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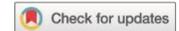


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## Title page

### **Local fat content and muscle quality measured by a new electrical impedance myography device: correlations with ultrasound variables**

Stefano Longo\*<sup>1</sup>, Giuseppe Coratella<sup>1</sup>, Susanna Rampichini<sup>1</sup>, Marta Borrelli<sup>1</sup>, Raffaele Scurati<sup>1</sup>,  
Eloisa Limonta<sup>1,2</sup>, Emiliano Cè<sup>1,2</sup>, Fabio Esposito<sup>1,2</sup>

<sup>1</sup>Department of Biomedical Sciences for Health, Università degli Studi di Milano, Via G. Colombo  
71, 20133, Milan, Italy

<sup>2</sup>IRCCS Istituto Ortopedico Galeazzi, Via R. Galeazzi 4, 20161, Milan, Italy.

\*Corresponding author:

Dr. Stefano Longo

Department of Biomedical Sciences for Health

Università degli Studi di Milano

Via G. Colombo 71, 20133, Milan, Italy

Phone: +39 02 503 14648

e-mail: stefano.longo@unimi.it

## **Local fat content and muscle quality measured by a new electrical impedance myography device: correlations with ultrasound variables**

### **Abstract**

The present study investigated the relationship between local fat percentage ( $SK_{fat}$ ) and muscle quality (MQ) estimated by a new hand-held electrical impedance myography ( $hEIM$ ) device or derived from ultrasound and strength assessments. The right anterior thigh of 90 healthy participants (mean $\pm$ SD; age=22.9 $\pm$ 2.9 years; 46 men: BMI=23.9  $\pm$  2.4 kgm<sup>-2</sup>; 46 women: BMI=21.1  $\pm$  1.9 kgm<sup>-2</sup>) was scanned by  $hEIM$  and ultrasound. Correlations between  $SK_{fat}$ , local subcutaneous fat ( $SUB_{fat}$ ), and echo intensity ( $EI_{us}$ ) were explored. Correlations between MQ,  $EI_{us}$ , quadriceps femoris anatomical cross-sectional area ( $ACSA_{QF}$ ), knee extensors maximum voluntary isometric torque (T), T/ $ACSA_{QF}$ ,  $EI_{us}/SUB_{fat}$ , and  $ACSA_{QF}/SUB_{fat}$  were also assessed.  $SK_{fat}$  correlated with  $SUB_{fat}$  ( $r=0.88$ ;  $p<0.001$ ) and  $EI_{us}$  ( $r=0.64$ ;  $p<0.001$ ). MQ correlated with  $EI_{us}$  ( $r=-0.66$ ;  $p<0.001$ ),  $ACSA_{QF}$  ( $r=0.37$ ;  $p<0.001$ ),  $EI_{us}/SUB_{fat}$  ( $r=0.37$ ;  $p<0.001$ ), and  $ACSA_{QF}/SUB_{fat}$  ( $r=0.81$ ;  $p<0.001$ ). Multiple regression analysis showed that  $SUB_{fat}$ ,  $EI_{us}$ , and sex explained 86% of  $SK_{fat}$  variance, whereas  $ACSA_{QF}/SUB_{fat}$ , sex and  $EI_{us}$  explained 75% of MQ variance. In conclusion, high  $hEIM$  local fat percentage relates to greater subcutaneous fat and intramuscular non-contractile tissue content. High  $hEIM$  muscle quality relates to greater muscle-size:subcutaneous-fat ratio and contractile tissue content. Sex influences the prediction of both parameters. This  $hEIM$  device seems to be useful to estimate local thigh composition.

**Keywords:** Body composition, assessment, technology, strength, musculoskeletal

**Word count:** ~ 4090

## Introduction

Electrical impedance myography (EIM) is a recent technique based on the analysis of local tissue impedance, which measures the obstruction to the flow of a painless, high-frequency low-amplitude electric current injected through a localized area (Rutkove, 2009). The resulting surface voltages are measured, and impedance, which includes resistance, reactance and phase angle, can be calculated. EIM has been employed to assess muscle health status in various pathological conditions, such as atrophy, disuse, amyotrophic lateral sclerosis, and muscular dystrophies, and to monitor disease progression (Rutkove, 2009; Sanchez & Rutkove, 2017). Being local impedance influenced by subcutaneous fat, lean mass, intramuscular fat and connective tissue content, EIM was employed also to assess local and whole-body fat percentage, as well as muscle tissue characteristics (Kapur, Nagy, Taylor, Sanchez, & Rutkove, 2018; L. Li, Li, Hu, Shin, & Zhou, 2016; Rutkove, 2009). Moreover, local EIM is relatively insensitive to the hydration status (J Li, Sanchez, & Rutkove, 2014).

Recently, a new handheld device based on EIM (*hEIM*) has been developed and used to evaluate body composition (Graybeal, Moore, Cruz, & Tinsley, 2018; McLester, Dewitt, Rooks, & McLester, 2018; van Rassel et al., 2019). This device is claimed to estimate local and/or whole-body fat percentage by taking into account primarily the impedance of subcutaneous fat (Skulpt performance training system). Moreover, an index of muscle quality purportedly indicating the health status of a muscle is provided. Expressed in arbitrary units, a high muscle quality index is claimed to reflect great muscle fibers' size, low fat content, and, in turn, high strength, thus indicating a healthy muscle (Skulpt performance training system). However, scientific studies aiming to investigate the validity of these claims are still lacking. Very few studies attempted to validate whole-body fat percentage estimation by comparing *hEIM*, DXA and other body composition measurements in different populations (Graybeal et al., 2018; McLester et al., 2018; van Rassel et al., 2019). At local level, only one study conducted in the elderly investigated thigh composition by comparing *hEIM* and ultrasound-derived variables (Hobson-Webb et al., 2018). Furthermore, *hEIM* reliability was investigated only in two studies, at whole-body (McLester et al., 2018) and local level (Hobson-Webb

et al., 2018). Both investigations reported *high* or *very high* reliability values (Hobson-Webb et al., 2018; McLester et al., 2018). However, only Hobson-Webb et al. (2018) included muscle quality, and McLester et al. (2018) found that the inter-day measurements of whole-body fat percentage in absolute values were statistically different. Thus, more reliability studies are needed including both fat percentage and muscle quality as measured by this *hEIM* device.

While subcutaneous fat (Fukumoto et al., 2012; Störchle et al., 2017) and muscle size (Franchi et al., 2018) can be directly measured on an ultrasound image, muscle quality can be estimated by two different methods that take into account the non-contractile tissue within the muscle: (i) the muscle belly echo intensity analysis from an ultrasound scan ( $EI_{us}$ ) (Fukumoto et al., 2012; Mota & Stock, 2017; Pillen et al., 2009), and (ii) the ratio of maximum muscle strength to its size, also named as specific tension (Delmonico et al., 2009; Goodpaster et al., 2006).  $EI_{us}$  has been associated directly to intramuscular fat (Young, Jenkins, Zhao, & McCully, 2015) and fibrous tissue (Pillen et al., 2009), i.e., the higher the  $EI_{us}$  value the more non-contractile tissue within the muscle.

Therefore, the aims of this study were to assess: (i) the *hEIM* reliability in both fat content and muscle quality; (ii) whether *hEIM*-derived fat content and muscle quality correlated with ultrasound-derived variables reflecting the local composition of the thigh; and (iii) the weight of the ultrasound-derived variables and sex in predicting both fat and muscle quality values.

## Methods

### *Participants*

Based on *a priori* F-test linear multiple regression fixed-model, with an  $\alpha$ -level =0.05, power >0.80,  $f^2$  =0.33 (*large* effect size), and a minimum number of predictors =3, the calculated sample size was  $n=37$  (G\*Power 3.1, Dusseldorf, Germany). To ensure sufficient statistical power for investigating multiple regressions in both sexes, 90 physically active participants (45 males and 45 females, hours of training  $week^{-1}=2.0\pm 1.5$ ) were recruited among the students of our University (Table 1).

Participants declared no lower limbs injuries in the previous six months or neuromuscular diseases. They were required to abstain from heavy intensity physical exercise for 48 hours, from drinking alcohol and ergogenic beverages for 24 hours, and from eating and drinking (with the exception of water) for a minimum of 3 hours prior to testing. After receiving a full explanation of the aim of the study and the experimental procedures, a written informed consent was provided. To minimize the circadian changes in strength, tests were performed in the afternoon hours. The local university ethical committee approved the project in accordance with the principles of the 1975 Declaration of Helsinki.

#### *Handheld electrical impedance myography*

The *hEIM* device (Skulpt Chisel<sup>TM</sup>, Myolex Inc., Boston, MA) has six pairs of current and voltage electrodes on its bottom surface oriented perpendicularly between each other (figure 1A), which injects the current at multiple frequencies. This configuration allows the current flow in a multidirectional way at various depths.

The participant rested supine on a medical bed with the knee in full extension (i.e., anatomical zero) for 20 minutes to allow a uniform fluid distribution. The device was connected to a smartphone with a dedicated app via Bluetooth® technology. After pairing, the single scan modality of the right anterior thigh was selected. This location was chosen since it represents an interesting measurement site for assessing sex differences due to different fat thickness accumulation (Störchle et al., 2017). Based on the app instruction, the center of the device was placed at 50% femur length for males (figure 1B), and at the first proximal third for females. Prior to each scan, the electrodes and the skin were sprayed with water. After positioning on the skin, fat percentage ( $SK_{fat}$ , %) and muscle quality (MQ, arbitrary units–AU) were estimated within 10 seconds based on proprietary multiple algorithms. Two measurements were performed, and the corresponding values saved on a personal computer. The two values for  $SK_{fat}$  were averaged (Hobson-Webb et al., 2018). The same calculation was computed for MQ. A rectangle corresponding to the Skulpt Chisel<sup>TM</sup> dimension was drawn on

the skin of the right anterior thigh region with a dermatographic pencil as reference for ultrasound measurements and reliability assessment.

Noticeably, MQ values are given on a positive integer scale accompanied by a qualitative description, as follows: <93: *needs work*; 93–102: *fair*; 103–115: *good*; 116–129: *fit*;  $\geq 130$ : *athletic*. It should be also underlined that details about the computation of  $SK_{\text{fat}}$  and MQ values are not known, and raw impedance data are not provided.

### *Ultrasound measurements*

B-mode ultrasound (LOGIQ S7, GE Healthcare, Milwaukee, WI, US) with a linear transducer (mod. 9L, 3.1–10 MHz, 4-mm field-of-view) was used for images acquisition. Brightness, gain, and frequency were adjusted to 50, 51 dB and 10 MHz, respectively, and kept constant for all participants. Scanning depth was set to a minimum of 6 cm. The focus position of the ultrasound beam was consistent across participants. An expert operator collected all the ultrasound images while the participant was resting supine on the medical bed after *hEIM* measurement. The marked rectangle (see above) was divided in four equal portions (figure 1C). A layer of  $\sim 5$  mm ultrasound gel (Aquasonic 100 ultrasound transmission gel, Parker Laboratories, Inc., Fairfield, NJ, USA) was applied over each portion before image acquisition to avoid measurement bias due to compression (Störchle et al., 2017). As little pressure as possible was exerted during image collection. Four images per participant were collected on the transverse plane and stored. For intra-session reliability analysis, all acquisitions were repeated twice at each site.

Subcutaneous fat thickness ( $SUB_{\text{fat}}$ , mm) was measured with ImageJ software (Version 1.46, National Institutes of Health, Bethesda, MD, USA) by drawing three straight lines (left, center, right) between the skin-muscle interface and the superficial muscle's aponeurosis (figure 1D). These three measures were averaged to obtain one value for each of the four analyzed images. Finally, the values of the four images were averaged to obtain a representative  $SUB_{\text{fat}}$  measure of the anterior thigh for each individual.

The echo intensity analysis was conducted only in the *rectus femoris* muscle (Fukumoto et al., 2012). The polygon tool of the ImageJ software was used to draw the contour of the largest region of interest excluding the fascia area (figure 1D). Mean grayscale level (black=0, white=255) within the region of interest was determined and used as  $EI_{us}$  measure. The values of the four images were averaged to obtain a representative  $EI_{us}$  measure for each individual.  $EI_{us}/SUB_{fat}$  was calculated.

To determine quadriceps femoris muscle anatomical cross-sectional area ( $ACSA_{QF}$ ), the ultrasound was set in extended-field-of-view mode. This technique has been previously validated against MRI for the same muscle group (Ahtiainen et al., 2010; Noorkoiv, Nosaka, & Blazevich, 2010). Two lines perpendicular to the longitudinal axis (i.e., across the thigh) were drawn at 50% and 40% (towards the knee) femur length, where the *quadriceps femoris* muscle exhibits the largest  $ACSA_{QF}$  (Erskine, Jones, Maganaris, & Degens, 2009). A continuous single scan was taken at each site along the drawn line. During image acquisition, care was taken to avoid identifiable muscle compression. For intra-session reliability analysis, all acquisitions were repeated twice at each site. Images were saved and stored on a personal computer for subsequent analysis. The contours of each single quadriceps femoris  $ACSA$  was digitized offline using Image J and summed to identify the largest  $ACSA_{QF}$ .  $ACSA_{QF}/SUB_{fat}$  was calculated.

#### *Maximum voluntary isometric contraction*

Knee extension MVIC was measured with a custom-built ergometer. Participants lay supine on a medical bed (hip joint angle  $160^\circ$ , with  $180^\circ$  being full extension) with both legs with a knee angle of  $90^\circ$ . The right ankle was firmly attached to a calibrated force transducer (mod. SM-2000 N, Interface, UK). The pelvis and the tested thigh were securely fixed to the ergometer (Longo et al., 2017). The supine position yields MVIC torque values comparable to the ones obtained in a seated position, and less antagonist coactivation (Bampouras, Reeves, Baltzopoulos, & Maganaris, 2017). The passive baseline trace from force was considered as limb weight and then subtracted. After a standardized warm-up (~10 submaximum isometric contractions), participants performed three

MVICs interspersed by 3 minutes of rest. Participants were instructed to contract as fast and as hard as possible. During trials, the arms remained crossed on the chest. Verbal encouragement was provided. Force signal was recorded, amplified ( $\times 200$ ), and stored at a sampling rate of 2048 Hz on a personal computer after A/D conversion (mod. EMG-USB, OtBioelettronica, Turin, Italy). The maximum torque (T) was obtained by multiplying the highest force value by the external moment arm length.  $T/ACSA_{QF}$  was calculated.

\*\*\* Figure 1 about here \*\*\*

### *Reliability*

Reliability was assessed intra-session for  $SK_{fat}$ , MQ,  $SUB_{fat}$ ,  $EI_{us}$ ,  $ACSA_{QF}$ , and T in a sub-group of 12 participants. After two days,  $SK_{fat}$  and MQ were re-assessed in the same sub-group for inter-day reliability calculation.

### *Statistical analysis*

Data are presented as mean (SD). Normality of data distribution was checked with the Komolgorov-Smirnov's test. Reliability was assessed with the intraclass correlation coefficient (ICC, two-way random, absolute agreement) with 95% confidence interval (95% CI) (Munro, 2004) and standard error of measurement as percentage (SEM%) calculations.

Sex differences were assessed by independent sample Student's *t*-test and Cohen's effect size. The effect size (ES) of the difference between males and females with 95% confidence interval (CI<sub>95%</sub>) was calculated for each parameter and interpreted as follows: 0.00–0.19: *trivial*; 0.20–0.59: *small*; 0.60–1.19: *moderate*; 1.20–1.99: *large* and  $>2.00$ : *very large* (Drinkwater, Hopkins, McKenna, Hunt, & Pyne, 2007). Pearson's correlation coefficient was calculated to define the association between the independent variables and the respective dependent variable for both *hEIM*- and ultrasound-derived fat content (independent:  $SUB_{fat}$  and  $EI_{us}$ ; dependent:  $SK_{fat}$ ) and muscle quality

(independent:  $El_{us}$ ,  $ACSA_{QF}$ , and  $T$ ; dependent:  $MQ$ ) measures. Moreover,  $MQ$  was correlated with further indexes of muscle quality ( $T/ACSA_{QF}$ ,  $El_{us}/SUB_{fat}$ , and  $ACSA_{QF}/SUB_{fat}$ ). The strength of the correlation was defined as follows:  $r < 0.10$ , *trivial*;  $0.10 < r < 0.30$ , *low*;  $0.31 < r < 0.50$ , *moderate*;  $0.51 < r < 0.70$ , *high*;  $0.71 < r < 0.90$ , *very high*;  $0.91 < r < 0.99$ , *nearly perfect*;  $r = 1$ , *perfect* (Hopkins, 2000). In case of significant correlations, a stepwise multiple regression model (separated for fat content and muscle quality measures) was computed to assess the weight of each independent variable on its respective dependent variable, considering sex as dummy variable. On the successful models, multiple regressions were run for validating the original models on a randomly selected sub-sample (*training* sample) corresponding to the 70% of the original sample balanced for sex. The remaining 30% (*confirmatory* sample) was used to cross-validate the regression models. Differences between the real and the predicted values in the *confirmatory* sample were also assessed with a paired Student's *t*-test. Multicollinearity among independent variables was assessed by calculating the variance inflation factor (VIF) prior to the multiple regression analysis, considering VIF  $< 3.0$  acceptable. The analysis was conducted with IBM SPSS® Statistics (v.24, Armonk, NY, USA), and the level of significance was set at  $\alpha < 0.05$ .

## Results

Raw values of participants' characteristics are reported in Table 1. Males and females were significantly different in all parameters ( $p < 0.001$ , *moderate-very large* ES) except for age ( $p = 0.10$ , *small*).

Mean  $SK_{fat}$  values were soundly distributed between sexes, whereas mean  $MQ$  values were above 100 AU in both male and female participants (Table 1). Given that individuals with  $MQ \geq 103$  AU are qualitatively rated from *good* to *athletic* by the device app, the  $MQ$  values highlight the homogeneity of our sample in fitness status.

All the investigated variables had very high intra-session reliability ( $ICC > 0.90$ ), with narrow confidence intervals and small SEM% (Table 1). Moreover, similar reliability values were found for inter-day *hEIM* parameters.

\*\*\* Table 1 about here \*\*\*

### Correlations

Correlations between  $SK_{fat}$  and both  $SUB_{fat}$  and  $EI_{us}$  are displayed in Table 2 for the whole sample and divided by sex.  $SK_{fat}$  was positively correlated ( $p < 0.001$ ) with both  $SUB_{fat}$  (*very high*) and  $EI_{us}$  (*high*) in the whole sample and in both sexes (males:  $SUB_{fat}$ , *very high*;  $EI_{us}$ , *high*; females:  $SUB_{fat}$ , *high*;  $EI_{us}$ , *high*). Based on these results,  $SUB_{fat}$ ,  $EI_{us}$  and sex (as dummy variable) were used for predicting  $SK_{fat}$ .

Correlations between MQ and  $EI_{us}$ ,  $ACSA_{QF}$ , and T are displayed in Table 2 for the whole sample and divided by sex, together with correlations between MQ and other indexes possibly related to muscle quality. MQ correlated negatively with  $EI_{us}$  ( $p < 0.001$ , *high*) and positively with  $ACSA_{QF}$  ( $p < 0.001$ , *moderate*). Moreover, MQ was positively correlated with  $EI_{us}/SUB_{fat}$  ( $p < 0.001$ , *moderate*) and  $ACSA_{QF}/SUB_{fat}$  ( $p < 0.001$ , *very high*). Although not significant ( $p = 0.06$ ), there was a tendency for a *low* correlation between MQ and T. However, no significant correlation ( $p > 0.05$ ) was found between MQ and T/ $ACSA_{QF}$  ( $p > 0.05$ , Table 2). In males, MQ correlated negatively with  $EI_{us}$  ( $p < 0.001$ , *high*), and positively with  $ACSA_{QF}/SUB_{fat}$  ( $p < 0.001$ , *very high*). In females, MQ correlated negatively with  $EI_{us}$  ( $p < 0.001$ , *moderate*), and positively with  $ACSA_{QF}$  ( $p = 0.05$ , *low*),  $EI_{us}/SUB_{fat}$  ( $p < 0.001$ , *moderate*), and  $ACSA_{QF}/SUB_{fat}$  ( $p < 0.001$ , *very high*). When divided by sex, MQ did not significantly correlate with T and T/ $ACSA_{QF}$  ( $p > 0.05$ ). Based on these results and on the high correlation between  $ACSA_{QF}$  and  $ACSA_{QF}/SUB_{fat}$  ( $r = 0.72$ ,  $p < 0.001$ ),  $EI_{us}$ ,  $EI_{us}/SUB_{fat}$ ,  $ACSA_{QF}/SUB_{fat}$ , and sex (as dummy variable) were used for predicting MQ.

\*\*\* Table 2 about here \*\*\*

### *Multiple regressions*

All SK<sub>fat</sub> predictors had an appropriate VIF (i.e., <3.0). A significant regression equation was found ( $F=175.11$ ,  $p<0.001$ , Table 2) with an adjusted  $R^2=0.86$ . The three predictors were retained in the model ( $p\leq 0.001$ ). The original regression equation is as follows:

$$SK_{fat} = 3.605 + 0.888 \times SUB_{fat} + 9.931 \times sex + 0.153 \times EI_{us}$$

where sex is dummy coded as 0=male and 1=female, SUB<sub>fat</sub> is measured in mm and EI<sub>us</sub> in AU. Measured *versus* predicted SK<sub>fat</sub> is shown for the whole sample (figure 2A) and divided by sex (figure 2B and 2C).

When the regression model was run on the *training* sample, all the predictors were included in the model ( $F=148.79$ ,  $p<0.001$ , Table 2) with an adjusted  $R^2=0.88$ , which was similar to the original model  $R^2$ . The  $\beta$  coefficients were in line with the original model (Table 2). The *training* and *confirmatory* sub-samples showed similar multiple  $r$  (0.91 and 0.94, respectively), similar  $R^2$  (0.87 and 0.84, respectively), and similar standard error ( $SE$ , 4.09 and 4.97, respectively) between the real and predicted values. The dependent samples  $t$ -test did not reveal significant differences between real and predicted values in the *confirmatory* sub-sample ( $26.85\pm 11.64$  *versus*  $25.32\pm 11.32$  %,  $t=1.42$ ,  $p=0.17$ ). Hence, the regression calculation was considered validated.

All the MQ predictors had an appropriate VIF (i.e., <3.0). A significant regression equation was found ( $F=87.769$ ,  $p<0.001$ , Table 2) with an adjusted  $R^2=0.75$ . EI<sub>us</sub>/SUB<sub>fat</sub> was excluded from the stepwise regression ( $p=0.11$ ). The original regression equation is as follows:

$$MQ = 103.289 + 2.871 \times ACSA_{QF}/SUB_{fat} + 10.080 \times sex - 0.237 \times EI_{us}$$

where sex is dummy coded as 0=male and 1=female, ACSA<sub>QF</sub>/SUB<sub>fat</sub> is measured in cm<sup>2</sup>/mm, and EI<sub>us</sub> in AU. Measured *versus* predicted MQ is shown for the whole sample (figure 2D) and divided by sex (figure 2E and 2F).

When the regression model was run on the *training* sample, all the predictors were included in the model ( $F=63.138, p<0.001$ , Table 2) with an adjusted  $R^2=0.74$ , which was similar to the original model  $R^2$ . The  $\beta$  coefficients were in line with the original model (Table 2). The *training* and *confirmatory* sub-samples showed similar  $r$  (0.87 and 0.89, respectively), similar  $R^2$  (0.74 and 0.75, respectively), and similar  $SE$  (7.96 and 7.13, respectively) between the real and predicted values. The dependent samples  $t$ -test did not reveal significant differences between real and predicted values in the *confirmatory* sub-sample ( $119.93\pm 14.40$  versus  $119.96\pm 13.01$  AU,  $t=-0.017, p=0.99$ ). Hence, the regression calculation was considered validated.

\*\*\* Figure 2 about here \*\*\*

## Discussion

The main results of the present study were that: (i) intra-session and inter-day reliability of the  $hEIM$  variables was very high; (ii)  $SK_{fat}$  correlated with both ultrasound-derived fat variables ( $SUB_{fat}$  and  $EI_{us}$ ), whereas  $MQ$  correlated with ultrasound-derived muscle quality ( $EI_{us}$ ), size ( $ACSA_{QF}$ ), and the indexes representing the ratio between contractile and fat/non contractile-tissue ( $EI_{us}/SUB_{fat}$  and  $ACSA_{QF}/SUB_{fat}$ ); and (iii)  $SUB_{fat}$  and sex had a heavier weight compared to  $EI_{us}$  in the prediction of  $SK_{fat}$ , while  $ACSA_{QF}/SUB_{fat}$  had a heavier weight compared to sex and  $EI_{us}$  in predicting  $MQ$ . These findings suggest that the SkulptChisel™ is a reliable device. Local fat estimation seems to be based predominantly on subcutaneous fat content, whereas high muscle quality seems to be related to high contractile tissue content and less subcutaneous fat, rather than muscle size and/or strength alone. Both parameters depend on sex.

The present results demonstrated that this device was highly reliable, showing low SEM% values in both intra- and inter-conditions. A previous study conducted in the elderly with the same device on the same location found comparable ICC values to the present ones for both  $SK_{fat}$  and  $MQ$  (Hobson-Webb et al., 2018). When estimating total body-fat percentage, another investigation

demonstrated high reliability of the SkulptChisel™, although the absolute difference between inter-day measurements was statistically significant (McLester et al., 2018). Therefore, further studies are needed to clarify the *hEIM* inter-day reliability in whole-body fat assessment.

### *Correlations*

The present investigation demonstrated a *very high* strength of the correlation between  $SK_{fat}$  and  $SUB_{fat}$ , as well as a *high* correlation between  $SK_{fat}$  and  $EI_{us}$ . These results are in line with another study that found *high* correlation ( $r=0.74$ ) between thigh fat percentage measured by *hEIM* and DXA in the elderly (Hobson-Webb et al., 2018). The difference in correlation coefficients with the present results could be due to the different sample size and selected population. The present correlations support the manufacturer's claim that local fat determined by *hEIM* reflects mostly subcutaneous fat, and highlight a relationship between  $SK_{fat}$  and intramuscular non-contractile tissue for the first time.

Despite not providing raw impedance data (resistance, reactance, and phase angle) to be compared with the literature, it is plausible that the SkulptChisel™ relates to the amount of  $SUB_{fat}$  and intramuscular non-contractile tissue by analyzing the resistance/phase parameters detected and computed by the internal algorithm. Based on measurements performed in a laboratory setting, it has been acknowledged that EIM phase values are reduced when high fat and connective tissue content are present, due to their low capacitance and, in the case of fat, high resistivity (Rutkove, 2009; Rutkove et al., 2014). Moreover, it has been shown that EIM resistance and phase in the *biceps brachii* (L. Li et al., 2016) and *gastrocnemius* (Sung, Spieker, Narayanaswami, & Rutkove, 2013) muscles measured with another EIM portable device correlated strongly with their respective local subcutaneous fat layer measured by ultrasound, supporting the laboratory-setting results (Rutkove et al., 2014). Likely, the SkulptChisel™ exploits the same technology. Indeed, the device was designed for allowing the flowing current to pass through both fat and muscle tissues by combining short inter-electrodes distance (Sung et al., 2013) with the injection of multifrequency current, which increases the accuracy in distinguishing between the two tissues (Schwartz et al., 2015). Nonetheless, further

studies are needed to clarify whether changes in  $SUB_{fat}$  and  $EI_{us}$  could be mirrored by changes in  $SK_{fat}$ , and to what extent  $SUB_{fat}$  can influence  $SK_{fat}$  compared to  $EI_{us}$  in these changes.

The bivariate correlation analysis revealed that MQ was related to  $EI_{us}$ ,  $ACSA_{QF}$ ,  $EI_{us}/SUB_{fat}$  and  $ACSA_{QF}/SUB_{fat}$ , excluding relationships between MQ and T in absolute or normalized values. Only one previous study investigated the relationship between thigh MQ determined by the SkulptChisel™, muscle mass, strength and fat mass, conducted in an older population (Hobson-Webb et al., 2018). In that investigation, no significant correlations between MQ and muscle mass measured by DXA, and MQ and isokinetic peak torque were found. These findings were partially in line with our results, where a relationship between MQ and muscle size was retrieved but no correlations between MQ and strength variables were found. The discrepancy between the present study and the one by Hobson-Webb et al. (2018) could be due to the different population investigated, sample size, and methods to determine muscle mass. Nonetheless, in the same study a significant negative correlation was found between MQ and DXA-derived fat mass (Hobson-Webb et al., 2018), which is in line with the present correlations between MQ and  $EI_{us}$ , and MQ and  $ACSA/SUB_{fat}$ . Collectively, the present correlations partially support the manufacturer's claims, in which MQ should reflect a greater muscle fibers' size, a smaller fat content, and, in turn, a greater strength expression and muscle health (Skulpt performance training system).

The principle by which MQ could be related to muscle mass and strength is likely based on the influence that these variables can have on the reactance and phase components of the EIM analysis. Indeed, a relationship between EIM parameters and muscle fiber diameter, and atrophy/hypertrophy in cellular (Rakhilin et al., 2011) and animal model (Kapur et al., 2018) has been previously described. Muscle fibers act as a network of capacitors; hence, changes in muscle fibers' size and number would alter the reactance value, and, in turn, phase (i.e., the greater the size the greater reactance/phase values). Additionally, the presence of non-contractile tissue within the muscle and subcutaneous fat would have an impact on both resistance and reactance values (Rutkove et al., 2010). Therefore, according also to the present results, it is plausible that the algorithm for calculating

MQ accounted for the amount of contractile and non-contractile tissue, as well as subcutaneous fat. Nonetheless, the association between EIM parameters and strength is less clear. Positive correlations between force output and phase were found in an animal model (Jia Li, Pacheck, Sanchez, & Rutkove, 2016) and in humans (Rutkove et al., 2010). High phase values are reached due to the combination of high reactance and low resistance, which reflects greater muscle size and intact muscle cells. Therefore, the authors hypothesized that the positive association between strength and phase could be explained by muscle size (Jia Li et al., 2016; Rutkove et al., 2010), implying a direct relationship between muscle mass and strength expression. However, the present results do not support this hypothesis. More studies are needed to investigate the supposed link between MQ and strength, and to assess whether changes in  $EI_{us}$ ,  $ACSA_{QF}$ , and  $SUB_{fat}$  could be mirrored by changes in MQ.

#### *Multiple regressions*

Concerning fat content, the multiple regression model retained all the inserted variables explaining 86% of the  $SK_{fat}$  variance. However, when examining the regression standardized  $\beta$ -coefficients it could be observed that  $EI_{us}$  ( $\beta=0.232$ ) had a lower weight compared to both  $SUB_{fat}$  ( $\beta=0.400$ ) and sex ( $\beta=0.436$ ) in predicting  $SK_{fat}$ . This model suggests that despite the significant bivariate correlations,  $SK_{fat}$  values likely reflect more the subcutaneous fat content compared to the intramuscular non-contractile tissue component, and that female sex influences fat percentage estimation.

Concerning muscle quality, the multiple regression model retained  $ACSA_{QF}/SUB_{fat}$ , sex and  $EI_{us}$ , explaining 75% of MQ variance, whereas  $EI_{us}/SUB_{fat}$  was excluded. Considering the standardized  $\beta$ -coefficients ( $\beta=0.921$ , 0.333, and -0.270,  $ACSA_{QF}/SUB_{fat}$ , sex, and  $EI_{us}$ , respectively), this model suggests that the MQ value was mainly predicted by greater muscle size and/or less subcutaneous fat, more than the amount of contractile tissue within the muscle, with female sex influencing MQ values. However, since ~25% of the variance remained unexplained, other factors could contribute to MQ determination.

## **Limitations**

This study has several limitations. Firstly, only young physically active participants were measured on one body location, thus limiting the applicability of the present results to this population and site. Secondly, T values were obtained at 90° knee flexion without correction for antagonist coactivation; therefore, since the correlation between MQ and T was almost significant, it cannot be excluded that testing MVIC at the best knee flexion angle and correcting T for antagonist coactivation could yield significant correlations between MQ and corrected T. Nonetheless, the supine position in which our participants were tested decreases antagonist coactivation. Therefore, the weight of corrected T in a multiple regression model for best predicting MQ needs further investigation. Lastly,  $El_{us}$  is a measurement depending on the ultrasound equipment, contrast, brightness, and analysis software; these characteristics limit the present results to the ultrasound settings used in this investigation. Further studies using DXA-derived local composition are needed to confirm the present results.

## **Conclusions**

In conclusion, the SkulptChisel™ is a reliable *h*EIM device for measuring local thigh fat percentage and muscle quality in young healthy individuals, and it is sufficiently sensitive for assessing differences in local body segment composition between males and females. Local fat percentage was mainly predicted by ultrasound-derived subcutaneous fat and, with less weight, by the intramuscular non-contractile tissue content. Local muscle quality was mainly predicted by the ratio between muscle size and subcutaneous fat, and, with less weight, by ultrasound-derived intramuscular contractile tissue content. Less clear is the role of strength in muscle quality determination. In both estimations, female sex had an influence in the prediction. Further studies are needed to investigate deeper the factors involved in muscle quality prediction, and whether estimated body composition by local electrical impedance myography could be applicable in longitudinal studies.

## **Disclosure statement**

No potential conflict of interest was reported by the authors.

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## Figures caption

### Figure 1

A: Electrodes disposition for the handheld electrical impedance myography (*hEIM*) device on the bottom side. B: *hEIM* device positioning on the right thigh of a male participant. C: Example of ultrasound scanning of the right anterior thigh region in a male participant. The area scanned with the *hEIM* device was marked by drawing the device's angles, and divided in four 3x7-cm rectangles. D: Example of ultrasound scan analysis for measuring subcutaneous fat thickness ( $SUB_{fat}$ ), and the region of interest (dotted line) for measuring echo intensity within *rectus femoris* muscle (RF). *Vastus intermedius* (VI) muscle and femur bone (FB) are also shown.

### Figure 2

A: Predicted *versus* measured local fat percentage of the anterior thigh region for the whole sample (gray circles). Predicted values ( $SK_{fat\_predicted}$ ) were calculated based on the significant independent variables (subcutaneous fat, echo intensity, and sex) included in the multiple regression equation for predicting fat percentage ( $SK_{fat}$ ) estimated by the handheld impedance myography device. B and C:  $SK_{fat\_predicted}$  *versus*  $SK_{fat}$  in males (filled circles) and females (empty circles), respectively. D: Predicted *versus* measured local muscle quality of the anterior thigh region for the whole sample (gray circles). Predicted values ( $MQ_{predicted}$ ) were calculated based on the significant independent variables (the ratio between anatomical cross-sectional area and subcutaneous fat, sex, and echo intensity) included in the multiple regression equation for predicting muscle quality (MQ) estimated by the handheld impedance myography device. E and F:  $MQ_{predicted}$  *versus* MQ in males (filled circles) and females (empty circles), respectively.

**Table 1.** Participants' characteristics divided by sex and measurements' reliability.

<i>Participants' characteristics</i>					
<i>Variable</i>	<b>Males (<i>n</i> = 45)</b>		<b>Females (<i>n</i> = 45)</b>		<b>ES (CI<sub>95%</sub>)</b>
	<b>Mean ± SD</b>		<b>Mean ± SD</b>		
Age (years)	23.3 ± 3.2		23.4 ± 2.4		0.34 (-0.07-0.75)
Height (cm)	177.2 ± 7.0		167.2 ± 5.4**		1.60 (1.12-2.06)
Body mass (kg)	75.2 ± 10.2		59.2 ± 7.1**		1.82 (1.32-2.29)
BMI (kg m <sup>-2</sup> )	23.9 ± 2.4		21.1 ± 1.9**		1.24 (0.79-1.68)
SK <sub>fat</sub> (%)	15.5 ± 5.4		34.1 ± 7.3**		2.95 (2.34-3.52)
MQ (AU)	127.4 ± 17.6		112.8 ± 5.9**		1.11 (0.66-1.53)
SUB <sub>fat</sub> (mm)	6.9 ± 1.9		14.8 ± 4.2**		2.41 (1.85-2.92)
EI <sub>us</sub> (AU)	36.1 ± 17.1		48.8 ± 15.2**		0.78 (0.35-1.20)
ACSA <sub>QF</sub> (cm <sup>2</sup> )	73.7 ± 9.7		51.8 ± 9.0**		2.34 (1.79-2.85)
T (Nm)	278.3 ± 57.6		169.9 ± 52.8**		1.96 (2.34-2.44)
<i>Measurements' reliability</i>					
<i>Intra-session</i>	<b>Measure 1</b>	<b>Measure 2</b>	<b>ICC</b>	<b>CI<sub>95%</sub></b>	<b>SEM%</b>
SK <sub>fat</sub> (%)	24.7 ± 8.4	24.5 ± 7.9	0.997	0.991 - 0.999	1.81
MQ (AU)	53.3 ± 15.0	52.5 ± 14.0	0.991	0.975 - 0.997	2.60
SUB <sub>fat</sub> (mm)	5.0 ± 2.1	5.0 ± 2.2	0.997	0.984 - 0.999	2.38
EI <sub>us</sub> (AU)	45.3 ± 16.4	45.9 ± 17.1	0.997	0.988 - 0.999	2.01
ACSA <sub>QF</sub> (cm <sup>2</sup> )	61.5 ± 13.3	61.2 ± 12.9	0.998	0.992 - 0.999	1.92
T (Nm)	198.4 ± 38.9	200.7 ± 39.7	0.994	0.969 - 0.999	1.53
<i>Inter-day</i>					
SK <sub>fat</sub> (%)	23.2 ± 8.4	24.0 ± 9.1	0.988	0.956 - 0.994	4.83
MQ (AU)	55.5 ± 21.1	54.5 ± 21.2	0.988	0.967 - 0.996	4.22

**Note:** *N*: sample size; SD: standard deviation; ES: effect size; CI<sub>95%</sub>: 95% confidence interval; BMI: body mass index; M: males; F: females; SK<sub>fat</sub>: percentage of local fat estimated by electrical impedance myography; MQ: local muscle quality in arbitrary units (AU) estimated by electrical impedance myography; SUB<sub>fat</sub>: local subcutaneous fat measured by ultrasound imaging; EI<sub>us</sub>: echo intensity; ACSA<sub>QF</sub>: anatomical-cross sectional area of quadriceps femoris; T: knee extensors torque; ICC: intra-class correlation coefficient; SEM%: standard error of measurement as percentage; T/ACSA<sub>QF</sub>: torque normalized for quadriceps femoris anatomical-cross sectional area; EI<sub>us</sub>/SUB<sub>fat</sub>: echo intensity: subcutaneous fat ratio; ACSA<sub>QF</sub>/SUB<sub>fat</sub>: quadriceps femoris anatomical-cross sectional area: subcutaneous fat ratio; *p*: level of significance; \*\*: statistically different from males with *p*<0.001.

**Table 2.** Bivariate correlations between local electrical impedance myography and ultrasound-derived measurements, and multiple regression models of the original and *training* samples (70% of the original sample) for validation analysis.

<i>Bivariate correlations</i>					
<b>Fat content</b>		<b>SUB<sub>fat</sub></b>	<b>EI<sub>us</sub></b>		
SK <sub>fat</sub> ( <i>N</i> = 90)	<i>r</i> ( <i>p</i> )	0.88 (<0.001)	0.64 (<0.001)		
M SK <sub>fat</sub> ( <i>N</i> = 45)	<i>r</i> ( <i>p</i> )	0.74 (<0.001)	0.66 (<0.001)		
F SK <sub>fat</sub> ( <i>N</i> = 45)	<i>r</i> ( <i>p</i> )	0.68 (<0.001)	0.64 (<0.001)		
<b>Muscle quality</b>					
<b>Muscle quality</b>		<b>EI<sub>us</sub></b>	<b>ACSA<sub>QF</sub></b>	<b>T</b>	
MQ ( <i>N</i> = 90)	<i>r</i> ( <i>p</i> )	-0.66 (<0.001)	0.37 (<0.001)	0.29 (0.06)	
M MQ ( <i>N</i> = 45)	<i>r</i> ( <i>p</i> )	-0.68 (<0.001)	-0.11 (0.45)	-0.15 (0.50)	
F MQ ( <i>N</i> = 45)	<i>r</i> ( <i>p</i> )	-0.50 (0.001)	0.30 (0.05)	0.34 (0.17)	
		<b>T/ACSA<sub>QF</sub></b>	<b>EI<sub>us</sub>/SUB<sub>fat</sub></b>	<b>ACSA<sub>QF</sub>/SUB<sub>fat</sub></b>	
MQ ( <i>N</i> = 90)	<i>r</i> ( <i>p</i> )	0.18 (0.27)	0.37 (<0.001)	0.81 (<0.001)	
M MQ ( <i>N</i> = 45)	<i>r</i> ( <i>p</i> )	-0.06 (0.78)	0.10 (0.52)	0.78 (<0.001)	
F MQ ( <i>N</i> = 45)	<i>r</i> ( <i>p</i> )	0.22 (0.38)	0.37 (0.012)	0.78 (<0.001)	
<i>Fat content: multiple regression analysis of the original sample</i>					
<b>Predictor</b>	<b><math>\beta</math></b>	<b><i>B</i></b>	<b><i>SE</i></b>	<b><i>t</i></b>	<b><i>p</i></b>
SUB <sub>fat</sub>	0.400	0.888	0.169	5.253	<0.001
Sex	0.436	9.931	1.478	6.718	<0.001
EI <sub>us</sub>	0.232	0.153	0.034	4.463	<0.001
<i>Fat content: multiple regression analysis of the “training” sample (70%) for validation</i>					
<b>Predictor</b>	<b><math>\beta</math></b>	<b><i>B</i></b>	<b><i>SE</i></b>	<b><i>t</i></b>	<b><i>p</i></b>
SUB <sub>fat</sub>	0.442	0.976	0.180	5.410	<0.001
Sex	0.426	9.600	1.592	6.029	<0.001
EI <sub>us</sub>	0.213	0.140	0.037	3.828	<0.001
<i>Muscle quality: multiple regression analysis of the original sample</i>					
<b>Predictor</b>	<b><math>\beta</math></b>	<b><i>B</i></b>	<b><i>SE</i></b>	<b><i>t</i></b>	<b><i>p</i></b>
ACSA <sub>QF</sub> /SUB <sub>fat</sub>	0.921	2.871	0.311	9.229	<0.001
Sex	0.333	10.080	2.701	3.732	<0.001
EI <sub>us</sub>	-0.270	-0.237	0.057	-4.147	<0.001
<i>Muscle quality: multiple regression analysis of the “training” sample (70%) for validation</i>					
<b>Predictor</b>	<b><math>\beta</math></b>	<b><i>B</i></b>	<b><i>SE</i></b>	<b><i>t</i></b>	<b><i>p</i></b>
ACSA <sub>QF</sub> /SUB <sub>fat</sub>	0.857	2.774	0.375	7.399	<0.001
Sex	0.301	9.337	3.150	2.964	0.004
EI <sub>us</sub>	-0.304	-0.260	0.068	-3.838	<0.001

**Note:** *N*: sample size; M: males; F: females; SK<sub>fat</sub>: percentage of local fat estimated by electrical impedance myography; MQ: local muscle quality estimated by electrical impedance myography; SUB<sub>fat</sub>: local subcutaneous fat measured by ultrasound imaging; EI<sub>us</sub>: echo intensity; ACSA<sub>QF</sub>: anatomical-cross sectional area of quadriceps femoris; T: knee extensors torque; T/ACSA<sub>QF</sub>: torque normalized for quadriceps femoris anatomical-cross sectional area; EI<sub>us</sub>/SUB<sub>fat</sub>: echo intensity: subcutaneous fat ratio; ACSA<sub>QF</sub>/SUB<sub>fat</sub>: quadriceps femoris anatomical-cross sectional area:

subcutaneous fat ratio;  $r$ : Pearson's correlation coefficient;  $\beta$ : standardized beta-coefficient (beta weight);  $B$ : non-standardized coefficient;  $SE$ : standard error;  $t$ : t-value;  $p$ : level of significance.

