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Review Article

Muscle Ultrasound and Sarcopenia in Older Individuals: A Clinical Perspective

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ABSTRACT

Introduction: A precise quantitative measurement of skeletal muscle mass is fundamental for diagnosing sarcopenia in older individuals. The current techniques of assessment, including dual-energy x-ray absorptiometry (DXA), bioimpedance analysis (BIA), and magnetic resonance imaging (MRI) are either difficult to perform in everyday clinical practice or biased by concurrent clinical confounders. B-mode muscle ultrasound can be helpful in assessing muscle mass and architecture, and thus possibly useful for diagnosing or screening sarcopenia.
Methods: A literature search of published articles on muscle ultrasound and sarcopenia in older individuals as of July 31, 2016, was made on PubMed and Scopus. Manual search and cross-referencing from reviews and original articles was also performed.
Results: Most of the existing studies were carried out on healthy well-fit subjects, with a low prevalence of sarcopenia. The main parameters that can be assessed through muscle ultrasound are muscle thickness, cross-sectional area, echo intensity, and, for pennate muscles, fascicle length and pennation angle. In older subjects, all these parameters show some degree of alteration compared to young adults, particularly in lower limb muscles with antigravitary function, such as the quadriceps femoris and gastrocnemius medialis. Each of these parameters may be theoretically useful for detecting the loss of muscle mass and functionality in geriatric patients. They are also poorly influenced by the presence of acute and chronic diseases and fluid balance, unlike DXA and BIA, but a high degree of standardization in ultrasound protocols is necessary. Frontier applications of ultrasound in the assessment of sarcopenia may include contrast-enhanced and diaphragm ultrasound.
Conclusions: The current literature does not allow to make conclusive recommendations about the use of muscle ultrasound in geriatric practice. However, this technique is very promising, and further studies should validate its applications in the context of sarcopenia assessment.

Why Muscle Ultrasound in Sarcopenia?

Since the first definition,[1] all experts and consensus groups have considered loss of skeletal muscle mass as the key element for diagnosing sarcopenia in older individuals.[2–6] Although functional parameters, such as gait speed and grip strength, have been emphasized in the most recent definitions,[7] quantitative measures of lean mass still represent an important part of the clinical assessment of sarcopenia in geriatric patients. This is particularly important when considering that skeletal muscle is the second largest store of energy in the body and plays an essential role in glucose homeostasis.

From a clinical perspective, the current definitions of sarcopenia may imply several pitfalls, particularly for quantitative measures of muscle mass. First, muscle mass thresholds considerably vary from one definition to another, making it very difficult to correctly classify patients as normal or sarcopenic.[8] The prevalence of sarcopenia in community-dwelling older individuals also depends on the definition adopted for assessment.[8–11]

Second, the method of muscle mass measurement is not completely standardized. Computed tomography (CT) and, most of all, magnetic resonance (MR) imaging are regarded as the gold standard techniques for measuring body composition, and thus for quantifying

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lean mass, in humans.[12] This concept also applies to the geriatric setting.[13,14] However, these imaging tests are not applicable in everyday clinical practice, since they are expensive and often uncomfortable for patients, and, according to the current healthcare policies, should not be prescribed for measuring muscle mass except in the context of research protocols.[12–14] Thus, alternative methods such as bioimpedance analysis (BIA) and dual-energy x-ray absorptiometry (DXA) seem preferable, although no wide consensus has been reached in favor of one or another test. For example, the International Working Group on Sarcopenia recommends the use of DXA in older subjects with a reduced gait speed,[6] but the European Working Group on Sarcopenia in Older People (EWGSOP) states that both BIA and DXA can be used interchangeably in this setting,[4] being both significantly related to gold standard measures in elderly people.[15–17]

Both of these techniques however imply a certain degree of inaccuracy in clinical practice. DXA measures may differ according to device manufacturer[18] and their degree of concordance with gold standard techniques may depend on age and gender.[19] BIA accuracy strongly relies on the type of equations used for appendicular skeletal mass estimation,[20] which should be validated in older populations,[21] and depends on the hydration status, with fluid overload acting as a strong confounding factor.[22] As such, the assessment of sarcopenia in hospitalized geriatric patients can be particularly difficult, given the limited availability of DXA and the alterations in fluid balance caused by acute disease and limited mobility. Interestingly, Reiss and colleagues recently reported a suboptimal degree of concordance of BIA and DXA when measuring muscle mass in hospitalized patients according to the EWGSOP algorithm, with a significant underestimation of sarcopenia by BIA, especially in overweight subjects.[23]

In this scenario, innovative tools for measuring muscle mass and quality are needed. Skeletal muscle ultrasound is a standardized and accurate imaging technique that has entered clinical practice for the diagnosis and follow-up of neuromuscular disorders.[24] Its main pros include simplicity, low costs, quickness of execution, and availability at bedside.[12] Conversely, specific training of operators and adherence to strict protocols are needed to obtain reliable and reproducible results.[12] Sonographic and MR assessments of muscle mass at different anatomic sites exhibit a high degree of concordance in both experimental animal models[25] and healthy humans.[26–30]

However, the role of skeletal muscle ultrasound for screening and diagnosing sarcopenia in elderly individuals remains speculative. None of the current definitions of sarcopenia includes it in diagnostic algorithms.[4–6] In spite of this, some experts acknowledge the potential usefulness of muscle ultrasound also in this setting,[12,13,31] basing mainly on pioneering studies assessing muscle mass and architecture by this technique on healthy aging subjects.[32–34]

Thus, in the present paper, we review the existing studies on the role of muscle ultrasound for detecting muscle mass loss in older individuals, using a clinically oriented approach in order to highlight possible advantages and pitfalls and areas for future research.

Methods of Literature Search and Results

A literature search of published articles as of July 31, 2016, was made on PubMed and Scopus. “Sarcopenia ultrasound,” “muscle ultrasound geriatric,” “muscle ultrasound elderly,” “muscle echointensity older,” “muscle cross-sectional area older,” “muscle thickness older,” and “pennation angle older” were used as search strings. Articles lacking original data, not in English language, or focused exclusively on young subjects or specific neuromuscular diseases were excluded.

The literature search revealed 44 papers eligible for inclusion, with a wide heterogeneity of aims, outcomes, and settings (healthy active subjects vs outpatients with chronic diseases). Most of the studies were carried out on small groups, thus, despite the scientific relevance, they had limited clinical value. However, because our aim was to provide insights into the possible future applications of muscle ultrasound in geriatrics and gerontology, they were not excluded, but their limitations were critically considered. Therefore, we chose to follow a narrative review approach, identifying 5 areas of interest: “studies of comparison with reference methods,” “regional differences in sarcopenia assessment,” “ultrasonographical evaluation of muscle structure,” “association of muscle ultrasound with functional parameters and clinical outcomes,” and “muscle ultrasound in intervention studies.” Finally, we identified possible pitfalls in the application of muscle ultrasound for diagnosing sarcopenia and provided a brief overview of frontier topics, such as the possible application of contrast-enhanced muscle ultrasound and diaphragm ultrasound in geriatrics.

Muscle Ultrasound Assessment in Older Individuals: State of the Art

Studies of Comparison with Reference Methods

Only 4 studies have compared muscle ultrasound vs DXA in order to measure muscle mass and detect sarcopenia (Table 1).[35–38] Three of them were focused on healthy community-dwelling elderly but also included middle-aged subjects,[35–37] whereas one, although aimed at diagnosing sarcopenia, was carried out exclusively on middle-aged females.[38] None of them focused on hospitalized individuals. All these studies agreed that the sonographic measurement of muscle thickness, obtained either at multiple sites or at the thigh, have a high concordance with DXA-predicted estimates of muscle mass.[35–38] In the largest of these studies,[35] the authors demonstrated that in 77 healthy subjects aged 52 to 78, the product of ultrasound-measured muscle thickness at 4 different sites and lower limb length is independently and strongly related to body fat-free mass (FFM) as calculated by DXA.

Three studies have instead compared muscle ultrasound and BIA for detecting muscle mass loss. Minetto et al performed both techniques in a cohort of 44 healthy older subjects aged on average 82, and compared their results with the values obtained, as a reference standard, in 60 young adults. They observed that the prevalence of low muscle mass in older subjects was highly variable, depending on the adopted BIA-derived cut-points and site of ultrasound measurement, being maximal at the proximal muscles of lower limb.[39] Conversely, Kuyumcu et al found that in a cohort of 100 community-dwelling older subjects, gastrocnemius muscle thickness and fascicle length showed high sensitivity and negative predictive value (approaching 100%) in detecting sarcopenia, diagnosed with BIA and handgrip strength as reference standards.[40] Finally, Seymour et al found a significant correlation between rectus femoris cross-sectional area measured by ultrasound and BIA-derived FFM in a cohort of 30 patients with chronic obstructive pulmonary disease (COPD) aged on average 67 years. However, in this study the correlation coefficient, although statistically significant, was not optimal ($r = 0.43$).[41]

Muscle ultrasound has been compared to CT in only one small study, performed on 45 patients (mean age 68) with coronary artery disease. Rectus femoris thickness measured by ultrasound was significantly correlated with CT-derived muscle volume of the midthigh region, although in a suboptimal way ($r = 0.49$).[42] Ultrasound was compared with CT also in a small subsample (18 subjects) of the Seymour et al study,[41] with rectus femoris cross-sectional area well correlated between the 2 techniques ($r = 0.88$).

Finally, 3 studies compared ultrasonographic assessment of muscle mass with MRI,[43–45] but 2 of them also enrolled adult subjects.[43,45] Sanada and colleagues measured muscle thickness by MRI and ultrasound in different anatomic sites of 72 young Japanese subjects. They demonstrated significant and strong site-matched skeletal

Table 1
Summary of Studies Comparing Muscle Ultrasound Parameters With Reference Techniques of Muscle Mass Measurement in Older Individuals

Reference	Country	Sample Size	Setting/Health Status	Male (%)	Mean Age (y)	Measured Ultrasound Parameters	Results	Correlation Coefficients (Where Available)
Muscle ultrasound vs DXA								
Takai Y et al, 2014[35]	Japan	77	Healthy volunteers	43	65 ± 7	Muscle thickness at 9 body sites (anterior upper arm, posterior upper arm, forearm, abdomen, anterior thigh, posterior thigh, anterior lower leg, posterior lower leg, subscapula)	Muscle thickness–derived equations (Eq 1 and Eq 2) are significantly related to DXA-measured total lean mass	$r^2 = 0.92$ (Eq 1) $r^2 = 0.95$ (Eq 2)
Abe T et al, 2014[36]	Japan	81	Healthy volunteers	51	60 ± 7	Anterior and posterior thigh muscle thickness	Anterior and posterior thigh muscle thickness is significantly related to DXA-measured appendicular lean mass	Anterior: $r = 0.59$ (men), 0.55 (women) Posterior: $r = 0.54$ (men), 0.69 (women)
Berger J et al, 2015[37]	Chile	51	Healthy volunteers	49	73 ± 6	Rectus femoris thickness and echo-intensity	Rectus femoris thickness is significantly related to DXA-measured total appendicular fat-free mass; echo-intensity is inversely related to thickness	r=0.70
Ismail C et al, 2015[38]	United States	20	Healthy female volunteers	0	43 ± 20	Muscle thickness and echo-intensity at 5 body sites (upper trapezius, upper pectoralis major, lateral deltoid, proximal forearm, rectus femoris)	Aggregate muscle thickness is significantly related with DXA-measured lean body mass; echo-intensity is inversely related with grip strength	$r = 0.64$ ($r^2 = 0.87$ in a model accounting for BMI and age)
Muscle ultrasound vs BIA								
Minetto MA et al, 2016[39]	Italy	44	Healthy volunteers	32	82 ± 7	Muscle thickness at 4 body sites (rectus femoris, vastus lateralis, tibialis anterior, medial gastrocnemius)	The prevalence of sarcopenia depends on the site of muscle thickness measurement and on the BIA-derived equations used	—
Kuyumcu ME et al, 2016[40]	Turkey	100	Subjects evaluated in a geriatric outpatient clinic	41	73 ± 6	Gastrocnemius medialis thickness, fascicle length and pennation angle	Muscle thickness and fascicle length, but not pennation angle, were significantly lower in subjects with sarcopenia diagnosed by BIA	$r = 0.59$ (right muscle thickness) $r = 0.52$ (right fascicle length)
Seymour JM et al, 2009[41]	United Kingdom	56	Healthy volunteers and patients with stable COPD	48	66 ± 9	Rectus femoris cross-sectional area	Rectus femoris cross-sectional area is significantly and strongly associated with BIA-derived fat-free mass in both patients with COPD and controls	$r = 0.66$ (healthy) $r = 0.43$ (COPD patients)
Muscle ultrasound vs CT								
Thomaes T et al, 2012[42]	Belgium	45	Patients with stable coronary artery disease	98	68 ± 6	Rectus femoris diameter	Rectus femoris diameter assessed by ultrasound is significantly related with rectus femoris diameter and volume measured by CT	$r = 0.67$ (diameter) $r = 0.49$ (volume)
Seymour JM et al, 2009[41]	United Kingdom	18	Healthy volunteers and patients with stable COPD	–	–	Rectus femoris cross-sectional area	Rectus femoris cross-sectional area measured by ultrasound is strongly associated with the same parameter measured by CT in both healthy volunteers and patients with COPD	$r = 0.88$ (healthy volunteers and COPD patients merged)
Muscle ultrasound vs MRI								
Tandon P et al, 2016[43]	Canada	159	Outpatients with cirrhosis	56	58 ± 10	Thigh muscle thickness	A bivariate model including thigh muscle thickness and BMI is able to predict the MRI-based diagnosis of sarcopenia in a significant way	—
Reeves ND et al, 2004[44]	United Kingdom	6	Healthy older individuals	50	77 ± 3	Right vastus lateralis muscle cross-sectional area	Ultrasound measurement of vastus lateralis cross-sectional area after a 20-min bed rest is strongly correlated to the same parameter measured by MRI	$r = 0.99$

muscle mass measure correlations between the 2 techniques and were able to develop and validate equations estimating the MRI-measured total body muscle mass from sonographic parameters with a high degree of accuracy ($r = 0.98$).[45] Similarly, Tandon et al demonstrated that a bivariate model, including thigh muscle thickness and BMI, was as accurate as MRI in identifying skeletal muscle loss in a large cohort of outpatients with cirrhosis.[43] Finally, in a small group of healthy elderly subjects, Reeves et al. found that muscle cross-sectional area (CSA) evaluated by ultrasound tomography of the human vastus lateralis muscle was highly correlated with CSA measured by MRI. The authors also reported intraclass correlation coefficients of 0.998 for the reliability of ultrasound and 0.999 for the validity of ultrasound against MRI.[44]

Hence, as highlighted in Table 1, most of the studies support the potential utility of muscle ultrasound for identifying sarcopenia in older individuals. However, because they were carried out on small samples and in different clinical settings (ranging from healthy subjects to patients with chronic diseases), no conclusive recommendations can be made about the applicability of muscle ultrasound on a large scale.

Regional Differences in Sarcopenia Assessment

The age-related decline in muscle mass does not proceed in all anatomic regions at the same pace, as confirmed by studies with MRI.[46] This is because sarcopenia is greater for the lower limb than for the upper limb muscles.[47] Thus, in the same patient there may be some muscles that are affected by sarcopenia and others that are not. The pioneering studies on muscle ultrasound by Abe and colleagues have contributed to enhance knowledge on this phenomenon and develop the concept of “regional” or “site-specific sarcopenia.” Namely, in both healthy Japanese and Caucasian adults, an age-related decline in quadriceps femoris and abdominal muscle thickness has been detected.[48,49] In thigh muscles of older individuals, a decline in ultrasound-based measures of CSA was also demonstrated[50] and confirmed by CT findings.[51] Conversely, other anatomic sites, such as upper limbs, were not affected by this phenomenon.[48,49]

These results were confirmed in a population-based cross-sectional study carried out on 1994 Japanese adults, where the prevalence of sarcopenia, estimated by a reduced ultrasound-based thigh muscle thickness, was higher than the one derived from measurements of muscle thickness at multiple sites.[52] Moreover, an age-related decline in thigh anterior/posterior muscle thickness ratio was also detected, possibly indicating that the rectus femoris is involved in muscle mass decline earlier than biceps femoris.[53] Thus,

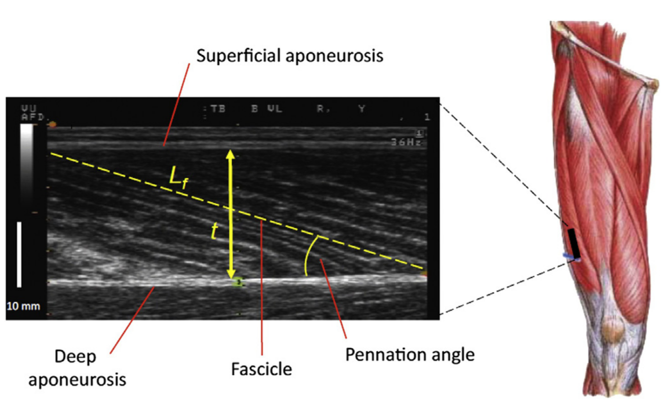

Fig. 1. Schematic representation of ultrasound measurements of gastrocnemius muscle architecture.

this parameter has been proposed as an early biomarker of sarcopenia in older individuals.

Abe and colleagues also developed and validated equations allowing to calculate total lean body mass from multiple ultrasonographic muscle thickness measurements in Japanese[54] and Caucasian subjects.[55] These proposed equations can be useful for diagnosing sarcopenia according to the Baumgartner definition.[1]

However, the relationship between whole-body sarcopenia and site-specific sarcopenia has not been completely understood. In fact, in a small cohort of 81 healthy adults, the same authors demonstrated that ultrasound-derived thigh anterior/posterior muscle thickness ratio is not related to appendicular lean mass measured by DXA.[36] These findings were confirmed on a larger cohort, where appendicular lean mass was calculated with the above-mentioned ultrasound-derived equations.[52] Moreover, the site-specific loss of thigh muscle, diagnosed by ultrasound, showed a poor or incomplete correlation with functional parameters, such as gait speed.[56] In both adult and older subjects, it was instead significantly associated with zigzag walking test performance,[56] which is not part of standard evaluations included in the comprehensive geriatric assessment. Thus, the clinical correlates of site-specific sarcopenia are not well understood. As such, the ultrasonographic evaluation of site-specific sarcopenia needs further clinical investigation and cannot be recommended in clinical geriatric practice.

Ultrasonographic Evaluation of Muscle Structure

Muscle ultrasound can also provide qualitative information about skeletal muscle. This may represent one of the main advantages of this technique over DXA, BIA, CT, and MRI, which primarily give quantitative data.

Fascicle length and pennation angle (ie, the angle formed at the fiber insertions into deep and superficial aponeurosis in pennate muscles such as gastrocnemius medialis) and their changes with passive movements and force load are the main structural parameters that can be measured by ultrasound in skeletal muscles,[57] as shown in Figure 1. Moreover, muscle echogenicity can give important information about the presence of inflammation, fibrosis, and adipose infiltration.[58]

Narici and colleagues demonstrated that ageing is associated with significant changes in gastrocnemius medialis ultrasonographic structure, with reduced fascicle length and pennation angle.[33] These alterations can result in a decrease of physiological cross-sectional area (PCSA; ie, muscle volume divided by fascicle length), a parameter that is better related to muscle function than anatomic cross-sectional area measured by CT or MRI.[33] Moreover, the pennation angle and PCSA, but not the fascicle length, of the lateral head of gastrocnemius muscle of a group of healthy elderly people were decreased compared to young controls.[34] These results allowed to conclude that the loss of muscle strength that occurs with aging is mostly explained by a decrease in intrinsic muscle force (ie, force/PCSA).[34] However, wider ultrasound studies showed that the total PCSA of the whole triceps surae muscle group is maintained with aging in healthy subjects, because the soleus PCSA is not different between young and older volunteers.[59] Although this may appear as a paradox, the reason why PCSA tends to remain constant with aging is simply that the decrease in muscle volume is similar to that of fascicle length; thus, their ratio (PCSA) tends to remain the same.

Other studies comparing muscle architecture, assessed by ultrasound, between sarcopenic vs nonsarcopenic healthy subjects raised some doubts about the clinical application of parameters such as fascicle length and pennation angle. For example, in a study carried out on 100 elderly community-dwellers, pennation angle of the gastrocnemius muscle was unaffected by the sarcopenic status, while a direct relationship between fascicle length and muscle thickness was detected.[40] Moreover, Strasser et al found that in a group of 26 healthy older subjects, pennation angle of quadriceps femoris was not significantly related with functional muscle parameters, although resulting significantly lower than that of a group of 26 matched young controls.[60] The authors however highlighted the low inter- and intraoperator reproducibility of pennation angle measurement, supporting instead the clinical value of measuring muscle thickness.[60]

In fact, the measurement of fascicle length and pennation angle of pennate muscles by ultrasound needs standardized protocols of assessment and trained operators. When these conditions are satisfied, a good reproducibility exists. This is confirmed by studies comparing ultrasound vs anatomic dissection in cadavers[61] and repeated in vivo measures, showing an intraclass coefficient (ICC) of 0.99.[62] Pennation angle and fascicle length in gastrocnemius medialis are strongly dependent on the ankle joint angle and degree of isometric voluntary contraction present during ultrasonographic assessment.[61] Similar results have recently been obtained also for the vastus intermedius and vastus lateralis muscles.[63] Thus, fascicle length and pennation angle should be measured at rest in a fixed standardized position. Otherwise, results of measurements should be corrected for degree of isometric contraction and joint angles.

Muscle echogenicity has been measured only in few studies carried out on elderly subjects. However, increase in intermuscular adipose tissue (IMAT), also called "myosteatosis," and intramyocellular lipid droplets has been demonstrated as an important histopathologic background for both age-related sarcopenia and cancer-related cachexia.[64–67] These rearrangements can result in an increased muscle echogenicity, similarly to what has been demonstrated in myopathies.[68]

Fukumoto et al demonstrated that quadriceps femoris echogenicity of a group of 92 Japanese healthy women (age range 51-87) was positively associated with age and negatively with muscle thickness and knee extensor isometric strength. No association was detected with BIA-derived estimates of total body fat.[69]

The same authors also demonstrated that in a larger cohort of healthy males, the echogenicity of the anterior muscular compartment of the thigh is related to muscle strength, irrespective of a series of covariates including age and body composition.[70] These results were confirmed by Ismail et al in a small group of healthy adult females.[38] Strasser et al found that muscle echogenicity is significantly higher in old sarcopenic than in young subjects, although they demonstrated a poor correlation with other ultrasound measures and functional parameters, possibly due to low inter- and intrarater reproducibility.[60] Similar results have also been obtained by Berger and colleagues in a group of Chilean healthy volunteers.[37] Finally, patients with COPD generally have a higher muscle echo-intensity than healthy age-matched controls, even if quantitative measures of muscle mass are not different.[71]

In summary, although representing a promising and potentially effective technique, the ultrasonographic measurement of muscle architectural parameters, including pennation angle, fascicle length, and echogenicity, still needs further assessment and validation to be included in standard imaging algorithms for the evaluation of sarcopenia in older subjects.

Association of Muscle Ultrasound with Functional Parameters and Clinical Outcomes

Surprisingly, the functional correlates of muscle ultrasound parameters have been poorly investigated by the existing medical literature, notwithstanding that in vivo ultrasound measures of muscle architecture have been shown to be key determinants of muscle force, velocity, and power output.[57,72] In fact, age-related muscle mass decline is a major determinant of muscle weakness, reduced endurance capacity, and insulin resistance in older individuals.[73] Inter- and intraindividual variations of muscle mass are good predictors of changes in muscle function.[74] However, muscle strength declines much more rapidly than muscle mass, suggesting age-related alterations of muscle quality and architecture.[74,75] These alterations have a strong negative impact on global functional performance of patients and are involved in the onset of physical disability, whereas the association between muscle mass and functional outcomes is still unclear.[76]

The evidence from cross-sectional studies supporting an association between ultrasonographic measures, such as muscle thickness and echo-intensity, and functional parameters, such as muscle strength, is summarized in Table 2. Notably, no association between muscle ultrasound and gait speed, the cornerstone parameter for functional assessment in geriatric patients, has been demonstrated so far.[77] This may not be surprising because most of the mechanical work during locomotion is not performed by the muscle itself, which basically maintains an isometric contraction, but by the tendon, which is stretched and recoils storing and releasing elastic energy used for forward propulsion.[78,79] Gait performance and balance indeed depend on a close interaction between muscle and tendon and, when both are accounted for, a close correlation exists between muscle-tendon characteristics, gait, and balance performance.[79,80]

Although gait speed is poorly correlated with muscle architecture, 100-m sprint performance is significantly correlated with fascicle length[81] because longer fascicles have a greater number of sarcomeres in series and thus a higher shortening velocity.[82]

A significant association between forearm muscle thickness and handgrip strength, another important functional parameter, was also demonstrated in both young and elderly volunteers.[77,83] Further studies are needed to evaluate these possible associations on larger cohorts.

The ability of muscle ultrasound parameters to prospectively predict clinical outcomes for adult and elderly patients has been, if possible, even less investigated. Ido and colleagues recently demonstrated that sonographic measures of abdominal muscle thickness predicted metabolic syndrome risk more accurately than DEXA-derived estimations of skeletal muscle mass in a small group of obese adults.[84] Moreover, the ultrasonographic measurement of rectus femoris cross-sectional area was demonstrated as an independent predictor of hospital length of stay, mortality, and nursing home discharge in a group of adults admitted in a surgical intensive care unit.[85] Similarly, a reduced rectus femoris cross-sectional area was significantly associated with mortality, length of stay, and readmissions in a cohort of 191 older patients admitted to hospital with COPD exacerbation.[86] Despite these reports, the relevance of muscle ultrasound measures in terms of clinical outcomes needs further investigation.

Muscle Ultrasound in Intervention Studies

Ultrasound parameters of muscle mass and structure have been used to monitor the physiological response to long-term bed rest and exercise in several studies. In young volunteers, significant decreases in gastrocnemius medialis and vastus lateralis muscle thickness, fascicle length, and pennation angle have been documented after 5 weeks of forced horizontal bed rest[87] and after 14 days of unilateral lower limb suspension.[88] Conversely, no structural changes have been detected by ultrasound in non-antigravity muscles such as tibialis anterior and biceps brachii after prolonged bed rest.[87] However, all alterations of ultrasonographic parameters induced by inactivity in young volunteers can be completely restored after resistance training intervention.[89]

Muscle ultrasound can also represent a valid tool for assessing ultrastructural changes that occur in muscle architecture with physical exercise. This application is particularly useful in older persons,

Table 2
Summary of the Associations Between Muscle Ultrasound and Parameters of Muscle Function Demonstrated in Cross-Sectional Studies Carried Out on Older Individuals

Ultrasound Parameter	Clinical/Functional Parameter	Correlation Coefficient	Reference	Setting	Number of Subjects	Mean Age
Rectus femoris diameter	Maximal voluntary muscle strength of hamstrings and quadriceps (a) Isometric quadriceps strength (b)	$r = 0.61$ (a) $r = 0.52$ (b)	Thomaes T et al, 2012[42]	Older outpatients with coronary artery disease	45	68 ± 6
Quadriceps muscle thickness	Isometric maximum voluntary contraction force of quadriceps muscle	$r = 0.88$ (young) $r = 0.83$ (old), rectus femoris	Strasser EM et al, 2013[60]	Outpatients from a physical medicine and rehabilitation clinic	26 young 26 old	24 ± 3 (young) 68 ± 5 (old)
Forearm-ulna muscle thickness	Handgrip strength	$r = 0.52$ (men) $r = 0.47$ (women)	Abe T et al, 2014[77]	Healthy volunteers	53	70–83
Rectus femoris cross-sectional area	Maximum voluntary quadriceps strength	$r = 0.69$	Seymour JM et al, 2009[41]	Outpatients with COPD	30	67 ± 9
Anterior/posterior thigh muscle thickness ratio	Zigzag walking time	$r = -0.29$	Abe T et al, 2012[56]	Healthy volunteers	53	52–83
Anterior/posterior thigh muscle thickness ratio	Zigzag walking time	$r = -0.36$ (men) $r = -0.30$ (women)	Abe T et al, 2014[36]	Healthy volunteers	81	50–74
Echo-intensity at different sites	Handgrip strength	$r = -0.67$	Ismail C et al, 2015[38]	Healthy volunteers	20	43 ± 21
Echo-intensity of the anterior compartment of the right thigh	Maximum isometric torque of knee extension	$r = -0.33$	Watanabe Y et al, 2013[70]	Elderly community dwellers	184	74 ± 6
Echo-intensity of quadriceps femoris	Knee extensor isometric strength	$r = -0.40$	Fukumoto Y et al, 2012[69]	Female community dwellers	92	70 ± 6

where exercise is considered a cornerstone prescription for the prevention of sarcopenia and mobility disability.[90] In a small randomized controlled trial carried out on 19 elderly male volunteers, Morse et al demonstrated that a 12-month physical training program resulted in a significant increase in gastrocnemius lateralis PCSA and pennation angle but not fascicle length.[91] These changes were also associated with significant improvement in functional parameters, such as muscle strength.[91] In another trial, 19 healthy elderly subjects were randomized to receive eccentric-only resistance training vs conventional resistance training, encompassing both concentric and eccentric exercises. The authors demonstrated that vastus lateralis muscle thickness and fascicle length increased in both groups, whereas pennation angle increased only in the conventionally trained group.[92] Similar results were obtained in older individuals after a 12-week intervention consisting of regular alpine skiing training.[93] Moreover, in a small group of patients with COPD, an 8-week bilateral high-intensity isokinetic knee extensor resistance training program resulted in significant improvement in ultrasonographic measures of rectus femoris cross-sectional area.[94]

Muscle ultrasound has been used to evaluate ultrastructural muscle changes after a pharmacologic treatment in only 1 study. Atkinson et al in fact demonstrated that, in a group of elderly pre-frail and frail males with borderline to low testosterone levels, a 6-month treatment with 50 mg transdermal testosterone resulted in preservation of gastrocnemius medialis muscle thickness, compared with control group where this parameter significantly declined. No effect of testosterone on pennation angle and fascicle length was detected.[95]

Finally, muscle ultrasound has been used to monitor the effects of neuromuscular electrical stimulation on muscle mass in one high-quality randomized double-blind placebo-controlled trial including 52 patients with COPD aged in average 70 years, demonstrating a significant improvement in the intervention group.[96]

These evidences support the possible utility of muscle ultrasound in clinical geriatric practice for monitoring both the deleterious effects of mobility limitations and the effectiveness of prescriptions, including physical exercise, diet, and medications, aimed at preventing the decline of muscle mass and functionality. However, further studies, carried out on larger groups and including also subjects with various degrees of frailty and disability, are needed before a wider application of muscle ultrasound can be recommended.

Muscle Ultrasound in Geriatrics: Pitfalls and Frontier Applications

Methodological Issues

The analysis of current medical literature highlights the absence of a standardized method of performing muscle ultrasonography in clinical practice. The main points of variance from one study to another regard the type of ultrasonographic probe employed, the anatomic sites of measurement, the posture patients should maintain during examinations, the position of the probe, and the type of parameters obtained.

Although in most studies muscle ultrasonography is performed with linear array probes, some authors recommend the utilization of convex probes also, especially for measuring muscle cross-sectional area.[32] For example, Hammond et al recently demonstrated that the use of curved-array probes for measuring rectus femoris CSA is equivalent to the use of linear probes.[97] Moreover, when linear-array probes were used, there were differences in the applied ultrasound frequencies, ranging from 5 MHz in the studies by Abe and colleagues[36] to 7.25 MHz in other studies.[60] More recently, the introduction of extended field-of-view ultrasound has been shown to enable the measurement of muscle CSA reliably, with a strong correlation with MRI.[98] This new technological advancement in ultrasound

Table 3
Summary of Parameters That Can Be Assessed by Muscle Ultrasound in Older Individuals, With Main Strengths and Limitations

Parameter	Anatomic Site of Assessment	Strengths	Limitations
Muscle thickness	Every muscular compartment (most studies on anterior and posterior thigh)	Simple and quick Reproducible Correlated with gold standard measures of muscle mass and muscle function	Estimations of total muscle mass need multiple measurements and complex population-specific equations Fixed anatomic landmarks needed Results depend on pressure applied with the probe Site-specific sarcopenia may bias results
Muscle cross-sectional area	Lower limb muscular compartments	Simple and quick Reproducible Correlated with gold standard measures of muscle mass Both linear- and curved-array probes can be used	Technique of assessment not completely standardized Fixed anatomic landmarks needed Correlation with functional parameters still unclear
Pennation angle	Pennate muscles of the lower limb (gastrocnemius medialis)	Gives information about muscle structure and strength generation capacity	Specific training of operators needed Requires collaboration from the patient Results are influenced by articular position and degree of muscle contraction Correlation with functional parameters still unclear
Fascicle length	Pennate muscles of the lower limb (gastrocnemius medialis)	Gives information about muscle structure and strength generation capacity	Specific training of operators needed Requires collaboration from the patient Results are influenced by articular position and degree of muscle contraction Correlation with functional parameters still unclear
Echo-intensity	Every muscular compartment (most studies on anterior and posterior thigh)	Gives information about myosteatosis Correlated with measures of muscle function	Specific training of operators needed Results depending on the type of software used to interpret images Technique of assessment not completely standardized
Contrast-enhanced assessment of vascularization	Quadriceps femoris	Identifies alterations in vascularization that may contribute to the onset of sarcopenia	Requires a high level of expertise Technique of assessment not completely standardized Studies focused on elderly subjects are lacking
Diaphragm thickness at tidal volume and total lung capacity and their ratio (ΔTdi)	Diaphragm	Simple and quick Reproducible Correlated with measures of respiratory function	Technique never been studied in geriatric patients with sarcopenia

imaging seems thus very promising for the assessment of muscle CSA in future clinical studies.

Most studies have measured ultrasonographic muscle parameters in lower limbs, since the effects of sarcopenia are generally more evident in antigravitary muscle groups[32,47] and sarcopenia of the lower limb muscles is expected to have a greater impact on mobility. Some of these studies focused on the gastrocnemius muscle because its pennate architecture makes it the ideal site of assessment of fascicle length and pennation angle.[34,59–61,91–93] Others have instead measured exclusively the anterior and/or posterior muscular compartment of the thigh.[35,37–42] Moreover, in their studies, Abe and colleagues have performed repeated measures at multiple anatomic sites, including also abdomen and upper limbs.[36,48–50,52–56]

The posture of patients during examinations is another relevant issue. Although in most studies measures were generally taken with the patient lying supine for the anterior thigh and prone for the posterior thigh and gastrocnemius evaluation, Abe and colleagues generally performed their measures with the patient standing in a rest position.[36,48–50,52–56] The need of the patient's collaboration to maintain a fixed posture during examination may represent a relevant limitation to the applicability of muscle ultrasound in clinical practice, especially for subjects with mobility-disability or cognitive impairment.

Ultrasound measures can also be significantly influenced by the position, pressure, and inclination of the probe.[32] For each anatomic site of measurement, fixed anatomic landmarks should be considered to allow reproducibility. For example, Narici and colleagues have identified 3 points of ultrasonographic assessment of the gastrocnemius muscle, respectively named proximal, central, and distal.[61] The inclination of the probe should be perpendicular to the skin, and the accuracy of this position is fundamental to obtain reliable and reproducible results, especially for measurements of fascicle length, pennation angle, and echo-intensity.[60] Tandon et al have instead measured the difference in muscle ultrasound thickness before and after applying a maximal pressure on the ultrasound probe.[43] Also, the length of the linear probe affects the accuracy of measurement of muscle architecture. Most commercial ultrasounds have linear probes of length 4–5 cm. However, some ultrasound machines are fitted with longer probes 6–10 cm in length. Such probes enable the visualization of entire fascicles in one image and thus afford greater accuracy for fascicle length measurement.

Finally, analysis of the existing literature highlights that 5 different parameters can be obtained by muscle ultrasound for assessment of sarcopenia in older individuals: muscle thickness, cross-sectional area, fascicle length, pennation angle, and echo-intensity (Table 3). However, in none of the available studies were all these parameters assessed together in the same individuals. Thus, further evidence is needed to assess the specific clinical value and applicability of each of them. Muscle thickness, however, seems the simplest and most easily reproducible of these measures, which nowadays can even be assessed automatically by specific softwares with a high degree of accuracy.[99] Conversely, echo-intensity assessment is the most troublesome measure and needs specific training to avoid low reproducibility.[37,60]

Ultrasound Muscle Vascularization Assessment

Reduced muscle blood flow due to microvascular damage and impaired nitric oxide production is generally regarded as one of the main physiopathologic mechanisms of sarcopenia.[100,101] However, the muscle vascularization and blood flow following metabolic stimuli has been poorly studied in older individuals, because of the absence of noninvasive methods of assessment. In a recent study, Mitchell et al reported that Sonovue contrast-enhanced muscle ultrasound was able to detect impaired circulatory responses to an oral feeding stimulus in older individuals, possibly reflecting lower muscle glucose disposal and thus suboptimal protein anabolism.[102] The utility of this technique in clinical practice should be verified by other studies, but contrast-enhanced ultrasonography represents a promising frontier method of assessing sarcopenia.

Diaphragm Ultrasound and Sarcopenia

Several studies carried out on mouse models have demonstrated that aging is associated with structural and functional changes in the diaphragm muscle as well. In particular, a reduction in fiber cross-sectional area and muscle strength and a rearrangement in fiber types and motor unit clustering have been documented.[103–106] However, the molecular and physiological mechanisms of sarcopenia in the diaphragm muscle of murine models of chronic heart failure consistently diverge from the mechanisms of skeletal muscle sarcopenia.[107] These differences have been postulated in humans also.[108]

An age-related reduction of diaphragm muscle strength is a well-known phenomenon in healthy volunteers,[109,110] that correlates with functional parameters such as exercise tolerance.[111] From a functional point of view, diaphragm sarcopenia does not affect physiologic ventilator behaviors, but mainly results in insufficient non-ventilatory behaviors, with impaired ability to perform expulsive airway clearance maneuvers.[112]

B-mode diaphragm ultrasound has been developed and validated for clinical use mainly for patients with severe respiratory failure undergoing invasive mechanical ventilation in intensive care units.[113] In particular, the measurement of diaphragm thickness at tidal volume or total lung capacity and their ratio (ΔTdi) can be considered reliable predictors of diaphragm atrophy during mechanical ventilation, and thus may be used as a guide for correct extubation timing.[114–116]

Diaphragm ultrasound has been poorly studied outside the intensive care setting. Diaphragm thickness and ΔTdi correlated with clinical variables and pulmonary function tests in patients with amyotrophic lateral sclerosis,[117] but not in patients with COPD.[118] In a group of older women with normal spirometry, ultrasound-based measures of diaphragm thickness improved after an 8-week period of moderate intensity inspiratory muscle training protocols.[119]

This technique is simple, quick, rapidly available, well-standardized and highly reproducible irrespective of the level of expertise.[120,121] Reference values of diaphragm thickness for normal subjects have also been established.[122] This makes diaphragm ultrasound a promising technique for clinical and research evaluation of sarcopenic elderly subjects.

Conclusions

Simple, reproducible, and cost-effective techniques are needed for an early diagnosis of sarcopenia in older patients in everyday clinical practice. Muscle ultrasound has a potential of becoming a reference method for this evaluation. However, the current body of knowledge derived from studies performed on healthy volunteers needs to be transferred to the "real life" of frail geriatric patients with reduced functional performance and high burden of multimorbidity. The measurement of ultrasound parameters, highlighted in Table 3, also needs to be standardized before the use of this diagnostic technique can be recommended outside the field of clinical research. Thus, future clinical studies should fill the existing gap between bench and bedside.

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