

## Original Article

# Assessing SARCopenia with ecHOgraphy in Community-Dwelling Older Adults: A Validation Study (SARCHO)

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## Abstract

**Objectives:** To prospectively evaluate the diagnostic accuracy of the sarcopenia echography (SARCHO) point-of-care ultrasound protocol for diagnosing sarcopenia, in accordance with the European Working Group on Sarcopenia in Older People 2. **Methods:** This study was conducted as a single-center prospective, feasibility diagnostic accuracy study among referred patients for falls assessment. They underwent an assessment for sarcopenia according to the EWGSOP2 criteria. Participants were subjected to physical testing: 30-second chair stand test, short physical performance battery (SPPB), timed-up and go (TUG) test, and imaging procedures using DXA(gold standard) and SARCHO. **Results:** 24 participants (15 women) with a mean age of 81 ( $\pm$  5.2) years were included. Nine participants were classified as sarcopenic by DXA and physical testing, whereas seven participants were classified as sarcopenic by SARCHO and physical testing, according to the EWGSOP2 criteria. SARCHO showed a diagnostic accuracy of 91.7% (95% CI: 81.9 - 97.2). When assessing the muscle architectural components, sarcopenic individuals showed lower muscle thickness, cross-sectional area, and pennation angle and a higher shear-wave kiloPascal value indicating higher degree of muscle stiffness. **Conclusion:** The SARCHO protocol is a promising point-of-care, bedside tool with high diagnostic accuracy, providing a valuable standardized and evidence-based approach for assessing sarcopenia.

**Keywords:** Geriatric, Muscle assessment, Point-of-care ultrasound, Sarcopenia, Ultrasound

## Introduction

Sarcopenia, defined as the loss of muscle strength, muscle mass, and impaired physical performance, has been recognized as one of the major syndromes in geriatric patients<sup>1</sup>. Sarcopenia is associated with functional decline, falls and fractures, decreased quality of life, increased hospital admissions, and mortality<sup>2</sup>. It is also a central parameter of the frailty syndrome in geriatric patients, i.e. the combined loss of functional reserves across different domains (e.g. physical ability, cognition, and energy) with a negative impact on health status<sup>3,4</sup>. When screening for a decline in functional capacity as a measure of the level of dependence in daily activities several frailty scores may

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be used. Many of these evaluate muscle function either via muscle strength and gait speed and/or muscle mass via bioimpedance (BIA) or whole-body dual-energy X-ray absorptiometry emphasizing the importance of muscle function evaluation among geriatric patients<sup>5-7</sup>.

The prevalence of sarcopenia differs across settings in older adults, with rates reported as high as 33% among nursing home residents and 26% among geriatric outpatients, using the lesser restrictive diagnostic criteria of 2010<sup>8,9</sup>. A recent study from Ireland, using the updated diagnostic criteria from 2019 show a prevalence of approximately 20% of probable sarcopenia in community dwelling older citizens<sup>10</sup>. It is of major importance to secure standardized assessment and diagnosis of sarcopenia to be able to provide evidence-based treatments such as nutrition and physical therapy.

An updated standardization and recommendation of assessment of sarcopenia has been suggested in the latest revision of the definition of sarcopenia by the European Working Group on Sarcopenia in Older People (EWGSOP)<sup>2</sup>. The diagnosis of sarcopenia is based on muscle quantity (muscle mass) and muscle quality (strength and endurance), while the severity of sarcopenia is based on physical performance<sup>2</sup>. Muscle quantity is typically assessed using the gold standard whole-body dual-energy X-ray absorptiometry (DXA) scan, with an established threshold for low appendicular skeletal muscle mass (ASM) of <20 kg for men and <15 kg for women<sup>2</sup>. However, assessment of muscle quality remains an area for debate since no current standardization nor consensus exist. Hence, no technical recommendation either in research nor in clinical practice on which part of the muscle architecture that can be proven valid for measurements and representation of muscle quality<sup>11</sup>.

The use of echography – also known as ultrasound – is an evolving area within imaging of the muscles and has several advantages compared to DXA, CT-, or MR scans. It is a non-invasive method without any radiation exposure, which can be carried out bedside using handheld equipment. Furthermore, it can validly measure muscle quantity like whole-body DXA<sup>12-14</sup> and, in contrast to whole-body DXA, it can also be used to assess muscle quality by examining the elements of muscle architecture (e.g. pennation angle and fascicle length)<sup>15,16</sup>. With the formation of the Sarcopenia Assessment Through Ultrasound (SARCUS) project<sup>17</sup>, great efforts have been made to standardize quantitative and qualitative ultrasound measurements for muscle assessment. Recently, an important tool for assessing muscle quality by ultrasound, shear-wave elastography<sup>18</sup>, has emerged. This technology is now incorporated in many ultrasound scanners and can be carried out as part of 2-D real time B-mode ultrasound<sup>18</sup>. Ultrasound can therefore be used to assess both muscle mass and muscle quality, i.e., sarcopenia.

As of today, no collective point-of-care protocol for

examining muscle quantity and quality exists. Thus, there is an urgent need for investigating potential point-of-care image modalities for evaluating muscle quantity and quality in the assessment of sarcopenia. Therefore, the aim of this study was to compare the diagnostic accuracy of the sarcopenia echo (SARCHO) point-of-care ultrasound protocol with whole-body DXA scans for diagnosing sarcopenia, based on the EWGSOP2 criteria on image modality, in a cohort of geriatric outpatients referred to a falls clinic, in a feasibility manner.

## Methods

### Study design

This study was carried out as a single-center prospective diagnostic accuracy study performed in a feasibility manner.

### Setting

Participants were recruited among patients referred to the outpatient falls clinic of the Department of Geriatric Medicine, Odense University Hospital (OUH)-Svendborg, Denmark. The study was conducted by a physician in geriatric medicine, who has formal education and expertise in the use of point-of-care ultrasound. Physical tests were carried out by experienced physiotherapists with knowledge of geriatric syndromes, e.g. functional decline, fall, postural instability, and impaired gait.

### Outcomes

The primary outcome was to compare the diagnostic accuracy of the SARCHO point-of-care ultrasound protocol for diagnosing sarcopenia, according to the EWGSOP2 criteria against whole-body DXA-scan in a cohort of falls patients in a geriatric outpatient clinic.

The secondary outcome was to compare the elements of muscle architecture and physical performance between participants with and without sarcopenia.

Elements for muscle architecture were muscle thickness (MT), fascicle length (FL), pennation angle (PA), cross-sectional area (CSA), fascicle length/muscle thickness ratio (FL/MT), quadriceps volume (QV) and shear wave elastography (SWE). Elements of physical performance were timed up and go (TUG), 30-second-Chair-Stand-Test and SPPB.

### Study population

Fallers (2 or more falls within the last year) ≥65 years of age with more than 2 diseases and categorized them as either sarcopenic or non-sarcopenic according to the following:

#### Sarcopenic participants

- 1) impaired chair stand test:
  - i) >15 seconds for 5 repetitions<sup>2</sup>
- 2) appendicular skeletal muscle mass (ASM) assessed by whole-body DXA scan <20 kg and <15 kg men and women, respectively.

3) a score of <8 points in the short physical performance battery (SPPB).

Conditions 1, 2, must apply for participants to be classified as sarcopenic. In addition, if condition 3 applies participants were categorized as severe sarcopenic.

#### Non-sarcopenic controls:

1) non-impaired chair stand test:

i)  $\leq 15$  seconds for 5 repetitions<sup>2</sup>

Or

2) ASM assessed by whole-body DXA scan  $>20$  kg and  $>15$  kg men and women, respectively.

In case participants had an abnormal score in SPPB and impaired chair stand test but a normal ASM in the whole-body DXA-scan, they were included as controls, hence non-sarcopenic, as defined by the FACS-algorithm by the EWGSOP2<sup>2</sup>.

Participants will have to undergo both ultrasound examination, physical testing and DXA scan in order to be included in this study.

### **Whole-body Dual-energy X-ray Absorptiometry (DXA)**

#### **Quantitative muscle measures**

The study participants underwent a whole body DXA scan to measure their appendicular muscle mass (ASM), as described in the EWGSOP criteria<sup>2</sup>.

All DXA scans were performed on a CE-certified DXA scanner (Hologic® Model: Horizon A (S/N 304635M)) located at Odense University Hospital locations in Nyborg or in Odense.

#### **The Ultrasound protocol**

The SARCHO protocol is based upon current evidence and recommendations regarding muscle ultrasound<sup>15,17-24</sup> with the purpose of creating a focused and easy point-of-care ultrasound protocol for stationary and handheld ultrasound devices. It evaluates muscle quantity and quality at specific muscle groups of the thigh, due to the importance of thigh muscles in physical function such as force generation, initiating the gait cycle, and balance adjustment<sup>25,26</sup>. When thigh muscles undergo atrophy, muscle function is impaired, ultimately resulting in falls<sup>27</sup>. The SARCHO ultrasound protocol is a two-sided protocol, which evaluates both muscle quantity and muscle quality (Figure 1 in the supplementary material). Below is a short overview of the performed muscle quantitative and qualitative measurements. For full overview of the SARCHO protocol please see the supplementary material.

#### **Quantitative muscle measures**

- 1) Appendicular lean muscle mass (using biceps brachii and the femoral rectus muscle)<sup>23</sup>
- 2) Muscle thickness (femoral rectus muscle)<sup>28,29</sup>
- 3) Cross sectional area (femoral rectus muscle)<sup>28,30</sup>
- 4) Quadriceps volume (femoral rectus muscle)<sup>24</sup>

If there is a discrepancy between DXA and ultrasound ASM results, where DXA classifies a patient as sarcopenic but ultrasound does not, the DXA ASM classification will be considered definitive.

### **Qualitative muscle measures**

#### Muscle of interest

The femoral rectus muscle, located at the anterior, middle part of the thigh and forming part of the femoral quadriceps muscle group, serves as an important flexor of the hip. The bipennate structure of the muscle makes it an important force generator during the gait cycle. During the loading response phase this muscle is essential due to its eccentric role at the knee during load bearing<sup>25</sup>. Additionally, it plays a role in the pre-swing and initial swing phase of the gait cycle because of its function as a hip flexor<sup>25,26</sup>. Therefore, this muscle was chosen for the study for its importance in the gait cycle, which correlates well with the physical tests performed by the participants.

#### Patient positioning

With the patient in a neutral, supine position, B-mode ultrasound was carried out on the rectus muscle at the middle part of the muscle, defined as half the length from the trochanter major (upper landmark) to the superior border of the patella (the lower landmark)<sup>15</sup>. A minimum of pressure was applied, and three sets of measurements was made, and the mean was noted. The following muscle quality parameters were measured:

- 1) Pennation angle
- 2) Fascicle length
- 3) Fascicle length/muscle thickness ratio<sup>31</sup>
- 4) Shear-Wave Elastography

### **Ultrasound Equipment**

A CE-certified GE LOGIQ ultrasound device was used for the 2-D real time B-mode ultrasound and shear-wave elastography scan. The assessor performing the SARCHO scan was blinded to the results of the physical tests. Time from inclusion in the study, where participants underwent SARCHO scan, to the time of DXA scan was approximately three months.

### **Physical test**

#### **Short Physical Performance Battery**

The SPPB comprises three tests that evaluate lower extremity functioning: gait speed (4 meters walking test), balance (tandem test), and chair stand (5 repetition chair stand test)<sup>32</sup>. The SBBP has been intensively validated<sup>33-35</sup> and the combined scores of the three SPPB tests varies from 0 (worst) to 12 (best). The EWGSOP2 recommends a cut-off score of 8 points for both genders to define the presence of severe sarcopenia<sup>2</sup>.

### Timed Up and Go test

TUG is a simple test for evaluating physical performance. The test is performed by asking the participants to rise from a chair and walk three meters, marked, and turn around, walk back to the chair, and sit down on the chair again<sup>36</sup>. The EWGSOP2 have defined cut-off value of 20 seconds for both genders<sup>2</sup>.

### 30 second Chair stand test

The number of repetitions to rise and sit participants could complete within the 30 seconds was measured using a chair with standardized seat height (43.2 cm)<sup>37</sup>. Danish reference values according to age and gender were used<sup>38</sup>.

### Sample-size

The known prevalence of sarcopenia in a population of Danish geriatric outpatients with no specific referral diagnosis 26%<sup>8</sup>. In this study, outpatients referred due to falls were included, aiming for a higher prevalence of sarcopenia at 33%, which has also been reported as a prevalence in other geriatric settings<sup>39</sup>. With an alpha value of 0.05 and a power of 0.8, a 10% sample of the total calculation was used in this study due to the feasibility design and setup.

The following equation was used:

$$N = \frac{0.26 \cdot 0.74 \left\{ 1.96 + 0.84 \sqrt{\frac{(0.33 \cdot 0.67)}{(0.26 \cdot 0.74)}} \right\}^2}{(0.33 - 0.26)^2} = 322 \cdot 0.10 \approx 32$$

Hence a total of 32 participants was selected as aim.

### Statistical Analysis

Normally distributed data are presented as a mean  $\pm$  standard deviation (SD). Normality was assessed using the Shapiro-Wilk test. Non-normally distributed data are presented as median with interquartile range (IQR). Results of numeric data were compared using paired t-test or Wilcoxon-rank sum test depending on the normality of the data. Diagnostic accuracy of SARCHO protocol was assessed through calculation of sensitivity and specificity, positive and negative predictive values as well as accuracy. 95% confidence intervals were calculated as Clopper-Pearson intervals<sup>40</sup>. All statistical analyses were carried out in R-statistics (version R-4.2.0 for Windows). As a limit for statistical significance a two-sided p-value of <0.05 was chosen.

### Ethics

Eligible participants were informed orally and in writing about the purpose of the study and all participants signed consent before inclusion. Participants could at any time withdraw from the study. The study is reported in line with STARD 2015 statements for diagnostic accuracy studies<sup>41,42</sup>. The study was registered at the Region of Southern Denmark record of data processing activities

Participants, n (%)	24 (100%)
Women, n (%)	15 (63%)
Age, years (mean, SD)	81 $\pm$ 5.2
Height, m (mean, SD)	1.65 $\pm$ 0.10
Weight, kg (mean, SD)	71.2 $\pm$ 13.0
BMI, kg/m <sup>2</sup> (mean)	26.0 $\pm$ 4.1
Prior falls within the last year, n (mean, SD)	1.0 $\pm$ 0.74
Walking aid, n (%)	13 (54%)
Single point stick	4 (17%)
Four-point stick	1 (4%)
Four wheeled walker	8 (33%)
Charlson Comorbidity Index, score (mean, SD)	5.6 $\pm$ 1.5
BMI: Body mass index, SD: Standard deviation.	

**Table 1.** Baseline characteristics.

(Project identification number: 21/50801). Data was processed and stored in accordance with EU General Data Protection Regulation (GDPR) and the Danish Data Protection Act. In addition, the study was approval by the Regional Committees on Health Research Ethics for Southern Denmark (Project identification number: S-20210100) and was conducted in accordance with the Declaration of Helsinki (1964) and its later amendments.

### Results

A total of 32 participants were included in the study, but eight participants did not attend the DXA-scan, leaving a total of 24 participants. The participants (n=24) had a mean age of 81  $\pm$  5.2 years, with the majority being women (63%) and having a mean BMI of 26.0  $\pm$  4.1. Half of the participants (54%) reported using walking aids, with preferred walking aid being a four-wheel walker (33%). Most participants had multiple comorbidities with a mean (SD) Charlson Comorbidity Index of 5.6  $\pm$  1.5. The baseline characteristics of the study population are summarized in Table 1.

Nine participants (37.5%) were identified as sarcopenic using DXA as image diagnostic modality and seven participants (29.2%) using SARCHO as image modality. Table 2 illustrates the differences in ASM between sarcopenic and non-sarcopenic groups, measured by DXA and SARCHO. Sarcopenic individuals demonstrated significant differences in muscle thickness (p=0.009), cross-sectional area (p=0.01), pennation angle (p=0.008), and SWE kPa (p=0.02) compared to

Group	DXA	Ultrasound	Median DXA	Median Ultrasound	IQR DXA	IQR Ultrasound	p
Control	15	17	17.32	17.44	6.265	5.450	0.69
Sarcopenia	9	7	13.12	14.65	5.980	5.695	0.17

Number of participants classified as sarcopenia using either DXA: dual x-ray absorptiometry or Ultrasound: Sarcopenia Echo (SARCHO) protocol. Appendicular skeletal muscle mass in kilograms (kg) was estimated from the two-imaging modality and reported as median, corresponding to the 50<sup>th</sup> percentile. IQR: Interquartile range, reporting the range in kg from the 25<sup>th</sup> to 75<sup>th</sup> percentile. Descriptive statistics were reported using the Wilcoxon-rank sum test.

**Table 2.** Classification of sarcopenia by DXA vs. SARCHO according to EWGSOP2.

Parameter	Sarcopenic (n=7)	Non-sarcopenic (n=17)	P
Muscle thickness (mean, SD) cm	1.49 ± 0.2	1.78 ± 0.3	<b>0.009*</b>
Cross-section area (mean, SD) cm <sup>2</sup>	5.84 ± 1.5	7.94 ± 2	<b>0.01*</b>
Fascicle length (mean, SD) cm	7.53 ± 1.3	6.41 ± 1.1	0.07
Pennation angle (mean, SD)°	11 ± 2.0	13.9 ± 1.7	<b>0.008*</b>
Fascicle length/muscle thickness ratio (mean, SD)	5.12 ± 1.3	3.69 ± 0.71	0.09
Quadriceps volume cm <sup>3</sup> (mean, SD)	1280.8 ± 74.8	1362.3 ± 115.2	0.54
SWE kPa (mean, SD)	13.9 ± 3.3	11.6 ± 2.1	<b>0.02*</b>
SWE velocity/ms (mean, SD)	2.15 ± 0.3	1.96 ± 0.2	0.15

Ultrasound: Sarcopenia Echo (SARCHO) protocol was used for ultrasound examination. Muscle thickness was reported as centimetres (cm). Cross-section area was reported as cm<sup>2</sup>. Fascicle length was reported as cm. Pennation angle was reported as degrees. Quadriceps volume was reported as cm<sup>3</sup>. SWE: Shear-wave elastography, ms: meters per second, kPa: kilo Pascal. SD: Standard deviation. A p-value of <0.05 was considered statistically significant and is indicated with an asterisk (\*).

**Table 3.** Muscle architecture characteristics including shear-wave elastography in sarcopenic and non-sarcopenic participants identified by SARCHO-protocol.

Parameter	Sarcopenic (n=7)	Non-sarcopenic (n=17)	P
<b>Short Physical Performance Battery (SPPB):</b>			
SPPB Chair stand test	1 ± 0.7	2 ± 1.5	0.001
SPPB Tandem test	2 ± 0.8	3 ± 1.5	0.14
SPPB Gait speed	2. ± 1.1	3 ± 0.7	0.11
SPPB Total	5. ± 1.6	9 ± 1.5	0.002
30-second Chair stand test	7 ± 2	9 ± 3	0.13
Timed Up and Go, s (mean, SD)	19 ± 4.8	12.7 ± 2.6	0.05

Ultrasound: Sarcopenia Echo (SARCHO) protocol was used for ultrasound examination, SD: Standard deviation. SPPB: Short Physical Performance Battery, where a score of <8 is considered significantly impaired physical function, as according to the European Working Group on Sarcopenia in Older People 2 criteria.

**Table 4.** Physical performance for sarcopenic and non-sarcopenic participants, identified by SARCHO-protocol.



Diagnostic accuracy (95% CI)	Sensitivity	Specificity	PPV	NPV	Accuracy
SARCHO	78 (55.4 to 88.7)	100 (100 to 100)	100 (100 to 100)	88.2 (75.0 to 90.3)	91.7 (81.9 to 97.2)

*Data are presented as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy with corresponding Clopper-Pearson intervals for SARCHO.*

**Tabel 5.** Diagnostic accuracy of the SARCHO protocol.

non-sarcopenic (Table 3).

The physical performance outcomes assessed using SPPB, TUG, and 30-seconds Chair Stand Test showed that sarcopenic participants exhibited significantly impaired performance in the total SPPB score  $5.1 \pm 1.6$  vs.  $9 \pm 1.5$ ,  $p=0.002$ ) and the TUG  $18.9 \pm 4.8$  vs.  $12.7 \pm 2.6$ ,  $p=0.05$  when compared to non-sarcopenic, but not in the 30-second Chair Stand Test  $6.8 \pm 2$  vs.  $9 \pm 2.7$ ,  $p=0.13$  (Table 4).

The diagnostic accuracy of the SARCHO protocol yielded a sensitivity of 78% (95% CI: 55.4 - 88.7), specificity of 100% (95% CI: 100 - 100), positive predictive value of 100% (95% CI: 100 - 100), negative predictive value of 88.2% (95% CI: 75.0 - 90.3), and an overall accuracy of 91.7% (95% CI: 81.9 - 97.2).

## Discussion

In this study, which included participants from a geriatric outpatient falls clinic, 37.5% of participants were identified as sarcopenic using DXA as the imaging modality, and 29.2% when using SARCHO. Sarcopenic participants showed significantly poorer physical performance in the SPPB and TUG tests. The overall diagnostic accuracy of SARCHO was high, reaching 91.7% when compared to DXA.

To our knowledge, this is the first study to explore and validate a comprehensive point-of-care ultrasound protocol for assessing sarcopenia, focusing on both muscle quantity and quality. The evaluation of the proposed protocol yielded promising results in terms of diagnostic accuracy regarding muscle quantity. When compared to the gold standard (DXA) the SARCHO protocol demonstrated a sensitivity of 78%, ensuring the identification of a substantial proportion of true-positive cases. The high specificity (100%) and positive predictive value (100%) underscore the tool's ability to correctly classify individuals without sarcopenia. The negative predictive value of 88.2% suggests that the SARCHO protocol is effective in ruling out sarcopenia in those individuals who are genuinely not affected. The overall accuracy of 91.7% indicates the robustness of the protocol in accurately identifying individuals with low muscle quantity as seen in sarcopenia, providing confidence in its clinical utility. These findings suggest that ultrasound could be a potential tool for bedside estimation

of ASM. However, its moderate sensitivity raises concerns about potential false negatives, underscoring the need for complementary diagnostic tools or follow-up testing in high-risk populations to ensure cases are not missed.

Interestingly, several prior studies have examined the diagnostic accuracy of another widely used bedside method for ASM estimation in older adults, bioimpedance analysis (BIA), in comparison to whole-body DXA<sup>43-45</sup>. In these studies, BIA showed lower overall accuracy and specificity compared to our results using SARCHO<sup>43-45</sup>. Additionally, unlike ultrasound, BIA cannot be performed on participants with a pacemaker device<sup>46</sup>, further limiting the potential of the method for ASM estimation in older adults.

SARCHO offers clear point-of-care advantages: it is bedside-ready, highly portable, and can be deployed by trained clinicians without specialized facilities. These features could lower per-patient costs and minimize logistical barriers compared with conventional imaging.

Together, these attributes strengthen its relevance as a pragmatic, scalable tool for routine frontline assessment.

In addition, this study made use of the fixed absolute ASM threshold values (e.g. <20 kg for men and <15 kg for women). This approach was used since a majority of studies are reporting ASM in regards to Sarcopenia assessment in this way. However, the height adjusted ASM (e.g. ASM/height<sup>2</sup>), which also have defined threshold values by the EWGSOP2, might have altered the results reported. Future studies should examine the differences in absolute ASM and height adjusted ASM in falls patients and the effect on the accuracy of the SARCHO protocol.

Although muscle quantity is a central part of sarcopenia assessment, muscle quality is widely acknowledged as an important feature to address, as it is strongly associated with muscle function<sup>47</sup>. However, evaluating muscle quality in a systematic and bed-side approach is challenged by the lack of standardization<sup>11</sup>. Thus, the need for an evidence-based, bed-side protocol for muscle quality evaluation is strong. SARCHO meets this need and has the advantage that it can be performed bedside by the attending physician. With SARCHO, the possibility of evaluating both muscle quantity and quality in a standardized and evidence-based approach could offer a deeper insight into sarcopenia at the muscle architectural level. This would facilitate a

better understanding of sarcopenia itself and highlight the importance of muscle quality in diagnostic process. This could help lay the groundwork for a future EWGSOP3 and an updated definition of sarcopenia.

The significant differences in MT, CSA, PA, and SWE between sarcopenic and non-sarcopenic groups are consistent with previous literature<sup>19,48</sup>. MT, CSA, and PA are all associated with muscle strength<sup>49</sup>, thus these findings are not surprising given the pathophysiology of sarcopenia. However, the higher SWE scores are interesting and somewhat surprising, indicating that stiffness of muscles due to sarcopenia-related alterations in muscle structure may play a central role in the manifestation of the condition. This may be due to the pathophysiological process of fibrosis of muscle components (myofibrosis) or fat infiltration in muscle tissue (myosteatosis), the latter often seen in sarco-obesity<sup>11,50,51</sup>.

Emerging ultrasound “muscle quality” metrics—e.g., echogenicity, texture, and stiffness—capture fatty infiltration and architectural disruption that are not reflected by muscle quantity alone, and have been linked to weakness, poorer physical performance, and adverse outcomes in sarcopenia.

Thus, integrating quality indices alongside mass (and strength/function) may improve risk stratification and clinical relevance, including monitoring response to interventions.

Nonetheless, broader adoption will require standardized acquisition/analysis protocols and consensus cutoffs to ensure comparability across centers.

The differences observed in mass between sarcopenic and non-sarcopenic participants, as measured by DXA and ultrasound, provide valuable insights into the structural alterations associated with sarcopenia. These findings highlight the intricate relationship between muscle architecture and the presence of sarcopenia as described in several previous studies<sup>2,16</sup>, reinforcing the need for a multifaceted approach in its assessment.

The baseline characteristics of the current study population underline the textbook characteristics of a geriatric cohort. This study showed a higher prevalence of sarcopenia in patients specifically referred to a Falls Clinic compared to patients referred to a general geriatric outpatient clinic reporting 26% with sarcopenia<sup>8</sup>. A major difference between the two studies is the diagnostic approach to sarcopenia. Whereas Christensen et al. solely relied on DXA as image diagnostics, they also made use of different physical tests such as handgrip and gait speed test compared to DXA, ultrasound, 30-Second chair stand test, SPPB and TUG in the current study. Theoretically, the differences in physical testing should not be a factor explaining the differences in reported prevalences of sarcopenia since these specific physical tests are all recommended by EWGSOP2. Since falls is associated with sarcopenia and sarcopenia is a key mechanism in falls<sup>52</sup>,

the higher percentage of patients with falls in this present study (100%) compared to the study by Christensen et al. (78%)<sup>8</sup> might also explain the prevalence difference. In addition, in the study by Christensen et al, participants were younger (79 vs. 81 years in the present study), which might also explain some of the difference since age is associated with sarcopenia<sup>2</sup>. Furthermore, differences in socioeconomic status and geographic areas could have a part in explaining these differences<sup>4</sup>. In addition, the observed differences in sarcopenia prevalence across studies may be attributable to the diagnostic criteria employed, particularly the distinctions between EWGSOP1 (Christensen et al.) and EWGSOP2 (this current study). These changes affect both the sensitivity and specificity of sarcopenia detection, resulting in variability in reported prevalence rates.

The significantly poorer performance of sarcopenic subjects compared to those without sarcopenia in SPPB and TUG tests ( $p=0.002$  and  $p=0.05$ , respectively) aligns with the previously reported studies (2). In contrast, no significant differences were found between the two groups in the 30-second Chair Stand Test ( $7\pm 2$  vs  $9\pm 3$ ,  $p=0.13$ ). The reason for this remains unknown but might be due to lack of power.

Overall, the findings in this study underscore the clinical relevance of a multifaceted approach when assessing sarcopenia to meet the complexity of the diagnosis: assessing muscle quantity, muscle quality, and physical performance. These findings contribute to the growing body of literature on sarcopenia assessment and offer potential avenues for further research. The use of ultrasound as a reliable, safe, and patient-centered tool for clinical examination and diagnosis of sarcopenia could ensure quick assessment, intervention, optimal treatment, and serve as a tool for follow-up and evaluating progress or regress. Patients often have to wait several months for a DXA scan but with the rapid and easy assessment of sarcopenia using ultrasound could ease the diagnostic route for the patients.

### Strengths and Limitations

The study has several strengths. First, it was conducted on a highly relevant cohort of geriatric patients with falls in an outpatient clinic. Since sarcopenia is associated with falls<sup>52</sup>, this cohort is representative for assessing the sarcopenia in geriatric patients. Second, the high overall diagnostic accuracy of 91.7% indicates a trustworthy, bedside protocol for clinical examination. It is also easy to perform. Third, the negative predictive value of 88.2% indicates that the SARCHO protocol is effective in ruling out sarcopenia. Finally, SARCHO was performed bedside at the patients' site and thus has the potential to provide the diagnosis of sarcopenia at the patient's first visit in the outpatient Falls Clinic. Quick and thorough diagnosis is important in sarcopenia, as early intervention and nutritional guidance are of great importance for effective treatment of the condition. SARCHO as a novel tool for

diagnosing Sarcopenia would enable for examining patients in a cross-sectional setting – more specific at home. This potential implementation and use of SARCHO could greatly benefit the patients, since quicker diagnosis allows for a more rapid intervention and hopefully a better outcome.

While this study provides valuable insights, some limitations could be considered. The relatively small sample size and the single-center nature of the study may limit the generalizability of our findings. Also, the decision of eight participants to decline the DXA-scan could potentially lead to a selection bias. However, the current dataset does not include information on the reasons for refusal, so this cannot be explored further. The differences in mass estimation between DXA and SARCHO might be due to elevated MT values by ultrasound, used in the muscle mass equation. This could be due to accumulation of the intramuscular connective tissue components such as enlarged epimysium (e.g. the connective tissue surrounding the muscle before merging with the fascia)<sup>53,54</sup>. Thus, including a relative proportion of non-functional muscle in the MT values. The role of accumulation of the intramuscular connective tissue components in older adults in physical function evaluation and muscle mass estimation should be investigated in future research. While the observed mean BMI and subsequent variance (mean (SD) 26.02 (4.08)) suggests a cohort with a certain variability in body composition, adding complexity to the interpretation of sarcopenia-related outcomes, we cannot pursue this further in our study due to lack of specific data on sarco-obesity.

In addition, We used absolute appendicular skeletal muscle mass (ASM) cutoffs (<20 kg for men, <15 kg for women) because they were specified a priori in our protocol, are straightforward to interpret clinically, and enable comparability with prior studies that operationalized sarcopenia using the same thresholds. However, absolute cutoffs may misclassify individuals at the extremes of body size—overestimating low muscle mass in shorter participants and underestimating it in taller or larger-framed participants—thereby limiting generalizability across diverse populations. To mitigate this, future studies should incorporate indexed measures such as ASM/height<sup>2</sup>, and ideally report sensitivity analyses using both absolute and height-adjusted definitions. This dual approach would reduce size-related misclassification, improve cross-cohort applicability (including across sexes, ages, and ethnic groups), and facilitate harmonization with consensus recommendations that increasingly favor height-adjusted metrics.

Despite that this study adhere to the STARD criteria, the lack of AUC analysis should also be considered as a limitation, when interpreting results presented in this article.

## Conclusion

This study provides a comprehensive examination of sarcopenia, including structural and functional

assessments as well as diagnostic accuracy. Our findings suggest that SARCHO shows potential as a promising bedside tool, although confirmation in larger cohorts is warranted. It offers deeper insights into sarcopenia at the muscular level, providing a valuable standardized and evidence-based approach for assessing sarcopenia in both clinical settings and research. The observed impairments in muscle characteristics and physical performance further highlight the clinical implications of sarcopenia in the ageing population. Future research should prioritize larger and more diverse cohorts, addressing inter and intra-rater reliability, test the association between muscle architectural properties and physical functioning. Future studies should also seek validation against CT or MRI in future studies since these methods are gold standards for muscle architecture. If possible this should be done in a cross-sectional setting, to enhance the external validity of these results before implementing the protocol in clinical practice.

### Ethical approval

*This study was approved by the Regional Committees on Health Research Ethics for Southern Denmark (approval ID: S-20210100) and was conducted in accordance with the Declaration of Helsinki (1964) and its later amendments.*

### Consent to participate

*Written informed consent was obtained from every participant.*

### Authors' contributions

*Kristoffer K. Brockhattingen conceptualized and designed the study, conducted participant recruitment and data analysis, and wrote the final manuscript. Karen Andersen-Ranberg and Jesper Ryg contributed to the study design and provided critical revisions to the final manuscript. Stany Perkisas contributed to the ultrasound analysis and participated in reviewing and editing the manuscript. Ditte Beck Jepsen and Freja Gram assisted in the final preparation and refinement of the manuscript. All authors read and approved the final version of the manuscript.*

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### Disclaimer

*Dr. Ditte Beck Jepsen is a member of the Editorial Board of the Journal of Frailty, Sarcopenia and Falls (JFSF). The manuscript underwent peer review process by independent experts.*



## References

- Cruz-Jentoft AJ, Landi F, Schneider SM, Zúñiga C, Arai H, Boirie Y, et al. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age and ageing*. 2014;43(6):748-59.
- Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age and ageing*. 2019;48(1):16-31.
- Rockwood K, Song X, MacKnight C, Bergman H, Hogan DB, McDowell I, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ*. 2005;173(5):489-95.
- Swan L, Warters A, O'Sullivan M. Socioeconomic Inequality and Risk of Sarcopenia in Community-Dwelling Older Adults. *Clin Interv Aging*. 2021;16:1119-29.
- Walston J, Buta B, Xue QL. Frailty Screening and Interventions: Considerations for Clinical Practice. *Clinics in geriatric medicine*. 2018;34(1):25-38.
- Ibrahim K, Howson FFA, Culliford DJ, Sayer AA, Roberts HC. The feasibility of assessing frailty and sarcopenia in hospitalised older people: a comparison of commonly used tools. *BMC Geriatrics*. 2019;19(1):42.
- Dodds R, Sayer AA. Sarcopenia and frailty: new challenges for clinical practice. *Clinical medicine (London, England)*. 2016;16(5):455-8.
- Christensen MG, Piper KS, Dreier R, Suetta C, Andersen HE. Prevalence of sarcopenia in a Danish geriatric out-patient population. *Dan Med J*. 2018;65(6).
- Landi F, Liperoti R, Fusco D, Mastropaolo S, Quattrociocchi D, Proia A, et al. Prevalence and risk factors of sarcopenia among nursing home older residents. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2012;67(1):48-55.
- Murphy CH, McCarthy SN, McMorrow AM, Egan B, McGowan MJ, Rafferty S, et al. Prevalence and determinants of sarcopenia in community-dwelling older adults in Ireland. *Aging clinical and experimental research*. 2023;35(8):1651-60.
- Correa-de-Araujo R, Harris-Love MO, Miljkovic I, Fragala MS, Anthony BW, Manini TM. The Need for Standardized Assessment of Muscle Quality in Skeletal Muscle Function Deficit and Other Aging-Related Muscle Dysfunctions: A Symposium Report. *Frontiers in physiology*. 2017;8:87.
- Abe T, Loenneke JP, Thiebaud RS, Fujita E, Akamine T, Loftin M. Prediction and Validation of DXA-Derived Appendicular Fat-Free Adipose Tissue by a Single Ultrasound Image of the Forearm in Japanese Older Adults. *J Ultrasound Med*. 2018;37(2):347-53.
- Abe T, Thiebaud RS, Loenneke JP, Young KC. Prediction and validation of DXA-derived appendicular lean soft tissue mass by ultrasound in older adults. *Age (Dordr)*. 2015;37(6):114.
- Abe T, Thiebaud RS, Loenneke JP, Fujita E, Akamine T. DXA-Rectified Appendicular Lean Mass: Development of Ultrasound Prediction Models in Older Adults. *J Nutr Health Aging*. 2018;22(9):1080-5.
- Perkisas S, Bastijns S, Baudry S, Bauer J, Beaudart C, Beckwée D, et al. Application of ultrasound for muscle assessment in sarcopenia: 2020 SARCUS update. *Eur Geriatr Med*. 2021;12(1):45-59.
- Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *The Lancet*. 2019;393(10191):2636-46.
- Perkisas S, Baudry S, Bauer J, Beckwée D, De Cock AM, Hobbelen H, et al. The SARCUS project: evidence-based muscle assessment through ultrasound. *European geriatric medicine*. 2019;10(1):157-8.
- Bastijns S, De Cock AM, Vandewoude M, Perkisas S. Usability and Pitfalls of Shear-Wave Elastography for Evaluation of Muscle Quality and Its Potential in Assessing Sarcopenia: A Review. *Ultrasound in medicine & biology*. 2020;46(11):2891-907.
- Narici M, McPhee J, Conte M, Franchi MV, Mitchell K, Tagliaferri S, et al. Age-related alterations in muscle architecture are a signature of sarcopenia: the ultrasound sarcopenia index. *Journal of cachexia, sarcopenia and muscle*. 2021;12(4):973-82.
- Narici M. Human skeletal muscle architecture studied in vivo by non-invasive imaging techniques: functional significance and applications. *J Electromyogr Kinesiol*. 1999;9(2):97-103.
- Narici MV, Maganaris CN, Reeves ND, Capodaglio P. Effect of aging on human muscle architecture. *J Appl Physiol (1985)*. 2003;95(6):2229-34.
- Săftoiu A, Gilja OH, Sidhu PS, Dietrich CF, Cantisani V, Amy D, et al. The EFSUMB Guidelines and Recommendations for the Clinical Practice of Elastography in Non-Hepatic Applications: Update 2018. *Ultraschall in der Medizin (Stuttgart, Germany : 1980)*. 2019;40(4):425-53.
- Barbosa-Silva TG, Gonzalez MC, Bielemann RM, Santos LP, Costa CdS, Menezes AMB. 2 + 2 (+ 2) = 4: A new approach for appendicular muscle mass assessment by ultrasound. *Nutrition*. 2021;83:111056.
- Rothwell DT, Fong DTP, Stapley SA, Williams DJ. A clinically applicable tool for rapidly estimating muscle volume using ultrasound images. *European journal of applied physiology*. 2019;119(11-12):2685-99.
- Annaswamy TM, Giddings CJ, Della Croce U, Kerrigan DC. Rectus femoris: its role in normal gait. *Archives of physical medicine and rehabilitation*. 1999;80(8):930-4.
- Shiavi R, Bugle HJ, Limbird T. Electromyographic gait assessment, Part 1: Adult EMG profiles and walking speed. *Journal of rehabilitation research and development*. 1987;24(2):13-23.
- Powers SK, Lynch GS, Murphy KT, Reid MB, Zijdwind I. Disease-Induced Skeletal Muscle Atrophy and Fatigue. *Med Sci Sports Exerc*. 2016;48(11):2307-19.
- Maden-Wilkinson TM, Balshaw TG, Massey GJ, Folland JP. Muscle architecture and morphology as determinants of explosive strength. *European journal of applied physiology*. 2021;121(4):1099-110.
- Franchi MV, Longo S, Mallinson J, Quinlan JI, Taylor T, Greenhaff PL, et al. Muscle thickness correlates to muscle cross-sectional area in the assessment of strength training-induced hypertrophy. *Scand J Med Sci Sports*. 2018;28(3):846-53.
- Maughan RJ, Watson JS, Weir J. Strength and cross-sectional area of human skeletal muscle. *The Journal of physiology*. 1983;338:37-49.
- Narici M, Franchi M, Maganaris C. Muscle structural assembly and functional consequences. *The Journal of experimental biology*. 2016;219(Pt 2):276-84.
- Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *Journal of gerontology*. 1994;49(2):M85-94.
- Freire AN, Guerra RO, Alvarado B, Guralnik JM, Zunzunegui MV. Validity and reliability of the short physical performance battery in two diverse older adult populations in Quebec and Brazil. *J Aging Health*. 2012;24(5):863-78.
- Phu S, Kirk B, Bani Hassan E, Vogrin S, Zanker J, Bernardo S, et al. The diagnostic value of the Short Physical Performance Battery for sarcopenia. *BMC geriatrics*. 2020;20(1):242.
- Berková M, Topinková E, Mádllová P, Klán J, Vlachová M, Běláček J. [The "Short Physical Performance Battery" in the Czech Republic - the pilot and validation study in older persons]. *Vnitr Lek*.

- 2013;59(4):256-63.
36. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. *Journal of the American Geriatrics Society*. 1991;39(2):142-8.
  37. Jones CJ, Rikli RE, Beam WC. A 30-s chair-stand test as a measure of lower body strength in community-residing older adults. *Res Q Exerc Sport*. 1999;70(2):113-9.
  38. Suetta C, Haddock B, Alcazar J, Noerst T, Hansen OM, Ludvig H, et al. The Copenhagen Sarcopenia Study: lean mass, strength, power, and physical function in a Danish cohort aged 20–93 years. *Journal of cachexia, sarcopenia and muscle*. 2019;10(6):1316-29.
  39. Landi F, Liperoti R, Fusco D, Mastropaolo S, Quattrocioni D, Proia A, et al. Sarcopenia and mortality among older nursing home residents. *J Am Med Dir Assoc*. 2012;13(2):121-6.
  40. Clopper CJ, Pearson, E. S. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. *Biometrika*. 1934;26:404-13.
  41. Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *Bmj*. 2016;355:i5239.
  42. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *Bmj*. 2015;351:h5527.
  43. van den Helder J, Verreijen AM, van Dronkelaar C, Memelink RG, Engberink MF, Engelbert RHH, et al. Bio-Electrical Impedance Analysis: A Valid Assessment Tool for Diagnosis of Low Appendicular Lean Mass in Older Adults? *Front Nutr*. 2022;9:874980.
  44. Tognon G, Malmros V, Freyer E, Bosaeus I, Mehlig K. Are segmental MF-BIA scales able to reliably assess fat mass and lean soft tissue in an elderly Swedish population? *Exp Gerontol*. 2015;72:239-43.
  45. Gába A, Kapuš O, Cuberek R, Botek M. Comparison of multi- and single-frequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry for assessment of body composition in post-menopausal women: effects of body mass index and accelerometer-determined physical activity. *Journal of Human Nutrition and Dietetics*. 2015;28(4):390-400.
  46. Cornier MA, Després JP, Davis N, Grossniklaus DA, Klein S, Lamarche B, et al. Assessing adiposity: a scientific statement from the American Heart Association. *Circulation*. 2011;124(18):1996-2019.
  47. Naimo MA, Varanoske AN, Hughes JM, Pasiakos SM. Skeletal Muscle Quality: A Biomarker for Assessing Physical Performance Capabilities in Young Populations. *Front Physiol*. 2021;12:706699.
  48. Narici MV, Maganaris CN, Reeves ND, Capodaglio P. Effect of aging on human muscle architecture. *Journal of Applied Physiology*. 2003;95(6):2229-34.
  49. Angleri V, Ugrinowitsch C, Libardi C. Crescent pyramid and drop-set systems do not promote greater strength gains, muscle hypertrophy and changes on muscle architecture compared with traditional resistance training in well-trained men. *European Journal of Applied Physiology*. 2017;117.
  50. Montano-Loza AJ, Angulo P, Meza-Junco J, Prado CM, Sawyer MB, Beaumont C, et al. Sarcopenic obesity and myosteotosis are associated with higher mortality in patients with cirrhosis. *Journal of cachexia, sarcopenia and muscle*. 2016;7(2):126-35.
  51. Meister FA, Lurje G, Verhoeven S, Wiltberger G, Heij L, Liu WJ, et al. The Role of Sarcopenia and Myosteotosis in Short- and Long-Term Outcomes Following Curative-Intent Surgery for Hepatocellular Carcinoma in a European Cohort. *Cancers (Basel)*. 2022;14(3):720.
  52. Yeung SSY, Reijnierse EM, Pham VK, Trappenburg MC, Lim WK, Meskers CGM, et al. Sarcopenia and its association with falls and fractures in older adults: A systematic review and meta-analysis. *Journal of cachexia, sarcopenia and muscle*. 2019;10(3):485-500.
  53. Olesen AT, Malchow-Møller L, Bendixen RD, Kjær M, Mackey AL, Magnusson SP, et al. Intramuscular connective tissue content and mechanical properties: Influence of aging and physical activity in mice. *Experimental Gerontology*. 2022;166:111893.
  54. Purslow PP. The Structure and Role of Intramuscular Connective Tissue in Muscle Function. *Frontiers in physiology*. 2020;11:495.

## Supplementary Material

### S1 SARCHO (SARcopenia eCHO) ultrasound protocol

#### *The Ultrasound protocol*

A specific ultrasound protocol (SARcopenia eCHO (SARCHO)) was developed by a team of researchers at the Geriatric Research Unit at Odense University Hospital led by first author (KKB) for the purpose of the current study. The SARCHO protocol is based upon current evidence and recommendations regarding muscle ultrasound<sup>14,16-21</sup>.

The SARCHO ultrasound protocol evaluates both muscle quantity and muscle quality and is presented in figure S1.

#### Quantitative muscle measures

##### 1) Appendicular lean muscle mass (ALM):

B-mode ultrasound is carried out to quantify muscle mass by estimating ALM, which corresponds approximately to appendicular skeletal muscle mass (ASM). In research the terms are used interchangeably<sup>55</sup>. A validated ultrasound scanning protocol and a validated regression model, by Barbosa-Silva et al was chosen<sup>22</sup>, as it is easily feasible for a clinician and has a high correlation to whole-body dual x ray absorptiometry (DXA) estimates of ALM. The protocol uses (A) two scanning sites (upper arm and thigh), and (B) three anthropometric measures.

(A) With the participant in a horizontal position, relaxed, and arms and legs in neutral position muscle thickness (MT) is assessed at the following two scanning sites:

1. On the dominant upper arm, at the anterior distal third of the arm measured by the length from the acromion to the olecranon.
2. On the dominant thigh, anterior midway of the measured length between the anterior superior iliac spine to the proximal top of the patella.

If individuals are left-handed the left site will be used. If individuals are ambidextrous the right side will be used as site for measurement, in accordance with the original study<sup>22</sup>.

(B) The three anthropometric measurements are carried out with the participant in standing position:

1. With the elbow flexed at 90 degrees and the palm facing upwards, the length (centimeters) of the upper arm is measured posteriorly from the acromion to the olecranon.
2. The circumference of the dominant upper arm (in centimeters) is measured at the distal third of the arm length, as described in "A-1".
3. The circumference (in centimeters) of the dominant thigh is measured midway of the length between the anterior superior iliac spine to the proximal top of the patella, as described in "A-2".

The participant's height in meters, weight in kilograms, and information about their gender, the measured MT and

anthropometric values are used in an equation to calculate ultrasound ALM, as follows:  $ALM (kg) = 3.27 \times \text{gender (women} = 0; \text{men} = 1) \times \text{height (m)} + 0.2 \times \text{arm length (cm)} + 0.09 \times \text{dominant arm circumference (cm)} + 0.04 \times \text{dominant thigh circumference (cm)} + 1.25 \times \text{dominant arm MT (cm)} + 0.72 \times \text{dominant thigh MT (cm)} - 24.9$

2) With the patient in a neutral and horizontal position, B-mode ultrasound is carried out on the rectus femur muscle at the middle part of the muscle, defined as half the length from the trochanter major (upper landmark of the muscle) to the superior border of the patella (the lower landmark of the muscle). A minimum of three sets of measurements is made with a minimum of pressure applied and the mean noted.

The following muscle quantitative parameters was measured:

##### *a. Muscle thickness (MT):*

Transducer is placed transversely with orientation marker facing left. MT is a simple measure of the thickness of the muscle. Although it is widely disputed whether MT is a good measure of muscle quantity<sup>10</sup>, it was chosen due to the strong correlation with muscle force<sup>10</sup>.

##### *b. Cross sectional area (CSA):*

Transducer is placed transversely with orientation marker left. CSA was chosen due to the strong correlation to MT<sup>28</sup> and the strong relation to maximum muscle strength<sup>29</sup>.

##### *c. Quadriceps volume (QV):*

QV is associated with late phase voluntary torque during knee extension and is a strong predictor for muscle strength<sup>27</sup>. To estimate QV, we used a modified version of the validated method by Rothwell et al<sup>23</sup>. In short, we used the MT of the rectus femur muscle at 50% length, with patients lying in neutral and horizontal position, and calculated the QV by the following equation:  $QV \text{ cm}^3 = 996.168 + (4.664 \times MT \times \text{body mass index (BMI)})$ .

#### Qualitative muscle measures

##### *Muscle of interest*

The rectus femur muscle is located at the anterior, middle part of thigh as a part of the quadriceps femoris and serves as an important flexor of the hip. The bipennate structure of the muscle makes it an important force generator during the gait cycle. The muscle has been described as essential during the loading response phase because of its eccentric role at the knee when load bearing<sup>24</sup>. In addition, the muscle also plays a role in the pre- and initial- swing phase of the gait cycle due to its function as a hip flexor<sup>24,25</sup>. Hence the muscle was chosen due to its importance in the gait cycle which correlates well with the physical tests performed by the participants.

##### *Patient positioning*

With the patient in a neutral and horizontal position, B-mode ultrasound is carried out on the rectus femur

<p><u>Muscle quantitative measures:</u></p> <ul style="list-style-type: none"> <li>- Appendicular lean (muscle) mass (aLM) →(Transducer positioning: Transverse. Location: Upper arm and upper leg)</li> <li>- Muscle thickness (MT) →(transducer positioning: Transverse. Location: m. rectus femoris)</li> <li>- Cross sectional area (CSA) →(transducer positioning: Transverse. Location: m. rectus femoris)</li> <li>- Quadriceps volume (QV) – calculated →(transducer positioning: Transverse. Location: m. rectus femoris)</li> </ul>		<p><u>Muscle qualitative measures:</u></p> <ul style="list-style-type: none"> <li>- Pennation angle (PA): →(Transducer positioning: longitudinal. Location m. rectus femoris)</li> <li>- Fascicle length (FL): →(Transducer positioning: longitudinal. Location: m. rectus femoris)</li> <li>- Fascicle length/muscle thickness ratio (FL/MT) – calculated: →(Transducer positioning: Transverse + longitudinal. Location: m. rectus femoris)</li> <li>- Shear-Wave Elastography (SWE): →(Transducer positioning: Transverse. Location m. rectus femoris)</li> </ul>
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**Figure S1.** Description of the SARCHO protocol.

muscle at the middle part of the muscle, defined as half the length from the trochanter major (upper landmark of the muscle) to the superior border of the patella (the lower landmark of the muscle). A minimum of three sets of measurements is made with a minimum of pressure applied and the mean noted.

The following muscle quality parameters was measured:

1) Pennation angle (PA):

Transducer is placed longitudinally with orientation marker towards the patient's head. PA was measured because of its importance in generating force and shortening velocity during contraction of the muscle<sup>48</sup>. PA is a parameter of sarcomeres placed in parallel within the muscle<sup>30</sup>. A low PA, due to a decrease in parallel sarcomeres, often present in sarcopenic patients, has shown to be associated with decrease in concentric strength<sup>48</sup>.

2) Fascicle length (FL):

Transducer is placed longitudinally with orientation marker towards the patient's head. FL is a component of the PA, and is the length of the muscle fascicle, a parameter of sarcomere placed in series. FL is an indicator of eccentric strength<sup>30</sup> and has shown to be decreased in sarcopenic patients<sup>48</sup>.

3) Fascicle length/muscle thickness ratio (FL/MT):

Proposed as a new muscle measurement by Narici

et al for the evaluation of sarcopenia via vastus lateralis muscle<sup>18</sup>, the FL/MT-ratio is a novel concept in evaluating muscle status. The notion being that muscle thickness is likely to decrease in sarcopenia, whereas fascicle length to a lesser extent experiences a decrease, due to the muscle's insertion into bone via tendon tissue. Hence, a large ratio would be found in individuals with sarcopenia.

4) Shear-Wave Elastography (SWE):

Transducer is placed longitudinally with orientation marker towards the patient's head. Real time 2-D B-mode ultrasound SWE measures the stiffness of tissue by applying an acoustic radiation force impulse (ARFI). The ARFI leads to shear, which is the change of shape in tissue without an additional change in volume and by the process of shear a wave is produced, which is traced back to the ultrasound transducer<sup>17,21</sup>. Measurements are expressed either by kPa as a measure of elasticity or SWE velocity (m/s). The changes in muscle fiber components (e.g. myofibrosis, myosteosis, or other) will lead to a change in signaling, hence changes in velocity. However, there is little standardization in using SWE in assessing muscle quality<sup>21</sup>. In this study we obtained SWE measurements in kPa and cm/s according to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) of the m. rectus femoris<sup>21</sup>.