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# Test-retest reliability of electrical impedance myography in hamstrings of healthy young men

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### ABSTRACT

Several techniques are available to assess muscle tissue status, including electrical impedance myography (EIM). Despite being used in the assessment of neuromuscular status in injury and response to exercise, reliability data for hamstrings muscles are limited. Therefore, this study aimed to determine the test-retest reliability of EIM components on hamstrings. Twenty-one healthy males  $(25.3 \pm 3.4 \text{ years}; 173 \pm 6.7 \text{ cm}; \text{ and } 79.7 \pm 15.9 \text{ kg})$  volunteered for this study. Subjects completed two visits, separated by seven days to collect EIM components (resistance, reactance, impedance, and phase angle) in the longitudinal and transversal axis of hamstrings in both thighs, using a bioimpedance device and Ag/AgCL adhesive contact electrodes. The electrode arrangement was in the muscular belly, half the distance between origin and insertion of the hamstrings. Reliability was determined by the intraclass correlation coefficient (ICC), minimal detectable change (MDC), and Bland-Altman plots. We observed high to excellent reliability (ICC > 0.85) between all EIM components, except for reactance with MDC ranged from 2.0 to 10.8 and the mean bias in Bland-Altman plots ranged from -0.02 to 2.48 (95% limits of agreement from -9.98 to 11.20). From our findings, the hamstrings assessment using EIM technique is reliable to assess muscle tissue; therefore, it enables the evaluation of changes/adaptations in clinical and applied contexts.

# 1. Introduction

The electrical impedance myography (EIM) has been used to assess the bundled fiber structure and cell membrane health status (Kyle et al., 2004; Shiffman et al., 2003). EIM, also referred to as segmental bioelectrical impedance, consists of electrodes located on the skin surface that apply a high-frequency with the low-intensity electrical current on tissue, that creates a circuit in which the intracellular and extracellular ionic fluids act as parallel resistors, and the cell membrane as the capacitor (Kyle et al., 2004; Martins et al., 2020). The EIM components—i.e., resistance, reactance, impedance, and phase angle —are associated with muscle strength (Yamada et al., 2014, 2017), and a variety of neuromuscular disorders (Rutkove and Sanchez, 2019).

In this sense, some investigators have found that these parameters

are sensitive to the diagnose and monitor the treatment of muscle injury (Francavilla et al., 2015; Nescolarde et al., 2011). For example, the magnitude of EIM components decreases in different muscles are directly related to the degree of severity of the injuries and after the treatment, the values return to the pre-injury moment (Nescolarde et al., 2013). Other applications are to understand the morphological and physiological changes that occur in skeletal muscle during contraction (Shiffman et al., 2003), in response to fatigue (Li et al., 2016b), and more recently, have been used to verify acute alterations induced by resistance exercise (Fu and Freeborn, 2018); characterizing it as a potential technique for measuring exercise-induced adaptations. Accordingly, because of this plethora of possibilities for use, it is necessary to verify the reliability of this technique in different muscles/sites.

Regarding the EIM technique, findings are pointing to the excellent

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reliability for some muscle groups such as quadriceps, tibialis anterior, and biceps brachii muscles (Martinez-Gonzalez et al., 2020; Rutkove et al., 2006), and only anecdotal information for other muscles groups (Martinez-Gonzalez et al., 2020). Since aspects such as muscle architecture, fluids within the tissue, and electrode positioning might modify the EIM components, these previous reliability values cannot be extrapolated for other muscle groups (Kyle et al., 2004; Sanchez et al., 2016). In this regard, hamstrings play an important role in motor tasks during exercise and sports, such as deceleration action, as well as this muscle group has a high prevalence of injuries (Nescolarde et al., 2013, 2011). However hamstrings have limited reliability data, showing only the inter-rater variation coefficient of resistance and reactance components of the proximal region in one soccer player (Nescolarde et al., 2013). Therefore, the objectives of this article were (a) to determine the test-retest reliability, (b) to test the possible influence of electrode arrangement, (c) and verify the minimal detectable change of EIM on the belly of the hamstrings.

# 2. Methods

# 2.1. Study design

The present research was delineated to quantify the reliability of the four EIM components in the hamstring muscle, namely: resistance (R), reactance (Xc), impedance (Z), and phase angle (PhA). The subjects were healthy, without symptoms of muscle disease/weakness, surgery, and a history of injury in the hamstrings. To test the reliability of the aforementioned components, the subjects visited our laboratory on two non-consecutive days, which were separated by seven days. The reliability was tested in the hamstrings of both thighs.

# 2.2. Participants

A group of 21 (all mean  $\pm$  SD, 25.3  $\pm$  3.4 years, 173  $\pm$  6.7 cm, 79.7  $\pm$  15.9 kg, and 27.1  $\pm$  3.8 kg/m<sup>2</sup>) healthy male participants, without previous muscle injury in hamstrings, recreationally active volunteered to participate in this study. All participants provided informed consent and completed an information sheet prior to data collection, including demographic and anthropometric data (height, weight). The subjects attended the laboratory at the same time of day on both occasions, to avoid alterations due to the circadian cycle. Subjects did not perform any physical exercise 48 h before each day of evaluation. All assessments were performed by the same rater. The procedures of our study were approved by the local institutional Ethics and Research Committee (Protocol number; 3.944.864).

# 2.3. Procedures

The Quantum V Segmental BIA® bioimpedance device (RJL Systems®) at a fixed frequency of 50 kHz was used for EIM measurements. The device was attached in disposable Ag/AgCL adhesive contact electrodes (Medi-trace®) at a specific electrode arrangement (Fig. 1) in the longitudinal and transverse axis of hamstrings, in the right and left thigh. Electrodes arrangement was adapted from other studies (Nescolarde et al., 2013, 2011) to evaluate the central region of hamstrings muscles.

For each EIM measurement, subjects remained to lie down for at least 10 min prior to the measurement. Before placing the electrodes, was performed a hair shaving followed by cleaning the skin area with alcohol. Participants were instructed not to contract the thigh muscles during the measurements. The arrangement of the electrodes on both thighs followed this sequence: a) Identified the fibular head (proximal region) and the sciatic tuberosity, through anatomical palpation; b) Marked at half the distance between the fibular head and the ischial tuberosity (point A); c) A 3 cm square was drawn, with the point A as the center of the square; d) The electrodes were placed on the longitudinal



Fig. 1. Electrodes arrangement in hamstrings.

and transverse axis, with two electrodes on each side of the square, 0.20 mm apart between their centers. The evaluations were made in an environment with a temperature of  $\sim$ 23 °C.

# 2.4. Data analysis

Participant information and EIM components data were summarized using means and standard deviations (SDs) or percentages, as appropriate. The test-retest reliability of the EIM components was calculated using intraclass correlation coefficients (ICC). For ICC values below 0.5 were indicative of poor reliability, values between 0.5 and 0.75 moderate reliability, values between 0.76 and 0.9 high reliability, and values greater than 0.9 excellent reliability (Koo and Li, 2016). The standard error of measurement (SEM) was determined and then used to calculate the minimal detectable change of each EIM component. We also calculated SEM% dividing the SEM with the average of the two measurements performed for each thigh and arrangement. The minimal detectable change (MDC) reflects the 95% CI of the difference in score between paired observations, calculated as MDC = SEM  $\times \sqrt{2} \times 1.96$  (Weir, 2005). An alpha level of 0.05 was used to determine statistical significance. Data are presented as mean  $\pm$  SD unless otherwise stated. All statistical analyses were conducted using R studio (Version 1.1.463) free software environment for statistical computing and graphics.

# 3. Results

Descriptive data of EIM variables in both days (test and re-test) are presented in Table 1. Precisely, the means and standard deviations of the R, Xc, Z, PhA of the right and left thigh in both longitudinal and transverse axes.

Between sessions, reliability data are presented in Table 2. High to excellent ICC for R (0.89–0.94), Z (0.85–0.94), and PhA (0.92–0.96), but moderate to high for Z (0.75–0.77), in right and left thigh, for both arrangements (longitudinal and transversal). The SEM% was below 6% for all EIM components, with the highest values for PhA (ranged from 4.26 to 5.38%) and lowest values for Xc (ranged from 1.91 to 2.85%). MDCs ranged from 0.63 to 6.33 among components.

Fig. 2A, B, C, and D show Bland-Altman plots of the test-retest

Table 1	
Descriptive data of EIM components in test-retest evaluations (n =	-

Leg	Axis	Resistance (Ω)		Reactance ( $\Omega$ )		Impedance (Ω)		Phase angle (°)		
		Test	Retest	Test	Retest	Test	Retest	Test	Retest	
R	Longitudinal	$\textbf{42.53} \pm \textbf{7.03}$	$40.19\pm 6.87$	$10.86 \pm 1.80$	$10.22 \pm 1.46$	$43.98 \pm 6.72$	$41.49 \pm 6.65$	$14.62\pm3.82$	$14.64\pm3.25$	
	Transversal	$\textbf{45.87} \pm \textbf{12.77}$	$\textbf{45.72} \pm \textbf{12.26}$	$11.73\pm1.48$	$11.39 \pm 1.39$	$\textbf{47.50} \pm \textbf{12.18}$	$\textbf{47.49} \pm \textbf{11.52}$	$15.41\pm4.96$	$15.00\pm4.67$	
L	Longitudinal	$\textbf{42.38} \pm \textbf{8.21}$	$41.10\pm7.45$	$10.54 \pm 1.78$	$\textbf{9.88} \pm \textbf{1.76}$	$\textbf{43.08} \pm \textbf{8.01}$	$42.37\pm7.04$	$14.58\pm3.71$	$14.05\pm4.04$	
	Transversal	$\textbf{46.26} \pm \textbf{13.71}$	$\textbf{46.26} \pm \textbf{13.71}$	$11.73 \pm 1.37$	$11.36 \pm 1.35$	$\textbf{47.40} \pm \textbf{13.02}$	$\textbf{48.52} \pm \textbf{13.28}$	$15.42 \pm 4.87$	$14.71 \pm 4.90$	

21).

Notes. R = right thigh; L = left thigh. The data are presented in mean  $\pm$  standard deviation.

Table 2

Test-retest ICC, SEM and MDC in both legs and arrangement (n = 21).

Leg	Axis	Resistance				Reactance			Impedance				Phase angle				
		ICC	SEM (Ω)	SEM (%)	MDC (Ω)	ICC	SEM (Ω)	SEM (%)	MDC (Ω)	ICC	SEM (Ω)	SEM (%)	MDC (Ω)	ICC	SEM (°)	SEM (%)	MDC (°)
R	Longitudinal	0.93	1.17	2.83	3.25	0.75	0.30	2.85	0.83	0.92	1.12	2.62	3.10	0.92	0.63	4.31	1.76
	Transversal	0.94	2.13	4.65	5.90	0.75	0.24	2.08	0.68	0.94	2.03	4.27	5.63	0.95	0.82	5.39	2.29
L	Longitudinal	0.89	1.37	3.28	3.79	0.76	0.29	2.84	0.82	0.85	1.33	3.11	3.70	0.92	0.61	4.26	1.71
	Transversal	0.92	2.28	4.89	6.33	0.77	0.22	1.91	0.63	0.91	2.17	4.52	6.02	0.96	0.81	5.38	2.25

Notes. R = right thigh; L = left thigh; ICC = intraclass correlation coefficient; SEM = standard error of measurement; MDC = minimal detectable change.



Fig. 2. Bland-Altman plot of phase angle (PhA).

agreement for PhA. The R, Xc, and Z Bland-Altman plots are presented in the supplementary material (omitted in the interest of brevity). These plots showed mean bias from -0.02 to 2.48, lowest for PhA, and highest for Z, respectively. The 95% limits of agreement ranged from -9.98 to 11.20, lowest for Z and highest for R, respectively.

## 4. Discussion

The objectives of the present study were (a) to test whether the measurement of the EIM components in the hamstrings is reliable; (b) test whether the arrangement (ie., longitudinal or transverse) would influence reliability; and (c) to determine the minimum difference to be

considered real for R, Xc, Z, and PhA. The main results of our study were that EIM components, assessed on hamstrings, have good to excellent reliability when evaluated on healthy young men, in a period of seven days. Precisely, the components that presented better reliability, organized according to the higher to lower ICC value, were PhA, R, Z, and Xc. In addition, the longitudinal arrangement showed lower values of SEM (absolute and percentage) and MDC than the transversal arrangement.

EIM has been focused on assessing different muscle groups, such as biceps brachii (Rutkove et al., 2006; Shiffman et al., 2003), quadriceps (Nescolarde et al., 2011; Rutkove et al., 2002; Sanchez et al., 2016), tibialis anterior (Rutkove et al., 2006), and gastrocnemius (Nescolarde et al., 2013, 2011) that has presented good to excellent reliability. For

example, the ICC of PhA for quadriceps, biceps brachii, and tibialis anterior are between 0.93 and 0.97 with a mean absolute change between days of 4.16 to 4.92% (Rutkove et al., 2006). However, regarding the hamstrings, only anecdotal data (i.e., case studies) existed (Francavilla et al., 2015; Nescolarde et al., 2014, 2011). Because each muscle has a distinct shape, size, depth, and fiber type, which makes it difficult to compare data of these different muscles. This fact reinforces the need to carry out studies in different muscle groups, standardizing positions for future comparisons for the development of longitudinal research using EIM components. Accordingly, in the present study, we demonstrated that the EIM components in the posterior thigh are reliable as well.

In this regard, the values of EIM components obtained here cannot be compared to previously reported normative values for healthy subjects by the lack of evidence for hamstrings. Despite this, there are anecdotal findings in the hamstrings that obtained values of resistance ( $\sim$ 39.6  $\Omega$ ), and reactance ( $\sim$ 14.2  $\Omega$ ) similar to those found in our study (Nescolarde et al., 2013, 2015), however with little or no details about the reliability. For example, Nescolarde et al. (2013) indicate the inter-tester reliability of R and Xc in hamstrings, five evaluators independently made R and Xc measurements on the proximal hamstrings of one soccer player, and provided little information about the arrangement, making it difficult to reproduce the method. The reliability data presented is restricted to the coefficient of variation for R and Xc that were 1.4% and 3.2%, respectively, making it difficult to compare data with our study.

By contrasting the transverse and longitudinal findings of our study, there seems to be a subtle advantage in the longitudinal arrangement. The longitudinal arrangement showed lower SEM and MDC values for R, Z, and PhA (see Table 2). In this context, there are some studies in the literature using one or both positions in different muscles (Nescolarde et al., 2013, 2015; Sanchez et al., 2016). Thus, future studies can be conducted to identify whether this fact is replicated in all muscles and if long-term monitoring indicates any substantial difference between these two positions for the outcomes investigated in the present study. Additionally, choosing which position to use is not a problem for EIM recent assessment equipment (Li et al., 2016a), which has fixed surface electrodes in both directions (longitudinal and transverse) and evaluates both simultaneously and in a short time.

Taking into account all the reliability metrics used in the present study, PhA was the variable that showed the greatest consistency between sessions. This variable is the most frequently used for the evaluation of the EIM; as well as whole-body bioimpedance (Di Vincenzo et al., 2019; Martins et al., 2020; Rutkove et al., 2002). The PhA is calculated as the arctangent of the directly measured reactance-toresistance ratio and has been associated with cellularity, cell size, and integrity of the cell membrane, besides that it seems to discriminate adaptations induced by exercise (Kyle et al., 2004; Martins et al., 2020; Nescolarde et al., 2013; Sardinha, 2018). On the other hand, the Xc presented the lowest ICC values (see Table 2). Our findings are in accordance with a previous study that quantified the reliability of this component in the quadriceps (Martinez-Gonzalez et al., 2020). A possible explanation may be that subtle variations in distance between electrodes are more likely to occur between sessions, and Xc appears to be more sensitive to these small changes when compared to other EIM components (Martinez-Gonzalez et al., 2020). However, further studies are needed to test this hypothesis.

To the best of our knowledge, this is the first study that sought to verify the reliability of the EIM components in the hamstring muscle. Our findings enable the use of this method in the posterior thigh either in the clinical context, diagnosis of injuries, and monitoring of the effects of treatments, as well as in applied environments, to measure acute and *possibly* chronic changes in response to resistance exercise (Fu and Freeborn, 2018; Shiffman et al., 2003). However, further studies are needed to contrast the components of EIM with the gold standard (eg., magnetic resonance imaging) to verify the sensitivity of these measures in detecting subtle changes (eg., a fluid shift that occurs during

exercise). Besides, we provide a standard of procedures that can be followed in future studies that aim to investigate the hamstrings. However, our study is not without limitations. First, our findings apply to the population with similar characteristics to the participants in our study. Therefore, there is a need for investigations that measure the reliability of this technique in other populations, such as young women, and older. Second, we measure the thigh posterior belly, and reliability was tested with controlled temperature and environmental factors, which implies the need for caution when extrapolating these findings to different muscle sites and contexts.

# 5. Conclusions

From our results, the EIM is a method with good to excellent reliability in assessing hamstrings muscle status in a healthy male, in both thighs, with subtle advantage for longitudinal versus and transverse axis. More precisely, concerning the posterior thigh, the PhA seems to be the variable that has the greatest consistency, among the four components measured in our study. In this regard, these findings open the possibility for future studies to test whether the components of EIM in hamstrings muscle are sensitive to verify subtle changes—e.g., fluid shift due to exercise, muscle tissue recovery status; thus, enabling the use of this method by clinicians and strength and conditioning professionals in different contexts.

# **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary material

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#### R.C. Honorato et al.

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