ASSESSING THE VALIDITY AND RELIABILITY OF A FREEHAND TOOL METHOD FOR ANALYSIS OF ULTRASOUND CROSS-SECTIONAL AREA IMAGES

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INTRODUCTION: Ultrasonography has been shown to be a valid and reliable method of assessing muscle size, measured as cross-sectional area (CSA), when compared to gold standard measurements such as magnetic resonance imaging and dual energy X-ray absorptiometry (Hides et al., 1995; Raadsheer et al., 1994; Waltonet al.,1997). Additionally, several studies have used this technology to examine training-induced changes in muscle size and have found associations between changes in muscle CSA and performance variables such as strength, jump height, and sprinting speed (Bazyler et al., 2017; Nimphius et al., 2012; Scanlon et al., 2014; Zaras et al., 2016). Therefore, as ultrasonography technology advances and becomes more widely available, it will likely become a more commonly used athlete monitoring tool for practitioners, coaches, and sport scientists who are interested in quantifying muscular changes related to sport performance. To be effectively used as an athlete monitoring tool, efficient methods of both collecting and analyzing the ultrasonography data must be developed.

Ultrasonography images are typically analyzed by tracing the inner boundary of the muscle aponeurosis using either the software available within the ultrasonography device or with image processing software such as ImageJ (Palmer et al., 2015). However, attempting to trace the muscle with ImageJ's polygon tool (PT) and a computer mouse or trackpad is difficult and may be prohibitively time intensive for the purposes of monitoring. To this end, increasing availability of touch-screen laptop and freehand stylus technology may offer a viable alternative for tracing ultrasonography images. Therefore, the purpose of this investigation is to assess the validity and reliability of a freehand tool (FT) method compared to the criterion PT method for analyzing ultrasound CSA images in athletes.

METHODS

Participants: Forty-three resistance-trained male athletes (20.7±1.7y; 182.8±9.1cm; 80.4±9.9kg) participated in the current study. Subjects were drawn from collegiate basketball, baseball, soccer, tennis, and weightlifting teams. Prior to data collection, all athletes were informed of the purpose of the study and provided written informed consent. This study was approved by the University's Institutional Review Board.

Ultrasonography: The same skilled technician (>500 ultrasound scans with athletes) collected 3 cross-sectional sonograms of the right vastus lateralis (VL) for each athlete using a 7.5 MHz ultrasound probe (LOGIQ P6, General Electric Healthcare, Wauwatosa, WI). The ultrasonography collection protocol previously described (Bazyler et al. 2017) was used for all testing sessions. All images were analyzed using the ImageJ Software (Version 1.47v, National Institutes of Health, Bethesda, MD, USA) by the same investigator and on the same computer. Prior to analysis, each image was individually calibrated from area in pixels to centimeters (cm) with the straight-line function using a distance of 1 cm. For the PT method, CSA was measured using a mouse to outline the inner portion of the aponeurosis of the VL region of interest (ROI), being sure to include as much muscle tissue as possible while excluding any non-muscle tissue (e.g., bone, fascia) (Wells et al., 2014). Adjustments were then made to the ROI outline using a spline tool. Alternatively, for the proposed FT method, a stylus was used by the investigator to

create a freehand tracing of the ROI. No further adjustments were made to the outline with the FT method (Figure 1).
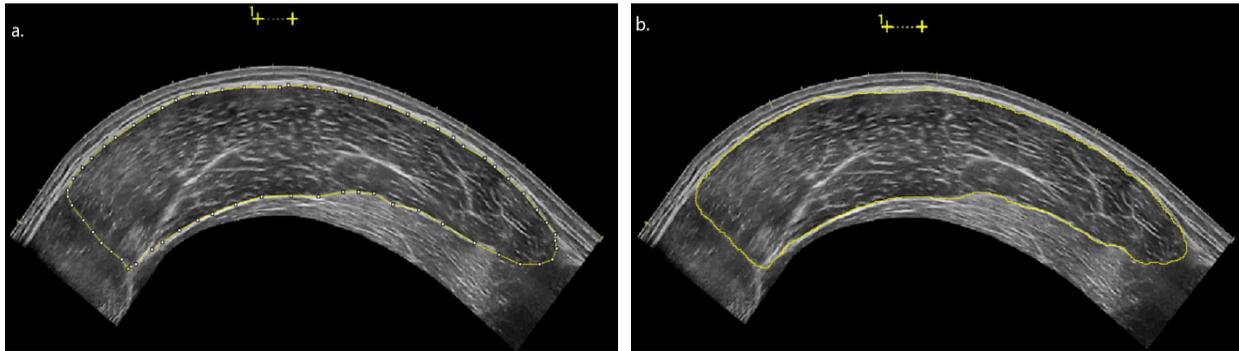
FIGURE 1. (a) CSA image of vastus lateralis tracing the ROI using the PT method. (b) CSA image of vastus lateralis tracing the ROI using the FT method.

Statistics: The data were first scanned for outliers (criteria: mean±3SD) followed by a Shapiro-Wilks test to assess normality. The minimum and maximum CSA values of the 3 images measured for each athlete were used for statistical analyses. Between-trial reliability for each method was assessed using intraclass correlation coefficients with 95% confidence intervals (CI), coefficient of variation (CV), and Bland-Altman plots with 95% Limits of Agreement and Pearson's product-moment correlation coefficients to assess heteroscedasticity within each method. Validity was assessed by comparing mean CSA between the criterion (PT) and alternative (FT) method using a paired-samples t-test, and Cohen's d_{av}. Additionally, a Bland-Altman plot with 95% Limits of Agreement and a Pearson's product-moment correlation coefficient was used to assess heteroscedasticity between methods. Critical alpha was set to $p<0.05$ for all analyses, which were calculated using Microsoft Office Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

TABLE 1. Measurement data for the polygon and freehand tool methods.

		Mean ± SD (cm^2)	Lower Range	Mean Difference	95% Limits of Agreement
PT	T1	31.36 ± 5.38	17.51 to 49.23	1.20	0.025 to 2.41
	T2	32.58 ± 5.61			
FT	T1	31.34 ± 5.39	17.38 to 48.53	1.20	-0.09 to 2.40
	T2	32.49 ± 5.49			

PT=polygon method. FT=freehand method. T1=trial 1. T2=trial 2.

RESULTS: Reliability analysis revealed that measurements of CSA in both methods were highly consistent (Table 1). Interclass correlations and coefficient of variation indicates that the agreement within the PT method was very high (ICC=0.994; 95% CI: 0.98-1.00; CV=3.00%). Similarly, interclass correlations and the coefficient of variation also indicate that the agreement within the FT method was very high (ICC=0.993; CI: 0.98-1.00; CV (between trials)=2.90%). Heteroscedasticity was present in the PT method (r=-0.384, p=0.011), but not the FT method (r=-0.156, p=0.317). In regard to criterion validity, the paired-samples t-test comparing PT

(31.97±5.49 cm²) and FT (31.92±5.43 cm²), mean CSA revealed no systematic bias (p=0.375; d_{av}=0.009). Also, no heteroscedasticity was present (r=0.187, p=0.229) (Figure 2).
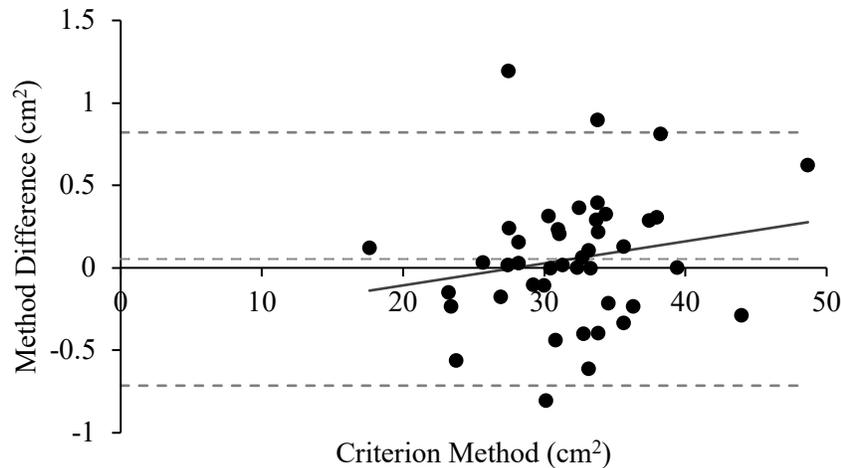
FIGURE 2. Bland-Altman plot with a non-significant correlation between the difference in means between polygon tool (PT) and freehand tool (FT) and the criterion method for each athlete. Solid black line represents the non-significant mean difference between methods (systematic bias) and the dashed gray lines represent the upper and lower 95% Limits of Agreement.

DISCUSSION: These results demonstrate that 1) both PT and FT are reliable methods for analyzing ultrasound CSA, and 2) FT is a valid method for analyzing ultrasound CSA in comparison to the PT criterion method. Reliability analysis revealed that the measurements of CSA in both methods were highly consistent, but heteroscedasticity was present for the PT method. This may indicate that the FT method is superior in tracing the curvature of the vastus lateralis, especially in larger CSA images. Regarding efficiency, the analyst noted that assessing 3 images for a single athlete using the PT method takes about double the time required for the FT method (PT= 4:25; FT = 2:05). This is partly due to the additional time needed to make further adjustments to the original trace using the PT method. The impact of this study provides sport scientists with a quicker and similarly accurate method for analyzing ultrasound CSA images. Specifically, the FT method can improve the turnover of CSA data in athlete-monitoring programs, which will translate to coaches getting the information they need faster without compromising accuracy. Overall, these findings demonstrate the FT method is a reliable and valid alternative for analyzing ultrasound CSA images.

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