**Statistical Analysis**

Data was imported into RStudio integrative development environment and analyzed with R statistical programming (R 3.0.1+) language with a custom-built script; the “dplyr” package was used for grammar, “ggplot2” and “ggpurb” for data visualization, “irr” for intra-class correlation analysis, and “blandr” Association between skinfold and ultrasound measures were determined via Pearson’s r correlation tests; the interpretation of the association between variables was interpreted as 0.3-0.5 as moderate, 0.5-0.7 as large, 0.7-0.9 as very large, and >0.9 as nearly perfect 1. Agreement between skinfold and ultrasound measures was assessed via a two-way random-effects model intra-class correlation coefficient (ICC2,k) with values < 0.70 denoting poor agreement, 0.70 – 0.89 moderate agreement, and > 0.90 excellent agreement 2, 3. Finally, in Bland-Altman plots were constructed for visual analysis of agreement in where fixed bias was considered when the data crossed the lower or upper 95% CI bounds.

*Exploratory Analyzes*

Data were analyzed into three steps: 1) Association of Skinfold and Ultrasound with skin and without skin, Skinfold vs Ultrasound with skin multiplied by 2, Skinfold vs Ultrasound without skin, and Skinfold vs Ultrasound without skin multiplied by 2, 2) A series of Bland-Altman analysis and Intra-Class Correlation were conducted for all participants, males, and females on Skinfold vs Ultrasound with skin, and Skinfold vs Ultrasound without skin, 3) individual variables for each segment were created from individual linear regression formulas (regression method: RM) and via multiplication of 2 (multiplication method: MM) for each segment of Ultrasound without skin; a series of associations, intra-class correlation, linear regressions, and Bland-Altman analysis were created for each of the segments and adipose tissue via the Deborah-Kerr method. Significance for all analyzes was set priori at an alpha level of 0.05.

**RESULTS**

*Associations*

Large to very large associations were found between Skinfold vs Ultrasound with skin (r = 0.75-0.95, p<0.01) and Skinfold vs Ultrasound without skin (r =0.77-0.94 , p<0.01). To the exception of Skinfold vs Ultrasound with skin and without skin for Abdominal segment (r = 0.67, p<0.01) (Table 1).

*Agreement Analysis*

Excellent agreements were found for Skinfold vs Ultrasound Biceps with skin for all participants (ICC2,k = 0.93), males (ICC2,k = 0.90), and females (ICC2,k = 0.91) with a mean bias of -0.94, -0.93, -1.26, and -1.40, respectively. Poor to moderate agreement was found for all segments reminder segments for all participants (ICC2,k = 0.36 – 0.73), males (ICC2,k = 0.34 – 0.74), and females (ICC2,k = 0.30 – 0.81) for Skinfold vs Ultrasound with Skin. Similarly, poor to moderate agreement were found for all participants (ICC2,k = 0.53 – 0.79), males (ICC2,k = 0.46 – 0.78), and females (ICC2,k = 0.34 – 0.69) for Skinfold vs Ultrasound without Skin (Table 2). Visual analysis of the Bland-Altman plots also indicates bias as the mean of the measurements increases for all the analyzes (Figures 1-3).

*New methods analysis*

Newly created data were constructed via linear regression for each segment; regression formulas (RM) for each segment are provided in Table 4. Excellent agreement (ICC2,k = 0.91-0.97), of the data generated via the RM was found on Skinfold vs Ultrasound Triceps, Subscapular, Biceps, Front Thigh, and Calf. Moderate agreement (ICC2,k = 0.77-85) were also found on Skinfold vs Ultrasound Iliac crest, Supraspinale, and Abdominal. There was no presence of mean bias for any of the segments (Table 4). Furthermore, there was excellent agreement using the multiplication method (MM) on Skinfold vs Ultrasound Calf (ICC2,k = 0.94). There was moderate agreement (ICC2,k = 0.66-89). on Skinfold vs Ultrasound Triceps, Subscapular, Biceps, Iliac crest, Supraspinale, and Front Thigh via the MM method. There was poor agreement between Skinfold and Ultrasound Abdominal via MM (ICC2,k = 0.49). The Bland-Altman plots indicate the presence of systematic bias as the mean of the two methods increases for each segment computed via RM or MM (Figure 4).

Lastly, there was a moderate agreement of adipose tissue using the MM (ICC2,k = 0.85) and excellent agreement (ICC2,k = 0.996) on muscle mass. Moreover, the artificially constructed data via LM method showed excellent agreement on adipose tissue (ICC2,k = 0.96) and muscle mass (ICC2,k = 0.992)

Table 1. Correlation Matrix

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | All | | Males | | Females | |
|  | r | p | r | p | r | p |
| US with Skin |  |  |  |  |  |  |
| Triceps | 0.84 | 0.00 | 0.90 | 0.00 | 0.66 | 0.00 |
| Subscapular | 0.80 | 0.00 | 0.73 | 0.00 | 0.87 | 0.00 |
| Biceps | 0.90 | 0.00 | 0.93 | 0.00 | 0.84 | 0.00 |
| Ileac crest | 0.75 | 0.00 | 0.87 | 0.00 | 0.68 | 0.00 |
| Supraspinale | 0.77 | 0.00 | 0.83 | 0.00 | 0.71 | 0.00 |
| Abdominal | 0.67 | 0.00 | 0.75 | 0.00 | 0.56 | 0.00 |
| Thigh | 0.93 | 0.00 | 0.93 | 0.00 | 0.87 | 0.00 |
| Calf | 0.95 | 0.00 | 0.95 | 0.00 | 0.92 | 0.00 |
| US without Skin |  |  |  |  |  |  |
| Triceps | 0.85 | 0.00 | 0.90 | 0.00 | 0.66 | 0.00 |
| Subscapular | 0.83 | 0.00 | 0.76 | 0.00 | 0.90 | 0.00 |
| Biceps | 0.90 | 0.00 | 0.92 | 0.00 | 0.85 | 0.00 |
| Ileac crest | 0.77 | 0.00 | 0.89 | 0.00 | 0.71 | 0.00 |
| Supraspinale | 0.78 | 0.00 | 0.84 | 0.00 | 0.73 | 0.00 |
| Abdominal | 0.68 | 0.00 | 0.76 | 0.00 | 0.56 | 0.00 |
| Thigh | 0.93 | 0.00 | 0.94 | 0.00 | 0.88 | 0.00 |
| Calf | 0.94 | 0.00 | 0.95 | 0.00 | 0.91 | 0.00 |
| US with Skin\*2 |  |  |  |  |  |  |
| Triceps | 0.84 | 0.00 | 0.90 | 0.00 | 0.66 | 0.00 |
| Subscapular | 0.80 | 0.00 | 0.73 | 0.00 | 0.87 | 0.00 |
| Biceps | 0.90 | 0.00 | 0.93 | 0.00 | 0.84 | 0.00 |
| Ileac crest | 0.75 | 0.00 | 0.87 | 0.00 | 0.68 | 0.00 |
| Supraspinale | 0.77 | 0.00 | 0.83 | 0.00 | 0.71 | 0.00 |
| Abdominal | 0.67 | 0.00 | 0.75 | 0.00 | 0.56 | 0.00 |
| Thigh | 0.93 | 0.00 | 0.93 | 0.00 | 0.87 | 0.00 |
| Calf | 0.95 | 0.00 | 0.95 | 0.00 | 0.92 | 0.00 |
| US without Skin\*2 |  |  |  |  |  |  |
| Triceps | 0.85 | 0.00 | 0.90 | 0.00 | 0.66 | 0.00 |
| Subscapular | 0.83 | 0.00 | 0.76 | 0.00 | 0.90 | 0.00 |
| Biceps | 0.90 | 0.00 | 0.92 | 0.00 | 0.85 | 0.00 |
| Ileac crest | 0.77 | 0.00 | 0.89 | 0.00 | 0.71 | 0.00 |
| Supraspinale | 0.78 | 0.00 | 0.84 | 0.00 | 0.73 | 0.00 |
| Abdominal | 0.68 | 0.00 | 0.76 | 0.00 | 0.56 | 0.00 |
| Thigh | 0.93 | 0.00 | 0.94 | 0.00 | 0.88 | 0.00 |
| Calf | 0.94 | 0.00 | 0.95 | 0.00 | 0.91 | 0.00 |

Table 2. Difference analysis through the Bland-Altman method and Intra-Class Correlation for Agreement

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | All | | | | Males | | | | Females | | | | |
|  | BA | | | ICC2,k | BA | | | ICC2,k | BA | | | ICC2,k | |
|  | Bias | Lower | Upper |  | Bias | Lower | Upper |  | Bias | Lower | Upper |  |
| *US with Skin* |  |  |  |  |  |  |  |  |  |  |  |  |
| Triceps | -5.68 | -6.97 | -4.39 | 0.73 | -5.10 | -6.68 | -3.53 | 0.69 | -6.26 | -8.39 | -4.13 | 0.51 |
| Subscapular | -11.25 | -13.06 | -9.44 | 0.36 | -11.07 | -13.81 | -8.32 | 0.34 | -11.43 | -13.98 | -8.88 | 0.37 |
| Biceps | -2.13 | -2.60 | -1.66 | 0.86 | -2.56 | -3.05 | -2.06 | 0.78 | -1.70 | -2.50 | -0.91 | 0.85 |
| Iliac crest | -11.07 | -13.10 | -9.04 | 0.46 | -12.62 | -15.76 | -9.49 | 0.47 | -9.52 | -12.18 | -6.86 | 0.46 |
| Supraspinale | -7.51 | -9.29 | -5.74 | 0.64 | -7.98 | -10.54 | -5.41 | 0.63 | -7.05 | -9.69 | -4.42 | 0.61 |
| Abdominal | -8.31 | -10.83 | -5.79 | 0.68 | -8.80 | -12.20 | -5.40 | 0.74 | -7.82 | -11.80 | -3.85 | 0.61 |
| Thigh | -13.20 | -15.50 | -10.90 | 0.48 | -9.36 | -11.89 | -6.83 | 0.54 | -17.04 | -20.37 | -13.71 | 0.30 |
| Calf | -6.84 | -8.01 | -5.67 | 0.68 | -5.27 | -6.57 | -3.97 | 0.70 | -8.41 | -10.23 | -6.58 | 0.54 |
| *US without Skin* | |  |  |  |  |  |  |  |  |  |  |  |
| Triceps | -4.23 | -5.51 | -2.96 | 0.79 | -3.62 | -5.17 | -2.07 | 0.78 | -4.85 | -6.96 | -2.74 | 0.59 |
| Subscapular | -8.62 | -10.36 | -6.89 | 0.48 | -8.23 | -10.88 | -5.58 | 0.46 | -9.02 | -11.44 | -6.59 | 0.49 |
| Biceps | -0.94 | -1.40 | -0.48 | 0.93 | -1.26 | -1.77 | -0.75 | 0.90 | -0.62 | -1.40 | 0.16 | 0.91 |
| Iliac crest | -9.21 | -11.20 | -7.23 | 0.54 | -10.82 | -13.86 | -7.77 | 0.54 | -7.61 | -10.20 | -5.02 | 0.55 |
| Supraspinale | -5.84 | -7.56 | -4.11 | 0.71 | -6.32 | -8.80 | -3.84 | 0.71 | -5.36 | -7.93 | -2.78 | 0.69 |
| Abdominal | -6.07 | -8.59 | -3.55 | 0.74 | -6.59 | -9.93 | -3.25 | 0.80 | -5.55 | -9.56 | -1.54 | 0.66 |
| Thigh | -11.69 | -13.96 | -9.43 | 0.53 | -7.80 | -10.27 | -5.33 | 0.62 | -15.59 | -18.84 | -12.34 | 0.34 |
| Calf | -5.59 | -6.76 | -4.43 | 0.74 | -3.98 | -5.23 | -2.72 | 0.78 | -7.21 | -9.04 | -5.38 | 0.60 |

**Diagram, engineering drawing

Description automatically generated**Figure 1. Bland-Altman plots in where all plots were constructed between Skinfold and (A) ultrasound triceps 1, (B) ultrasound triceps 2, (C) ultrasound subscapular 1, (D) ultrasound subscapular 2, (E) ultrasound Biceps 1, (F) ultrasound Biceps 2, (G) ultrasound Ileac Crest 1, (H) ultrasound Ileac Crest 2, (I) ultrasound Supraspinale 1, (J) ultrasound Supraspinale 2, (K) ultrasound Abdominal 1, (L) ultrasound Abdominal 2, (M) ultrasound Front Thigh 1, (N) ultrasound Front Thigh 2, (O) ultrasound Medial Calf 1, (P) ultrasound Medial Calf 2.

Diagram, engineering drawing

Description automatically generated

Figure 2. Bland-Altman plots of Male participants in where all plots were constructed between Skinfold and (A) ultrasound triceps 1, (B) ultrasound triceps 2, (C) ultrasound subscapular 1, (D) ultrasound subscapular 2, (E) ultrasound Biceps 1, (F) ultrasound Biceps 2, (G) ultrasound Ileac Crest 1, (H) ultrasound Ileac Crest 2, (I) ultrasound Supraspinale 1, (J) ultrasound Supraspinale 2, (K) ultrasound Abdominal 1, (L) ultrasound Abdominal 2, (M) ultrasound Front Thigh 1, (N) ultrasound Front Thigh 2, (O) ultrasound Medial Calf 1, (P) ultrasound Medial Calf 2.

Diagram, engineering drawing

Description automatically generated

Figure 3. Bland-Altman plots of Male participants in where all plots were constructed between Skinfold and (A) ultrasound triceps 1, (B) ultrasound triceps 2, (C) ultrasound subscapular 1, (D) ultrasound subscapular 2, (E) ultrasound Biceps 1, (F) ultrasound Biceps 2, (G) ultrasound Ileac Crest 1, (H) ultrasound Ileac Crest 2, (I) ultrasound Supraspinale 1, (J) ultrasound Supraspinale 2, (K) ultrasound Abdominal 1, (L) ultrasound Abdominal 2, (M) ultrasound Front Thigh 1, (N) ultrasound Front Thigh 2, (O) ultrasound Medial Calf 1, (P) ultrasound Medial Calf 2.

Diagram, schematic

Description automatically generated

Figure 4. Bland-Altman plots of each computed segment from the regression method (RM) or via the multiplication method (MM), and in where all plots were constructed between Skinfold and (A) ultrasound triceps RM, (B) ultrasound triceps MM, (C) ultrasound subscapular RM, (D) ultrasound subscapular MM, (E) ultrasound Biceps RM, (F) ultrasound Biceps MM, (G) ultrasound Ileac Crest RM, (H) ultrasound Ileac Crest MM, (I) ultrasound Supraspinale RM, (J) ultrasound Supraspinale MM, (K) ultrasound Abdominal RM, (L) ultrasound Abdominal RM, (M) ultrasound Front Thigh RM, (N) ultrasound Front Thigh MM, (O) ultrasound Medial Calf RM, (P) ultrasound Medial Calf MM.

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