Justifications and formulas

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Comparison Between Methods for Analyzing the Reproducibility of the Muscle Architecture of the Vastus Lateralis Using Ultrasound

1 - Typical Measurement Error and Coefficient of Variation

1.1 - To assess the reproducibility of the measurements, the Typical Error (TE) of measurement was calculated, as shown in **Equation 1**. This error was also expressed as a coefficient of variation (CV), presented as a percentage (Equation 2):

$$TE = \frac{\text{SDdiff}(T_{d1} - T_{d2})}{\sqrt{2}} \qquad (1)$$

Equation 1. SDdif(Td1-Td2) represents the standard deviation (SD) associated with the mean difference between the values of Td1 and Td2. √2 is the constant used to adjust the estimate obtained through the use of repeated measurements (Td1 and Td2). Td1 refers to the evaluation time for quantification 1, and Td2 refers to the evaluation time for quantification 2.

$$CV = \frac{TE}{\text{Mean } (T_{d1};T_{d2})} \times 100 \qquad (2)$$

Equation 2. TE represents the estimate of the typical error of the measurement presented in Equation 1. Mean (Td1; Td2) represents the average of all observed values in Td1 and Td2. Td1 refers to the evaluation time for quantification 1, and Td2 refers to the evaluation time for quantification 2.

1.2 - In each protocol (i.e., 1X and 2X), the CV was determined for each variable (i.e., Cross-Sectional Area [CSA], Muscle Thickness [MT], Fascicle Length [FL], and Pennation Angle [PA]) through the analysis of values obtained from repeated measurements (HOPKINS, 2000).

2 - Mean of the Differences Between Day 2 and Day 1 Measurements for Each Protocol

2.1 - Both the TE and the CV are uncommon scores for statistical comparison. Therefore, to directly compare the 1X and 2X protocols, the mean differences between the measurements ($T_{d1} - T_{d2}$) for each protocol were initially calculated. After obtaining these data, they were expressed as mean and standard deviation through the following equations:

$$\Delta 1\mathrm{X} = \frac{\sum_{i=1}^{n}\left(1X_{d2,i} - 1X_{d1,i}\right)}{n} \qquad (3)$$

Equation 3. Equation for the mean of the differences in the 1X protocol. Where 1Xd2 represents the value obtained in quantification 2, 1Xd1 represents the value obtained in quantification 1. The n represents the sample size, and i represents the index corresponding to each participant, from the first to the last.

$$\Delta 2\mathrm{X} = \frac{\sum_{i=1}^{n}\left(2X_{d2,i} - 2X_{d1,i}\right)}{n} \qquad (4)$$

Equation 4. Equation for the mean of the differences in the 2X protocol. Where 2Xd2 represents the value obtained in quantification 2, and 2Xd1 represents the value obtained in quantification 1. The *n* represents the sample size, and *i* represents the index corresponding to each participant, from the first to the last.

3 - Normality of the Data and Comparisons of the Means Derived from the Differences Between Day 2 and Day 1 Measurements in Each Protocol

3.1 - The normality of the data was initially assessed using the Shapiro–Wilk test. To compare the mean differences of the 1X protocol (**Equation 3**) with the mean differences of the 2X protocol (**Equation 4**).

3.2 - Paired t-tests (for normally distributed data) or Wilcoxon tests (for non-normally distributed data) were applied. The significance level was set at $p < 0.05$.

3.3 - Statistical analyses and graphs were generated using GraphPad Prism software (v. 9.5, GraphPad Software, San Diego, CA).

4 - Variance Analysis

4.1 - For each protocol, the variance was calculated based on the mean differences between quantifications 1 and 2 (**Equations 3 and 4**) for each protocol. In this context, the difference between the variances of the 1X and 2X protocols was analyzed using the Levene test, and the distribution of the data was graphically illustrated using Equations 3 and 4.

4.2 - All calculations and graphs were performed using a code developed in Python (version 3.11), an open-source programming language.

4.3 - A step-by-step guide for using this tool on a conventional computer will be available in this repository to facilitate the reader's use.

5 - Bland-Altman Analysis, Bias, Agreement, and Emphasis on the Illustration of Data Dispersion

5.1 - Finally, the Bland-Altman technique (BLAND; ALTMAN, 1986) was applied to complement the analysis, visualizing the variability of the data in each protocol. In addition to evaluating agreement and bias, the goal was to highlight the analysis of the limits of agreement range, allowing a clear visualization of the impact of data variability in each protocol.

5.2 - Statistical analyses and graphs were generated using GraphPad Prism software (v. 9.5, GraphPad Software, San Diego, CA).