European Journal of Human Movement, 2018: **40**, 15-28

RELIABILITY AND NORMALIZATION OF LONG-TERM VERSUS SUBSTITUTED ELECTROMYOGRAPHY ELECTRODES SET-UPS

Stefan Kratzenstein; Jan-Peter Brueckner; Christian Schlenstedt; Manfred Wegner

Institute for Sport Science, Department of Neurology, Kiel University, Germany.

ABSTRACT

Normalization methods are used to minimize the influence of external disturbances in the analysis of myo-electrical signals. Whether these methods actually minimize the error or only relocate it has not been evaluated so far. This verification is only possible in comparison to constant discharge conditions. The aims of this study were (I) to compare the inter-day test-retest reliability of myo-electrical signals from long-term with substituted electrodes and (II) the impact of different normalization methods on test-retest reliability. Myo-electrical activity of the tibialis anterior (TA) and vastus medialis (VA) was recorded from 14 cyclists during 1min cycling with permanent and substituted electrodes and then normalized by selected methods. A good reliability (ICC>0.86) was shown for both muscles with the long-term electrodes whereas poor reliability (ICC=0.32) was found for the substituted electrodes set-up (VM). Only the sprint normalization improved the reliability of the long-term electrode set-up, while the other functional approaches improved at least the reliability from substituted electrodes. Only the sprint method achieved the reliability of permanently attached electrodes. Thus, the results rather suggest the use of permanent electrode set ups. If this is not possible due to the study design, the use of functional normalization procedures is recommended.

Key words: amplitude normalization, cycling, surface electromyography, reliability

FIABILIDAD Y NORMALIZACION DE LA PREPARACIÓN CON ELECTRODOS DE LARGA DURACIÓN, FRENTE A LOS ELECTRODOS ELECTROMIOGRÁFICOS DESECHABLES

RESUMEN

Los métodos de normalización se utilizan para minimizar la influencia de las perturbaciones externas en el análisis de señales mioeléctricas. No se ha evaluado hasta el momento si estos métodos realmente minimizan el error o solo lo reubican. Esta verificación solo es posible en comparación con las condiciones de descarga constantes. Los objetivos de este estudio fueron (I) comparar la fiabilidad test-retest de señales mioeléctricas de larga duración con electrodos desechables y (II) el impacto de diferentes métodos de normalización en la fiabilidad test-retest. La actividad mioeléctrica del tibial anterior (TA) y el vasto medial (VA) se recogió de 14 ciclistas, durante un ciclo de 1min con electrodos de larga duración y desechables y luego se normalizó mediante métodos seleccionados. Se observó una buena fiabilidad (ICC> 0,86) para ambos músculos con los electrodos de larga duración, mientras que se encontró una fiabilidad deficiente (ICC = 0,32) para la configuración de los electrodos desechables (VM). Solamente la normalización del sprint mejoró la confiabilidad de la configuración del electrodo de larga duración, mientras que los otros enfoques funcionales mejoraron al menos la confiabilidad de los electrodos desechables. Solo el método de sprint logró la fiabilidad de los electrodos conectados permanentemente. Por lo tanto, los resultados sugieren más bien el uso de configuraciones permanentes de electrodos. Si esto no es posible debido al diseño del estudio, se recomienda el uso de procedimientos de normalización funcional.

Palabras clave: amplitud de normalización, ciclismo, electromiografía superficial, fiabilidad

Correspondence: Stefan Kratzenstein Stefan.Kratzenstein@email.uni-kiel.de
Institute for Sport Science, Department of Neurology, Kiel University, Germany.
Submitted: 26/03/2018 *Accepted:* 13/06/2018

INTRODUCTION

Electromyography (EMG) is a common method to study neuro-muscular activity investigating clinical treatments (Barbosa, Santos & Martins, 2015; Hogrel, 2005; R. Merletti & Farina, 2016; Yang et al., 2014) and sports performances (Ertl, Kruse & Tilp 2016). Especially in cycling, it is often used to analyze muscle activation strategies in sports and therapy (Hug & Dorel, 2009). The main focus here is on the change of activation patterns after training or therapy interventions, which requires a reliable inter-day reproducibility.

EMG reliability can be impacted by physical (internal) and non-physical (external) factors (Burden & Bartlett, 1999; Farina, 2006; de Luca, 1997). Physical factors (i.e. muscle fiber spectrum, geometrical changes due to joint motion, anthropometry) usually cannot be controlled by the observer and contribute to an unknown magnitude to the stochastically signal (Farina, Macaluso, Ferguson & Vito, 2004; Rouffet & Hautier, 2008).

On the other hand, non-physical factors (i.e. inter-electrode distance, electrode placement, EMG device) seem to be well investigated (Albertus-Kajee, Tucker, Derman & Lambert, 2010; Beck et al., 2006; Campanini et al., 2007; Farina, Merletti & Enoka, 2004; Mesin, Merletti & Rainoldi, 2009; Ochia & Cavanagh, 2007). The findings of those studies complement and the recommendations of the SENIAM (Surface EMG for Non Invasive Assessment of Muscles,) project (Hermens, Freriks, Disselhorst-Klug & Rau, 2000) and improve the reliability of the signal.

The influence of different factors contributing to inter-day reproducibility of neuromuscular activity during cycling was investigated by Laplaud et al. (2006). In an incremental test protocol, they found that signals were reproducible with a few exceptions (e.g. the m. rectus femoris). Dorel et al. (2008) completed the picture and investigated the intra-session reliability during a sub-maximal cycling task, which was also found to be given. However, they concluded that amplitude-related parameters show limited reliability. A reference to external factors is necessary to understand which factors impact reliability of EMG signals. Chapman (2010) addresses this problem and compares signals form a surface EMG with a fine-wire EMG. As a result, a reduction of the reliability by amplitude normalization has been shown and the use of fine-wire EMG was recommended. Although, this conclusion emphasizes the limitation of the surface EMG, it does not explain the key limiters.

Especially in longitudinal studies the impact of external factors is crucial as it has been shown that the substitution of electrodes between the measurements can create new measurement configurations. Even a small deviation in the electrodes' position and orientation will lead to the quantification of different motor units that contribute to skeletal motion (de Luca, 1997). Although the assumption that a change in the electrode position results in the analysis of different and at the same time unknown motor units appears as a logical construct, to our best knowledge, the inter-day reliability after electrode substitution has never been analyzed.

Normalization of EMG amplitudes is a standard procedure to overcome the limitation of electrode replacement and other external factors. However, there is no agreement which normalization procedure is most reliable. Recent studies developed and compared normalization methods that considered isometric (Ericson, 1986; Marsh & Martin, 1995; Potvin & Bent, 1997) and functional approaches (Albertus-Kajee et al., 2010; Hug & Dorel, 2009; Norcross, Blackburn & Goerger, 2010; Rouffet & Hautier, 2008). The studies leave the impression that the suitability of normalization procedures is strongly dependent on examination design, including the respective activities and participants. On the other hand, all studies aim at achieving a degree of reliability, which theoretically can only be reached by excluding the above-mentioned external and internal disturbances. However, this benchmark has not been quantified.

In this study the application and comparison of fixed and regular electrode arrangements, should allow a quantification of the influence of electrode repositioning. The study has two aims: (I) First, the comparison of the inter-day test-retest reliability of myo-electrical signals from fixed with regular electrodes and (II) second, the impact of different normalization methods on test-retest reliability.

METHOD

Participants

14 healthy adults (male n=8; female n=6; mean age: 25 ± 2.6 years) participated in this study. Participants were excluded if they reported any history of lower limb injuries and more than 7 hours of physical activity per week. All participants were informed about the given risks and gave their written consent to participate in this study, previously approved by the institutional ethical committee.

Testing procedure

Participants were assessed at 3 consecutive days. EMG recordings were obtained during cycling and during different normalization exercises to produce reference values in order to normalize absolute EMG parameters into so-hypothesized reproducible relative values. Participants attended at the same daytime to reduce the influence of circadian rhythms (Thun, Bjorvatn, Flo, Harris & Pallesen, 2015) and were requested to avoid athletic activity during the investigation period that would result in different levels of fatigue prior to the study. The individual warm up prior to testing included low intensity exercises and stretching. The order of cycling and normalization exercises was randomized.

Cycling

Cycling was performed on a bicycle ergometer (Cyclus 2™, RBM elektronik-automation GmbH, Germany). The road bike geometry was adjusted to individual anthropometry. No clip-less pedals were used, as it is expected in clinical settings. Crank position was measured using a multiaxial accelerometer (1000 Hz, myon 320, prophysics AG, Switzerland). It was attached at the furthest position relative to the bottom bracket. A monitor was used to provide visual feedback regarding cadence, power output and cycling time. Participants cycled 1 min at a permanent load of 150/170 W (female/male) and a cadence of 90 min^{-1}. Duration and load of cycling trial were chosen to avoid any muscular fatigue. Prior the study, three non-cyclists were asked to test the set-up. Pre-tests were conducted to ensure, the non-fatigue characteristic of the set-up and the appropriateness of the cycling parameters.

Normalization methods

Maximal voluntary isometric contraction (MVC), sprint, squat jumps and squats served as normalization exercises to cover the spectrum from maximal isometric contractions to sub-maximal till maximal functional exercises.

According to SENIAM recommendations, MVC of the *m. vastus medialis* (VM) during an isolated knee extension was performed at a 90° knee angle. During the exercise participants were sitting in an upright position with their arms hanging straight down and their hands holding designated grips. MVC of the *m. tibialis anterior* (TA) was measured with isometric dorsal flexion of the right foot in a standing upright position. For both MVC measurements, participants received the same instructions in each session. The isometric force had to be increased slowly until the maximal level and then be maintained for 5 s.

The *sprint method* was conducted by using the same set-up as described for cycling. All participants accelerated from standstill to the maximum of their sprint ability. After achieving a maximal cadence of 100 min^{-1}, the ergometer increased the resistance automatically to prevent a further increase in cadence. The participants, however, were instructed to provide maximal effort to further accelerate. According to Albertus-Kajee et al. (2010) the duration was 10s. Participants were told to remain seated on the bike for the entire test. Hand positions were documented and kept consistent during the test.

Squat jumps were performed as described in Ball and Scurr (2010) with a starting position of 90° knee flexion and forearms crossed in front of the chest. The *squats* exercises included the same posture. A visual scale provided a permanent feedback of the joint position during 10 repetitions of squats, while the exercise velocity was controlled using a metronome (0.5 beats/s).

Electromyography (EMG) and muscles

The skin preparation procedure was implemented after the SENIAM recommendations (Hermens et al., 2000). EMG signals were obtained from TA and VM. Both muscles were shown to contribute to cycling motion (Bijker, Groot & Hollander, 2002; Hug & Dorel, 2009). Instructions for localization and orientation of electrodes' position had to be adjusted according to the purpose of this study. After the regular center of electrodes had been identified, two pairs of long-term and substituted electrodes (Ag/AgCl, Ambu™, blue sensor N, inter-electrode distance 20mm) were attached parallel along the muscle fibers (Fig.1). The mid-line of both electrode pairs corresponded to the concrete SENIAM recommendation. The long-term electrodes were extensively covered with breathable tape to protect the set-up against exterior damage (e.g. clothes). Substituted electrodes were outlined to enable a reproducible replacement of electrodes for each re-test session. After the connection with EMG sensors (myon 320, prophysics AG, Switzerland), wires were fixed, using ordinary tape.

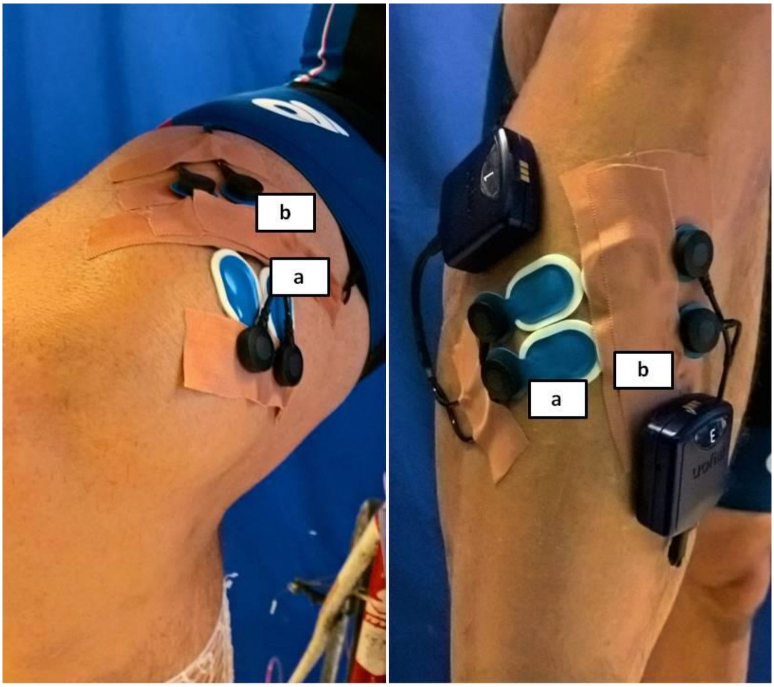

FIGURE 1: *EMG electrode set-ups at the m. vastus medialis (left) and m. tibialis anterior. (a) Electrodes were replaced between measurements after the position has been marked for reproducible replacement. (b) Electrodes were kept permanently at the same position.*

Myo-electrical signals were collected at 1000 Hz. Signal post-processing included 10-500 Hz band pass filtering (Butterworth, 2nd order) and rectification. Efforts in terms of isolated muscle contractions were undertaken to identify and minimize crosstalk prior to the measurement.

EMG analysis

MVC value was extracted from the central 3 s section of the 5 s maximum isometric contraction. The calculation procedure of the root mean square (RMS) corresponds to Hunter et al. (2002) who recommend sub-maximal parameters to be more reliable, when analyzing sub-maximal exercises.

According to Albertus-Kajee et al. (2010), 3 full cycle periods of the central period of the *10 s sprint* were isolated, each RMS calculated and averaged. Start and end of each cycle were defined by extracting the crank position from acceleration data (Myon 320, 1000Hz).

Squat reference value was calculated as the average RMS of the middle five of ten repetitions. Start and end of each squat activity were defined at passing the amplitude at two standard deviations of the signal in rest. *Squat jump* reference value was defined as the peak of signal amplitude.

Statistical analysis

Intra-class correlation coefficient (*ICC*) was applied to compare inter-day reliability between myo-electrical signals obtained from substituted versus long-term electrodes, whereby RMS of 90 cycles were used in each case. Intra-class correlation parameters were set at two-way mixed and absolute agreements.

Reliability of myo-electrical measurements was assessed calculating split-half reliability for all (14 participants x 2 muscles x 2 pairs of electrodes) 1minute-cycling trials. Here, the correlation between the RMS of the first 30 seconds and the RMS of second 30 seconds was calculated for each trial over all days. The average overall correlation coefficients (Pearson) were considered to assess whether cycling provides a reliable basis for the evaluation of normalization methods. The standard error of measurement (*SEM*) was calculated for each subject's set of repetitions and averaged over all 14 participants. Values were output as relatives to the mean of each subject's set of repetition. In order to gain a better understanding of the influence of the normalization methods, the same procedures were also applied on the normalization values itself. The significance of improvements due to application of normalization methods was proved by using a Student t-Test (p=0.05).

ICC was calculated to assess the reliability of normalized values and reference values. According to Sleivert and Wenger (1994) ranges of reliability were set at: 1-0.8 as "good", 0.79-0.6 as "fair" and less than 0.6 as "poor" reproducibility. SPSS (IBM) version 22.0 was used for statistical analysis.

RESULTS

Test-Retest reliability of long-term and substituted electrode set-ups

The internal consistency of cycling data over all applications was analyzed using split-half-reliability. A correlation coefficient of r=0.98 (p<0.01) stated an excellent reliability within each trial.

TABLE 1

Mean intra-class correlation coefficients for 1min sub-maximal cycling from absolute data (long-term and substituted electrode set-ups) surface EMG electrodes and normalized applying maximal voluntary contraction, sprint, squat jump and squat methods for each muscle; m. vastus medialis and m. tibialis anterior. Values are presented as the average of n=14 (95%CI). SEM are presented as relatives to the mean of each subject's set of 3 repetitions.

		Intra-class correlation coefficient			
		M. Vastus Medialis	SEM	**M. Tibialis Anterior**	SEM
Absolute RMS	**Cycling_substituted**	0.32 (0.00-0.62)	21%	0.82 (0.58-0.94)	5%
	Cycling_long-term	0.86 (0.65-0.95)	6%	0.88 (0.71-0.96)	7%
Normalized RMS	**MVC**	0.66 (0.23-0.88)	13%	0.86 (0.66-0.95)	5%
	Sprint	0.91 (0.78-0.97)	3%	0.76 (0.42-0.91)	7%
	Squats	0.78 (0.47-0.92)	5%	0.84 (0.61-0.94)	9%
	Squat jump	0.29 (0.00-0.66)	37%	0.73 (0.34-0.91)	11%

The application of the long-term electrodes set-up (Table 1) achieved an *ICC* of 0.86 whereas poor reliability (*ICC*=0.32) was found for the substituted method for the VM. *ICC* was slightly higher for the long-term electrode method (*ICC*=0.88) in comparison to the substituted electrode method (*ICC*=0.82) for the TA.

Test-Retest reliability after amplitude normalization

For the VM, only the sprint method was able to improve the reliability (*ICC*=0.91) over the 3 days. While the analysis of the regular set-up's absolute RMS values was found to be "poor", the application of sprint and squat normalization methods could at least improve this reliability deficit (Table 1). However, none of both methods achieved higher reliability than "fair", while normalization through the squat jump remained in "poor" reliability (*ICC*=0.29). None of the normalization of the TA activity resulted in a better reliability than achieved by the long-term set-up. Although MVC and squat normalization (TA) produced at least almost equal *ICC* and *SEM* (*ICC*=0.86 and 0.84). *ICC*s (TA) for the sprint and squat jump were found to be "fair". However, none of the applications at the TA resulted in a better reliability as it is obtained from the analysis of absolute RMS values.

Analysis of normalization values' consistency

While all normalization methods achieved at least "fair" till "good" reproducibility of reference values for both electrode arrangements at the TA, only the MVC method provided a reproducible outcome at the VM (Table 2). All non-isometric approaches resulted in low *ICC* coefficients and therefore in poor reliabilities. The analysis of the *SEM* (Table 2) confirmed these findings and indicated that within the generation of normalization values a deviation around the average of up to 27% needs to be considered.

TABLE 2

Mean intra-class correlation coefficients for normalization exercises, comparing long-term with regularly attached pairs of EMG surface electrodes for each muscle; m. vastus medialis (VM) and m. tibialis anterior (TA). Values are presented as the average of n=14 (95%CI). The standard error of measurement (SEM) are presented as relatives to the mean of each subject's set of 3 repetitions.

	Intra-class correlation coefficient							
	M. Vastus Medialis				M. Tibialis Anterior			
	ICC (long-term)	SEM	ICC (substituted)	SEM	ICC (long-term)	SEM	ICC (substituted)	SEM
MVC	0.74 (0.48-0.90)	10%	0.84 (0.66-0.94)	7%	0.77 (0.53-0.91)	7%	0.76 (0.52-0.91)	7%
Sprint	0.30 (0.00-0.51)	25%	0.60 (0.29-0.83)	8%	0.77 (0.54-0.91)	7%	0.81 (0.60-0.93)	5%
Squats	0.29 (0.00-0.53)	22%	0.57 (0.25-0.82)	11%	0.85 (0.68-0.95)	8%	0.86 (0.88-0.98)	9%
Squat jump	0.23 (0.00-0.56)	27%	0.41 (0.08-0.72)	17%	0.71 (0.44-0.88)	5%	0.74 (0.50-0.90)	8%

DISCUSSION

Myo-electrical signals of a 1minute-cycling trial were utilized to compare the reliability of long-term against substituted electrodes over three days and normalization methods against non-normalized data. The eligibility of the data was tested and approved to provide a reliable basis (r=0.98) for further analysis. However, the high correlation coefficient led to assume that even a smaller number of cycles (n<90) would be sufficient.

The effect of electrode substitution

The first aim of the study investigated whether the substitution of electrodes results in a loss of reliability. This was investigated by comparing the myo-electrical signals of a substituted electrode set-up with those of a long-term set-up. A "good" reliability in combination with an *SEM* of 6-7% was shown for both muscles (TA and VM) with the long-term electrode method whereas poor reliability was found for the VM for the substituted method. A long-term electrode method should be used rather than new substituted electrodes when investigating muscle activity during shorter periods of time (≤3 days), e.g. short-term interventions. Furthermore, although normalization improves reliability, normalization methods are not always feasible as people with cardio-vascular or neurological disease are unable to perform normalization exercises, supporting the use of a long-term electrode method under these circumstances.

Our findings confirm the results made by de Luca (1997), who stated that a repositioning of the electrodes is associated with a fundamental change in the discharge conditions. Furthermore, reliability seems to depend on the muscle type since good reliability was found for the TA but not for the VM for the substituted method. Laplaud et al (2006) also analyzed test-retest reliability and found strong correlations between the days. Although the reproducibility of the TA corresponds to different reliability levels (ICC=0.74 and 0.82), they confirm that reproducibility of absolute RMS is possible. Contrary, this study could not confirm reliability of the VM. This difference may be due to a different level of motor skills, which according to Laplaud et al. (2006), has a significant effect on muscle activation.

The benefit of normalization methods

With respect to our second aim of the study, our results support that the use of normalization procedures is justified and improves reliability. They are intended to provide a reliable comparability if the examination is not possible without a change of the electrodes. This study revealed two different pictures according to the muscles. The application of the normalization procedures for the TA signals did not lead to any improvements in the reliability characteristics compared to the substituted electrode set-ups. However, as measured by the similar levels of reliability, there is also no clear decrease of reliability. The MVC method and the squats led to slightly higher values compared to the sprint and the squat jump. This can be explained by the fact that both methods do not require an extensive learning process. Additionally, advanced coordinative skills sprint ability cannot always be performed by recreational cyclists without a motor learning process (Laplaud et al., 2006). The squat jump's execution and movement variability is strongly dependent on the motor skills of the subject (Panoutsakopoulos, Papachatzis & Kollias, 2014).

While the non-normalized reliability analysis results in "poor" parameters, it would require a significant improvement in the reliability through the normalization process. The application of the sprint method led to an almost "very good" reproducibility. This even improved the result of the long-term electrode set-ups. As measured by the reproducibility of the reference values themselves, which is not given in this study, it can be assumed that functional normalization procedures are capable of transporting a progress in motor learning. Thus, while the participants learned to sprint over the examination period, the reference cycling became also economized. Therefore, in this case, the application of the sprint method also appears to be more appropriate than the RMS analysis of the long-term electrode set-up. This means that the application of this functional normalization can transport or compensate motor progression and day-form-dependent variability in the performance, too. This finding, however, is only apparent for the VM, which, however, is rather a power producer in cycling, than the TA. A similar result, but on a "fair" level of reliability with a lower improvement, is shown for the squat method. The application of the MVC method and the squat jumps failed to produce a reproducible result. Here, it can only be assumed, that the MVC test cannot represent the dynamic activation pattern of cycling. The conflict of connecting concentric and isometric movement patterns has already been discussed by a number of authors (Albertus-Kajee et al., 2010; Burden & Bartlett, 1999; Jobson, Hopker, Arkesteijn & Passfield, 2013).

Limitations of the study

The authors are aware of some limitations in the study design. The use of EMG electrodes over a period of three days can lead to a change in the conducting properties. According to a consultation with the manufacturer (Ambu™ blue sensor N), this change is only to be expected from the fifth day. Care was taken that the gel pad is completely enclosed by the adhesive surface. The gel of all electrodes was still moist when removed. The placement of the electrode pairs was based on the SENIAM guidelines. However, since this guideline is appropriate for bipolar derivatives, a compromise had to be found here. According to the investigations of Jensen et al. (1993), only a displacement along the muscle fiber would have influenced the signal derivation. In this study, the placement was parallel to the optimal position, similar to that used in studies with electrode clusters (Pereira et al., 2013).

The examination of only two selected muscles cannot depict the entire muscular interplay during the pedaling movement. The examination of this muscular section, however, could already reflect the necessity of normalization procedures. The authors are aware of the problems that are related to the small sample size (n=14) and lack of reliability. Contrary, this small number of subject emphasizes the range of variance that can be expected in individual analysis of muscle activity.

CONCLUSIONS

In summary, the use of long-term electrodes is recommended since they provided better reliability than substituted electrodes. Long-term electrodes should be used for short-term investigations not longer than 3 days and are especially useful when normalization exercises cannot be conducted due to injuries or diseases of the participants.

Normalization methods improved reliability for both, the long-term and the substituted electrode method. The choice of the normalization procedure must be adapted to the motor skills of the subject. This study utilized the commonly used MVC procedures, maximum (sprint), sub-maximum (squats) functional methods and a maximum effort method (squat jump). The squat jump seemed to be the least suitable in all comparisons whereas the sprint method best improved reliability for the VM.

REFERENCES

Albertus-Kajee, Y., Tucker, R., Derman, W. & Lambert, M. (2010). Alternative methods of normalising EMG during cycling. *Journal of Electromyography and Kinesiology*, *20*(6), 1036–1043. http://doi.org/10.1016/j.jelekin.2010.07.011

Ball, N. & Scurr, J. (2010). An assessment of the reliability and standardisation of tests used to elicit reference muscular actions for electromyographical normalisation. *Journal of Electromyography and Kinesiology*, *20*(1), 81–88. http:// doi.org/10.1016/j.jelekin.2008.09.004

Barbosa, D., Santos, C. P. & Martins, M. (2015). The Application of Cycling and Cycling Combined with Feedback in the Rehabilitation of Stroke Patients: A Review. *Journal of Stroke and Cerebrovascular Diseases*, *24*(2), 253–273. http://doi.org/10.1016/j.jstrokecerebrovasdis.2014.09.006

Beck, T. W., Housh, T. J., Johnson, G. O., Cramer, J. T., Weir, J. P., Coburn, J. W. & Malek, M. H. (2006). Electromyographic instantaneous amplitude and instantaneous mean power frequency patterns across a range of motion during a concentric isokinetic muscle action of the biceps brachii. *Journal of Electromyography and Kinesiology*, *16*(5), 531–539. https://doi.org/10.1016/j.jelekin.2005.10.002

Bijker, K., Groot, G. d. & Hollander, A. (2002). Differences in leg muscle activity during running and cycling in humans. *European Journal of Applied Physiology*, *87*(6), 556–561. http:// doi.org/10.1007/s00421-002-0663-8

Burden, A. & Bartlett, R. (1999). Normalisation of EMG amplitude: an evaluation and comparison of old and new methods. *Medical Engineering & Physics*. (21), 247–257. https://doi.org/10.1016/S1350-4533(99)00054-5

Campanini, I., Merlo, A., Degola, P., Merletti, R., Vezzosi, G. & Farina, D. (2007). Effect of electrode location on EMG signal envelope in leg muscles during gait. *Journal of Electromyography and Kinesiology*, *17*(4), 515–526. https://doi.org/10.1016/j.jelekin.2006.06.001

Chapman, A. R., Vicenzino, B., Blanch, P., Knox, J. J. & Hodges, P. W. (2010). Intramuscular fine-wire electromyography during cycling: repeatability, normalisation and a comparison to surface electromyography. *Journal of Electromyography and Kinesiology*, *20*(1), 108–117. https://doi.org/10.1016/j.jelekin.2008.11.013

De Luca, C. J. (1997). The Use of Surface Electromyography in Biomechanics. *Journal of Applied Biomechanics*, *13*(2), 135–163. https://doi.org/10.1123/jab.13.2.135

Dorel, S., Couturier, A. & Hug, F. (2008). Intra-session repeatability of lower limb muscles activation pattern during pedaling. *Journal of Electromyography and Kinesiology*, *18*(5), 857–865. https://doi.org/10.1016/j.jelekin.2007.03.002

Ericson, M. (1986). On the biomechanics of cycling. A study of joint and muscle load during exercise on the bicycle ergometer. *Scandinavian Journal of Rehabilitation Medicine. Supplement*, *16*, 1–43.

Ertl, P., Kruse, A. & Tilp, M. (2016). Detecting fatigue thresholds from electromyographic signals: A systematic review on approaches and methodologies. *Journal of Electromyography and Kinesiology*, *30*, 216–230. https://doi.org/10.1016/j.jelekin.2016.08.002

Farina, D. (2006). Interpretation of the surface electromyogram in dynamic contractions. *Exercise and Sport Sciences Reviews*, *34*(3), 121–127.http://doi.org/10.1249/00003677-200607000-00006

Farina, D., Macaluso, A., Ferguson, R. A. & Vito, G. de. (2004). Effect of power, pedal rate, and force on average muscle fiber conduction velocity during cycling. *Journal of Applied Physiology*, *97*(6), 2035–2041. http://doi.org/10.1152/jappl- physiol.00606.2004

Farina, D., Merletti, R. & Enoka, R. M. (2004). The extraction of neural strategies from the surface EMG. *Journal of Applied Physiology*, *96*(4), 1486–1495. http://doi.org/10.1152/japplphysiol.00162.2014

Hermens, H. J., Freriks, B., Disselhorst-Klug, C. & Rau, G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of Electromyography and Kinesiology*, *10*(5), 361–http://doi.org/374. 10.1016/S1050-6411(00)00027-4

Hogrel, J.-Y. (2005). Clinical applications of surface electromyography in neuromuscular disorders. *Neurophysiologie Clinique/Clinical Neurophysiology*, *35*(2-3), 59–71.
https://doi.org/10.1016/j.neucli.2005.03.001

Hug, F. & Dorel, S. (2009). Electromyographic analysis of pedaling: A review. *Journal of Electromyography and Kinesiology*, *19*(2), 182–198. https://doi.org/10.1016/j.jelekin.2007.10.010

Hunter, A. M., St Clair Gibson, A., Lambert, M. & Noakes, T. D. (2002). Electromyographic (EMG) normalization method for cycle fatigue protocols. *Medicine and Science in Sports and Exercise*, *34*(5), 857–861. https://doi.org/10.1097/00005768-200205000-00020

Jensen, C., Vasseljen, O. & Westgaard, R. H. (1993). The influence of electrode position on bipolar surface electromyogram recordings of the upper trapezius muscle. *European Journal of Applied Physiology and Occupational Physiology*, *67*(3), 266–273. https://doi.org/10.1007/BF00864227

Jobson, S. A., Hopker, J., Arkesteijn, M. & Passfield, L. (2013). Inter- and intra-session reliability of muscle activity patterns during cycling. *Journal of Electromyography and Kinesiology*, *23*(1), 230–237. https://doi.org/10.1016/j.jelekin.2012.08.013

Laplaud, D., Hug, F. & Grelot, L. (2006). Reproducibility of eight lower limb muscles activity level in the course of an incremental pedaling exercise. *Journal of Electromyography and Kinesiology*, *16*(2), 158–166. https://doi.org/10.1016/j.jelekin.2005.04.002

Marsh, A. P. & Martin, P. E. (1995). The relationship between cadence and lower extremity EMG in cyclists and noncyclists. *Medicine and Science in Sports and Exercise*, *27*(2), 217–225. http://doi.org/10.1249/00005768-199502000-00011

Merletti, R. & Farina, D. (Eds.). (2016). *IEEE Press series in biomedical engineering: Vol. 26*. *Surface electromyography: Physiology, engineering and applications*. Piscataway, NJ, Hoboken, NJ: IEEE Press; Wiley.

Mesin, L., Merletti, R. & Rainoldi, A. (2009). Surface EMG: the issue of electrode location. *Journal of Electromyography and Kinesiology*, *19*(5), 719–726. https://doi.org/10.1016/j.jelekin.2008.07.006

Norcross, M. F., Blackburn, J. T. & Goerger, B. M. (2010). Reliability and interpretation of single leg stance and maximum voluntary isometric contraction methods of electromyography normalization. *Journal of Electromyography and Kinesiology*, *20*(3), 420–425. https://doi.org/10.1016/j.jelekin.2009.08.003

Ochia, R. S. & Cavanagh, P. R. (2007). Reliability of surface EMG measurements over 12 hours. *Journal of Electromyography and Kinesiology*, *17*(3), 365–371. https://doi.org/10.1016/j.jelekin.2006.01.003

Panoutsakopoulos, V., Papachatzis, N. & Kollias, I. A. (2014). Sport specificity background affects the principal component structure of vertical squat jump performance of young adult female athletes. *Journal of Sport and Health Science*, *3*(3), 239–247. https://doi.org/10.1016/j.jelekin.2009.08.003

Pereira, M. C. C., Rocha Junior, V. d. A., Bottaro, M., Andrade, M. M. de, Schwartz, F. P., Martorelli, A., Celes, R. & Carmo, J. C. (2013). Relationship between ventilatory threshold and muscle fiber conduction velocity responses in trained cyclists. *Journal of Electromyography and Kinesiology*, *23*(2), 448–454. https://doi.org/10.1016/j.jelekin.2012.10.005

Potvin, J. R. & Bent, L. R. (1997). A validation of techniques using surface EMG signals from dynamic contractions to quantify muscle fatigue during repetitive tasks. *Journal of Electromyography and Kinesiology*, *7*(2), 131–139. https://doi.org/10.1016/S1050-6411(96)00025-9

Rouffet, D. M. & Hautier, C. A. (2008). EMG normalization to study muscle activation in cycling. *Journal of Electromyography and Kinesiology*, *18*(5), 866–878. https://doi.org/10.1016/j.jelekin.2007.03.008

Sleivert, G. G. & Wenger, H. A. (1994). Reliability of measuring isometric and isokinetic peak torque, rate of torque development, integrated electromyography, and tibial nerve conduction velocity. *Archives of Physical Medicine and Rehabilitation*, *75*(12), 1315–1321.

Thun, E., Bjorvatn, B., Flo, E., Harris, A. & Pallesen, S. (2015). Sleep, circadian rhythms, and athletic performance. *Sleep Medicine Reviews*, *23*, 1–9. https://doi.org/10.1016/j.smrv.2014.11.003

Yang, H.-C., Lee, C.-L., Lin, R., Hsu, M.-J., Chen, C.-H., Lin, J.-H. & Lo, S. K. (2014). Effect of biofeedback cycling training on functional recovery and walking ability of lower extremity in patients with stroke. *The Kaohsiung Journal of Medical Sciences*, *30*(1), 35–42. https://doi.org/10.1016/j.kjms.2013.07.006.