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Reproducibility of upper leg EMG frequency content during cycling

Rodrigo Rico Bini, Camila Peter Hoefelmann, Vitor Pereira Costa and Fernando Diefenthaeler

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ABSTRACT
Reproducibility of frequency content from surface electromyography (sEMG) signals has not been assessed and it is unknown if incremental load testing design could affect sEMG in cycling. The goals of this study were to assess the reproducibility of measures from sEMG frequency content between sessions and to compare these frequency components between a ramp and a step incremental cycling test. Eighteen cyclists performed four incremental load cycling tests to exhaustion. Two tests were performed using a step increment (load started at 100 W for 3 min followed by increments of 30 W every 3 min) and two were performed using a ramp increment (load started at 100 W for 1 min followed by increments of 30 W·min−1). sEMG was monitored bilaterally for the rectus femoris and vastus lateralis throughout the tests and converted into overall activation (whole signal bandwidth), high- and low-frequency contents. The reproducibility of the frequency content ranged from none to strong (ICC = 0.07–0.90). Vastus lateralis activation was larger at the step compared to the ramp test (P < 0.01), without differences for rectus femoris (P = 0.22–0.91) and for the high-frequency (P = 0.28–0.95) and low-frequency contents (P = 0.13–0.94). sEMG from vastus lateralis and rectus femoris presented none to strong reproducibility. Vastus lateralis is more activated in step test design.

1. Introduction
Surface electromyography (sEMG) enables the assessment of muscle activation during various exercise modes. In cycling, sEMG has been used to detect potential changes in neural drive to skeletal muscles due to changes in exercise intensity (Diefenthaeler, Bini, & Vaz, 2012; Macdonald, Farina, & Marcora, 2008), pedalling cadence (Sanderson, Martin, Honeyman, & Keefer, 2006), body position on the bicycle (Dorel, Couturier, & Hug, 2009) and changes in fatigue state (Dorel, Drouet, Couturier, Champoux, & Hug, 2009). The reason for a link between many factors and sEMG involves the connection between neural drive and muscle adenosine triphosphate (ATP) usage during muscle contraction, which implies that larger muscle activation is directly involved with an increase in energy cost (Blake & Wakeling, 2013).

Although greater exercise intensity leads to larger ATP usage and potentially to increased sEMG, studies have shown that, in incremental load tests, sEMG presents a non-linear profile of increase, similar to the one observed for ventilation (Hug, Laplaud, Savin, & Grélot, 2003). This non-linear increase was associated to changes in metabolic activity from aerobic to anaerobic priority (Racinais, Buchheit, & Girard, 2014). This non-linear response also led authors to verify that break points in sEMG during incremental cycling tests do match those observed in lactate (Candotti et al., 2008) and ventilation (Hug et al., 2003). Indeed, the link between sEMG and physiological break points was supported by potential increases in recruitment of larger size motor units, which are driven at greater frequency and intensity (Holt, Wakeling, & Biewener, 2014). Therefore, in order to sustain longer at incremental load tests, cyclists may be required to recruit larger size motor units at the final stages of the test.

The issue of recruiting larger size motor units at the final stages of an incremental load test is that these motor units are more prone to fatigue (Defreitas, Beck, Ye, & Stock, 2014), which could limit their support for force production in longer duration exercises. With that in mind, Macdonald et al. (2008) assessed vastus lateralis and vastus medialis activation using sEMG and observed an increase in activation associated to a faster muscle fibre-conduction velocity, suggesting an increased recruitment of faster motor units at latter stages of the test. Latter, Priego-Quesada, Bini, Diefenthaeler, and Carpes (2015) decomposed the sEMG signal using frequency band analysis in order to assess changes in high- and low-frequency components of the signal during an incremental maximal cycling test to exhaustion. This method has been shown effective to highlight changes in muscle recruitment when fatigue state is modified (i.e., larger fatigue) (Diefenthaeler, Bini, et al., 2012; von Tscharner, 2002). Priego-Quesada et al. (2015) however, observed any increases in high-frequency components during the test, which was explained by the opposite effects from increases in intensity (larger demand for greater motor units) and increases in fatigue (reduced recruitment of greater motor units).

A pending issue is the reproducibility of measures taken from sEMG, due to changes in position of electrodes in relation to the muscle belly, which could potentially limit spectral properties from sEMG (Merletti, Botter, Troiano, Merlo, & Minetto, 2009). The overall sEMG signal (before partition into

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frequency bands) presented varied reproducibility during cycling (variation between testing sessions of 11%-43%; Jobson, Hopker, Arkestein, & Passfield, 2013; Macdonald et al., 2008; Travis, Arthmire, Baig, Goldberg, & Malek, 2011). However, no study was found providing information on the reproducibility of the frequency band analysis method during cycling. In addition, although Zuniga et al. (2013) compared a ramp (15 W.min\(^{-1}\) – 1 W every 4 s) to a step test (30 W every 2 min), there is no prior study comparing sEMG signals in terms of time–frequency content (e.g., via frequency band analysis or wavelets). This information is important given changes in time–frequency content of the sEMG have been associated with changes in fatigue state (Diefenthaeler, Bini, et al., 2012; von Tscharner, 2002) and with a combination of load and fatigue effects (Priego-Quesada et al., 2015).

In order to address the aforementioned questions, the goals of this study were to (1) assess the between session reproducibility of measures from the frequency band analysis method (high- and low-frequency content in sEMG) and (2) compare these frequency components between a ramp and a step incremental cycling test to exhaustion.

2. Methods

2.1. Participants

Eighteen competitive male cyclists (Category 2 according to Ansley and Cangley 2009) with 25 ± 8 years of age, 71 ± 7.7 kg of body mass and 177 ± 4.7 cm of standing height participated in the study. All cyclists have been regularly involved in national cycling competitions for the last 2 years at the time of the study. Trained cyclists were assessed because they are regularly involved in performance testing and are largely familiarised with the effort involved in maximum aerobic tests.

Before the start of the evaluation session, all procedures were presented to cyclists who signed a consent form to participate in the study that was approved by the Ethics Committee of Human Research where the study was conducted (#2346/2011), in agreement with the Declaration of Helsinki.

2.2. Data collection

After reading and signing the consent form, cyclists’ body mass and standing height were assessed at the first session using a weight scale (2096 PP, Toledo, Rio de Janeiro, Brazil) and a stadiometer (ES2030, Sanny, São Bernardo do Campo, Brazil), respectively. After that, cyclists’ right and left legs were prepared for recording muscle activation using sEMG. Pairs of Ag/AgCl electrodes (Kendall™ 100 Foam Electrodes, Conductive Adhesive Hydrogel with 22 mm diameter, bipolar configuration with 2.2 cm of interelectrode distance (differential mode), Covidien, Mansfield, MA, USA) were positioned on the skin after carefully shaving and cleaning the area using an abrasive cleaner and alcohol swabs to reduce the skin impedance as recommended by the International Society of Electrophysiology and Kinesiology (Merletti et al., 2009). Electrodes were placed over the belly of the rectus femoris and vastus lateralis muscles and the skin where the electrodes were primarily attached was marked using a skin pen. A map for electrodes replacement was developed for each leg of each participant using acetate sheets measuring distances between the electrode site and anatomical landmarks (Konrad, 2005) in order to enable a proper replacement of each electrode at the following sessions. Cables connecting electrodes to the main amplifier were fixed to the leg using straps to minimise motion artefacts in EMG signals.

Participants were then positioned on a cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), matching the dimensions of their bicycles. They performed, in four separate sessions, two ramp and two step incremental load tests to exhaustion, assigned in random order. All tests were separated by 24 h at least, in which cyclists were instructed to refrain from training and to avoid drinking alcohol and caffeine. Ramp tests involved cycling at a load of 20 W for 4 min, for warm-up purposes, then an increase in load for 100 W was imposed for starting the test. Load was incremented by 1 W every 2 s (30 W·min\(^{-1}\)), in order to provide smooth increments in pedalling resistance. Step tests started at 100 W for 3 min followed by increments in load of 30 W every 3 min, until exhaustion. Muscle activation was measured continuously, during all testing sessions, at 2 kHz per channel using a sEMG system (Miotool 400, Miotec, Porto Alegre, Brazil), with common mode rejection ratio of the EMG system was 115 dB at 60 Hz with a 14-bit analog-to-digital converter controlled by commercial software (Miograph, Miotec, Porto Alegre, Brazil).

2.3. Data analyses

Peak power output (PO\(_{\text{PEAK}}\)) was also determined for each testing session. Muscle activation signals were corrected for offset drift and smoothed with a digital second-order zero lag band-pass Butterworth filter, with cut-off frequencies defined at 20 and 500 Hz.

Procedures of frequency band analyses usually followed discrete analyses of band-pass filtered signals (Diefenthaeler, Bini, et al., 2012; Priego, Bini, Lanferdini, & Carpes, 2014; Priego-Quesada et al., 2015). Each muscle’s EMG signal was filtered using each of the nine combinations of high (193.45–300.80 Hz) and low (26.95–48.45 Hz) band stop (frequency bands) filters (Table 1). EMG signals of each of the nine frequency bands that resulted from the filtering process were then converted into their root mean square values (RMS) computed using moving average windows of 40 ms (Neptune, Kautz, & Hull, 1997) to determine the intensity of muscle activation throughout the test.

<table>
<thead>
<tr>
<th>Band</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>48.45</td>
<td>75.75</td>
<td>110.00</td>
<td>149.00</td>
<td>193.45</td>
<td>244.45</td>
<td>300.80</td>
<td>363.80</td>
<td>431.65</td>
</tr>
<tr>
<td>Low</td>
<td>26.95</td>
<td>48.45</td>
<td>74.80</td>
<td>108.00</td>
<td>146.95</td>
<td>191.75</td>
<td>242.20</td>
<td>297.40</td>
<td>359.35</td>
</tr>
</tbody>
</table>
The sum of the nine average frequency bands was calculated for the computation of the magnitude of the overall RMS of each muscle (i.e., activation of all frequency bands of the EMG signal). The fifth, sixth and seventh bands were averaged to compute the RMS from high-frequency components of the signals, which would potentially represent the response of greater motor units (Wakeling & Horn, 2008). The first and the second bands were averaged to compute the RMS from low-frequency components of the signals, which would represent the response of smaller motor units (Wakeling & Horn, 2008).

RMS signals were selected from the full data set for each muscle from each cyclist at four instants of the test: 10%, 40%, 70% and 90% (averaged from 10% to 20%, 40% to 50%, 70% to 80% and 90% to 100% of the total test time) (Hug, Decherchi, Marqueste, & Jammes, 2004). The overall RMS in both legs were normalised to their individual responses at the start of the test (10–20%), where minimal fatigue effects are expected in order to reduce between-participants variability in EMG data (Diefenthaler, Coyle, Bini, Carpes, & Vaz, 2012). High- and low-frequency RMS values were normalised by cyclists’ overall RMS from each muscle in order to provide a percentage of the overall signal from each frequency content during the test. All signal processing were conducted using custom-made scripts in MATLAB® (Mathworks Inc., Natick, MA, USA).

### 2.4. Statistical analyses

Data were averaged for the 18 cyclists for the overall, high- and low-frequency bands for each muscle in each time window (i.e., 40%, 70% and 90% of the total time of each test). In order to assess the reproducibility between days of testing of these measures in each type of test (ramp and step), typical errors were computed as the ratio between the standard deviation from differences between days and the square root of “2” (TE = SDdiff/√2) (Hopkins, 2000), along with intraclass correlation coefficients (ICCs). Given most guidelines for labeling ICC values are subjective (Kottner et al., 2011), we decided ranking ICCs following methods from Dancey and Reidy (2004) for bivariate correlations (i.e., ICC ≤ 1.0 indicates perfect reproducibility, ICC between 0.7 and 0.99 indicates strong reproducibility, ICC between 0.4 and 0.69 indicates moderate reproducibility and ICC smaller than 0.39 indicates small to none reproducibility). Typical errors and ICCs were computed using a custom made spreadsheet (Excel, Microsoft, Inc., Redmond, WA, USA).

After that, student t-tests were conducted (assuming heteroscedasticity in data distribution) for comparison of the overall, high- and low-frequency bands in each time window between the ramp and step tests. In parallel, Cohen’s effect sizes were computed to test the magnitude of changes (Hopkins, Marshall, Batterham, & Hanin, 2009). Significant differences were assumed when $P < 0.05$ and ES > 0.8, to ascertain a non-overlap in mean scores greater than 55% (Cohen, 1988). Student t-tests and statistical analyses were conducted using custom made spreadsheets (Excel, Microsoft, Inc., Redmond, WA, USA).

### 3. Results

Larger maximal power output was observed for the ramp (408 ± 32 W) compared to the step test (353 ± 36 W, $P < 0.01$ and ES = 1.65), which was also reflected into larger power output at 40% (ramp = 223 ± 13 W vs. step = 201 ± 14 W), 70% (ramp = 316 ± 22 W vs. step = 277 ± 25 W) and 90% of the total time of the test (ramp = 378 ± 29 W vs. step = 327 ± 32 W) for the ramp compared to the step test. Total time for the step test (26 ± 3.33 min) was larger than the total time for the ramp test (10.30 ± 1.07 min, $P < 0.01$ and ES = 7.18).

Reproducibility of the overall, high- and low-frequency content of muscles activation (right and left rectus femoris and vastus lateralis) ranged from none (ICC = 0.07) to strong (ICC = 0.90) when comparing testing sessions, between days, for the ramp and step tests (see Tables 2 and 3).

The comparison of testing design (ramp vs. step) indicated that overall activation from the right vastus lateralis was larger at the step compared to the ramp test (40% of the test – $P < 0.01$ and ES = 1.05). Likewise, the overall activation from the left vastus lateralis was larger at the step compared to the ramp test (40% of the test – $P < 0.01$ and ES = 1.01; 70% of the test – $P < 0.01$ and ES = 0.97; 90% of the test – $P = 0.02$ and ES = 0.91). Differently, rectus femoris overall activation did not differ between testing designs ($P = 0.22$–0.91 and ES = 0.04–0.46). Moreover, high- and low-frequency bands did not differ between testing designs for the rectus femoris ($P = 0.51$–0.86 and ES = 0.06–0.24 for high bands and $P = 0.13$–0.88 and ES = 0.05–0.54 for low bands) and for the vastus lateralis ($P = 0.28$–0.95 and ES = 0.02–0.39 for high bands and

| Table 2. Typical errors (TE) and intraclass correlation coefficients (ICC) for the overall, high and low frequency bands from the right and left rectus femoris muscles (RF) activation measured at the 40%, 70% and 90% of the total time of the ramp and step tests. |
|-------------------------------|-----------------|-----------------|-----------------|
|                               | 40%            | 70%            | 90%            |
|                               | High           | Low            |
|                               | 40%            | 70%            | 90%            |
| RF left                       |                |                |                |
| Ramp – TE                    | 0.23 (0.34)    | 0.34 (0.44)    | 0.22 (0.34)    |
| Ramp – ICC                   | 0.03 (0.03)    | 0.07 (0.09)    | 0.07 (0.09)    |
| Step – TE                    | 0.21 (0.31)    | 0.23 (0.32)    | 0.21 (0.31)    |
| Step – ICC                   | 0.07 (0.07)    | 0.09 (0.10)    | 0.07 (0.07)    |
| RF right                     |                |                |                |
| Ramp – TE                    | 0.08 (0.11)    | 0.18 (0.22)    | 0.07 (0.10)    |
| Ramp – ICC                   | 0.06 (0.07)    | 0.08 (0.09)    | 0.06 (0.07)    |
| Step – TE                    | 0.06 (0.07)    | 0.07 (0.10)    | 0.06 (0.07)    |
| Step – ICC                   | 0.06 (0.07)    | 0.07 (0.10)    | 0.06 (0.07)    |
P = 0.59–0.94 and ES = 0.03–0.19 for low bands) as shown in Figures 1 and 2.

4. Discussion

This study aimed at assessing the reproducibility of sEMG during cycling taken at separate sessions and to compare the responses of rectus femoris and vastus lateralis between two incremental loading tests (ramp vs. step). The partition of sEMG signals into their high- and low-frequency content enhanced the novelty of the present study given that the reproducibility of these measures has not been assessed previously. Moreover, the responses of high- and low-frequency content of sEMG signals have not been compared in incremental cycling tests to exhaustion using different load increments (ramp vs. step). The main findings from the present study were that the reproducibility of the overall activation from vastus lateralis was greater than the reproducibility of the overall activation from rectus femoris, with a better reproducibility for the step compared to the ramp test. This result is aligned with findings from Travis et al. (2011), who observed increased reproducibility for the vastus lateralis compared to the rectus femoris. In addition, high- and low-frequency content of these muscles presented moderate to good reproducibility. For the comparison between test designs, larger

![Table 3](image)

### Table 3. Typical errors (TE) and intraclass correlation coefficients (ICC) for the overall, high and low frequencies from the right and left vastus lateralis muscles (VL) activation measured at the 40%, 70% and 90% of the total time of the ramp and step tests.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40%</td>
<td>70%</td>
<td>90%</td>
</tr>
<tr>
<td>Ramp – TE</td>
<td>7%</td>
<td>11%</td>
<td>20%</td>
</tr>
<tr>
<td>Ramp – ICC</td>
<td>0.62</td>
<td>0.79</td>
<td>0.67</td>
</tr>
<tr>
<td>Step – TE</td>
<td>8%</td>
<td>17%</td>
<td>23%</td>
</tr>
<tr>
<td>Step – ICC</td>
<td>0.60</td>
<td>0.57</td>
<td>0.62</td>
</tr>
<tr>
<td>VL right</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>70%</td>
<td>90%</td>
</tr>
<tr>
<td>Ramp – TE</td>
<td>7%</td>
<td>13%</td>
<td>20%</td>
</tr>
<tr>
<td>Ramp – ICC</td>
<td>0.44</td>
<td>0.51</td>
<td>0.53</td>
</tr>
<tr>
<td>Step – TE</td>
<td>8%</td>
<td>13%</td>
<td>17%</td>
</tr>
<tr>
<td>Step – ICC</td>
<td>0.68</td>
<td>0.70</td>
<td>0.78</td>
</tr>
<tr>
<td>VL left</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>70%</td>
<td>90%</td>
</tr>
<tr>
<td>Ramp – TE</td>
<td>2%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Ramp – ICC</td>
<td>0.90</td>
<td>0.91</td>
<td>0.90</td>
</tr>
<tr>
<td>Step – TE</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>Step – ICC</td>
<td>0.86</td>
<td>0.83</td>
<td>0.85</td>
</tr>
</tbody>
</table>

![Figure 1](image)

Figure 1. Right and left rectus femoris activation (presented as mean ± SD of RMS) at 40%, 70% and 90% of the total time of the test. No differences between testing designs (ramp vs. step) were observed for overall, high- or low-frequency bands.
activation was observed for the vastus lateralis overall activation at the step compared to the ramp test, without differences for the high- and low-frequency content. These findings are novel because although sEMG has been used to determine metabolic thresholds (Candotti et al., 2008; Hug et al., 2003), the influence of testing design (ramp vs. step) on time–frequency content of sEMG measures has not been assessed previously.

Differences in testing design to determine maximal aerobic capacity have indicated that cyclists produce larger peak power output at shorter incremented tests (i.e., 25 W·min⁻¹ vs. 50 W every 3 min) at similar maximal oxygen uptake (Amann, Subudhi, & Foster, 2004). This result suggests that potential differences in muscle recruitment would exist, which was partially confirmed by our study through larger activation of vastus lateralis at the step compared to the ramp test. This finding would indicate that larger percentage of small size motor units would be recruited at the step test, which would lead to lower power production. Moreover, a longer duration for the step test would mitigate the contribution from large size motor units, but no difference in frequency content was observed between testing designs. This result implies that a larger recruitment of both high- and low-frequency content would be needed for sustaining a larger activation at the step test. We can infer that, at the step test, the longer duration at each load increment would have limited power output production, leading to less power and larger muscle activation, therefore, a reduced muscle efficiency. This is somewhat in line with observations from Zuniga et al. (2013), who observed that the step test may induce greater fatigue influence in muscle recruitment and/or firing rate due to the longer time in each load step compared to the ramp.

The lack of differences, between testing designs, for the rectus femoris is potentially due to the role of this bi-articular muscle as a power transfer between joints (Van Ingen Schenau, Pratt, & Macpherson, 1994). This role implies that a fine tune in activation of this muscle is needed in order to control the transition between phases of pedalling cycle (downstroke and upstroke – see Zajac, Neptune, and Kautz (2002)), which would lead to a larger variability in activation of this muscle between cyclists. Therefore, although, rectus femoris is sensitive to changes in power output (Bini, Carpes, Diefenthaeler, Mota, & Guimarães, 2008), the control of leg motion would be a primary role of this muscle.

sEMG frequency content has been used in an attempt to infer on changes in recruitment of small and large size motor units (Holt et al., 2014). However, no previous study showed that the time–frequency content of sEMG signal has moderate to good reproducibility, as the present study. This result is important because previous studies observed that changes in fatigue state affect sEMG frequency content (Diefenthaeler, Bini, et al., 2012; von Tscharner, 2002). Moreover, Priego-Quesada et al. (2015) highlighted a greater contribution from the low-frequency component for biceps femoris during incremental load test in cycling, which is aligned to the reduced content of small
size motor units in this muscle (Johnson, Polgar, Weightman, & Appleton, 1973). Further studies could assess if fibre type and motor unit sizes could be detected by the assessment of time–frequency content of sEMG. The reason for that is associated to findings from Priego et al. (2014), who observed that cyclists presented larger proportion of high-frequency components in tibialis anterior and gastrocnemius medialis, compared to upper leg muscles (e.g., vastus lateralis). However, no studies presented fibre type proportion in these muscles for cyclists.

The current study was limited because surface EMG is limited in capturing muscle activation signals (i.e., adipose tissue filtering effect, crosstalk recording from nearby muscles and muscle temperature in frequency contents of EMG signals). A correction for differences between muscles in subcutaneous adipose tissue could be an alternative for assessment of differences in frequency content between muscles. This correction could indicate whether differences are due to motor unit profile or to the distance from the muscle fibres to the skin electrodes. An additional potential limitation from our experimental design was that, we were unable to assess cyclists in three session for each load increment (ramp vs. step), which could have strengthened the outcomes of our study.

As practical applications, we can suggest that overall sEMG signals could be varied across different days, which should be used with caution as a measure of adaptation from intervention and/or training. Moreover, although reproducibility of frequency content from sEMG signal could be good, further studies are needed to assess their association with metabolic thresholds. For the type of test, the ramp test potentially elicited reduced fatigue component compared to the step test, due to the larger maximum power output. However, step tests are needed when biomechanical (Bini, Senger, Lanferdini, & Lopes, 2012) or lactate variables (Candotti et al., 2008) are assessed due to the steady state period required to acquire consistent measures.

5. Conclusion

In summary, the sEMG frequency content from vastus lateralis and rectus femoris presented moderate to good reproducibility during step and ramp incremented load tests to exhaustion in cycling. Larger activation was observed for vastus lateralis at the step compared to the ramp test for a lower power production, without differences in frequency content for this muscle and for the rectus femoris. This result implies that, although maximal power output is lower for step tests, vastus lateralis is more activated in this testing design.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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