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VARIABILITY AND RELIABILITY OF ELBOW FLEXORS AND RECTUS FEMORIS ECHO INTENSITY IN HEALTHY ADULTS

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INTRODUCTION

The sonographic image of the skeletal muscle can be easily distinguished from other structures such as bone and subcutaneous fat [1]. The healthy skeletal muscle is characterized as a hypoechoic structure [2].

Neuromuscular disorders can increase muscle echo intensity (EI), for example, muscle damage induced by strength training [3]. Basically, the average of the grey-scale distribution histogram of the pixels at a region of interest has been used to quantify the EI in these studies [1, 3]. The histogram is available in many commercial software for image editing and are currently used to quantify muscle damage [2, 4] induced by a high intensity strength intervention (as for example, eccentric training [4]) when other biochemical and structural markers changes are also measured [4-6]. This muscle structural damage is produced by Z disk rupture [7] and it is directly associated to training intensity and volume [8].

Two studies showed good reliability of single-EI measure on two different days [5, 6], but there is no reports about the variability of this measure at different time periods among different days.

The aim of this study is to investigate the variability of the EI grey-scale in health elbow flexors (*biceps brachii* and *brachii*) and *rectus femoris* muscles, considering image acquisition in different days.

METHODS

Five health adults (four women and one man) were recruited for this study (age 28.8±4.66 years, body mass 62.46±10.18 kg and height 1.67±0.66 m). B-mode ultrasound (US) images were acquired by an US (MyLab25 ® Gold, ESAOTE SpA, Italy) with a 10-MHz linear probe.

For the elbow flexors (EF), the US probe was oriented transversally to the elbow flexors of the right arm at 60% of the distance between the posterior crista of the acromion and the olecranon, the participants were relaxed with their elbows extended. For the *rectus femoris* (ReF) image acquisition, participants lay supine with hip and knee fully extended and relaxed, the probe was oriented on the transverse-plane of the segment at 50% of the entire right thigh length – distance from the superior border of the patella and great trochanter.

All the ultrasound configurations were maintained the same during the experiment. The images were acquired in two non-consecutive days. Each day, four images were taken, with one hour interval approximately. Eight images were acquired for each participant for each muscle.

The parameter EI was considered the average of the grey-scale histogram (0: Black and 255: White) determined over a 1cm^2 region of interest [4, 6] using a freeware software (Image J, National Institutes of Health, USA, v.1.42).

The Shapiro-Wilk test confirmed normality for echo intensity average for each measurement. The coefficient of variation (CV = 100 * standard deviation / average) and absolute and relative typical error of the mean were determined to investigate the variability of the measurement intra and inter-day. The reliability of the measurement intra-day was calculated by intra-class correlation coefficients (ICC) between days. The significance level was set at $\alpha \leq 0.05$. Statistical analyses were carried out with the commercial statistical software (Statistica 7.0, Statsoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Our EI data for elbow flexors are similar to those reported in the literature, (aproximadely 53-57AU) for pre-intervention [5, 9]. Chen et al. [9] found higher values for the ReF EI, of 78.7±1.7AU, using the same protocol, except the frequency rate (7.5-MHz), and with a single male sample.

The ReF and EF EI variability and reliability are demonstrated in Table 1. The CV and TEM inter-day revealed high variability of the EI. This was expected due to the large CV variation among the five subjects, as described by [3]. Chen et al. [5] and Radaelli et al. [6] reported an EF CV around 5.2% and 2.2%, much less than our results. They focused on muscle damage after resistance training and the reliability protocol consisted of only one measure each day (two days) which cannot provide sufficient information for this determination.

Our higher variability results can also be related to the small region of interest /area (1cm^2) adopted. This is in accordance with some studies [6], but is smaller than others that use

$4cm^2$ [5, 9]. Future studies involving the entire area of the muscle with a panoramic US image [10], could enhance the EI results.

The reliability (R = 0.746 and 0.297 of EF and ReF, respectively) are considered weak and not significantly ($p>0.05$) (Table 1). These findings corroborate the high variability and is expected with small sample sizes (our N=5). Contrary to our results, Chen et al. [5] presented a good reliability (r = 0.92) after one measure each day to EF EI after low intensity of eccentric exercise using $4cm^2$ of region of interest.

Based on our data, some published results of muscle damage, using this methodology have to be accepted with caution. For example, Chen at al. [9] showed a 25% variation between the elbow flexors EI at baseline (high intensity eccentric exercise) and post two days. In the same study, ReF EI varied only 5%. These variations are close or very inferior to the CV% determined in our study (Table 1).

The study of muscle condition with ultrasound images is yet in its beginning and the variability of the results indicates that there is a long way to go in terms of finding the best way to apply such imaging method. US Images do not have standard protocol for positioning subjects as other imaging modalities do so the first challenge would be finding the most stable acquisition protocol. Image histograms are limited in the sense that they disregard spatial information. More sophisticated quantitative parameters have to be used like texture descriptors [11]

CONCLUSIONS

The EI base on the average of grey-scale distribution histogram of a region of interest resulted in a high variability and a weak reliability for the elbow flexors and rectus femoris muscles, which is yet an important methodological limitation for quantifying muscle damage. Next steps are the development of an acquisition protocol and the use of more robust image descriptors.

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Table 1: Mean and standard deviation, typical error of the measurement inter-day absolute (TEM) and relative (TEM%), coefficient of variation (CV) and intra-class correlation coefficient (ICC) of the elbow flexors and rectus femoris IE grey-scale.

		Mean±SD (AU)	ICC (r)	TEM	TEM%	CV% (min-max)
Elbow flexors	**Day 1**	55.68±8.63	0.746 (p=0.106)	8.89	13.42	18.98 (13.07- 35.01)
	Day 2	70.16±14.48				
Rectus femoris	**Day 1**	38.40±10.75	0.297 (p=0.370)	15.18	26.10	36.92 (21.53-55.69)
	Day 2	64.49±25.48				