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SPECIAL ARTICLE

Application of ultrasound for muscle assessment in sarcopenia: towards standardized measurements

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Abstract

Purpose Measurement of muscle mass is paramount in the screening and diagnosis of sarcopenia. Besides muscle quantity however, also quality assessment is important. Ultrasonography (US) has the advantage over dual-energy X-ray absorptiometry (DEXA) and bio-impedance analysis (BIA) to give both quantitative and qualitative information on muscle. However, before its use in clinical practice, several methodological aspects still need to be addressed. Both standardization in measurement techniques and the availability of reference values are currently lacking. This review aims to provide an evidence-based standardization of assessing appendicular muscle with the use of US.

Methods A systematic review was performed for ultrasonography to assess muscle in older people. Pubmed, SCOPUS and Web of Sciences were searched. All articles regarding the use of US in assessing appendicular muscle were used. Description of US-specific parameters and localization of the measurement were retrieved.

Results Through this process, five items of muscle assessment were identified in the evaluated articles: thickness, cross-sectional area, echogenicity, fascicle length and pennation angle. Different techniques for measurement and location of measurement used were noted, as also the different muscles in which this was evaluated. Then, a translation for a clinical setting in a standardized way was proposed.

Conclusions The results of this review provide thus an evidence base for an ultrasound protocol in the assessment of skeletal muscle. This standardization of measurements is the first step in creating conditions to further test the applicability of US for use on a large scale as a routine assessment and follow-up tool for appendicular muscle.

Keywords Sarcopenia · Ultrasound · Muscle assessment

Introduction

Sarcopenia is becoming one of the biggest health care challenges that arises together with increasing age expectations [1]. It is associated with mortality, functional decline, disability, a higher rate of falls, a higher incidence of hospitalizations, increased health care costs and a lower quality of life [2]. Therefore, clear cutoffs are desirable for an early diagnosis. However, there is still some debate about a universal definition. The currently most used operational definition is the age-related decline of muscle mass, together with the decline of strength and/or function [3]. This definition can still be regarded as largely heterogeneous. This debate is continued in the use of specific cutoff points for muscle mass, as is clearly seen in the many criteria that are proposed by various international organizations [3–8].

When applying these criteria, it is evident that in diagnosing sarcopenia, muscle mass still holds the most weight, more than strength or function. This also finds its way in a case finding algorithm, published by the European Working Group for Sarcopenia in Older People [3], where there

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can be no diagnosis of sarcopenia without the assessment of muscle mass. This focus on muscle mass, however, gives way to a few practical implications, as there seems to be a large cleft between guidelines and clinical practice.

First of all, the prevalence of sarcopenia is highly dependent on the diagnostic method used [9]. As stated, there are many different cutoff points for diagnosing sarcopenia through muscle mass. All are either based on dual-energy X-ray absorptiometry (DXA) or bio-impedancemetry (BIA); currently, there are no cutoff values for either CT or MRI. DXA cutoff points are based on skeletal muscle mass index (SMI), which is the sum of the all the appendicular muscle mass, divided by height. For DXA SMI, three groups of cutoff points are used [10–12]. BIA also uses SMI, with one cutoff point proposition based on BIA-predicted skeletal muscle mass [13] and the other based on absolute muscle mass [14, 15]. It is unclear in how far both DXA and BIA are available in clinical practice.

Secondly, it has become clear that measuring muscle quantity, i.e., muscle mass, in itself is only part of the problem of age-related muscle degradation. Also the muscle architectural qualities—here used synonymously as muscle quality—need to be assessed, as volume in itself has no linear relation with either strength or function. Muscle quality is a broad term and is in current literature used in two meanings. On one hand, it can mean the muscle strength or muscle power per unit of muscle mass [16–18]. On the other hand—as in this study—it can be used as description of the components of the muscle described [19]. The neural component in the process of sarcopenia could possibly amount up to 50% [19], but it is clear that shear volume alone does not explain the rest. Measuring the components of this volume is vital [20, 21]. The assessment of these components cannot be done either by DXA or BIA [22], although there is some evidence that multi-frequency bio-impedance and segmental bioelectrical impedance spectroscopy could measure muscle composition to a certain degree and give information regarding skeletal muscle physiology [23, 24]. For assessing muscle quality, computerized tomography (CT) and magnetic resonance imaging (MRI) are considered 'gold standards' [25], in as far this is possible, because many different scanning techniques exist: CT muscle attenuation [26], diffusion tensor MRI [27], Dixon MRI [28], proton magnetic resonance spectroscopy (MRS) [29], 13C-MRS [30] and 31P-MRS [31]. For both CT and MRI, whole body scanning can be done, as also single/multiple slice scanning [32–34]. The former are considered as superior, but adequate data are present to use the latter as a convenient alternative [3]. In clinical practice, however, CT and MRI are not practical, due to the limited availability, lack of portability, high cost and radiation exposure (CT). Also, currently there are no cutoff values for either CT or MRI. This way, current guidelines and criteria fall short, in the way that they are often not applicable in a clinical routine setting due to practical reasons as stated above, and do not yet have incorporated the quality aspect of muscle mass. There is an important need for an instrument that can give information about both muscle quantity and quality, that is cheap and easily available in routine practice, and that can be used in large population-based screenings.

Ultrasonography or ultrasound (US) could fill this gap.

US is a well-studied technique that has already proven its worth in the detection of neuromuscular pathology with positive predictive values of up to 90% [35] and in the assessment of muscle–tendon interactions [36]. US is a portable, inexpensive, non-invasive technique without using ionising radiation that also has a high repeatability. Regarding muscle mass assessment, US-based measurements have a strong positive correlation with DEXA [37–40], CT [41] and MRI-based measurements [42–44]. For the estimation of muscle quality and quantity, US is a valid and accurate technique [45, 46]. The validity of ultrasound to discern architectural properties has also been demonstrated in cadaver validation studies [47–51]. It has good intra- and interrater reliability [45], as well as test–retest reliability [52], both in an elderly people [45] as in a younger population [53]. However, it is unclear which anatomical site is best to be used for specific outcomes, e.g., prediction of total skeletal muscle mass [54]. It must be taken into account that not all peripheral muscles decline alike [55, 56]. In this regard, prediction equations for total skeletal muscle mass need further validation [45]. Also, there are hardly any reference data currently available, as there is no standardization of the measurement technique. To our knowledge, there are only four studies giving limited reference data, for muscle thickness and echo intensity of selected muscles [57–60].

This review aims to provide a standardization of ultrasound measurements for assessing muscle mass in the assessment of sarcopenia. To the best of our knowledge, there are no hard clinical data to prefer assessing one muscle or set of muscles over another, or to choose one type of measurement and disregards the others. Therefore, an overview will be given of how appendicular muscles are measured in the literature so far, followed by a proposition of how to do a muscle assessment in a standardized way. These propositions will be based on the literature and consensus within the review group. Among these propositions, there will be a list summarizing which information needs to be minimally included in a protocol when planning future studies. This way, we hope to advance the study of the application of ultrasound in sarcopenia assessment in different settings [61, 62].

Methods

Registration

The protocol for this systematic review has been registered at PROSPERO (Registration number CRD42018085587).

Search strategy

The search strategy was set up based on three main components: elderly (population) [63], ultrasound (exposure), and muscle (outcome). For this, a modified PECO model for clinical questions was used. The search was performed in Pubmed, Web of Science and Cochrane Library, up until the 20th of January 2018. All eligible studies in English, German, French and Dutch were screened for their applicability. Studies regarding the use of ultrasound in the assessment of muscle mass were considered for this review. Bibliographic lists of included papers were hand-searched for additional studies. Animal studies, studies using cadaver specimens, studies assessing non-appendicular muscle, case reports, letters to the editor, editorials and (systematic) reviews were excluded.

Search structure for PubMed was as follows: ((((Elderly[tiab] OR community-dwelling[tiab] OR geriatric[tiab] OR Frailty[tiab] OR Ageing[tiab] OR elders[tiab] OR Frail[tiab] OR "postmenopausal women"[tiab] OR aging[tiab] OR older[tiab] OR residents[tiab] OR "old people"[tiab] OR nursing homes[mh] OR aging[mh] OR frail elderly[mh] OR homes for the aged[mh] OR aged, 80 and over[mh])))) AND ((((((((Ultrasonography[Mesh]) OR Ultrasound) OR Echograph*) OR Ultrasonograph*) OR Ultrasonic) OR Echotomograph*) OR Sonograph*)) AND (("Muscles"[Mesh] OR "Lean tissue" OR "Lean mass" OR "Lean body mass" OR muscle OR "Fat free mass"))).

Search structure for SCOPUS was as follows: ((TITLE-ABS-KEY (elder*)) OR (TITLE-ABS-KEY (community-dwelling)) OR (TITLE-ABS-KEY (geriatric)) OR (TITLE-ABSKEY (frail*)) OR (TITLE-ABS-KEY (ag*ing)) OR (TITLE-ABSKEY ("postmenopausal women")) OR (TITLE-ABS-KEY (old*)) OR (TITLE-ABSKEY (resident*))) OR (TITLE-ABS-KEY ("nursing homes"))) AND ((TITLE-ABSKEY (ultrasound)) OR (TITLE-ABS-KEY (echograph*)) OR (TITLE-ABSKEY (ultrasonograph*)) OR (TITLE-ABS-KEY (ultrasonic)) OR (TITLE-ABSKEY (echotomograph*)) OR (TITLE-ABS-KEY (sonograph*))) AND ((TITLE-ABSKEY (muscle*)) OR (TITLE-ABS-KEY ("Lean tissue")) OR (TITLE-ABS-KEY ("Lean mass")) OR (TITLE-ABS-KEY ("Lean body mass")) OR (TITLE-ABS-KEY ("Fat free mass"))).

Search structure for Web of Science was as follows: Elder* OR community-dwelling OR geriatric OR Frail* OR Ageing OR Frail OR "postmenopausal women" OR old* OR resident* OR "nursing homes". Ultrasound OR Echograph* OR Ultrasonograph* OR Ultrasonic OR Echotomograph* OR Sonograph*. Muscle* OR "Lean tissue" OR "Lean mass" OR "Lean body mass" OR "Fat free mass".

An overview of the study selection process is shown in Fig. 1. After deleting duplicates, abstracts were gathered. Abstracts were divided among 12 independent reviewers, experienced in either geriatrics, physical therapy, radiology or body composition. One other reviewer (SP) screened all the abstracts. Title and abstract of all manuscripts were screened for eligibility, reviewers being blinded from each other, using the Rayyan web-based software [64]. Disagreements were resolved by consensus within the group. All review articles, case reports, letters to the editor, and editorials were excluded. Then, the selected articles were used for full-text reading. Again, the full-text articles were divided among the same 12 independent reviewers as in the previous step. However, for the reviewers, the full texts to be reviewed were not the same as the abstracts that were reviewed. In other words, each reviewer reviewed two different sets of publications. Reasons for exclusion of an article after full-text assessment were: absence of clear description of location of measuring point, absence of clear description of muscle measured, content being article outside the scope of the manuscript, or referencing to another article regarding measurement technique/location. For the last category, the article to which was referred for measurement technique/location, was checked for inclusion. If not already included, it was added.

Results

Search strategy

The initial search yielded 17.579 abstracts (PubMed = 5565, SCOPUS = 7255, Web of Science = 4759). There was one additional record identified through other sources. After deleting duplicates ($n = 2751$), 14.829 abstracts were screened. Of these, 359 articles were withheld. After full-text assessment, 76 articles were used in this review. See also Fig. 1 for details about the study selection process.

All articles included in this paper are detailed in a supplemental volume (Table S1), with cohort size, age (median or range) and ethnicity.

Mean cohort size was 58 (median 44, range 9–347). One study did not mention the cohort size. Two papers did not have cohorts as they were suggestions for US protocols.

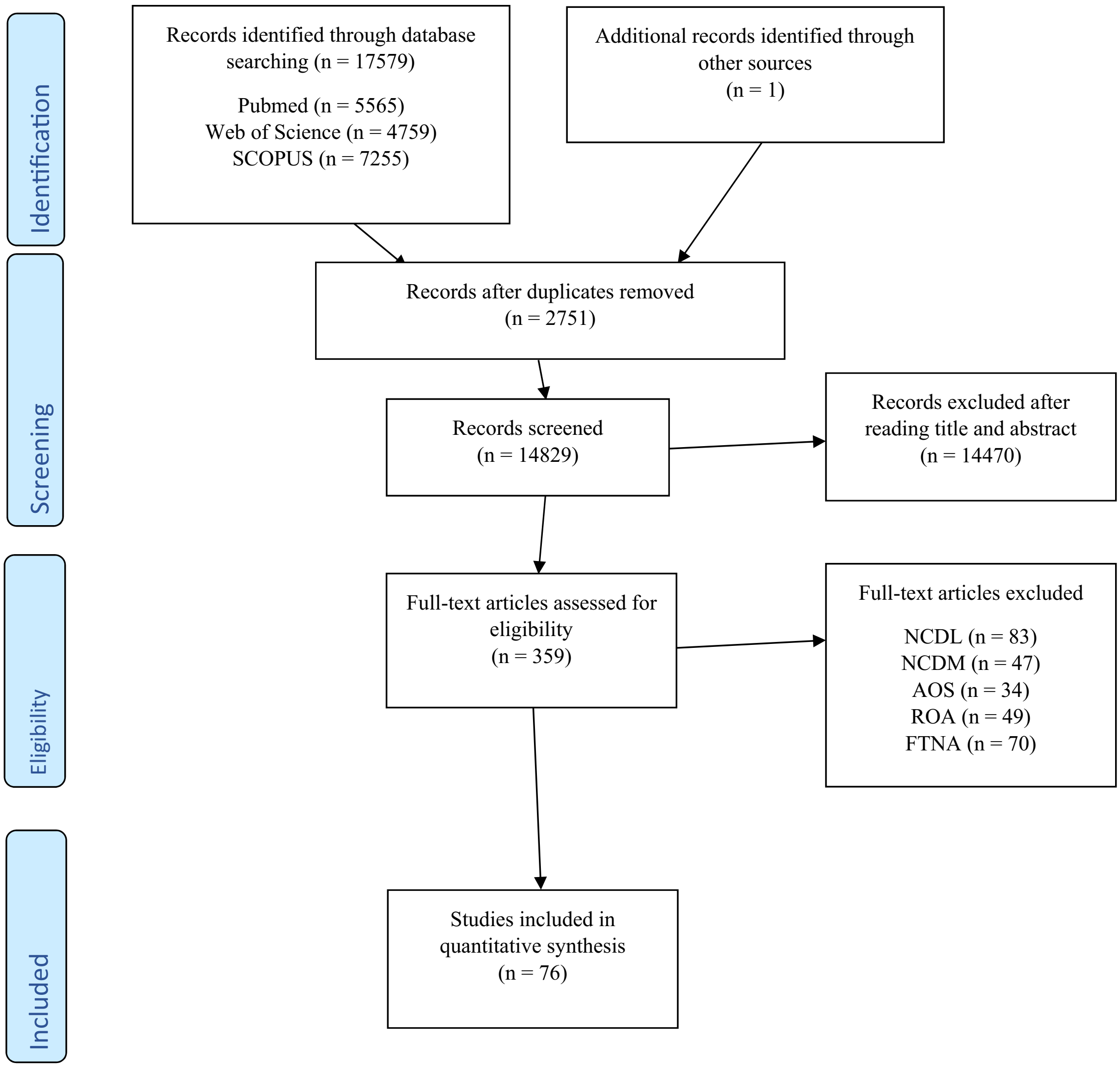

Fig. 1 Overview of the study selection process using the PRISMA 2009 flow chart [166]. *NCDL* no clear description of location of measuring point, *NCDM* no clear description of muscle measured, *AOS* article outside of scope of manuscript, *ROA* referencing to other articles for measurement technique, *FTNA* full-text not available

Age was clearly described in 38 studies. In 33 studies, only ranges were given. Three studies did not mention age. In the two protocol studies, no patients were used so no age was noted.

Ethnicity was very poorly described explicitly. Eight studies mentioned Asian ethnicity (one Chinese, seven Japanese), one study mentioned Caucasian ethnicity, and one study mentioned white/Indian ethnicity. One study mentioned different ethnicities: Caucasian 87%, Asian 29%, native American 3%, African 1%. In two studies (protocol), ethnicity was not applicable.

The sex of study subjects was not included in this review.

As there is no gold standard in US muscle assessment, it was difficult to have a good quality assessment of the included articles. Although a multitude of research questions were present, it must be noted that no studies compared different US techniques in the assessment of muscle.

Patient positioning pre-investigation

Only seven studies stated clearly which subject side was assessed. In five studies, the dominant side was used [65–69]. Only one study specified using the non-dominant side [70]. One study used only the right side, not mentioning if this was the dominant side or not [71].

Regarding the state of the muscle assessed, one article mentioned letting the test subject perform three maximum contractions of the muscle assessed before measurement

Table 1 Overview of different patient positioning used in muscle assessment

	Supine					Lying on non-dominant side, knees 10°	Prone	Sitting			Standing
	Full extension	Knees at 10°	Semi-Fowler position	Legs at 50°	Knee angle 115° and hip angle 140°			Hips 90°, knees 90°	Hips 90°, knees 60°	Hips 85°, knees 0°	
Lower limb											
Rectus femoris	X	X	X	X				X	X		X
Vastus lateralis	X	X		X	X	X		X			
Vastus medialis	X										
Vastus intermedius	X		X						X		X
Quadriceps (all 4 muscles)	X								X		
Biceps femoris							X				
Tibialis anterior	X										
Gastrocnemius medialis							X	X		X	
Soleus							X	X			
Upper limb											
Biceps brachii	X	X									
Triceps brachii											X

[65]. Two studies had the test subjects being relaxed for 15 min, one to allow fluid shifts to stabilize [72], the other to avoid muscle contraction-induced fluid shifts and muscle blood flow during the measurements [73]. The latter, therefore, also specified to have done all ultrasound measurement before any functional testing. Two groups refrained the study subjects from rigorous exercises, one only for the same day [74], one for the last 48 h [75].

The position of the study subject was dependent on the specific muscle investigated, but also herein there were some differences. For an overview of the different positions used in selected muscles, see Table 1. In the supplemental material, a table is included that indicates which study assessed which muscle at a certain position (Table S2).

For supine and prone positions, study subject is regarded as having hips and shoulders in neutral position, knees and elbows in full extension, ankles at 90°, unless specified otherwise. It has to be mentioned that it is important to correctly describe the positioning, as the angle of joints is not standardized in one direction, e.g., knees in 150° or 30° could mean the same. In none of the studies, it was described whether the supine position included the feet being of the table or not. One study, assessing the gastrocnemius medialis, specified having the tibiotalar joint angle at 115°, for which they had a cast specially made for all participants [69].

Upper leg muscles

- Rectus femoris was assessed in supine position in full extension [65, 68, 72, 76–86], with knees at 10° [87–89], in semi-Fowler position [90–92]. It was assessed in sitting position with hips and knees at 90° [66, 93] and with hips at 90° and knees at 60° [94]. Lastly, it was also assessed standing [95, 96].
- Vastus lateralis was assessed in supine position in full extension [72, 78, 84, 85, 89, 97–101], with knees at 10° [88], with legs at 50° [71] and with knee angle at 115° and hip angle at 140° [102]. It was assessed lying in the non-dominant side with knees at 10° lateralis [87]. Lastly, it was assessed sitting with hips and knees at 90° [103].
- Vastus medialis was assessed in supine position in full extension [72, 84, 104].
- Vastus intermedius was assessed in supine position in full extension [72, 76–79, 84] and in semi-Fowler position [90]. It was assessed in sitting position with hips at 90° and knees at 60° [94]. Lastly, it was also assessed standing [96].
- Quadriceps—comprising all 4 muscles: rectus femoris, vastus medialis, vastus intermedius and vastus lateralis—were assessed in supine position in full extension [105]. It was also assessed in sitting position with hips at 90° and knees at 60° [106].
- Biceps femoris was only assessed in prone position [78].

Lower leg muscles

- Tibialis anterior was only assessed in full extension [97].
- Gastrocnemius medialis was assessed in prone position [78, 82, 97, 102, 107–111] and in sitting position with hips and knees at 90° [69, 103, 112].
- Soleus was assessed in prone position [78] and in sitting position with hips and knees at 90° [112].

Upper arm muscles

- Biceps brachii was only assessed in full extension [57, 97, 113].
- Triceps brachii was assessed standing [114, 115]. One study that assessed triceps brachii did not mention the patient position [116].

System and system settings

Many different types of ultrasound machines were used, from various manufacturers (SonoSite, GE Healthcare, Siemens, Mindray, Philips, Toshiba, Hitachi Aloka Medical, Esaote, Fukuda Denshi, Hewlett-Packard, Telemed). A summary of the different types used is beyond the scope of this article.

Every research group used B-mode ultrasound, and all but one used a linear transducer probe. The only article using a curved transducer had as preposition the validation of the use of a curved versus a linear transducer [91].

The length of the transducer varied from 3.8 to 7 cm. The frequency used was described in 63 cases, and ranged from 3 to 15 MHz.

In 13 cases, the bandwidth instead of the exact frequency was described: 10–15 MHz ($n=1$), 7–12 MHz ($n=3$), 6–13 MHz ($n=1$), 5–12 MHz ($n=2$), 5–10 MHz ($n=3$), 3–13 MHz ($n=1$), 3–12 MHz ($n=1$), 3–11 MHz ($n=1$).

In 50 articles, a clear frequency was described. The most common used frequency was 7.5 mHz ($n=23$); further frequencies used were 15 MHz ($n=1$), 13.6 MHz ($n=1$), 12 MHz ($n=7$), 10 MHz ($n=6$), 9 MHz ($n=3$), 8 MHz ($n=7$), 5 MHz (linear array, $n=6$), 5 MHz (curved array, $n=1$).

No information on the inclination of the probe was noted.

Some additional system settings were described. Most were on image depth (focus point) and general gain.

Image depth was described in 7 cases: 45 mm (mm) [71], 50 mm [87, 88, 117], 60 mm [101, 118] and 70 mm [72].

General gain (in decibels, dB) was described in 15 cases: was either set to 50 dB [82, 83, 87, 117, 119], 58 dB [120], 68 dB [71, 108, 111, 121], 85 dB [94], 86 dB [122] or 90 dB [72, 84, 123].

Time gain compensation was described as being in a neutral position in two cases [72, 124].

Dynamic range was described in five cases: being set to 40 [121], set to 69 [120], or set to 72 [83, 117, 119].

Compression—which is altering the display of the range of echo intensities to end up with a lower amount of different shades of gray—was described in one study as set to 70 dB [122].

No further system settings were described.

For image post-processing and measurement, 35 of the 75 articles mentioned the use of additional software. These included Image J [125] ($n=26$), Matlab (The MathWorks, Inc., Natick, Massachusetts, United States) ($n=2$) and Photoshop (Adobe, Adobe Systems Incorporated, San Jose, California, United States) ($n=7$).

Components and measuring points

Five main components were distilled from the literature: muscle thickness (MT), pennation angle (PA), fascicle length (Lf), echo intensity (EI) and cross-sectional area (CSA).

Four studies mentioned that all measurements were done three times [66, 67, 91, 97], and after that, the mean value was taken. Two other studies mentioned doing all measurements five times [89, 97]. One of the latter two discarded the highest and lowest value, and then took the mean value of the remaining three [89].

Muscle thickness was defined as the distance between deep and superficial aponeurosis [97, 109]. It can be expressed either in centimetres (cm) or in millimetres (mm).

Cross-sectional area was divided into two: anatomical cross-sectional area (ACSA) and physiological cross-sectional area (PCSA). Anatomical cross-sectional area was defined as the area of cross-section of a muscle perpendicular to its longitudinal axis. Physiological cross-sectional area was defined as the area of cross-section of a muscle perpendicular to its fibers. In non-pennate muscle, ACSA and PCSA are the same; in pennate muscles they are not. ACSA underestimates the number of total fibers in a pennated muscle. Muscle strength is more correlated with PCSA than with ACSA because the former represents the maximal number of acto-myosin crossbridges that can be activated in parallel during contraction [126]. Therefore, when studying muscle strength, it is not advised to only measure ACSA.

Table 2 Measuring points and components of quadriceps muscles used in assessment

Proximal point	Distal point	Distance	MT	CSA	ACSA	PCSA	FL	PA	EI
Greater trochanter	Popliteal crease	60–70%	RF						
	Lateral condyle	30%	VM, VL	RF			VL	VM, VL	VM, VL
		50%	RF, VL, VI	RF, VL	VL		VL	RF, VL, VI	RF, VL, VI
		2/3	RF, VL	VL			VL	VL	VL
	Articular cleft of knee	50%	RF, VI						
	Proximal border of patella	50%			RF	RF			
Anterior superior iliac spine	Lateral condyle	2/3	RF, VI						RF, VI
	Proximal border of patella	50%	RF, VI, VL						RF, VI
		60%	RF, VL						
		2/3	RF, VL, VI						VL
		3/5		RF					
		75%				RF			
	Midpoint of patella	50%	RF, VI						
	Distal border of patella	50%	RF		RF				
Anterior inferior iliac spine	Proximal border of patella	50%	RF, VI	RF	RF		RF	RF	RF, VI
–	Proximal border of patella	10 cm proximal to distal point	RF						
–		15 cm proximal to distal point		RF	RF				

MT muscle thickness, *CSA* cross-sectional area, *ACSA* anatomical cross-sectional area, *PCSA* physiological cross-sectional area, *FL* fascicle length, *PA* pennation angle, *EI* echo intensity, *RF* rectus femoris, *VL* vastus lateralis, *VM* vastus medialis, *VI* vastus intermedius

Measurements of CSA were usually presented in square centimeter. Some studies mentioned to measure the CSA by manually drawing the circumference of the muscle with a cursor [66, 91, 127]. One study normalized the CSA to body mass to represent it as a relative measure of quadriceps muscle [71].

The pennation angle (Ap) was defined as the angle of insertion of muscle fiber fascicles into the deep aponeurosis [69, 97]. The angle at which fibers in a pennate arrangement are oriented relative to the longitudinal axis varies from muscle to muscle [128]. The pennation angle is proportional to the number of sarcomeres packed in parallel along the aponeurosis and closely related to the force-generating capacity of the muscle [109].

Fascicle length (Lf) was defined as the length of the fascicular path between the insertions of the fascicle into the superficial and deep aponeuroses. In the cases where the fascicle extended outside of the acquired ultrasound image, the length of the missing portion of the fascicle was estimated by extrapolating linearly both the fascicular path, visible in the image, and the aponeurosis [65, 69, 97, 129, 130]. Another method of estimating the fascicle length is using a formula: multiplying the muscle thickness times the hypotenuse of the pennation angle inversed [131] or, stated differently, dividing the muscle thickness by the hypotenuse of the pennation angle [99]. These formulae do not account for fascicle curvature [73]. Fascicle length is proportional to the number of sarcomeres arranged in series and the excursion range of the muscle fiber [109].

Echo intensity was defined as the brightness of the image acquired through ultrasound. It is expressed in gray scales (0–255). Some studies used a gray scale analysis for determining the echo intensity [72, 119]. Different programs were used for this analysis, which came down to post-producing the images.

Components and specific measuring points will be given per individual muscle.

Upper leg muscles

The largest part of information on anatomical landmarks is found on the four bellies of the quadriceps muscle (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius). As common landmarks can be used for the identification of these four muscles, the data gathered are taken together. All measuring points and components of quadriceps muscles are also represented in Table 2. Besides the quadriceps muscle, the only other upper leg muscle assessed was the biceps femoris (data in the text, not in the table).

At a distance of 60–70% between the greater trochanter and the popliteal crease, rectus femoris was assessed for muscle thickness [65].

At a distance of 30% between the greater trochanter and the lateral condyle, vastus medialis was assessed for muscle thickness [84, 131, 132], pennation angle [131] and echo intensity [84, 123]. Vastus lateralis was assessed for muscle thickness [85, 132, 133], fascicle length [85, 133], pennation angle [85] and echo intensity [133]. Rectus femoris was assessed for cross-sectional area [85].

At a distance of 50% between the greater trochanter and the lateral condyle, rectus femoris was assessed for muscle thickness [41, 75, 84, 89, 95, 131, 134], cross-sectional area [71, 97], pennation angle [89, 131] and echo intensity [71, 84, 123]. Vastus lateralis was assessed for muscle thickness [70, 80, 84, 88, 89, 99–101, 103, 115, 131, 135], cross-sectional area [88], anatomical cross-sectional area [71, 87, 98, 119, 135], fascicle length [70, 88, 99–101, 103, 115], pennation angle [70, 88, 89, 99–101, 103, 115] and echo intensity [71, 84, 88, 119, 135, 136]. Vastus intermedius was assessed for muscle thickness [84, 131, 134], pennation angle [131] and echo intensity [84, 136]. Quadriceps (all 4 bellies) were assessed for muscle thickness [137].

At a two-third distance between the greater trochanter and the lateral condyle, rectus femoris was assessed for muscle thickness [67]. Vastus lateralis was assessed for muscle thickness [86], cross-sectional area [86], fascicle length [86], pennation angle [86] and echo intensity [86].

At a distance of 50% between the greater trochanter and the articular cleft of the knee, rectus femoris and vastus intermedius were assessed for muscle thickness [96].

At a distance of 50% between the greater trochanter and the proximal border of the patella, rectus femoris was assessed for anatomical cross-sectional area [138] and physiological cross-sectional area [127].

At a two-third distance between the anterior superior iliac spine and the lateral condyle, both rectus femoris and vastus intermedius were assessed for muscle thickness and echo intensity [90].

At a distance of 50% between the anterior superior iliac spine and the proximal border of the patella, rectus femoris was assessed for muscle thickness [43, 78, 79, 82, 117, 118, 120–122, 139, 140] and echo intensity [68, 83, 118, 120, 122]. Vastus intermedius was assessed for muscle thickness [78, 79, 120–122, 139] and echo intensity [120, 122]. Vastus lateralis was assessed for muscle thickness [140].

At a distance of 60% between the anterior superior iliac spine and the proximal border of the patella, rectus femoris was assessed for muscle thickness [92] as was the vastus lateralis [78].

At a two-third distance between the anterior superior iliac spine and the proximal border of the patella, rectus femoris was assessed for muscle thickness [43, 77, 141]. Vastus lateralis was assessed for muscle thickness [118] and echo intensity [118]. Vastus intermedius was assessed for muscle thickness [77, 141].

At a three-fifth distance between the anterior superior iliac spine and the proximal border of the patella, rectus femoris was assessed for cross-sectional area [81].

At a distance of 75% between the anterior superior iliac spine and the proximal border of the patella, rectus femoris was assessed for physiological cross-sectional area [91].

At a distance of 50% between the anterior superior iliac spine and the midpoint of the patella, rectus femoris was assessed for muscle thickness [76], as was the vastus intermedius [76].

At a distance of 50% between the anterior superior iliac spine and the distal border of the patella, rectus femoris was assessed for muscle thickness and anatomical cross-sectional area [93].

At a distance of 50% between the anterior inferior iliac spine and the proximal border of the patella, rectus femoris was assessed for muscle thickness [88, 94], cross-sectional area [87, 88], anatomical cross-sectional area [119], fascicle length [88], pennation angle [88] and echo intensity [88, 94, 119]. Vastus intermedius was assessed for muscle thickness [94] and echo intensity [94].

At 10 cm proximal of the proximal border of the patella, rectus femoris was assessed for muscle thickness [142].

At 15 cm proximal of the proximal border of the patella, rectus femoris was assessed for muscle thickness [74] and anatomical cross-sectional area [143].

At 50% between the ischial tuberosity and the lateral condyle of the tibia, biceps femoris was assessed for muscle thickness [78].

Lower leg muscles

The lower leg muscles assessed were the gastrocnemius medialis, the soleus, and the tibialis anterior muscle. One study mentioned that as a bipennate muscle, both the deep and superficial part of the tibialis anterior was measured [97].

At 30% proximal between the medial condyle of the tibia and the medial malleolus of the fibula, the gastrocnemius medialis was assessed for muscle thickness [78, 103, 115], cross-sectional area [111], anatomical cross-sectional area [108], pennation angle [108, 115], fascicle length [108, 115] and echo intensity [108, 111].

Further measurements use landmarks from the muscle itself.

At 50% between the proximal and distal tendon insertion of the muscle, gastrocnemius medialis was assessed for muscle thickness [82, 102, 109], fascicle length [69, 97, 102, 110] and pennation angle [69, 97, 102, 107, 110].

At the most bulky area of the leg, gastrocnemius medialis was assessed for muscle thickness [70, 80, 112, 144], cross-sectional area [145], fascicle length [70, 145] and pennation angle [70, 145].

No studies assessed the gastrocnemius lateralis.

At 30% proximal between the medial condyle of the tibia and the medial malleolus of the fibula, the soleus was assessed for muscle thickness [78].

At 50% between the proximal and distal tendon insertion of the muscle, soleus was assessed for pennation angle [146].

At the most bulky area of the leg, soleus was assessed for muscle thickness [112].

For the tibialis anterior, there was only one study, assessing pennation angle and fascicle length at 50% of muscle length, without giving clear anatomical landmarks [97].

Upper arm muscles

The upper arm muscles assessed were the biceps brachii and triceps brachii. Less studies used clear anatomical landmarks for the upper arm muscles than for the upper leg muscles.

At 50% between the acromion and the cubital fossa with the elbows flexed at 90 degrees, biceps brachii was assessed for muscle thickness [113].

At two-thirds between the acromion and the antecubital crease with the arms fully stretched, biceps brachii was assessed for muscle thickness [57].

At 50% of muscle length (not defined how this is determined), biceps brachii was assessed for fascicle length [97] and pennation angle [97]. This article also mentions that since the fascicles were almost parallel to the superficial aponeurosis, no fascicle length was measured as too much extrapolation was needed [97].

At maximal girth of the upper arm, biceps brachii was assessed for anatomical cross-sectional area [143].

At 40% between the acromion process of the scapula and the lateral epicondyle of the humerus (starting at the lateral epicondyle), triceps brachii was assessed for muscle thickness [114] and pennation angle [114].

At 40% between the acromion process of the scapula and the lateral epicondyle of the humerus (starting at the lateral epicondyle), triceps brachii was assessed for muscle thickness [115] and pennation angle [115].

At 50% between the posterior crista of the acromion and the olecranon triceps brachii was assessed for muscle thickness [116] and pennation angle [116].

Discussion

It is clear from the multitude of different measuring points that there is little consistency in the current ultrasonographic muscle assessment. To advance US as a routine technique

Table 3 Consensus proposition, shortcomings in knowledge and protocol listings for patient positioning pre-investigation

Consensus proposition:
No exercise 30 min before investigation
Preferably minimum 30 min (maximum 60 min) in the same position before
Investigation, for measurements in recumbent position
Muscle should be assessed in a relaxed state
If the patient is placed in a recumbent position, it is recommended to use the full extension position (either supine or prone)
Shortcomings in knowledge:
Exact influence of (minor) muscle exercise on measurements
To be mentioned in the protocol:
Preparations in advance of the investigation (amount of minutes rest, in which position)
State of muscle being investigated (relaxed, contracted)
Which position the patient is placed in, including the angles of the relevant joints, clearly describing which angle is meant exactly
Whether left/right side was taken and whether this was the dominant/non-dominant side
Sex and age of patient

to be used for muscle quality and quantity assessment in old age, standardization is paramount. In the following paragraphs, the possible points of discussion will be addressed regarding the different aspects of measurement. A consensus proposition will be given at the end of each paragraph. Shortcomings in knowledge will also be addressed, as more studies need to be done before able to give recommendations about this subject. We will also propose what information should minimally be mentioned in a study protocol.

Patient positioning pre-investigation

Since there is a clear difference between relaxed and contracted muscle [147], one state should be chosen. As it is easier to keep a muscle in a relaxed state than in a certain degree of contraction, it seems logical to choose for the former. For the record, it must be said that there is some evidence that contracted muscle correlates better with muscle function than muscle in a relaxed state [147]. Ideally, this should be studied for better intra- and interrater reliability and test–retest reliability. Also the state of contraction before an examination could possibly have an influence on the component measurements. Refraining study subjects from exercises for 24–48 h is only practical in a purely scientific setting [74, 75], not a clinical one, where one would want its patients to exercise as much as possible. Also letting patients performing an amount of maximal contractions [65] could possibly influence measurements. An older study has shown that intensive exercise can give a 15% increase in water content in a given muscle bulk [148], as also giving way to a possible large measuring error. As it is unclear to what degree minor contractions and light-to-moderate exercises influence muscle volume, it is advised not to let the study subjects do exercises before taking measurements. As there is no clear data on a timeframe, a period of minimal 30 min is proposed. Ideally, this period is spent laying down.

There are little data on how long patients should be in the same position in order to allow fluid shifts to stabilize [72], or to avoid muscle contraction-induced fluid shifts and muscle blood flow during the measurements [73]. Differences in measurements made in the standing and recumbent positions for example may be due to postural or positional forces acting on muscle shape (for instance joint angle), or due to physiological changes [149]. When going from standing to recumbent position, the most significant changes in thigh muscle size occurred within the first 15–20 min of recumbency, with a stabilization after 60 min of bed rest [150, 151]. When measuring a subject in a recumbent position, a minimum of 30 min and a maximum of 60 min in the same position prior to the measurements are thus advisable.

The patient positioning in itself is less important for measuring the five components, because depending on what one wants to measure, the positioning could change. However, it must be mentioned that muscle components are significantly different if measured in a standing or in a recumbent position. This is seen more in CSA than in muscle thickness or pennation angle [152]. If the patient is placed in a recumbent position, it is recommended to place the patient in full extension (described above), as in this position, most muscles are relaxed maximally. Mentioning the position in a protocol is thus strongly advised. Regarding dominant/non-dominant side, there is some discussion about the relevance of functional asymmetry and strength differences in dominant versus non-dominant side [153]. In non-athletic populations, inter-limb differences in strength are possibly more related to neural factors than pure muscle-related factors [86]. Since this is not clear in literature, it is advised to clearly indicate whether the dominant or non-dominant side is assessed.

Table 4 Consensus proposition, shortcomings in knowledge and protocol listings for system and system settings

Consensus proposition:
All types of ultrasound machine can be used, as long as B-mode is present
Extended field of view is not necessary but recommended
A linear transducer probe is recommended. A minimum length of 5 cm is advised
Inclination of the probe should be neutral, which is perpendicular to the skin
Using a generous amount of transmission gel is recommended
Maintaining the most minimal pressure possible between transducer and skin is recommended
Shortcomings in knowledge:
Exact influence of different system settings on measurements of echo intensity
To be mentioned in the protocol:
Manufacturer and type of US machine
Type of probe, including length of probe
Frequency of beam (other system setting, see "Components and measuring points: echo intensity")
Any additional software used in post-production of images

Consensus proposition, shortcomings in knowledge and protocol listings for patient positioning pre-investigation are addressed in Table 3.

System and system settings

The brand of ultrasonographic machine used is of no relevance for most of the measurements.

Standard B-mode is applied in all studies to visualize the different muscle components and is available on most machines.

Although a curved probe can be used [91], a linear transducer probe is more adapted to assess muscle anatomy.

The length of the transducer is less important for measuring the five main components. However, for assessing cross-sectional area and echo intensity, larger (longer) probes can potentially visualize more tissue, which can be helpful if a large muscle bulk is present. Therefore, a minimum length of 5 cm seems advisable. Extended field of view techniques could help solve this problem.

As no information on inclination of the probe is at hand, it is advised to keep the probe as much perpendicular to the skin as possible.

When applying the probe to the skin, it is important to avoid compression of dermal surface and distortion of muscle surface [72, 104, 154, 155]. Dupont et al. found that applying strong pressure with the ultrasound transducer could flatten the deltoid muscle by 50% or more and because this error is proportional to muscle size, the absolute error in muscle thickness measurement would be even greater for larger muscles [155]. To minimize both beam loss/scatter and need for dermal/muscle compression, the use of a transmission gel is standard in ultrasonographic investigations. There is no defined standard amount of gel to be used. It is advised to use a generous amount and maintain the minimal pressure possible/necessary between transducer and the skin [155].

The frequency of the transducer beam is less relevant for measuring components, except for assessing echo intensity. However, the higher the frequency, the better is the visualization of anatomical structures. Using ultrasound, there is therefore a constant compromise between image resolution and depth of penetration of the sound waves. Higher frequency transducers provide better spatial resolution, but these transducers have a shallower depth of penetrance than a lower frequency transducer [156].

To our knowledge, other system settings do not seem to be relevant for measuring the components, except for assessing echo intensity. Depth focus, general gain, time-gain compensation, dynamic range, etc. can be set in order to have the best possible view of the muscle that is to be assessed.

The use of software for post-processing and measurement is less relevant for measuring the components, except for assessing echo intensity. Most modern machines have software that allows measurements to be directly done during the investigation; otherwise, all other picture post-processing software can be used.

Measuring echo intensity is dependent on many factors. These are discussed under the paragraphs "Components" and "Measuring points".

Consensus proposition, shortcomings in knowledge and protocol listings for system and system settings are addressed in Table 4.

Components

The five components that can be easily measured when assessing muscle components are already mentioned: muscle thickness, pennation angle, fascicle length, echo intensity and cross-sectional area. The technique to measure these items is relative easy. The most difficult part is to define where to do the measurements. As there is no information available on the most ideal location within the muscle, there exists a multitude of measuring points. Since no scientific substantiated "best" point can be defined, consensus locations will be provided for each muscle described in literature.

It seems logical to use the thickest zone of a muscle when wanting to assess muscle thickness, as this will be the place that the muscle will generate the most contractive power. However, there are no studies to our knowledge that have looked at the evolution of muscle thickness throughout a specific muscle. Therefore, we advise different approaches for different kinds of muscles.

Theoretically, muscles like the four bellies from the quadriceps are the thickest at 50% of their length, measured from tendon to tendon [157]. Therefore, we propose to mark and use the point at 50% of the muscle's length (50% rule).

Unfortunately, not every muscle is shaped like the bellies of the quadriceps, e.g., the gastrocnemius medialis. This poses a certain difficulty, and a possible reason for measurement bias. In the literature, in this type of muscle the "maximal bulk" was most often noted as measuring point. We propose that in more asymmetrical muscles, the point of 50% length between tendons is visualized, and then 4 additional points are checked: at 30, 40, 60 and 70% length between

Table 5 Consensus proposition, shortcomings in knowledge and protocol listings for components

Consensus proposition:
Five components can be measured: muscle thickness, pennation angle, fascicle length, echo intensity and cross-sectional area
Measurements are ideally done at maximal muscle bulk
Depending on muscle anatomy, different techniques are advised for determining maximal muscle bulk
Panoramic vision and extended-field-of-view software are not absolutely necessary but recommended
In pennate muscles, measuring physiological CSA rather than anatomical CSA is recommended
When the fascicle length cannot be directly measured, it can be calculated using the standard formula
When measuring echo intensity, all system settings need to be kept the same. Currently, no proposition for specific system settings based upon literature can be done for echo intensity
Shortcomings in knowledge:
Exact point of maximal muscle thickness for each muscle
Changes of the main components (MT, CSA, FL, PA, EI) throughout the muscle bulk
A good measure for comparing echo intensity between different US machines/systems
To be mentioned in the protocol:
The muscle that is assessed, with inclusion of the anatomical landmarks that are used and the exact point in between the landmarks. If not the midpoint, clearly describe whether the proximal or distal end is meant
The components that are measured. If CSA is measured, define if anatomical or physiological CSA is meant
Total length of muscle (to calculate relative muscle thickness values)
The technique that is used to determine the position of maximal bulk

Table 6 Proposed anatomical landmarks for each muscle discussed

	Proximal landmark	Distal landmark	Asymmetry
Lower limb			
Rectus femoris	Greater trochanter	Proximal border of patella	Minimal
Vastus lateralis	Greater trochanter	Proximal border of patella	Minimal
Vastus medialis	Greater trochanter	Proximal border of patella	Minimal
Vastus intermedius	Greater trochanter	Proximal border of patella	Minimal
Biceps femoris (long head)	Ischial tuberosity	Proximal head of fibula	Minimal
Tibialis anterior	Lateral condyle (anterior) of tibia	US-measurement dependant	Minimal
Gastrocnemius (medialis)	Medial condyle (posterior) of the femur	US-measurement dependant	Minimal
Gastrocnemius (lateralis)	Medial condyle (posterior) of the femur	US-measurement dependant	Minimal
Soleus	Proximal head of fibula (posterior part)	Posterior superior part of calcaneus	Yes
Upper limb			
Biceps brachii	Anterior part of acromion process (acromioclavicular joint)	Elbow crease where tendon can be palpated	Yes
Triceps brachii	Most lateral distal part of acromion	Tip of olecranon	Yes

tendons. Then, the maximal muscle thickness can be chosen from these measurements. Without having large studies that provide reference data per muscle, this will have to be done per muscle, per patient. In muscles with severe asymmetry, e.g., biceps femoris, it is best to specify which part of the muscle is assessed, e.g., biceps femoris—long head.

Another variety is the muscle with a long tendon, which has no clear anatomical landmarks to indicate where the muscle bulk ends, e.g., tibialis anterior. In these muscles, it is advised to locate the proximal and distal border of the muscle through ultrasound before referring to the 50% rule.

Noting down the 100% length of the muscle from tendon to tendon is advised, as a longer muscle can potentially generate more power. It is not known whether absolute muscle thickness or relative muscle thickness (= muscle thickness/length of the muscle) is more representative [158]. To be complete, there could be other reasons to choose for a different measurement site in specific muscles. Ticinesi et al. [86] suggested to use the distal point of 65% of the length of the vastus lateralis, as this site is most free of vessels and muscle biopsies can be easily made, avoiding major vessels and nerves. However, without specific arguments for clinical correlates, this is not recommended. For this, studies are needed.

For measuring the maximal thickness of a muscle, it is advised not only to take the midpoint of the muscle in between the tendons, but at this point of the longitudinal axis, to use the point at 50% between the medial and lateral border of the muscle bulk. This will also have to be visualized through ultrasound and marked for easy Ref. [86].

The argument of using the thickest zone of the muscle bulk is also valid for measuring the cross-sectional area. One of the disadvantages of this point could be that in some cases it will be difficult to get a complete image of the CSA, in the case of a large muscle bulk. Panoramic vision and extended-field-of-view software that are almost standard on most US machines can nullify this problem. Also, since it is unknown how the muscle volume diminishes exactly towards the tendons, submaximal measurements do not weigh up to the maximal CSA. For this, studies are needed.

As said, cross-sectional area can be divided into anatomical CSA and physiological CSA. The former underestimates the number of total fibers in a pennated muscle, so in these types of muscles the use of the PCSA is advised. In non-pennate muscles, the ASCA is the same as the PCSA.

As there is no hard information available to our knowledge about the pattern of echo intensity or pennation angle throughout a given muscle—homogenous versus heterogenous [159]—the proposition is to take all measurements (including the fascicle length) at the point of maximal bulk, as discussed for muscle thickness and CSA.

There is no doubt that echo intensity is an important parameter. As a parameter of fatty infiltration of the muscle (myosteatosis), it helps unravel an important aspect of the process of sarcopenia. Myosteatosis is linked with increased mortality in specific populations [20, 160–162], with a strong need for further clinical investigation. However, the standardisation and comparability of measurements of echo intensity between different US system brands are appalling. Defined as the brightness of the image and expressed in gray scales, there are many factors (system relates) that influence the image. A small difference in beam frequency or gain—or many other settings—can give completely different results, and comparing system settings between different manufacturers is currently impossible. No good calibration model exists to date. Even the age of a probe can influence results by influencing the strength of the beam emitted. Also, although the analysis of muscle tissue acquired via biopsy suggests that echogenicity is more strongly associated with intramuscular adipose tissue rather than fibrosis [163, 164], biopsies are still needed to determine the percentage both components [165]. More studies are needed on both the comparability between systems and the differentiation of fat and fibrosis. Ideally, a universally accepted calibration dummy will be developed in the near future.

Consensus proposition, shortcomings in knowledge and protocol listings for components are addressed in Table 5.

Measuring points

For the appendicular muscle described, we refer to Table 6 for an overview of the proposed anatomical landmarks. These landmarks were selected in view of the discussion above, and with regard to the possible use of good-identifiable anatomical landmarks. As discussed in the paragraph about muscle thickness, some muscles show an asymmetry, either in length, width, or both. Table 6 also indicates whether a certain asymmetry exists and, thus, caution has to be taken in use of the 50% rule. When there are multiple origins, or if there is a larger area of insertion, the most identifiable/representative landmark is chosen. Future studies will have to confirm the usability of these landmarks.

Method of measurement

As a short overview, the proposed procedure of measurement will be shortly addressed. This procedure is based on the discussion above. It will be represented schematically as a sort of checklist.

- Place the patient in the desired position, preferably 30 min before investigation.
- Select the muscle that is to be assessed, and check for the desired technique to locate the maximal muscle bulk.

- Use the appropriate technique and the anatomical landmarks provided to locate the longitudinal measuring point. Mark this with a dermographic pencil.
- Locate the medial and lateral side of the muscle and mark these with a dermographic pencil. Use the middle point of these marks. Now the correct measuring point is found.
- Keeping the transducer probe in a longitudinal direction in line with the muscle fiber fascicles. At this position, measure muscle thickness (from aponeurosis to aponeurosis), pennation angle (angle of muscle fiber fascicles into the deep aponeurosis) and fascicle length.
- Turn the transducer probe 90°. At this position, measure cross-sectional area. As this will probably not be a perfect ellipse, the circumference of the muscle can be manually drawn with a cursor. Use this maximal area to also measure echo intensity.
- Repeat all measurements three times and use the mean value of these measurements.

Limitations

There are some limitations to this review. Although the search strategy was very broad, we have focused on appendicular muscle mass, and have not included facial, thoracic, abdominal and pelvic muscle. Also the incidental reports from smaller muscle groups from the hand were not included, because too little information was available per muscle. The muscle groups that were not included in this review could be the subject of future investigations.

Also some ultrasound-based techniques were not included in this review, such as elastography. This could also be the subject of a review in itself.

Future direction

The ultimate goal would be to have not only a standardized way to assess muscle characteristics, but also to have a workable algorithm to diagnose sarcopenia using ultrasound. For this, some barriers have to be taken.

First of all more insights should be gained into the age-related evolution of the different muscle characteristics. It seems reasonable to assume that muscle thickness and cross-sectional area for instance will decrease with age, but perhaps the pennation angle will start shifting before any thickness or volume dimensions change. This is important because making a diagnosis as early as possible also means that treatment can start earlier.

Secondly, reference values of a 'normal population' are lacking. In 2016, Minetto et al. [82] have already done some work on this, using muscle thickness values 2 standard deviations below the gender-specific means of a sample of younger subjects to diagnose sarcopenia. In this regard, one could discuss about using one set of cutoff points derived from a younger population, or if age-related reference values should be used. It is also clear that all reference points should be specific for each muscle. This means that every muscle should be examined separately, because cutoff points for, e.g., the vastus lateralis are different than those for the gastrocnemius.

Thirdly, it is too early to say if diagnosing sarcopenia with ultrasound should be done by a composite score or by a single measurement. In this stage, it seems advisable to measure all muscle characteristics and link this with diagnostic criteria that are currently used (e.g., BIA, DEXA). In a later stage, perhaps it will become clear that certain ultrasound-based characteristics are more important than others. For this, more studies have to be done.

Fourthly, the measurements proposed in this manuscript are not exhaustive. Perhaps other parameters are equal or more important, e.g., elastography of the muscle or tendon. Although an area that should certainly be explored, too little is known of this in the context of sarcopenia to currently make any statements.

In summary, there is certainly a dynamic towards using ultrasound for diagnosing sarcopenia, but more studies need to be done, preferentially starting with the creation of reference values.

Conclusion

To compare studies and advance the use of ultrasound in the assessment of sarcopenia, certain standardization has to be done. However, it is not yet clear which measuring points are more relevant than others. Therefore, the recommendations proposed in this review should not be regarded as set in stone. They are rather intended as a reference point or as a guideline, from which comparative studies can be initiated. Future directions can certainly involve changing some of the recommendations, if there is new evidence to support these changes. The limitations of this review are clear since there are a lot of unknown factors: the exact spreading pattern and evolution of the different architectural components throughout the muscle, which muscles or muscle measurements are most clinically relevant, influence of position of the patient, influence of pre-investigation activity, etcetera. Therefore, this consensus approach is used as a starting point. Hopefully, this way future studies will have an extra support to build upon. Also, this way interested researchers can collaborate towards an ultrasonographic diagnosis of sarcopenia.

In conclusion, this review offers a guideline for investigators wanting to set up a study using ultrasound in muscle assessment. Studies in clinical settings are needed to validate the effectiveness of these propositions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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