms per muscle (5 trials x 3 activities x 3 normalizations). The peak normalized magnitudes were then averaged from each normalized signal of interest to produce a grand mean of the peak EMG magnitudes across the 5 trials. The resulting values were expressed as %MVIC, %sprint cycle, %sprint run.

2.7 Statistical Analysis

All statistics were performed using SAS Studio Statistical software System (SAS Institute Inc., Cary, NC, USA). Differences between normalization values among normalization methods were detected using separate repeated-measures one-way ANOVAs (one per muscle) and differences between mean relative muscle activations among normalization methods were detected using separate one-way ANOVAs (one per muscle). Significance was determined with an alpha level of 0.05 and Bonferroni post-hoc tests were performed for pairwise comparisons. The peak values

of each of the normalization trials (3 per normalization method) were used to compute the within-day intra-subject coefficient of variation (CoV) for each participant. CoVs were averaged across participants to produce a grand mean CoV for each muscle. A CoV greater than 20% indicated large variability of peak EMG values across the three trials of each normalization method [Albertus-kajee et al., 2010] and has been previously used as a threshold for unacceptable repeatability. Conversely, a CoV less than 12% indicated low variability of peak EMG values [Albertus-Kajee et al., 2010; Taylor & Bronks, 1995] and has been previously used as a threshold to identify acceptable repeatability [Albertus-Kajee et al., 2010; Taylor & Bronks, 1995]. In cases where one or more of the three normalization method trials were excluded (as per the visually inspected data outlined in *Signal Processing*), that participant's CoV was not calculated for that muscle. The peak normalized EMG magnitudes from the moderate trials of interest were compared between normalization methods using separate one-way repeated measures ANOVAs were performed for each muscle and activity. Significance was determined with an alpha level of 0.05 and Bonferroni post-hoc tests were performed for pairwise comparisons.

3. Results

Less than 1% of all signals were removed due to signal drop, signal spike or a non-biological waveform (depolarization of signal or wireless interference).

3.1 Normalization Values

The normalization values differed between at least two of the methods for each muscle investigated (Table 1). TFL was the only muscle different between all three normalization methods. VL was only different between MVIC and sprint cycle and TA was only different

between sprint cycle and sprint run. All other muscles had differences between both MVIC and sprint run as well as sprint cycle and sprint run. MVICs produced mean relative muscle activations between 26.8% and 86.1%, sprint cycle produced mean relative muscle activations between 41.3 and 91.5% and sprint run produced mean relative muscle activations between 89.8% and 100.0% (Table 2). The percentage of participants that produced the greatest normalization values ranged from 0.0% to 45.0% for MVIC, 0.0% to 35.0% for sprint cycle and 40.0% to 100.0% for sprint run (Table 2). For all muscles except TA, sprint running was the method in which the most participants produced a maximum normalization value between methods. Across all participants, MVIC never resulted in the greatest normalization value for VL, LG or MG, with sprint cycle never resulting in the greatest normalization value for LG. For all other muscles, each method resulted in the greatest normalization value for at least one person. Sprint run produced the greatest normalization value for LG in all participants.

[Insert Table 1 near here]

[Insert Table 2 near here]

3.2 Coefficients of Variation

CoV values ranged from 8.8% - 20.1% for MVICs, 11.7% - 25.5% for sprint cycle and 10.8% - 34.00% for sprint run (Table 3). MVIC produced the best overall repeatability, with the lowest CoV for 6/9 muscles.

[Insert Table 3 near here]

3.3 Peak Normalized Values

During moderate walking (Table 4a), when comparing normalization methods, there was a significant difference in the mean of the peak normalized magnitude of all muscles except VL, TA and ST ($p > 0.063$ for these three muscles). During moderate cycling (Table 4b), when comparing normalization methods, there was a significant difference in the mean of the peak normalized magnitude of all muscles except VL and TA ($p > 0.073$ for these two muscles). During moderate running (Table 4c), when comparing normalization methods, there was a significant difference the mean of the peak normalized magnitude of all muscles except TA ($p = 0.079$ for TA). Furthermore, LG and MG, when normalized to MVIC for moderate running, both resulted in normalized peaks over 100% at 156.6% (128.1) and 151.1% (61.5) respectively.

[Insert Table 4a near here]

[Insert Table 4b near here]

[Insert Table 4c near here]

4. Discussion

The purpose of the current study was to investigate how the magnitude and repeatability of obtaining normalization values differed when obtained from three different proposed normalization methods as well as how normalizing to these different normalization values affected EMG signals of interest from three moderate-intensity activities. Results revealed that sprint run produced the largest normalization values in six of nine muscles investigated (LG, MG, BF, ST, GM and TFL) and larger than at least one of the other methods in two other muscles (VM and TA). Additionally, sprint run produced the greatest normalization value for the most participants in each muscle, except TA. The repeatability of peak values of the normalization trials when obtaining normalization values was greater than 20% in one muscle

during MVIC, two muscles during sprint cycle and five muscles during sprint run. When comparing normalized signals of interest between normalization methods, there were differences in peak normalized values in six of the muscles investigated during moderate walking, in seven muscles during moderate cycling and in eight muscles during moderate running, indicating that normalization method could have an impact on interpretation of normalized EMG signal peaks.

4.1 Normalization Value

Based on the normalization values (Table 1), our first hypothesis, that sprint running would produce the greatest normalization values for all muscles is accepted. Sprint running produced a significantly higher normalization value compared to MVIC and sprint cycle in all but VL, VM and TA. In these three muscles, sprint running was not significantly different from one or more of the other normalization methods. There were no cases where another normalization method was significantly greater than sprint running. These findings are supported by the analysis of mean relative muscle activations (Table 2), which take into account both the magnitude of the normalization factors as well as the number of participants who achieved a maximum normalization value with each method.

In eight of nine muscles measured, sprint run elicited higher mean relative muscle activations than either MVICs or sprint cycle with all nine muscles having over 87% mean relative muscle activation (Table 2). Sprint run elicited the greatest normalization value in LG for all 20 participants and in MG for 19 participants. These two muscles had the highest proportion of participants produce the greatest normalization value during sprint run. This is thought to be due to the large eccentric contraction of LG and MG during running that are not seen during an isometric MVIC [Mann et al., 1986]. As would be expected, synergistic muscle pairings (VL/VM, LG/MG, BF/ST) produced both similar mean relative muscle activation values (Table

2) and had a similar number of participants (within 3) who elicited the greatest normalization value from each method (Table 2). Though TA has been shown to be active during both the stance and swing phase of gait [Fernandes et al., 2017], it is not a primary mover (i.e. does not contribute to forward propulsion), and works to support the leg during stance and dorsiflex the foot during swing for obstacle avoidance, which has a relatively low mass. This may explain why it was the only muscle during sprint running to not produce the greatest normalization value. All other muscles studied, except TFL, provide much of the work and forward propulsion in the gait stride [Sousa et al., 2012b]. TFL is a stabilizing muscle that inserts into the iliotibial band and works to stabilize the hip joint about the frontal plane by providing hip abduction [Selkowitz et al., 2013]. Due to the inertia of the body segments while running and the weight of the upper body that it has to support, TFL was more active than TA during sprint running, even though both are not primary movers.

4.2 Coefficient of Variation

The second hypothesis, that sprint cycle and sprint run will produce more repeatable normalization values for all muscles compared to MVIC, was rejected. CoV values for the current study (Table 2) show that MVIC had the lowest variability for all muscles, except VL, VM and MG. Sprint cycle produced the lowest variability for VM and MG, while sprint run resulted in the lowest variability for VL. The highest variability occurred for TFL during sprint run. According to the thresholds defined by Albertus-Kajee et al. (2010), normalization values obtained for VM during MVIC, BF and TFL during sprint cycle and TA, LG, MG, GM and TFL during sprint run are considered unrepeatable, since their CoV are greater than 20%.

These within-day CoV are similar to the ranges previously found for between-day analyses of MVIC and sprint running of MVIC and sprint cycling for 6 lower limb muscles (VL, VM, RF,

LG, MG and BF) [Albertus-Kajee et al., 2010; Albertus-Kajee et al., 2011], (MVIC (11.4% - 20.1 vs. 15% - 27%, respectively), sprint cycle (13.7% - 17.9% vs. 14% - 21%, respectively) and sprint run (10.8% - 29.0% vs. 15% - 20%, respectively)). However, in this previous work [Albertus-Kajee et al., 2010; Albertus-Kajee et al., 2011], between-day CoV was lowest for dynamic normalization methods, while in the current study, within-day CoV was lowest for MVICs. Within-day CoV was also investigated previously for MVICs and sprint cycling for 6 lower limb muscles [Norcross et al., 2010; Rouffet & Hautier, 2008] (Table 5). The MVIC CoV from the current study are very similar to Norcross et al. (2010) (Table 5), which could be due to similarities between the MVIC protocols in which testing positions, testing duration and rest periods were similar. MVIC CoV differs slightly from values reported by Rouffet & Hautier (2007), specifically for VL and GM. These differences may be attributed to differences in MVIC protocols (90° knee flexion vs. 45° knee flexion for VL and 45° hip flexion vs. 0° hip flexion for GM). The sprint cycle CoV reported by Rouffet & Hautier (2007) differ slightly from ours (for VL, MG and BF), which may be due to their use of a resistance workload on the cycle ergometer compared to an increasing resistance in the current study.

[Insert Table 5 near here]

4.3 Effect on Signals of Interest

The third hypothesis is rejected; peak normalized EMG magnitude were not always significantly different across normalization methods. For walking, all muscles except VL, TA, ST were found to have in significant differences in normalized peak values. For cycling, all muscles except VL, TA resulted in different normalized peaks. For running, all muscles except TA resulted in different normalized peaks. Trends indicate that the greater the magnitude of the EMG signal of the trial of interest, the greater the difference in normalized peak values between normalization

methods. Walking trials were found to have relatively lower EMG magnitudes than observed in running and as such, smaller differences in peak normalized EMG between methods. This supports the findings of Sousa & Tavares (2012a) who found increasing EMG magnitude with increasing gait speed. These findings also emphasize the importance of EMG normalization method, since at the most severe, in the case of LG during running, normalized values for MVIC and sprint run normalized data was 156.6% compared to 34.8%. In addition to the large magnitude difference between methods, EMG activations greater than 100% may be undesirable for applications such as for modelling or performance analyses. If activations are over 100%, interpretations of the data cannot be in terms of the muscle's maximum voluntary capacity [Halaki & Ginn, 2012].

4.4 Limitations

Some limitations were present in the study. During the sprint cycle and sprint run trials, cycling power output and running speed, respectively, were not monitored or controlled. This may have affected the repeatability measures of the normalization trials, however, it was assumed that all trials were performed at the participant's best ability each time. In addition, it was not possible to determine whether a participant truly reached their maximum EMG activity levels during the normalization trials. Maximum EMG is often identified using M-wave stimulation, [Merletti, 1990], however, during a complex, non-stationary task such as cycling or running is not feasible. Fatigue was also not assessed. 1-3 minutes rest was offered to participants between trials [Albertus-kajee et al., 2010; Albertus-Kajee et al. 2011; Billaut et al., 2006; Hubley-Kozey et al., 2009; Mathiassen et al. 1995; Norcross et al., 2010; Rouffet & Hautier, 2008; Sinclair et al., 2015] and 3-5 minutes rest was offered between testing blocks [Rouffet & Hautier, 2008]. In the aforementioned studies, fatigue was not assessed explicitly, however, these rest periods were

assumed to reduce the effects of fatigue on recorded EMG data. All normalization trials were block randomized to ensure that if, despite the best efforts to avoid fatigue, fatigue still occurred, it did not systematically bias the outcome. Lastly, the broader applications of the current study for future use may be limited by available equipment. The current study employed the use of a wireless EMG system, allowing more unconstrained movement compared to some wired/tethered EMG set-ups.

4.5 Future Directions

To conclude, for studies that investigate comparisons between walking, cycling and/or running, especially those that wish to express activity with respect to the muscle's maximum voluntary capacity, it is recommended that a sprint run task be used to obtain normalization values for EMG processing. Note, however, that the lower within-participant repeatability for sprint running normalization values may mean that this method is not ideal for day-to-day comparisons. When maximum repeatability of normalization methods is sought, MVIC is recommended for the normalization of EMG values. Further work should be done to assess any age-related effects on obtaining normalization values since age-related changes in fibre type distribution and firing rates play a role in one's ability to produce maximal force [Merletti et al., 2002]. Additional work should also be done to investigate the effectiveness or practicality of using the sprint run for special populations. Though MVICs were found to be highly repeatable in the current study, when normalization to a maximal effort is desired, the use of this normalization method for walking, cycling and running is discouraged since they may result in biologically unlikely interpretations (greater than 100% maximum effort). The sprint running protocol, consisting of 3 sets of 30m sprints, due to its short duration and its lack of need for specialized equipment, may make it ideal for field tests when space or time is limited.

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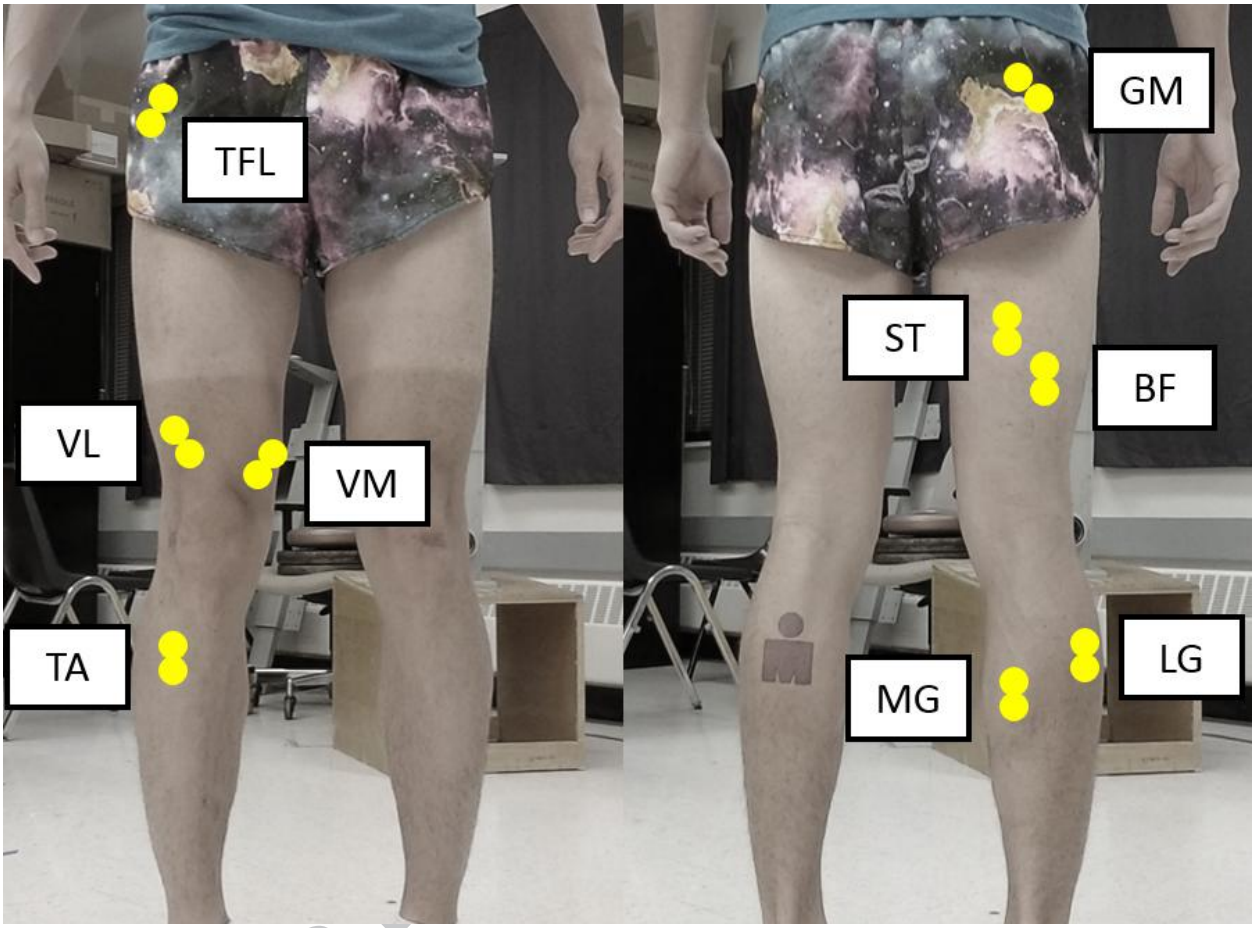
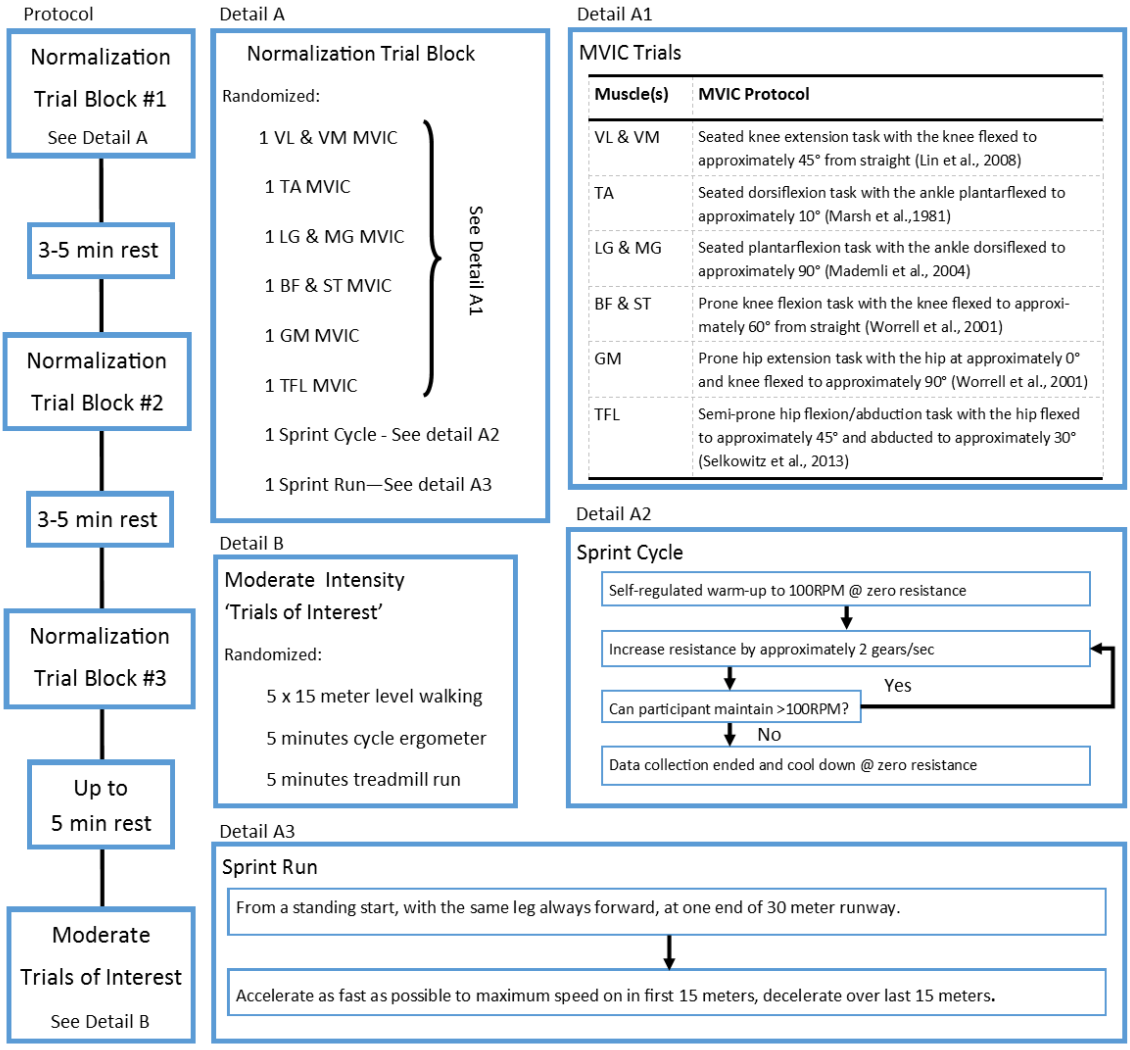
Captions to Figures

Figure 1: EMG Instrumentation for nine lower limb muscles: vastus lateralis (VL), vastus medialis (VM), tibialis anterior (TA), gastrocnemius lateralis (LG), gastrocnemius medialis (MG), biceps femoris (BF), semitendinosis (ST), gluteus maximus (GM) and tensor fascia latae (TFL).

Figure 2: Flowchart of the experimental protocol. Detail A1, A2 and A3 outlines the normalization method protocols and Detail B outlines the trials of interest.

Figure 3: Cycle ergometer set-up. The cycle ergometer was adjusted to the participant such that when the crank arm was placed at the 3 o'clock position, the participant's knee was flexed to 60 degrees and the plumb line of the knee was in line with the middle of the pedal spindle (Bini et al., 2016)




Protocol
Normalization Trial Block #1
See Detail A
3-5 min rest
Normalization Trial Block #2
3-5 min rest
Normalization Trial Block #3
Up to 5 min rest
Moderate Trials of Interest
See Detail B
Detail A
Normalization Trial Block
Randomized:
1 VL & VM MVIC
1 TA MVIC
1 LG & MG MVIC
1 BF & ST MVIC
1 GM MVIC
1 TFL MVIC
See Detail A1
1 Sprint Cycle - See detail A2
1 Sprint Run—See detail A3
Detail A1
MVIC Trials
Muscle(s) | MVIC Protocol
VL & VM | Seated knee extension task with the knee flexed to approximately 45° from straight (Lin et al., 2008)
TA | Seated dorsiflexion task with the ankle plantarflexed to approximately 10° (Marsh et al.,1981)
LG & MG | Seated plantarflexion task with the ankle dorsiflexed to approximately 90° (Mademli et al., 2004)
BF & ST | Prone knee flexion task with the knee flexed to approximately 60° from straight (Worrell et al., 2001)
GM | Prone hip extension task with the hip at approximately 0° and knee flexed to approximately 90° (Worrell et al., 2001)
TFL | Semi-prone hip flexion/abduction task with the hip flexed to approximately 45° and abducted to approximately 30° (Selkowitz et al., 2013)
Detail B
Moderate Intensity 'Trials of Interest'
Randomized:
5 x 15 meter level walking
5 minutes cycle ergometer
5 minutes treadmill run
Detail A2
Sprint Cycle
Self-regulated warm-up to 100RPM @ zero resistance
Increase resistance by approximately 2 gears/sec
Yes
Can participant maintain >100RPM?
No
Data collection ended and cool down @ zero resistance
Detail A3
Sprint Run
From a standing start, with the same leg always forward, at one end of 30 meter runway.
Accelerate as fast as possible to maximum speed on in first 15 meters, decelerate over last 15 meters.

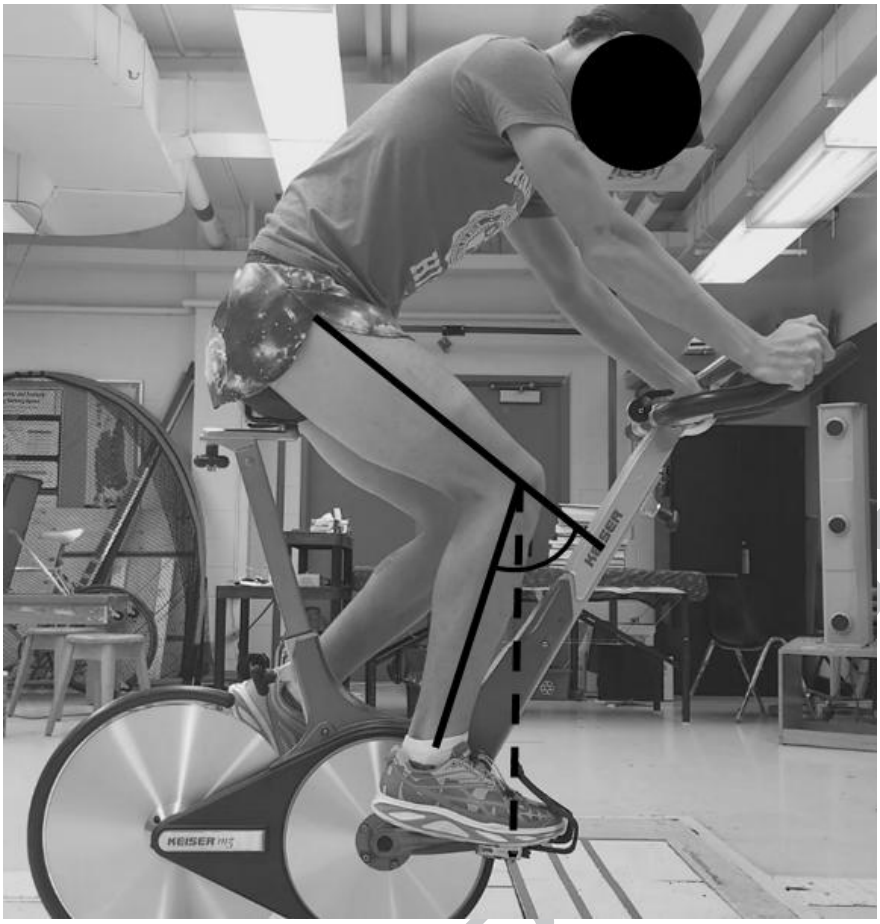


Table 1 – Repeated measures p-values comparing normalization values between methods. Bold values indicate a significant difference between the two indicated normalization methods ($p<0.05$)

Muscle	MVIC / Sprint Cycle	MVIC / Sprint Run	Sprint Cycle / Sprint Run
VL	**0.0158**	0.7036	0.5250
VM	**0.0002**	**0.0003**	0.3854
TA	0.08288	0.6505	**0.0421**
LG	0.2374	**< 0.0001**	**< 0.0001**
MG	0.0724	**< 0.0001**	**0.0001**
BF	0.8268	**0.0408**	**0.0002**
ST	0.2132	**0.0201**	**< 0.0001**
GM	1.0000	**0.0039**	**0.0037**
TFL	**0.0127**	**0.0331**	**< 0.0001**

Table 2 - Mean relative muscle activations (with SD in brackets) and number of participants (% of total) who produced the greatest normalization value from each normalization method, for each muscle. Bold indicates methods that produced the greatest relative muscle activations ($p<0.05$, where applicable) and in which the most participants produced a maximum for each muscle.

	MVIC		Sprint Cycle		Sprint Run	
Muscle	Relative Muscle Activation	Number of participants	Relative Muscle Activation	Number of participants	Relative Muscle Activation	Number of participants
VL	55.71 (20.23)	0 (0.0%)	**91.47 (10.06)**	9 (45.0%)	**87.09 (21.10)**	**11 (55.0%)**
VM	46.22 (23.64)	1 (5.0%)	**78.07 (23.27)**	7 (35.0%)	**88.16 (17.60)**	**12 (60.0%)**
TA	86.13 (21.21)	**9 (45.0%)**	77.16 (22.69)	3 (15.0%)	89.78 (11.67)	8 (40%)
LG	26.78 (16.15)	0 (0.0%)	48.46 (25.46)	0 (0.0%)	**100.00 (0.00)**	**20 (100.0%)**
MG	33.03 (14.88)	0 (0.0%)	54.05 (22.79)	1 (5.0%)	**99.57 (1.93)**	**19 (95.0%)**
BF	72.86 (25.81)	6 (30.0%)	59.15 (21.80)	2 (10.0%)	**92.93 (14.94)**	**12 (60.0%)**
ST	72.59 (28.42)	3 (15.0%)	50.39 (26.69)	2 (10.0%)	**93.82 (12.15)**	**15 (75.0%)**
GM	53.46 (30.67)	2 (10.0%)	61.32 (31.32)	6 (30.0%)	**92.38 (13.82)**	**12 (60.0%)**

TFL	70.81 (25.70)	5 (25.0%)	41.34 (22.48)	1 (5.0%)	**93.59 (13.90)**	**14 (70.0%)**

Table 3 – Coefficient of Variation (with SD in brackets) for reference values for each method and muscle. Bolded values indicate the method that produced the lowest CoV for each muscle.

Muscle	MVIC			Sprint Cycle			Sprint Run		
	Mean%	SD%	Range%	Mean %	SD%	Range%	Mean %	SD%	Range%
VL	16.5	13.4	2.7-62.9	15.0	11.0	1.0-42.8	**10.8**	**6.0**	**2.9-29.2**
VM	20.1	17.7	4.6-69.8	**13.7**	**9.7**	**4.6-41.3**	19.4	15.6	2.6-55.0
TA	**8.8**	**5.9**	**1.7-20.8**	11.7	8.9	1.9-38.8	21.3	19.1	3.9-62.0
LG	**14.5**	**11.4**	**3.1-20.8**	14.6	11.6	2.7-44.1	29.0	23.1	3.3-73.7
MG	15.4	9.2	4.0-47.2	**14.3**	**21.1**	**2.6-25.5**	26.0	14.5	6.1-53.1
BF	**11.1**	**5.3**	**2.7-25.6**	25.5	28.2	3.8-81.1	14.6	12.5	1.4-43.6
ST	**11.4**	**8.8**	**2.6-31.6**	17.9	16.2	4.4-69.5	19.9	15.0	5.9-63.6
GM	**13.2**	**6.6**	**3.4-30.4**	19.6	12.7	4.3-48.4	27.1	19.7	6.3-69.9
TFL	**19.9**	**10.1**	**5.8-47.0**	23.3	15.6	5.9-68.6	34.0	22.3	6.8-87.8

Table 4a – Mean peak normalized values (SD) using each normalization method for walking trials, as % of normalization method. [A] are different between MVIC and Cycle, those indicated by [B] are different between MVIC and Run, while those indicated by [C] are different between Cycle and Run ($p < 0.05$).

Method	VL	VM [AB]	TA	LG [ABC]	MG [ABC]	BF [C]	ST	GM [B]	TFL [C]
MVIC	19.37 (7.66)	21.91 (21.92)	37.95 (8.09)	64.93 (27.43)	67.15 (18.73)	19.44 (9.05)	26.26 (32.65)	22.45 (15.87)	19.65 (19.19)
Sprint Cycle	11.22 (5.36)	15.42 (22.60)	44.33 (14.77)	35.27 (14.06)	42.07 (16.38)	24.23 (11.51)	44.21 (84.89)	17.15 (11.87)	23.99 (18.52)
Sprint Run	15.86 (23.67)	13.32 (22.32)	37.65 (14.35)	16.27 (9.85)	21.47 (10.53)	14.92 (7.11)	17.43 (17.56)	13.09 (10.51)	10.53 (9.10)

Table 4b – Mean peak normalized values (SD) using each normalization method for cycling trials, as % of normalization method. [A] are different between MVIC and Cycle, those indicated by [B] are different between MVIC and Run, while those indicated by [C] are different between Cycle and Run ($p < 0.05$).

Method	VL	VM [AB]	TA	LG [ABC]	MG [ABC]	BF [C]	ST [AC]	GM [BC]	TFL [AC]
MVIC	62.32 (28.18)	64.73 (28.77)	29.21 (14.57)	55.78 (36.06)	77.18 (39.96)	23.23 (13.01)	18.39 (11.49)	16.63 (8.05)	20.88 (13.72)
Sprint Cycle	35.17 (17.25)	34.94 (17.99)	33.72 (17.13)	28.78 (14.78)	47.16 (21.71)	27.89 (14.80)	25.81 (12.12)	14.25 (8.38)	42.32 (34.24)
Sprint Run	50.31 (23.61)	33.76 (23.61)	28.34 (15.78)	13.63 (9.18)	24.92 (15.64)	18.29 (12.15)	13.41 (8.77)	8.76 (6.01)	16.48 (13.53)

Table 4c – Mean peak normalized values (SD) using each normalization method for running trials, as % of normalization method. [A] are different between MVIC and Cycle, those indicated by [B] are different between MVIC and Run, while those indicated by [C] are different between Cycle and Run ($p < 0.05$).

Method	VL [AB]	VM [AB]	TA	LG [AB]	MG [ABC]	BF [C]	ST [C]	GM [B]	TFL [AC]
MVIC	81.09 (27.52)	91.85 (51.26)	52.56 (17.55)	156.63 (128.10)	151.07 (61.53)	54.93 (26.02)	46.40 (42.19)	44.48 (32.73)	42.48 (23.11)
Sprint Cycle	47.21 (20.53)	57.50 (42.96)	58.65 (19.03)	77.33 (32.17)	93.36 (40.09)	66.52 (30.19)	75.64 (86.42)	41.35 (18.18)	71.04 (42.15)
Sprint Run	55.80 (42.53)	43.08 (22.53)	50.37 (19.19)	34.21 (16.67)	47.23 (19.53)	39.23 (13.88)	30.94 (17.40)	26.62 (12.14)	32.40 (16.30)

Table 5 - Comparison of within-day CoV (Mean % (SD, where available)) of normalization methods from the current study to Rouffet & Hautier (2007) and Norcross et al. (2010).

Muscle	MVIC			Sprint Cycle	
	Chuang et al. (2018)	Rouffet & Hautier (2007)	Norcross et al.,(2010)	Chuang et al. (2018)	Rouffet & Hautier (2007)
VL	16.5 (13.4)	19 (18)	14.5	15.0 (11.0)	9 (9)
MG	15.4 (9.2)	17 (20)	-	14.3 (21.1)	8 (8)
BF	11.1 (5.3)	10 (7)	9.0	25.5 (28.2)	19 (31)
GM	13.2 (6.6)	23 (25)	13.6	23.3 (15.6)	20 (12)