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Original Article

Muscle measurements in daily clinical practice: correlations between ultrasound, bioelectrical impedance analysis and hand grip strength

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Abstract

Objectives: Muscle ultrasound is gaining importance as a measurement tool to evaluate sarcopenia in daily clinical practice. This study sought to collect reference values of the biceps brachii (BB) in healthy subjects, and to correlate them to bioelectrical impedance analysis (BIA) and hand grip strength (HGS). **Methods**: Ultrasound was used to measure muscle thickness (MT), cross-sectional area (CSA) and muscle stiffness (EG). Lean mass (LM), fat mass (FM) and phase angle (PhA) were measured by BIA. HGS was measured using a Jamar dynamometer. Intra-rater reliability was calculated using intraclass correlation (ICC). Correlations were investigated using Pearson correlation (PCC). **Results**: One-hundred-twenty-three Caucasian individuals (51 male and 72 female subjects) between 18 and 69 years old were included. ICC for ultrasound was 0.99 (CI 0.98-0.99). LM, PhA and HGS showed significant correlations with MT and CSA in the entire population (PCC>0.684). These correlations were more significant and stronger in male than in female subjects (PCC>0.419 and >0.279 resp.). Significant correlations with respect to FM and EG were seen exclusively in female subjects. **Conclusion**: Significant correlations were observed between parameters of muscle quantity measured by ultrasound, BIA and HGS. Intra-rater reliability was excellent. Future studies in larger populations are needed to clarify the observed gender differences.

Keywords: Sarcopenia, Muscle ultrasound, Bioelectrical impedance analysis, Hand grip strength

Introduction

Over the past years, sarcopenia has become an increasingly important topic. The condition is a major contributor to illness, loss of independence and mortality in the ageing population[1-4]. In high-income societies, where the median life expectancy is increasing, this means a significant burden on national health expenditure[1-3]. In 2018, the European Working Group on Sarcopenia in Older People (EWGSOP) published their revised definition of sarcopenia as a generalized decrease in skeletal muscle strength, quality and quantity[4]. Muscle quantity refers to total body muscle mass (MM)[4,5]. Muscle quality can refer to the micro- and macroscopic aspects of muscle architecture and composition, but is also frequently expressed as a ratio of muscle strength per unit of MM[4,6]. Most likely both characteristics play an important role in the generation of muscle force, as previous studies have already established that the decrease in muscle strength during aging is substantially greater than the decrease in MM[7,8].

Presently, clinicians have multiple validated questionnaires and tools at their disposal to predict the presence or absence of sarcopenia[4,9,10]. In order to diagnose sarcopenia in daily clinical practice, a measurement tool capable of evaluating both muscle quantity and quality is needed, while preferentially being easily accessible,

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inexpensive and non-invasive[11]. None of the currently validated tools to diagnose sarcopenia comply to all of these demands[11-14]. Dual-energy X-ray absorptiometry (DXA), for example, has been proved efficient to measure muscle quantity, but gives no information about muscle quality[4,11,12]. Computed tomography (CT) and magnetic resonance imaging (MRI) can measure both muscle quantity and quality, but these tools are expensive and not always available for use in daily clinical practice[11-14]. Bioelectrical impedance analysis (BIA) is capable of measuring not only lean mass (LM), but other characteristics of body composition as well, such as fat mass (FM) and phase angle (PhA)[7,8]. PhA is considered to be a parameter of intact cell function and cell membrane integrity, and therefore a measurement of tissue quality[4,7,8]. A disadvantage of BIA, however, is that a patient's hydration status might influence measurements and lead to unreliable results[8,11]. It is also not recommended to use BIA in patients with implantable cardiac devices, joint prostheses or osteosynthetic material, due to possible interference with the instrument's electrical current[8].

In contrast, ultrasound possesses the qualities of being available, inexpensive and non-invasive, and has shown to have good validity as compared to DXA, MRI and CT[4,11-14]. It is capable of measuring quantitative parameters, such as muscle thickness (MT), cross-sectional area (CSA) and muscle volume, as well as more qualitative parameters, such as the measurement of muscle stiffness through elastography (EG), fascicle length, pennation angle, muscle contraction and microcirculation[13]. The SARCUS working group already made efforts to propose standardized ultrasonographic measurement techniques for 39 muscles[13,14]. The only factor still hampering the implementation of muscle ultrasound in daily clinical practice, is the absence of reference and cut-off values derived from healthy subjects[15,16]. These would not only guide the use of muscle ultrasound as a diagnostic tool for sarcopenia and other muscle conditions, but would also provide an interesting opportunity to investigate age-, gender- and weight-related influences on muscular components[13].

The primary aim of this study was therefore to obtain ultrasonographic reference values of the biceps brachii (BB) in healthy subjects. Secondly, ultrasound parameters were correlated to BIA, a validated measurement tool for sarcopenia, and hand grip strength (HGS), a proxy for total body strength[4].

Materials and methods

Study population

The study was approved by the ethical committee of the Hospital Network Antwerp (ZNA). Healthy subjects between 18 and 70 years old were included between the 1st of January 2021 and the 30th of September 2021. Each participant signed an informed consent form. The group was comprised of hospital staff and acquaintances of the researchers. Subject's health status was evaluated by the Cumulative Illness Rating Scale (CIRS), a measurement tool that rates disease severity in 13 different organ systems[17]. For each system, disease severity is given a score ranging from 0 (no problems) to 4 (life-threatening problems). Only subjects with a maximum score of 1 (mild problems, no interference with daily functioning) in 2 organ systems at the most were included, in order to guarantee a good, global health status.

Figure 1. Demonstration of the measurement location at 75% of the distance between acromion and elbow crease (distally). Photo used with the participant's consent.

Subjects on chronic corticosteroid therapy were excluded due to possible influence on MM.

Subjects with contraindications for BIA, such as implanted cardiac devices (cardiac pacemaker, implantable cardioverter-defibrillator), joint prostheses, osteosynthetic material, pregnancy or peripheral edema were also excluded.

Measurements

Subject characteristics

Subjects' gender, age (years), length (cm), weight (kg) and body mass index (BMI, kg/m^2) were noted.

Ultrasonographic measurements

All measurements were performed by the same investigator, by use of a linear probe of 5 cm width, with a beam frequency of 12 MHz. Subjects lay supine, with their

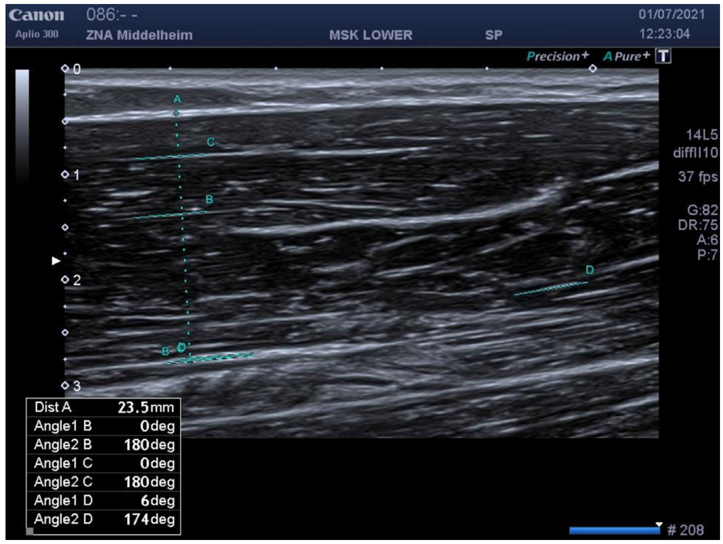

Figure 2. Example of an ultrasound image taken at 75% of the distance between acromion and elbow crease (distally). "A" represents the distance between the superficial and deep fascia of the biceps brachii (BB), or in other words, the muscle thickness (MT). "B, C and D" represent attempts at measuring the angles between the deep fascia and individual muscle fibers of the BB, also known as the pennation angles (PA). Given that nearly each measurement resulted in a PA of 0°, they were not considered to be conducive to this study and not withheld for further analyses.

dominant arm in a neutral position (lower arm between pronation and supination). First, the intended measurement location at 75% of the distance between acromion and elbow crease was marked on the BB, as can be seen in Figure 1. These anatomical landmarks were based on the SARCUS protocol from Perkisas et al.[14]. At this location, MT, CSA and EG were taken. Figure 2 demonstrates the ultrasonographic measurement of MT in one of the study subjects. Every muscle parameter was measured three times, after which the mean value was noted to use in statistical analysis.

Muscle strength

HGS was evaluated by using the Jamar® dynamometer in the dominant hand, elbow flexed at 90°. Subjects were instructed to squeeze the dynamometer once with maximum strength[18].

Bioelectrical impedance analysis

LM, FM and PhA were measured using BIA (Inbody®).

Statistical analysis

IBM®'s SPSS version 28.0 was used for statistical analysis. Normality was tested through the Shapiro-Wilk test. In case of a normal distribution, unpaired student's t-test was used. As non-parametric test, Mann-Whitney U was used. Intra-rater reliability was studied by use of intraclass correlation coefficients (ICC) and 95% confidence intervals, by implementation of a mean-rating, absolute-agreement, 2-way mixed-effects model. An ICC of >0.9 was considered as indicative of "excellent" reliability[19].

Correlations were investigated using Pearson correlation coefficients (PCC). A PCC between ±0,1 and ±0,3 was considered "weak", a PCC between ±0,3 and ±0,6 "moderate" and a PCC > ±0,6 "strong"[20]. A p-value of <0.05 was considered statistically significant.

Results

General characteristics of the study population and ultrasonographic reference values

One-hundred-twenty-three Caucasian individuals (51 male and 72 female subjects) were included. Besides age, weight and BMI, all parameters were distributed normally.

The population's general characteristics and mean values of the ultrasound parameters were summarized in Table 1. Mean MT, CSA and EG differed significantly between the two sexes.

Intra-rater reliability

Muscle ultrasound showed an excellent intra-rater reliability, as illustrated by an ICC of 0.99 (95% CI 0.98-0.99).

Correlations between bioelectrical impedance analysis, hand grip strength and muscle ultrasound

PCC's between BIA and muscle ultrasound values were summarized in Table 2.

	Men (n=51)	Women (n=72)	p-value
Age (years)	33.8 ± 11.4 (18; 69)	37.1 ± 12.7 (21; 65)	0.058
Weight (kg)	77.7 ± 10.5 (54; 100)	66.8 ± 12.9 (41; 103)	<0.001
Length (cm)	179.9 ± 6.2 (164; 193)	167.1 ± 6.5 (150; 186)	<0.001
BMI (kg/m²)	24.0 ± 2.9 (18.3; 31.7)	24.0 ± 4.5 (16.6; 35.6)	<0.001
MT (mm)	22.6 ± 3.0 (16.1; 30.8)	16.6 ± 2.5 (10.1; 22.8)	<0.001
CSA (cm²)	12.0 ± 2.4 (5.7; 17.1)	6.4 ± 1.1 (4.0; 8.9)	<0.001
EG (kPa)	9.0 ± 1.9 (5.2; 13.4)	8.3 ± 1.9 (4.6; 13.0)	0.040

Table 1. Age, weight, length and body mass index (BMI) of the study population. Muscle thickness (MT) in mm, cross-sectional area (CSA) in cm² and elastography (EG) in kilopascal (kPa). Display of mean values ± standard deviation and lowest and highest values of each parameter. Differences between means expressed as p-value.

		Bioelectrical impedance analysis								
		LM (kg)			FM (kg)			PhA (°)		
		Men (n=51)	Women (n=72)	Total (n=123)	Men (n=51)	Women (n=72)	Total (n=123)	Men (n=51)	Women (n=72)	Total (n=123)
Muscle Ultrasound	MT (mm)	0.510	0.389	0.787	-0.085	0.371	-0.171	0.467	0.283	0.688
	p	<0.001	<0.001	<0.001	0.552	0.001	0.059	<0.001	0.016	<0.001
	CSA (cm²)	0.530	0.360	0.841	-0.133	0.093	-0.341	0.435	0.279	0.714
	p	<0.001	0.002	<0.001	0.352	0.438	<0.001	0.001	0.018	<0.001
	EG (kPa)	-0.026	-0.170	0.102	-0.217	-0.253	-0.288	0.110	0.182	0.234
	p	0.857	0.154	0.262	0.126	0.032	0.001	0.443	0.126	0.009

Table 2. Pearson correlations between bioelectrical impedance analysis and muscle ultrasound. Muscle thickness (MT) in mm, cross-sectional area (CSA) in cm² and elastography (EG) in kilopascal (kPa). Lean mass (LM) and fat mass (FM) in kg, phase angle (PhA) in degrees.

		Hand grip strength		
		Men (n=51)	Women (n=72)	Total (n=123)
Muscle ultrasound	MT (mm)	0.430	0.129	0.709
	p	0.002	0.279	<0.001
	CSA (cm²)	0.481	0.339	0.808
	p	<0.001	0.004	<0.001
	EG (kPa)	0.125	0.139	0.224
	p	0.384	0.246	0.013

Table 3. Pearson correlations between hand grip strength in kg, and muscle ultrasound. Muscle thickness (MT) in mm, cross-sectional area (CSA) in cm² and elastography (EG) in kilopascal (kPa).

LM showed moderate to strong positive correlations with MT and CSA in male subjects (PCC >0.500), female subjects (PCC>0.300) and the entire study population (PCC>0.700). FM had a moderate positive correlation with MT in female subjects (PCC 0.371) and a moderate negative correlation with CSA in the entire population (PCC-0.341). PhA showed moderate to strong positive correlations with MT and CSA in male subjects

(PCC>0.400) and the entire population (PCC>0.600).

PCC's between HGS and muscle ultrasound values were summarized in Table 3.

HGS had moderate to strong positive correlations with MT in male subjects (PCC>0.400) and the entire population (PCC>0.700), and with CSA in male subjects (PCC>0.400), female subjects (PCC>0.300) and the entire population (PCC>0.800).

Discussion

Ultrasonographic reference values for different muscles remain scarce, and limited to relatively small study samples[21-26]. The use of different measurement techniques and study populations, and the investigation of different muscles and muscle parameters within this limited number of studies, make it very difficult to extrapolate their results to the general population[14]. The standardization efforts of the SARCUS working group are an important first step to rectify this situation[13,14], but even so, reference values derived from large populations with different demographic backgrounds remain to be collected. Additionally, much is still to be learned about the influence of age, sex and weight on ultrasonographic parameters[13,14].

The reason for collecting reference values of the BB in the current study, was that previous research regarding muscle ultrasound has focused mainly on lower limb muscles, making the upper limb muscles relatively unmarked territory[13]. Ata et al. (2019) measured MT of the BB and other muscles in 145 healthy subjects, and compared two different age categories to one another[23]. The investigators observed a decrease in MT of the BB in the age group above 50 years, but described a greater age-related decline of MT in the lower limb muscles as compared to the upper limb muscles. Alfuraih et al. (2019) performed EG to evaluate muscle stiffness of the BB and other muscles in 77 healthy subjects, divided into three different age categories[24]. The investigators observed an age-related decrease in muscle stiffness. Limitations to the aforementioned studies are the fact that in both cases the investigators implemented their own, unstandardized measurement protocol, and the fact that they investigated no other parameters besides MT and EG, respectively. Neither did they compare the two sexes to one another, or investigate the effect of body weight on their measurements.

In the current study, reference values for MT, CSA and EG of the BB were collected in healthy men and women by use of the proposed measurement protocol by the SARCUS working group[13]. The measurement location at 75% of the distance between acromioclavicular joint and elbow crease was reported to be comfortable by all test subjects. Indeed, none of the subjects had to undress themselves or remain in an uncomfortable position throughout the testing. The location yielded reliable results, as demonstrated by the excellent ICC of 0.99. In an accepted manuscript concerning the same study population as in the current study, gender-specific differences in age-related muscle changes were described[27]. Even though both male and female subjects showed an age-related decline in HGS, male subjects seemed to have a greater age-related decline in MT as compared to female subjects, suggesting that parameters of muscle quantity are not solely responsible for the generation of muscle strength. Especially in females, parameters of muscle quality, such as fat infiltration and stiffness, are probably equally important. The influence of body weight on ultrasonographic parameters could unfortunately not be investigated due to the relatively limited study population, which made it impossible to divide the group into different weight categories. Pereira et al. (2020) did find a negative correlation between BMI and MT of the BB and other muscles in a study population of 117 overweight subjects[28], but much is still to be investigated with respect to this subject.

The second goal of this study was to calculate correlations between ultrasound parameters, BIA and HGS, both validated measurement tools for sarcopenia[4]. The significant, moderate to strong correlations between LM, HGS, MT and CSA, both in male and female subjects separately as in the entire study population, speak in favor of BB ultrasound as a potential measurement tool for sarcopenia. These results are also in accordance with previous investigations regarding ultrasonography of the lower limb muscles. For example, in the study by Hida et al. (2018) with 201 healthy subjects, moderate positive correlations were found between LM and the combined MT of the rectus femoris and vastus intermedius muscles[29]. In two studies with patients undergoing hemodialysis, weak to moderate positive correlations were observed between LM, PhA, CSA and MT of the rectus femoris muscle[30,31]. Lastly, in the study by Madden et al. (2021) with 150 ageing people, moderate positive correlations between HGS and MT of the vastus medialis muscle were observed[32].

Interestingly, in the current study, the significant correlations between LM, PhA, MT and CSA seemed to be less pronounced in female than in male subjects. On the other hand, significant correlations between FM, MT and EG were observed exclusively in female subjects. The male subjects showed no significant correlations regarding FM and EG. These observations suggest that correlations between BIA and muscle ultrasound might differ between the two sexes. Evidently, men have a relatively larger MM than women, which could explain why correlations regarding quantitative parameters are more pronounced in the former[33]. Women, on the other hand, have a relatively larger FM than men[33]. A higher FM, together with hyperlipidaemia and physical inactivity, is associated with an increased muscular fat infiltration or myosteatosis[34]. Although this phenomenon has been observed to increase during ageing in both men and women, it appears to be present to a greater degree in women[35]. Myosteatosis has been found to be inversely correlated to muscle specific force, which can be defined as the maximum isometric force normalized to the muscle CSA, and thus considered to be a parameter of muscle quality[35]. Myosteatosis is also related to changes in muscular architecture and stiffness, which could explain the correlations between FM and EG in the current study[24]. In conclusion, the observed gender differences could mean that muscular changes during ageing should be evaluated differently in men and women.

Strengths and limitations

Needless to say, the inclusion of an elderly population to this study would have been extremely valuable, but due to the COVID-19 pandemic it was deemed unsafe and irresponsible to invite this vulnerable population to the hospital. It would've also been preferable if the current study population were larger, in order to make it possible to compare different age and weight categories to one another. Still, the establishment of reference values in 123 healthy subjects will help to define cut-off values for sarcopenia based on ultrasonographic measurements of the BB through future studies.

One of the strengths of this study was the intent to examine correlations between multiple muscle parameters collected by three different measurement tools. This might shed some light on the complex pathophysiology of sarcopenia, in which muscle strength, quantity and quality all play an important role. Despite the relatively small and young study population, interesting gender differences were observed concerning quantitative and qualitative parameters.

Another positive aspect of this study were the significant correlations found between the three measurement tools, and the excellent intra-rater reliability of the ultrasound measurements. These speak in favor of BB ultrasound as a potential diagnostic tool for sarcopenia, although the observations still need to be validated in a sarcopenic population.

Conclusion

The primary aim of this study was to collect ultrasonographic reference values of the BB in a healthy population by use of a standardized measurement protocol. 51 male and 72 female subjects were included for this purpose. Secondly, correlations between ultrasound, BIA and HGS data were investigated. The measurement location at 75% of the distance between acromioclavicular joint and elbow crease was easily accessible and required no removal of clothing. Furthermore, intra-rater reliability was excellent. Ultrasonographic parameters of muscle quantity showed moderate to strong correlations to BIA and HGS data in both male and female subjects separately, as in the entire study population. Interesting gender differences were observed, suggesting that correlations regarding quantitative parameters are stronger in a male population, and significant correlations regarding more qualitative parameters, such as FM and EG, are solely present in a female population. Future research in larger study populations will surely provide more insight on this subject. Hopefully, more and more researchers will be inspired to contribute to the promising field of muscle ultrasound.

Authors' contributions

All authors contributed substantially to the development of this manuscript: E. Cassiers, who will take responsibility for the integrity of the data analysis, performed data acquisition, analysis and interpretation, and drafted the manuscript. S. Bastijns, S. Perkisas, M. Vandewoude, and A.M. De Cock contributed to the conception and design of the work, the analysis and interpretation of the data and regularly revised the drafts. All authors agreed to the current version of the manuscript.

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