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# Quantitative assessment of volumetric muscle loss: Dual-energy X-ray absorptiometry and ultrasonography

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**Abstract**

The generalized skeletal muscle disorder that involves (in elderly subjects) the progressive loss of muscle mass and function has been defined sarcopenia, whereas the rapid-onset (traumatic or surgical) and focal (unilateral) loss of skeletal muscle with resultant functional impairment has been defined volumetric muscle loss. Different tools and approaches are commonly used in the clinical settings to quantify the loss of muscle or lean mass and to assess the consequent motor impairment. This review describes the technical principles and provides a summary of the main parameters that can be obtained to assess lean mass (and its distribution) or muscle size (and its structure) through the two imaging techniques most easily accessible and therefore frequently adopted in the clinical practice: dual-energy X-ray absorptiometry and muscle ultrasonography.

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**Keywords**

Lean mass, Muscle echo intensity, Muscle thickness, Sarcopenia, Volumetric muscle loss.

## Introduction

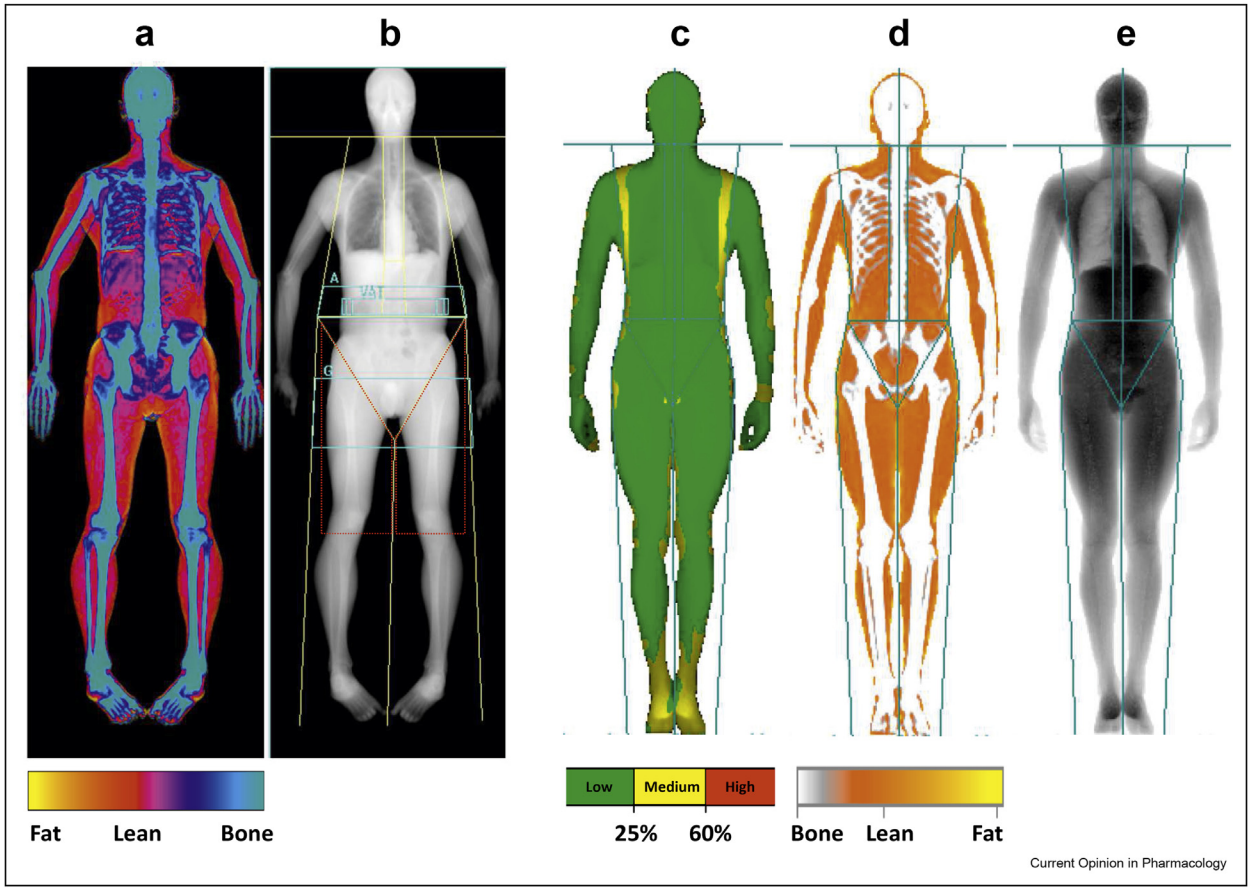
Skeletal muscles can be regarded as the largest organ of the human body, accounting for approximately 20–40% of the total body weight in women and 30–50% in men, exhibiting major metabolic activity by contributing up to 40% of the resting metabolic rate in adults, and serving as the largest body protein pool. The two main components of skeletal muscles are represented by muscle fibers and connective tissue. Individual muscle fibers are grouped together in bundles, which are commonly known as fascicles, and several fascicles join together to form an individual muscle. Muscle fibers are highly adaptable cells, responding to numerous environmental, physiological stimuli (e.g., mechanical loading, nutrient availability, disuse, and inactivity), and pathological challenges (e.g., neuromuscular disorders, drugs, aging) by changing their phenotypic profile in terms of size, composition, biochemical, and metabolic properties [1–3]. The generalized (although not uniform among muscles) skeletal muscle disorder that involves in elderly subjects the progressive loss of muscle mass and function has been defined sarcopenia [4, 5**, 6**, 7], whereas the rapid-onset (traumatic or surgical) and focal (unilateral) loss of skeletal muscle with resultant functional impairment has been defined volumetric muscle loss (VML) [8,9]. The latter disorder represents an emerging focus area among orthopedic and rehabilitation medicine fields [10,11]. Although no standardized evaluation protocol exists for the characterization and quantification of VML, and no guidelines have been produced for a disease-specific evaluation of muscle impairment, the following assessment tools are commonly used in the clinical settings: clinical photographs and video recordings, range of motion measurements, manual muscle strength testing, and isometric or isokinetic muscle function testing [8]. Moreover, the whole spectrum of radiological imaging modalities can also be useful in documenting VML: dual-energy X-ray absorptiometry (DXA), ultrasonography (US), computed tomography, and magnetic resonance.

The aim of this narrative review is to describe the technical principles and provide a summary of the main parameters that can be obtained to assess lean and muscle mass through the two imaging techniques most easily accessible and therefore frequently adopted in the clinical practice: DXA and US.

## Dual-energy X-ray absorptiometry

DXA is a two-dimensional technique originally developed to evaluate bone mineral content (BMC) and bone mineral density. In recent years, it gained popularity also as an accurate and reliable method to investigate whole body and regional soft tissue composition [12, 13, 14**, 15*]. The technique is based on the principle that X-rays of different energies are differentially attenuated when passing through different tissues: the beam of X-rays is attenuated in proportion to the composition and thickness of the material. The X-ray attenuations enable to estimate the mass of the different compartments, given simple algebra and the physical properties of the compartment materials. By radiating the body in the anterior–posterior direction and assuming a two-compartment model in each measurement point (pixel), a two-dimensional (coronal) projection of the body can be reconstructed (representative images of body scans obtained with different instruments are shown in Figure 1). The model assumes that pixels containing bone (that represent approximately 30–40% of the pixels of the projected body) depend on BMC and soft tissue ratio, while pixels not containing bone depend on fat mass (FM) and lean mass (LM) ratio. In other words, DXA 'simplifies' the body into two compartments: bone and soft tissue. The attenuation in the soft tissue can be resolved further to yield FM and LM, as fat and lean tissues have different attenuation characteristics. In fact, DXA manufacturers use calibration phantoms made of biologically equivalent materials that have known compositions to derive equations that predict BMC, FM, and LM from measured pixel attenuation values. Taken together, DXA provides an estimate of the composition of three body compartments: bone, fat, and lean.

**Figure 1**




Dual-energy X-ray absorptiometry images of body composition for two representative subjects (subject 1: panels A–B; subject 2: panels: C-D-E) studied with different instruments. Color image maps show in panels A and D the relative distribution of fat (yellow in both panels), lean tissue (orange and red in panel A, orange in panel D), and bone (blue in panel A and white in panel D), whereas the relative distribution of fat (low, medium, high) in shown in panel C. Crystal image maps (panels B and E) show the cut lines (yellow in panel B and blue in panel E) used to distinguish the standard regions of interest considered for body composition assessment (upper limbs, lower limbs, trunk). Panel B also highlights the gynoid (G)/android (A) areas and the visceral adipose tissue (VAT) slice (light blue regions). Dotted red lines in panel B delimit the thigh region of interest used to quantify the thigh lean mass (right thigh, 5.0 kg; left thigh, 4.9 kg).

A limitation of this technique is represented by the assumption of uniform and constant (73% of body water) LM hydration, which is not always true as hydration varies with age, gender, physical activity, and health status. Other limitations of the technique are that DXA does not assess the LM quality (i.e., the extent of fat infiltration of skeletal muscles, which is also known as myosteatosis) and that single body regions (i.e., individual muscles) cannot be assessed separately. Systematic variation in the absolute estimates of LM and FM can also arise from different hardware and software accommodations to several factors, including treatment of pixels for which a small portion is bone and interpolations for soft tissues located over bone. Briefly, the soft tissue composition is assumed to be comparable between soft tissues over bones and bone-free tissues: the amount of LM and FM in soft tissues over bones is therefore based on neighboring pixels not containing bone.

Total body LM and appendicular lean mass ([ALM]: the lean mass of the upper and lower limbs) are the main parameters obtained through DXA to assess LM. The absolute values of LM and ALM can be normalized to height$^2$ (or to the body mass index) to account for allometric differences in body size, thus obtaining the lean mass index (LMI: kg/m$^2$) or the appendicular lean mass index (ALMI: kg/m$^2$) that enable the comparisons among different subjects independently of their body size [16]. Different cut-off points have been proposed to discriminate between normal and low LM: (i) Baumgartner et al. [17] identified the cut-off points for low LM (i.e., values two standard deviations below the sex-specific means observed in Caucasian young adults) as ALMI $<$7.26 kg/m$^2$ in men and $<$5.45 kg/m$^2$ in women; (ii) Cawthon at al. [18] more recently proposed the cut-off points for low LM (i.e., values discriminating between normal and weak elderly subjects of different ethnic groups) as ALM $<$19.75 kg (or ALM normalized to BMI $<$0.789) in men and $<$15.02 kg (or ALM normalized to BMI $<$0.512) in women; (iii) the last revision of the European Working Group on Sarcopenia in Older People [5**] criteria provided the following recommendations for the cut-off points of different parameters: ALM $<$20 kg in men and $<$15 kg in women; ALMI $<$7.0 kg/m$^2$ in men and $<$5.5 kg/m$^2$ in women; (iv) the 2019 consensus of the Asian Working Group for Sarcopenia [19] provided the following recommendations for the cut-off points: ALMI $<$7.0 kg/m$^2$ in men and $<$5.4 kg/m$^2$ in women; (v) Walowski et al. [20] recently identified ethnic-specific (i.e., Caucasian) body mass index–dependent cut-off points for low LMI (only the cut-off points for normal-weight and overweight subjects are reported as follows: $<$15.6 kg/m$^2$ and $<$13.6 kg/m$^2$ in normal-weight men and women, respectively; $<$19.0 kg/m$^2$ and $<$15.8 kg/m$^2$ in overweight men and women, respectively). These authors also found discrepancies among the published reference values for different (i.e., obtained using different methods and reference populations) parameters of lean or muscle mass [20]. Consistently, it has previously been observed that the prevalence of sarcopenia is highly dependent on the applied diagnostic criteria [21–23]; (vi) Suetta et al. [24] identified ethnic-specific (i.e., Caucasian) cut-off points for low LM (i.e., values two standard deviations below the sex-specific means observed in Caucasian young adults) as LMI $<$14.58 kg/m$^2$ in men and $<$12.14 kg/m$^2$ in women and ALMI $<$6.60 kg/m$^2$ in men and $<$5.03 kg/m$^2$ in women. Given that the cut-off thresholds derived in this study differed from earlier reference data, the authors underlined the importance of obtaining updated and local reference materials [24].

Given that the ALM has a high muscle content that constitutes a large fraction of the total body skeletal muscle mass (TBSMM), different prediction equations were developed for children (aged $>$ 5 years and Tanner stage $\leq$4) [25] and adults (through calibration analyses performed between DXA-derived values of ALM and CT- or MRI-derived values of TBSMM) [26–28] to estimate the TBSMM value from the DXA-derived ALM value (Table 1). The authors of all these prediction equations suggested that they should prove useful in assessing the skeletal muscle compartment *in vivo* and that the relatively simple models (models 1 and 2 in Table 1) should prove practical to apply in the clinical setting [28]. Which of these equations is the most accurate and precise for the estimation of TBSMM remains to be established in further investigations. In addition to the above-mentioned studies, a large number of other comparative studies [29–33] were conducted between DXA and MRI/CT and reported good to strong correlations not only for whole-body scans but also for regional scans. For example, Hansen et al. [30] showed a good correlation between DXA-derived midthigh LM (estimated for a 1.3-cm midthigh region of interest) and CT-derived midthigh cross-sectional area (determination coefficient of 0.73). More recently, Tavoian et al. [32] showed in a group of young athletes a strong correlation (correlation coefficient of 0.89) between DXA-derived thigh LM and five-slice MRI-derived thigh muscle volume. Cameron et al. [33] also showed a good correlation between DXA-derived thigh LM and single-slice MRI-derived thigh muscle mass (determination coefficient of 0.86). However, it is worth mentioning that DXA values of LM and FM relate to the 'molecular' level of body composition, whereas MRI and CT measures relate to the 'tissue' level [34]: therefore, LM and FM measured by DXA are somewhat higher and lower, respectively, than the corresponding values measured by cross-sectional imaging techniques [35]. Besides its accuracy for the whole-body and regional body composition assessment, other strengths of DXA are represented by the high reproducibility and widespread availability.

**Table 1**

**Prediction equations to estimate the total body skeletal muscle mass (TBSMM: kg) from the DXA-derived appendicular lean mass (ALM: kg) [25–28] and from the ultrasound-derived muscle thickness [45].**

| Prediction equations for children and adolescents | Reference |
|---|---|
| TBSMM = (1.115 * ALM) − 1.135 | [25] model 1 |
| TBSMM = (1.003 * ALM) + (0.039 * WEIGHT) − 1.315 | [25] model 2 |
| TBSMM = (0.483 * ALM) + (0.042 * WEIGHT) − (0.015 * HEIGHT) + (0.003 * ALM * HEIGHT) + 1.734 | [25] model 3 |
| **Prediction equation for adults** | **Reference** |
| TBSMM = 1.33 * ALM | [26] |
| TBSMM = (1.19 * ALM) − 1.0 | [27] model 1 |
| TBSMM = (1.17 * ALM) − (0.02 * AGE) + 0.35 | [27] model 2 |
| TBSMM = (1.13 * ALM) − (0.02 * AGE) + (0.61 * SEX) + 0.97 | [27] model 3 |
| TBSMM = (1.19 * ALM) − 1.65 | [28] model 1 |
| TBSMM = (1.18 * ALM) − (0.03 * AGE) - 0.14 | [28] model 2 |
| TBSMM = $10^{(0.0115 \cdot ALM)+(-0.0034 \cdot AGE)+(0.0001 \cdot AGE \cdot ALM)+(-01866 \cdot SEX)+(0.0063 \cdot SEX \cdot ALM)+(0.0007 \cdot SEX \cdot AGE)+RACE+(RACE' \cdot ALM)+1.1932}$ | [28] model 3 |
| TBSMM (men) = 0.641 * 9 MT * HEIGHT − 12.087 | [45] |
| TBSMM (women) = 0.594 * 9 MT * HEIGHT − 11.320 | |

Equation coefficients. SEX, female = 0; male = 1; RACE, African American = 0.0350; Asian = –0.468; Caucasian = 0; RACE', African American = –0.0015; Asian = 0.0011; Caucasian = 0.
9 MT (cm): sum of the muscle thickness for nine sites (lateral forearm, anterior and posterior upper arm, abdomen, subscapula, anterior and posterior thigh, anterior and posterior leg) of the right side of the body. HEIGHT (m); TBSMM, total body skeletal muscle mass.

On the basis of its strengths and despite the previously discussed weaknesses and limitations, DXA must be considered the reference technique [16,36] for assessing body composition (i.e. the amount and distribution of LM and FM) in both clinical practice and research studies performed in healthy subjects (e.g., athletes) and in musculoskeletal disorders (sarcopenia, sarcopenic obesity, disuse atrophy). Moreover, DXA can also be used in patients with VML to assess the differences between the affected and unaffected side. Panel B of Figure 1 shows an example of segmental (right and left thighs) assessment of LM in one representative healthy subject showing comparable LM values between the two thighs: similarly, LM asymmetry can be quantified and longitudinally assessed also in patients with VML.

## Muscle ultrasonography

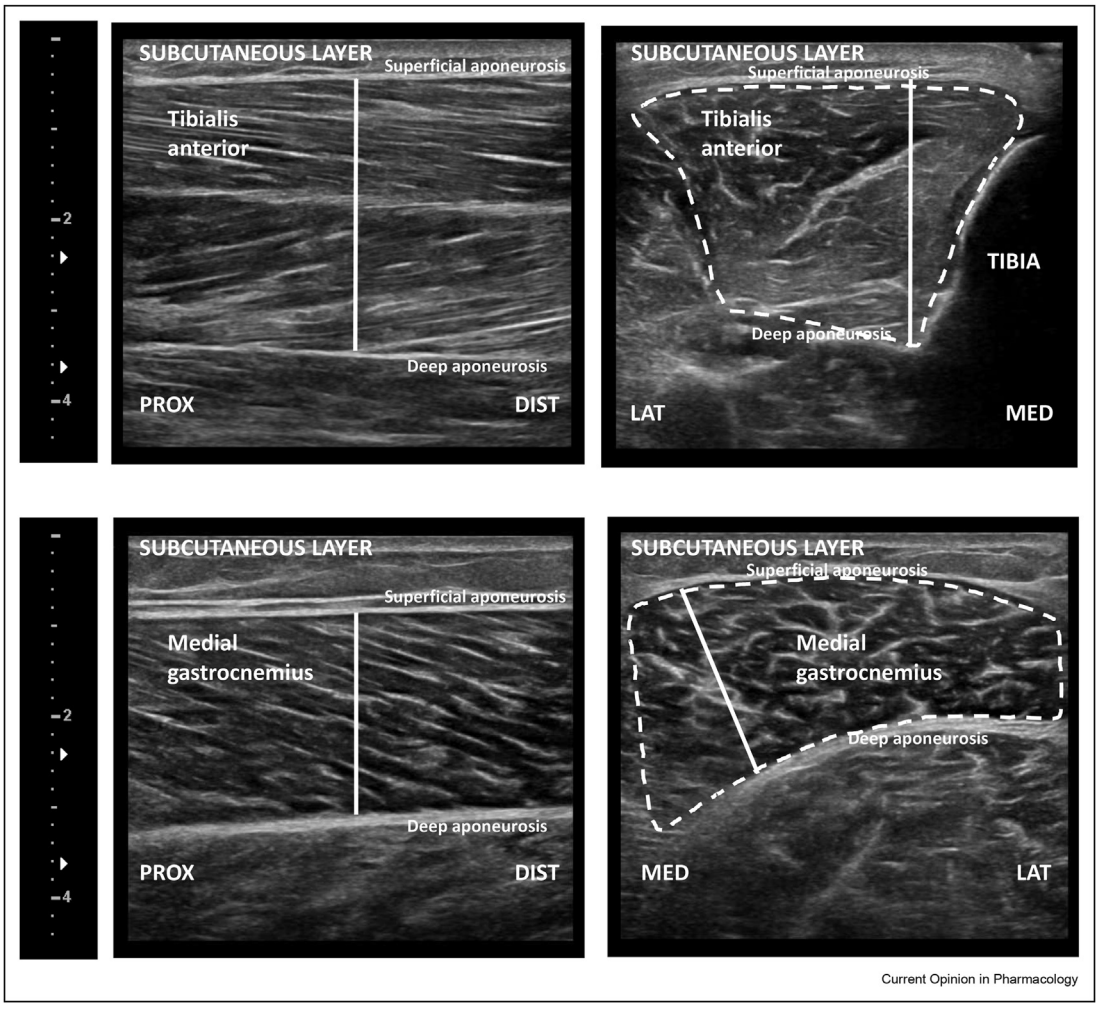
Ultrasound has been used in medicine since the early 1950s, when Wild and Neal [37] and Dussik [38] showed the ability of high-frequency ultrasonic waves to visualize human tissues. The fundamental principle that creates the ultrasound image is the echo: a transducer sends out pulses of high-frequency sound waves and receives their echoes. The image creation from all returning echoes is based on the analysis of their acoustic and temporal properties [39]. The sonographic appearance of a muscle is fairly distinct and can easily be distinguished from surrounding structures such skin, subcutaneous adipose tissue, bone, nerves, and vessels [40].

Normal muscle tissue appears as a structure with low echo intensity (i.e., it is black in appearance) surrounded and divided by echogenic sheaths of connective tissue known as epimysium (i.e. the envelope surrounding the whole muscle) and perimysium (i.e. the sheath grouping muscle fibers into fascicles), respectively. All superficial skeletal muscles can easily be investigated with ultrasound, whereas deep muscles can be more difficult to visualize because of the absorption and reflection of sound by superficial tissue layers. Muscles can be investigated in both the sagittal and axial plan and in different conditions (rest, isometric contraction, dynamic movement) [39,40]. Figure 2 reports representative images of the tibialis anterior and medial gastrocnemius muscles of a healthy subject. In all the images, the boundaries of the muscle are clearly visible as the epimysium is a highly echogenic (reflective) structure, the subcutaneous fat has a low echo intensity, and fascicles are distinct and clearly detectable (in sagittal images).

The optimal insonation of a muscle can be obtained by ensuring the best representation of superficial and deep aponeuroses, muscle fascicles, and bone boundary. Aponeuroses and bones can easily be detected in images acquired in both the axial and sagittal axis, whereas fascicles can be detected in the sagittal images only. The simplest use of ultrasound in evaluating skeletal muscles is represented by the size assessment: the extent of both muscle hypertrophy and atrophy can be quantified. Care must be taken, however, when measuring muscle size to ensure that the transducer pressure on the skin is minimal (and constant), as the muscle can easily be displaced (especially in resting conditions) with excessive force on the transducer.

The main parameters adopted for the muscle size assessment are represented by thickness and cross-

**Figure 2**




Sagittal (left panels) and axial (right panel) ultrasound scans of the tibialis anterior (top panels) and medial gastrocnemius (bottom panels) muscles for one representative subject. Continuous white lines in all panels were used to calculate the muscle thickness (tibialis anterior, 3.0 cm in the sagittal image, 2.9 cm in the axial image; medial gastrocnemius, 2.3 cm in the sagittal image, 2.2 cm in the axial image), whereas white dashed lines delimit the regions of interest used to quantify the tibialis anterior cross-sectional area (7.78 $cm^2$) and the echo intensity of both muscles (tibialis anterior, 70.0 a.u.; medial gastrocnemius, 58.0 a.u.). Proximal and distal portions of the muscles are indicated as PROX and DIST, respectively. Medial and lateral portions of the muscles are indicated as MED and LAT, respectively.

sectional area [40,41]. As shown in the representative images of the tibialis anterior muscle reported in Figure 2, the former parameter can be assessed in both axial and sagittal images, whereas the latter parameter can be assessed in axial images only and for a muscle width equal or lower than the length of the ultrasound transducer. We recently proposed muscle-specific cut-off points for the ultrasound-based assessment of low muscle thickness, and we found that the prevalence of low muscle mass in older adults was highly dependent on the muscle being investigated: in fact, proximal muscles of the lower limb are more affected than distal muscles [42]. Consistently, Agyapong-Badu et al. [43] and Loenneke et al. [44] suggested that the ultrasound estimate of the anterior thigh thickness can represent a biomarker for musculoskeletal health as it enables to quantify the relative amount of muscle and noncontractile tissue. Therefore, this parameter could be useful for the assessment of the age-related, disuse-related, or disease-related loss of muscle mass. Similarly, limb thickness asymmetry could also be useful in patients with VML to quantify and longitudinally investigate the extent of muscle loss and its recovery. Moreover, muscle thickness can easily be measured in the same subject for different body sites and the summed total thickness can be used to estimate the TBSMM through the prediction equation proposed by Sanada et al. [45] (Table 1) that was recently validated by Abe et al. [46]. To our

knowledge, no previous study has been performed to compare the TBSMM estimations obtained from the ultrasound-derived muscle thickness and from the DXA-derived ALM value.

Other parameters can also be obtained, for pennate muscles, through the analysis of sagittal ultrasound images such as muscle fascicle length and pennation angle [41,47–51]. These are key parameters of muscle architecture that has attracted considerable research interest in recent years because of its relation with muscle force-producing capability and energetics [49–51*] and its relevance for the evaluation of the muscle adaptations to training [52]. However, the ultrasound assessment of muscle architecture is not yet a standardized technique and does not yet have validated cut-off points [53]. Therefore, its application for the detection of low muscle mass and muscle changes in the clinical setting is currently limited.

Axial ultrasound images enable to investigate not only the muscle cross-sectional area but also the muscle echo intensity that has been proposed as a clinically relevant noninvasive marker of muscle quality [40,54,55]. This parameter increases with age and in presence of neuromuscular and myopathic disorders because of the age- and disease-related muscle replacement by fat and fibrous tissue (i.e. myosteatosis) [40,56,57]. These tissues have an acoustic impedance different from that of the surrounding muscle: therefore, an increased number of reflecting interfaces in the muscle gives the muscle a whiter appearance (i.e. an increased echo intensity) [40]. Young et al. [58] developed muscle- and gender-specific prediction equations to estimate the percent intramuscular fat from muscle echo intensity and subcutaneous fat thickness. They also found significant associations between the ultrasound-derived estimation of intramuscular fat and physical activity level [59]. However, the ultrasound approach for estimating the intramuscular fat needs further development and validation. A limitation of the muscle echo intensity assessment is that this quantitative variable is highly dependent on the ultrasound scanner settings [60]. Therefore, different patients must be evaluated with the same ultrasound device and with the same system-setting parameters. Alternatively, a calibration procedure [57,61] is required for echo intensity comparison between different ultrasound devices. Despite this limitation, the muscle echo intensity can be considered a useful descriptor of the muscle quality and its changes that may present a time course different from the changes of muscle mass. Consistently, we found in steroid myopathy patients with active and remitted Cushing's disease a muscle thickness comparable between the two groups and a higher echo intensity in the former compared with the latter group [57]. On this basis, we suggested that the muscle mass recovery after resolution of the hypercortisolemic state seems longer than the muscle structure recovery [57]. It may be hypothesized that similar differences between the muscle mass and structure recovery could also occur in patients with VML. Thus, future studies are required to investigate the extent and time evolution of muscle thickness reduction and echo intensity increase in patients with VML.

## Conclusions

The progressive (in patients with sarcopenia) as well as the rapid-onset (in patients with VML) loss of muscle mass (and the associated changes of muscle quality) result in neuromuscular function impairment that significantly affects the state of health, especially in elderly subjects, because it is associated with pain, mobility disorders, increased risk of falls and fractures, and impaired ability or disability to perform activities of daily living [4,7]. Although not only peripheral (muscular) but also central (neural) mechanisms can underlie the age- and disease-related neuromuscular function impairment, from a pathophysiological

**Table 2**

**Strength and limitations of dual-energy X-ray absorptiometry (DXA) and ultrasonography (US) to assess lean and muscle mass.**

| Properties and measurements | DXA | US |
|---|---|---|
| Cost/time | Strengths: easy to use, quick | Strengths: inexpensive, portable, simple, safe, and quick |
| Radiation | Strengths: low radiation exposure (~5 μSv for a whole-body scan using a last-generation densitometer) | Strengths: no radiation exposure |
| Accuracy and precision | Strengths | Strengths |
| Primary measurements for lean/muscle mass | Total body lean mass (kg)<br>Appendicular lean mass (kg) | Muscle thickness (cm)<br>Muscle cross-sectional area ($cm^2$)<br>Muscle fascicle length (cm)<br>Fascicle pennation angle (°)<br>Echo intensity (a.u.) |
| Assessment of myosteatosis | Limitations | Limitations |

perspective sarcopenia and VML can be considered an 'organ failure'. In fact, previous authors proposed the pathophysiologic constructs of 'skeletal muscle function deficit' [62] and 'muscle insufficiency' [63]. Therefore, the reductions in LM and muscle size may predict (and may also precede) the organ failure-associated impairment of neuromuscular function and should be quantified when a patient reports suggestive symptoms or signs (i.e. feeling weak, slow walking speed, difficulty rising from a chair, falling). Methods and approaches for the assessment of LM quantity (and its distribution) and muscle size (and its structure) continue to evolve providing a wide array of choices for use in research and clinical settings [12]. Table 2 summarizes the strengths and limitations of the DXA and US methods discussed in this review. The systematic incorporation of these methods and approaches into routine examinations of patients with sarcopenia and VML is recommended for identifying patients at risk for neuromuscular impairment-related comorbidities and evaluating the effectiveness of pharmacological and rehabilitative interventions. Consistently, the association between skeletal muscle volume loss and poor prognosis has already been observed in different patient populations (i.e., cirrhotic and oncologic patients) [64–67] and deserves future investigations also in patients with VML. In this patient population, beyond promoting *de novo* regeneration of lost muscle tissue, amelioration of the secondary pathophysiological changes induced by muscle loss within the remaining musculature (i.e., disuse atrophy, myosteatosis) and surrounding tissues (i.e., bone loss, increased subcutaneous and visceral fat) may promote functional improvements.

## Conflict of interest statement

Nothing declared.

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