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Anatomical Variation in Rectus Femoris Muscle Stiffness Assessed by Shear Wave Elastography: Implications for Standardized Assessments

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Abstract

Objectives: Elastography offers a non-invasive method for evaluating muscle stiffness, but standardized protocols are lacking. We investigated age and site variation in rectus femoris stiffness and its associations with sarcopenia components. **Methods**: We assessed rectus femoris stiffness in the dominant leg using shear wave elastography at three sites 25%, 50%, and 75% of thigh length. We examined correlations between muscle stiffness, strength, body composition assessed by bioelectrical impedance, and cellular health measured by phase angle. **Results**: Sixty-eight participants were included (42 aged ≥60 years; 30 females). In older adults, stiffness increased significantly in the distal regions (median: 12.0 kPa proximal, 13.9 mid-thigh and 18.3 distal; p <0.001). In contrast, younger adults showed a significant decrease in stiffness from the proximal to mid-thigh regions (16.4 proximal, 12.7 mid-thigh, and 14.2 distal; p <0.001). At the proximal site, stiffness correlated strongly with phase angle (r = 0.53), moderately with muscle strength, and weakly with muscle mass. These associations became reversed at the mid and distal sites. **Conclusions**: Muscle stiffness varied by anatomical site and age. Its associations with muscle strength and phase angle also differed in magnitude and direction between measurement sites, highlighting the need for standardized site selection in elastography protocols.

Keywords: Sarcopenia, Shear wave elastography, Rectus Femoris, Muscle quality

Introduction

Sarcopenia is a generalized skeletal muscle disease defined by the presence of both low muscle mass and low muscle strength[1]. Its prevalence increases with age and represents a global health problem[1]. It is also associated with a range of adverse health outcomes, including falls, fractures, loss of independence and mortality[2].

According to the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), the diagnosis of sarcopenia requires the presence of reduced muscle strength and low muscle quantity or quality[3]. While there are well-

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established methods for assessing muscle strength and quantity, there is currently no global consensus on how to measure muscle quality. Muscle quality is a broad concept that includes micro- and macroscopic muscle characteristics. Proposed measures include intramuscular fat infiltration, muscle attenuation, strength-to-muscle mass ratios, and phase angle[3]. Passive mechanical stiffness is a biomechanical property of muscle that may be related to muscle quality[4-6]. Passive muscle stiffness (E) describes the relationship between applied force and the resulting tissue deformation[7]. It is linked to aging and muscle weakness[8-10]. Shear Wave Elastography (SWE), a non-invasive ultrasound-based technique, estimates tissue elasticity by analyzing the propagation speed of shear waves within tissue[4,7]. SWE is the most commonly used elastography method for assessing muscle stiffness[5].

Many studies have examined muscle stiffness, but inconsistent methodologies make cross-study comparisons difficult[4,5]. Factors such as the elastography type (e.g. strain or shear wave), ultrasound probe positioning, participant posture, the degree of muscle stretch, imaging depth, and differences in muscle architecture may affect measurements[4,5,11-14].

As shear wave elastography is an available, non-invasive, ultrasound-based technique that is free of ionizing radiation and can be performed rapidly at the point of care without waiting times, it has the potential to become an attractive tool for routine clinical assessment[4,5]. However, because the examination can be performed by different clinicians, detailed and standardized measurement protocols are essential to ensure reliable and comparable results. Therefore, establishing standardized measurement protocols that account for factors influencing muscle stiffness measurements is highly important.

The SARCUS Working Group has proposed standardized protocols for muscle ultrasound assessment[6,15]. The rectus femoris (RF) is among the most commonly assessed muscles in sarcopenia research, with ultrasound parameters showing associations with muscle mass, strength, and physical function[5,16-18]. Its accessibility and established use make it a suitable target for standardized assessment. The vastus lateralis (VL), another commonly assessed muscle, was included for comparison, with measurements performed at the mid-thigh site as recommended by SARCUS[6,15].

While some studies explored stiffness differences along a muscle's length, none have compared these patterns between younger and older adults. Therefore, the primary aim of this study was to characterize age- and longitudinal-axis-related variation in RF stiffness to help standardize SWE measurement protocols. As secondary aims, we examined stiffness across muscle layers and explored associations with sarcopenia components and phase angle.

Methods

Study Design and Sample

This prospective cross-sectional study was conducted between March and May 2025 in accordance with the Declaration of Helsinki and all relevant guidelines and regulations. All participants provided informed consent prior to participation. Older adults were recruited from the geriatric day clinic and geriatric wards at ZAS-Middelheim Hospital in Antwerp, Belgium. The younger adult group consisted of community-dwelling healthy volunteers. Due to the exploratory design and limited comparable data for this measurement protocol, an *a priori* sample size calculation was not performed; however, post hoc power analyses were conducted. Exclusion criteria included inability or unwillingness to provide informed consent, significant mobility impairments, physical limitations preventing the participant from lying supine during the examination or significant leg edema.

Data Collection

Baseline characteristics

Demographic data included sex and age of the participants. For the age-based comparison, a cutoff of 60 years was selected to distinguish between younger and older adults, as structural and electrophysiological alterations in skeletal muscle are commonly observed after the sixth decade of life, followed by a progressive decline with advancing age[19,20]. Because this study focused on biological differences in muscle stiffness, a threshold of 60 years was selected to better reflect biological changes in skeletal muscle associated with older age than the conventional administrative threshold of 65 years. In older adults aged ≥65 years, the Clinical Frailty Scale (CFS) and Parker Mobility Score (PMS) were additionally assessed.

Sarcopenia components

Muscle strength was evaluated using handgrip strength (HGS) and the chair stand test (CST). HGS was measured in both hands using a dynamometer. The highest value from three attempts per hand was recorded. The CST, which measures the time to rise from a chair five times without using the hands, assesses lower-body strength[3]. Before testing, all procedures were explained and demonstrated to the participants by the examiner, after which the participants performed the tests themselves.

Body composition was assessed using bioelectrical impedance analysis (BIA) with the InBody S10 device. Measurements were performed in the supine position using tetrapolar electrode placement (thumb and middle finger of both hands and both ankles). Participants were instructed not to eat or drink for at least two hours prior to the examination. Individuals with severe leg edema or pacemakers were excluded. Parameters including fat mass (FM), appendicular skeletal muscle mass (ASM), segmental

Parameter	Specification
Examined Muscles	Rectus femoris (RF), Vastus lateralis (VL) of the dominant leg
Muscle sites	RF: Proximal, Middle, and distal segments measured at 25%, 50%, and 75% of the thigh length (from the greater trochanter to the superior edge of the patella); VL: Mid segment measured at 50% of the thigh length
Measurement Points	Midpoint between medial and lateral borders of the muscle
Patient position	Supine on a flat examination table
Muscle relaxation	Fully relaxed; participants instructed to avoid any voluntary contraction. Younger participants were instructed to refrain from strenuous physical activity for at least 24 hours prior to testing
Ultrasound Machine	Canon Aplio 300
Ultrasound Probe	Linear transduce (5.8 cm- PLT-1005BT)
Probe pressure	Minimal pressure using a generous amount of ultrasound gel
Orientation	Longitudinal
Probe Inclination	Perpendicular to the skin
ROI: Main Analysis	0.79 cm^2, ROI positioned centrally within the muscle belly
ROI: Depth Analysis	0.071 cm^2
Acquisition depth	Adjusted individually to visualize the entire muscle with the ROI positioned centrally
Value	Mean value of three repetitions
Outcome	Young modulus (E)
Measurement Unit	Kilopascal (kPa)

Table 1. Shear Wave Elastography Protocol.

lean mass of the legs, and phase angle were calculated using the LookinBody120 software. ASM values were corrected for the extracellular water to total body water ratio to account for fluid overload[21].

Elastography Protocol

Muscle stiffness was assessed by measuring Young's modulus using shear wave elastography (SWE)[4,5]. The detailed SWE protocol is presented in Table 1.

The RF was examined at three anatomical sites: 25%, 50%, and 75% of the thigh length (measured from the greater trochanter to the upper edge of the patella)[15], representing the proximal, mid, and distal segments, respectively (Figure 1). The stiffness of the vastus lateralis (VL) was additionally measured at 50% of the thigh length. For the primary analysis, a larger region of interest (ROI) of 0.79 cm^2 was used[4] (Figure 2). To examine how muscle stiffness changes with depth, an additional depth-layer analysis was performed at the proximal and midpoint regions of the RF and the midpoint of the VL. Due to insufficient muscle thickness, the distal RF was excluded. A smaller ROI (0.071 cm^2) was used to measure stiffness in the superficial, intermediate, and deep muscle layers along the longitudinal axis from skin to bone, as presented in

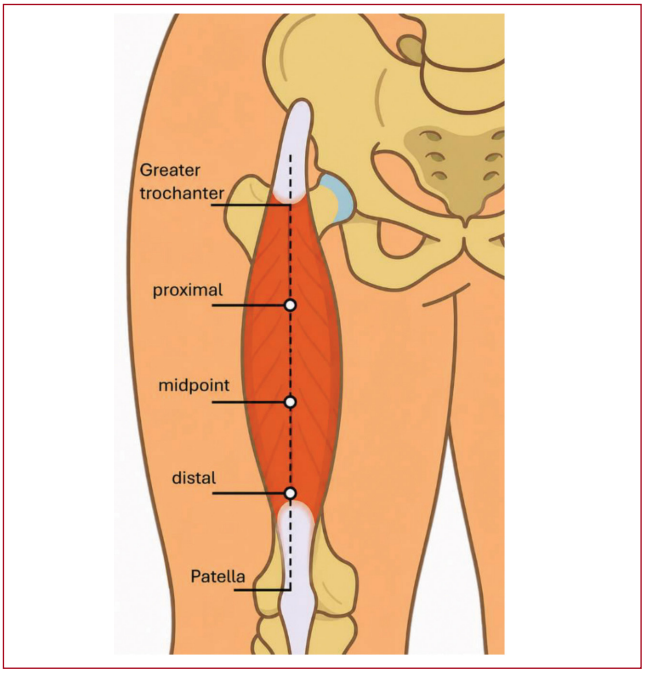

Figure 1. Longitudinal probe positions and anatomical sites along the rectus femoris muscle.

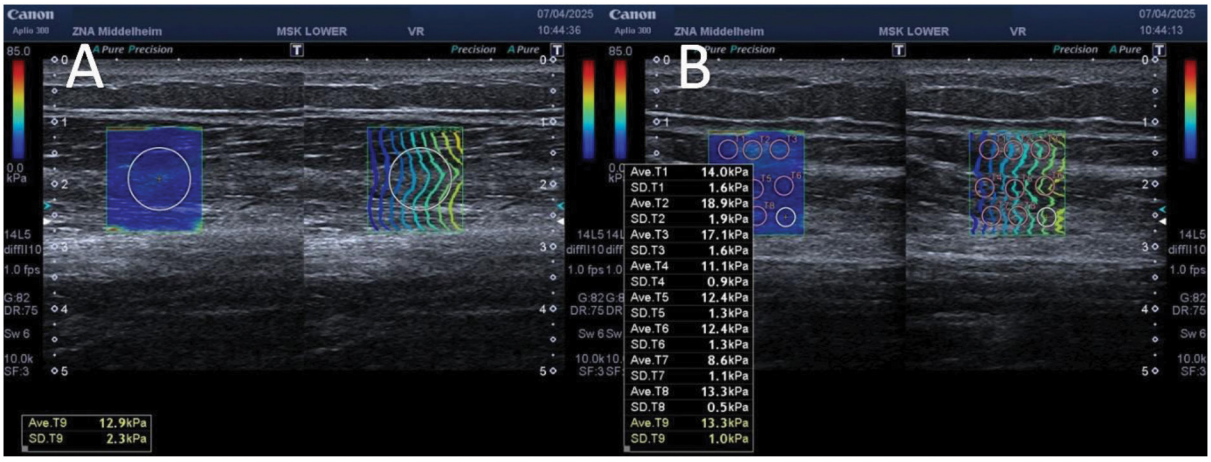

Figure 2. Rectus Femoris SWE Measurement. (A) Example shear wave elastography (SWE) measurement at the rectus femoris mid-portion in a 64-year-old male using the standard 0.79 cm^2 region of interest (ROI). (B) Example SWE measurements at the rectus femoris mid-portion in the same participant using small 0.071 cm^2 ROIs to compare muscle stiffness across three depth layers.

Figure 2. At each depth layer, measurements were repeated three times, and the mean value was used for analysis.

Statistical Analysis

Continuous variables are presented as mean (SD) or median (IQR), with group comparisons performed using independent *t*-tests or Mann–Whitney *U* tests, as appropriate. Muscle stiffness was analyzed using an aligned rank transform (ART) ANOVA due to the skewed distribution[22]. The primary analyses examined differences in RF stiffness across measurement sites separately in younger and older adults. A Bonferroni correction was applied to these two primary analyses, resulting in an adjusted significance threshold of $p < 0.025$. Effect sizes were estimated using partial eta squared. Post hoc comparisons were performed using aligned rank transform contrasts with Tukey adjustment for multiple comparisons[23]. Post hoc power calculations were based on a conventional ANOVA framework. All other analyses had an exploratory character and used a significance threshold of $p < 0.05$. Spearman rank correlations (ρ) assessed associations between muscle stiffness across anatomical sites, depth layers, and sarcopenia components. To identify factors influencing muscle stiffness, a multilevel mixed-effects model using measurements from the RF at its three anatomical sites was constructed. Model details are presented in Supplementary Material 1.

Missing data was minimal and assumed to be missing completely at random; therefore, no imputation or adjustments were performed. All analyses were conducted using **R** (version 4.4.1).

Results

Sixty-eight participants were recruited, including 42 aged 60 years or older. Overall, males (n = 38, 56%) had higher HGS and greater ASM, while female participants had higher FM. CST was comparable between males and females. Table 2 presents the sample characteristics stratified by sex. Stratification by sex and age group is provided in the Table S1. In the subgroup aged ≥65 years (n = 38), the median CFS was 4 [IQR 3–5], with 12 participants (31.6%) classified as frail (CFS ≥5). The median PMS was 7 [IQR 6–9]. Nine participants (23.7%) met the EWGSOP2 criteria for sarcopenia[3].

Four participants were excluded from body composition analysis because they had a pacemaker. SWE data was missing from one participant at the distal site of the RF due to a technical issue. Another participant lacked SWE depth measurements at the proximal RF site, and a participant was missing these measurements for the VL.

Effects of longitudinal probe positioning

Age-stratified muscle stiffness

ART-ANOVA showed that stiffness differed significantly across measurement sites in both younger ($F(2,50) = 8.24$, $p < 0.001$, $\eta p^2 = 0.25$) and older adults ($F(2,80) = 19.77$, $p < 0.001$, $\eta p^2 = 0.33$), with large effect sizes in both groups. Post hoc contrasts showed that, in

	Females (N=30)	Males (N=38)	P-value	Effect size
Age (years)				
Mean (SD)	60.6 (26.5)	60.5 (27.8)	0.985 [a]	0.003
Median [Q1, Q3]	70.5 [29.3, 82.8]	72.5 [28.5, 84.5]		
HGS (kg)				
Mean (SD)	22.4 (8.78)	34.0 (11.1)	**<0.001** [b]	**1.14**
Median [Q1, Q3]	21.0 [14.3, 29.0]	32.5 [28.0, 40.0]		
CST (seconds)				
Mean (SD)	12.5 (5.69)	13.7 (8.57)	0.977 [a]	0.005
Median [Q1, Q3]	12.2 [7.80, 15.8]	11.3 [7.42, 17.1]		
Not possible	2 (6.7%)	4 (10.5%)		
ASM (kg)				
Mean (SD)	18.5 (4.36)	25.7 (4.90)	**<0.001** [b]	**1.56**
Median [Q1, Q3]	19.1 [15.2, 21.3]	25.2 [21.4, 28.9]		
Missing	1 (3.3%)	3 (7.9%)		
ASMI (kg)				
Mean (SD)	6.94 (1.15)	8.38 (1.16)	**<0.001** [b]	**1.25**
Median [Q1, Q3]	7.08 [6.15, 7.56]	8.45 [7.72, 9.08]		
Missing	1 (3.3%)	3 (7.9%)		
Leg SMM (kg)				
Mean (SD)	7.18 (1.76)	9.86 (1.98)	**<0.001** [b]	**1.42**
Median [Q1, Q3]	7.57 [5.79, 8.68]	9.49 [8.40, 11.3]		
Missing	1 (3.3%)	3 (7.9%)		
FM (kg)				
Mean (SD)	24.4 (10.5)	16.6 (10.1)	**0.0042** [b]	**0.75**
Median [Q1, Q3]	22.9 [19.3, 28.9]	17.1 [7.60, 22.0]		
Missing	1 (3.3%)	3 (7.9%)		
Phase angle (degree)				
Mean (SD)	4.96 (1.11)	5.55 (1.56)	0.119 [a]	0.2
Median [Q1, Q3]	4.90 [4.30, 5.80]	5.60 [3.95, 6.90]		
Missing	1 (3.3%)	3 (7.9%)		

HGS – Maximum Handgrip Strength, CST – Chair Stand Test, ASM – Appendicular Skeletal Muscle Mass, ASMI – (ASM/height^2) Appendicular Skeletal Muscle Mass Index (normalized by height squared), Leg SMM – Dominant Leg Segmental Muscle Mass, FM – Fat Mass. Kg - Kilogram.
[a] Mann–Whitney U test; effect size reported as Wilcoxon effect size (r): 0.10–<0.30 = small, 0.30–<0.50 = moderate, ≥0.50 = large.
*[b] Independent t-test; effect size reported as |**d**| (Cohen's d): <0.20 = negligible, <0.50 = small, <0.80 = medium, ≥0.80 = large. Bold values indicate statistical significance (p < 0.05).*

Table 2. Sample characteristics.

younger adults, proximal stiffness was higher than at the midpoint (p < 0.001). In older adults, stiffness increased progressively toward the distal region, with significant differences between all measurement sites. These results are illustrated in Figure 3. Post hoc power analysis indicated excellent statistical power to detect differences in both age groups (power > 0.99).

Younger adults tended to have higher VL stiffness than older adults, with median values of 13.7 [IQR 12.9–17.4] and 13.1 [IQR 10.5–14.8], respectively (p = 0.058).

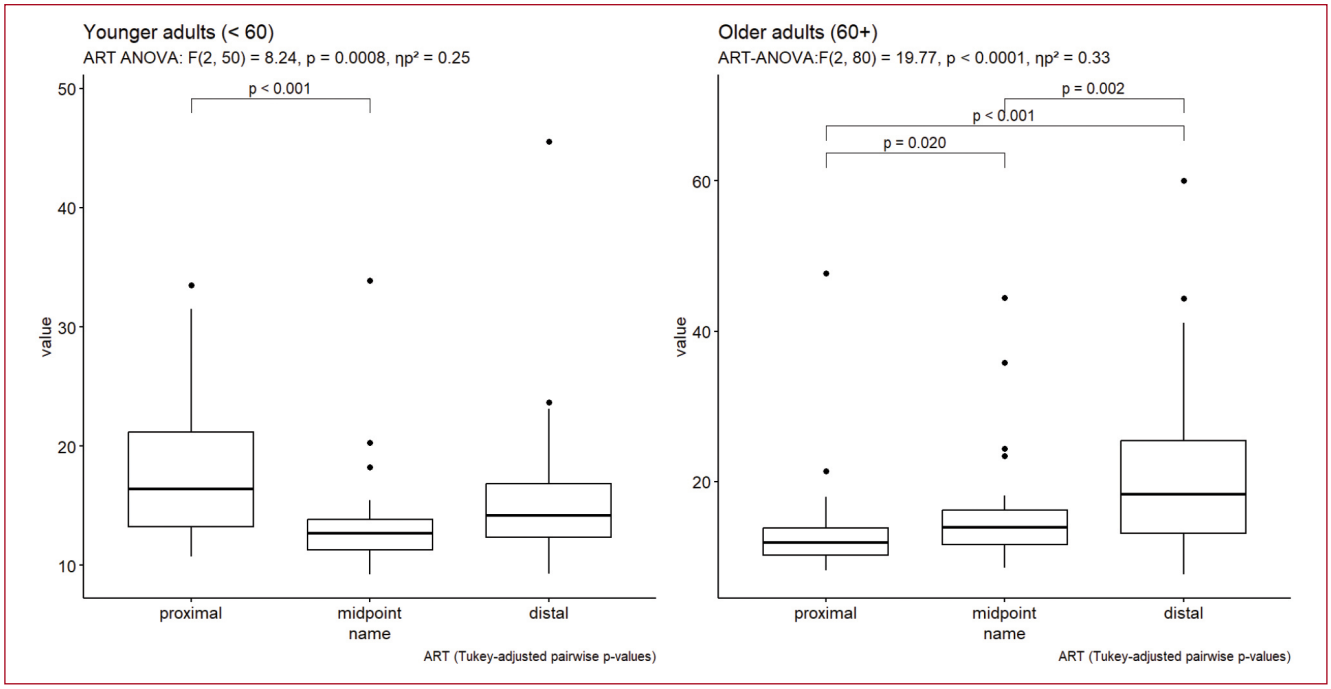

Figure 3. Regional Rectus Femoris Stiffness in Younger and Older Adults.

	<60 years (N=26)	>60 years (N=42)	P-value	Effect size
Proximal RF E (kPa)				
Mean (SD)	18.0 (6.05)	13.2 (6.15)	**<0.001** [a]	**0.51**
Median [Q1, Q3]	16.4 [13.2, 21.2]	12.0 [10.2, 13.7]		
Mid RF E (kPa)				
Mean (SD)	13.7 (4.77)	15.1 (6.65)	0.228 [a]	0.15
Median [Q1, Q3]	12.7 [11.3, 13.9]	13.9 [11.5, 16.1]		
Distal RF E (kPa)				
Mean (SD)	16.0 (7.12)	20.9 (10.9)	**0.0327** [a]	**0.26**
Median [Q1, Q3]	14.2 [12.3, 16.9]	18.3 [13.1, 25.4]		
Missing	0 (0%)	1 (2.4%)		
Mid VL E (kPa)				
Mean (SD)	15.5 (4.35)	14.6 (8.30)	0.0575 [a]	0.23
Median [Q1, Q3]	13.7 [12.9, 17.4]	13.1 [10.5, 14.8]		

Proximal RF E – Proximal Rectus Femoris Stiffness, Mid RF E – Midpoint Rectus Femoris Stiffness, Distal RF E – Distal Rectus Femoris Stiffness, Mid VL E – Midpoint Vastus Lateralis Stiffness. kPa - Kilopascal (kPa). [a] Mann–Whitney U test; effect size reported as Wilcoxon effect size (r): 0.10–<0.30 = small, 0.30–<0.50 = moderate, ≥0.50 = large. Bold values indicate statistical significance (p < 0.05).

Table 3. Rectus femoris (RF) and vastus lateralis (VL) muscle stiffness stratified by age group.

Fixed Effect	Estimate	Std. Error	95% CI (Lower)	95% CI (Upper)	p value
(Intercept)	3.01	0.22	2.59	3.43	**< 0.0001**
Age 60+	-0.47	0.12	-0.69	-0.24	**0.0001**
Midpoint	-0.27	0.08	-0.42	-0.11	**0.0013**
Distal	-0.13	0.08	-0.29	0.03	0.1052
Males	-0.07	0.10	-0.26	0.12	0.4793
FM (Kg)	0.01	0.003	0.001	0.02	**0.0244**
ASM (Kg)	-0.01	0.01	-0.03	0.01	0.3266
Interaction: Age 60+ & Midpoint	0.39	0.11	0.19	0.60	**0.0003**
Interaction: Age 60 + & Distal	0.57	0.11	0.35	0.76	**< 0.0001**

ASM – Appendicular Skeletal Muscle Mass, FM – Fat Mass. Bold values indicate statistical significance (p < 0.05).

Table 4. Multilevel regression model of factors associated with rectus femoris stiffness across measurement sites.

	HGS	CST	ASMI	ASM	leg SMM	FM	PhA
Proximal RF E	**0.32 (0.09, 0.50); p = 0.01**	**-0.36 (-0.54, -0.10); p < 0.01**	**0.34 (0.09, 0.55); p = 0.01**	**0.29 (0.06, 0.52); p = 0.02**	**0.27 (0.00, 0.48); p = 0.04**	-0.10 (-0.34, 0.17); p = 0.51	**0.53 (0.33, 0.68); p < 0.01**
Mid RF E	**-0.35 (-0.55, -0.09); p = 0.01**	**0.28 (0.05, 0.47); p = 0.03**	**-0.35 (-0.53, -0.12); p = 0.01**	**-0.42 (-0.58, -0.19); p < 0.01**	**-0.42 (-0.58, -0.19); p < 0.01**	**0.47 (0.28, 0.61); p < 0.01**	-0.24 (-0.41, 0.03); p = 0.07
Distal RF E	**-0.34 (-0.54, -0.08); p = 0.02**	**0.44 (0.24, 0.63); p < 0.01**	**-0.48 (-0.65, -0.26); p < 0.01**	**-0.49 (-0.66, -0.25); p < 0.01**	**-0.47 (-0.64, -0.24); p < 0.01**	**0.28 (0.01, 0.47); p = 0.03**	**-0.46 (-0.65, -0.19); p < 0.01**
Mid VL E	0.04 (-0.28, 0.28); p = 0.81	-0.20 (-0.41, 0.03); p = 0.11	0.01 (-0.27, 0.29); p = 0.94	-0.04 (-0.32, 0.24); p = 0.77	-0.06 (-0.35, 0.18); p = 0.62	**0.32 (0.07, 0.54); p = 0.01**	0.12 (-0.13, 0.37); p = 0.38

Proximal RF E – Proximal Rectus Femoris Stiffness, Mid RF E – Midpoint Rectus Femoris Stiffness, Distal RF E – Distal Rectus Femoris Stiffness, Mid VL E – Midpoint Vastus Lateralis Stiffness, HGS – Handgrip Strength, CST – Chair Stand Test, ASMI – Appendicular Skeletal Muscle Mass Index, ASM – Appendicular Skeletal Muscle Mass, Leg SMM – Examined Leg Segmental Muscle Mass, FM – Fat Mass, PhA – Phase Angle. Bold values indicate statistical significance (p < 0.05).

Table 5. Correlations between rectus femoris (RF) and vastus lateralis (VL) stiffness and components of sarcopenia.

In the RF, older adults exhibited higher distal stiffness (median 18.3 [IQR 13.1–25.4] vs 14.2 [12.3–16.9]; p = 0.033), whereas younger adults showed higher proximal stiffness (16.4 [13.2–21.2] vs 12.0 [10.2–13.7]; p < 0.001) at the midpoint, both groups had comparable stiffness (Table 3).

Sex-stratified muscle stiffness

Significant sex differences in muscle stiffness were observed at the RF midpoint (median 14.1 [IQR 12.3–17.4] in females vs 12.2 [11.1–14.2] in males, p = 0.006) and at the VL midpoint (14.6 [12.5–18.0] vs 13.0 [11.4–14.4], p = 0.027), whereas proximal and distal RF stiffness values were comparable (Table S2).

Factors influencing muscle stiffness

A multilevel regression examined factors influencing the log-transformed RF stiffness at all three anatomical sites (Table 4). Adjustment for body composition attenuated the observed sex differences in muscle stiffness, implying that variations in fat and muscle mass underlie these differences. Females had a mean fat mass of 24.4 kg compared with 16.6 kg in males, whereas males had a mean ASM of 25.7 kg compared with 18.5 kg in females.

Three factors significantly influenced RF stiffness. Fat mass showed a positive association with stiffness. Older adults had relatively lower RF stiffness than younger adults, however that changes in the distal region, where older adults have stiffer muscles. In the younger group, stiffness was significantly lower at the midpoint of the RF compared to the proximal part, but there was no significant difference between the distal and proximal parts. In contrast, older participants showed higher stiffness in the distal part of the muscle and lower stiffness in the proximal part.

Correlation between muscle stiffness and longitudinal locations

Muscle stiffness had low to moderate correlations between different measurement sites. The RF stiffness at the midpoint moderately correlated with the distal site ($\rho = 0.46$). In contrast, the proximal site showed only weak correlations with both the midpoint ($\rho = 0.21$) and the distal site ($\rho = -0.12$). VL midpoint stiffness exhibited low correlations with the RF stiffness at the proximal ($\rho = 0.25$) and midpoint ($\rho = 0.29$) sites, and negligible correlation with the distal site ($\rho = 0.08$).

Effect of in-depth probe positioning

Muscle stiffness showed generally strong correlations across depth layers (Table S3, Figure S4).

Muscle stiffness and sarcopenia components

At the proximal RF site, muscle stiffness showed a moderate positive correlation with muscle strength, a positive correlation with ASM, and a strong positive correlation with cellular health measured by PhA ($\rho = 0.53$). These relationships were reversed at the mid and distal regions of the RF (Table 5). The strongest correlation with FM was observed at the midpoint of the RF ($\rho = 0.47$), where stiffness increased with higher FM. Correlations between VL stiffness and sarcopenia components were mostly negligible.

Discussion

Factors influencing muscle stiffness

Age and longitudinal changes in stiffness

This study identified distinct regional patterns of passive RF stiffness along the proximal-distal axis in younger and older adults. Older adults showed increasing stiffness toward the distal region, whereas younger adults showed a decrease from proximal to midpoint. Age-related differences were also site-specific, with higher proximal stiffness in younger adults and higher distal stiffness in older adults.

Consistent with our findings, higher proximal rectus femoris stiffness in younger individuals has been reported previously and were attributed to a stiffer proximal aponeurosis with a possible stabilizing role[24]. In this study, older adults showed a different trend, with stiffness increasing toward the distal region. Several factors may contribute to this pattern. One possibility relates to regional differences in muscle cross-sectional area (CSA), which tend to be smaller distally. A reduced CSA may influence localized stiffness measurements[25], as surrounding structures such as aponeuroses, bone, or adjacent connective tissues may contribute more where muscle mass is lower. Tendons, which are inherently stiffer than muscle tissue, may also partly influence distal stiffness measurements[26]. These mechanisms remain speculative but may be more relevant in older adults due to their reduced muscle mass[27]. In younger adults, greater muscle mass may attenuate such influences. Another possible explanation relates to regional differences in intramuscular fat infiltration, which is known to be higher in distal regions of the quadriceps femoris in older adult[28]. As the proposed mechanisms were not directly investigated, these explanations remain hypothetical.

Our results suggest that age-related changes in muscle stiffness are region-specific within the muscle. This variability was observed despite measurements being performed by the same examiner using a standardized ultrasound protocol, highlighting the importance of considering anatomical location when evaluating muscle stiffness. To our knowledge, this is the first study to examine regional muscle stiffness from proximal-to-distal regions in a fully rested state across both younger and older adults.

Fat mass

Fat mass was associated with increased muscle stiffness. This association may reflect greater intramuscular fat infiltration or increased subcutaneous tissue thickness, both of which could influence elastography measurements[4,11,29-31].

Sex

Women showed higher muscle stiffness than men at the midpoints of the RF and VL, whereas no sex differences were observed at the proximal or distal RF sites. However, these differences were no longer significant after adjusting for body composition, suggesting that they were explained by differences in fat and muscle mass rather than sex itself. Given the limited sample size and the strong correlation between sex and body composition, these findings should be interpreted with caution. Previous studies have reported

inconsistent findings regarding sex differences in muscle stiffness[32-34]. Hormonal influences have been proposed as one potential explanation for these discrepancies[35-37], although their role in muscle mechanical properties remains incompletely understood.

Depth layers

A review from our research group aimed to standardize SWE. They recommended using a larger region of interest (ROI) to encompass more muscle tissue[4]. In this study, a broader ROI approach was compared with segmented analyses focusing on the superficial, intermediate, and deep layers of the muscle. The results suggest a stronger correlation between values obtained from the depth layers and those obtained using a larger ROI.

Passive muscle stiffness in relation to markers of muscle quality

Muscle quality is a multidimensional construct that lacks a universally accepted definition. The literature suggests several candidate markers for muscle quality, including intramuscular fat infiltration (assessed through Magnetic Resonance Imaging or Computed Tomography), the ratio of muscle strength to mass, and phase angle measured via BIA[38-40]. Passive mechanical stiffness is a biomechanical property of muscle that may provide additional information related to muscle quality[4-6]. There is still no global consensus on how to assess muscle quality in routine clinical settings[3].

RF muscle stiffness showed weak to moderate correlations with muscle strength and mass, and moderate to strong associations with phase angle. This suggests that elastography may reflect certain aspects of muscle quality and cellular health. Interestingly, the direction of these associations differed by measurement site, with proximal stiffness showing positive and mid/distal stiffness predominantly negative associations. This pattern may partly reflect differences in age, body composition, and subcutaneous fat, all of which can influence SWE measurements[4,11,14,28].

Overall, the findings highlight the need for further research to better understand the relationship between muscle stiffness and muscle quality, and to evaluate its potential role in more comprehensive assessments of muscle health.

From a clinical and scientific perspective, this study reinforces the importance of standardized SWE measurement protocols. As muscle stiffness varies with anatomical location and age, these factors should be considered when comparing SWE measurements across individuals and studies. To facilitate future research and improve comparability across studies, we suggest assessing RF stiffness at 50% of the thigh length, in accordance with SARCUS recommendations[6].

Strength and limitations

Due to the limited available literature, no a priori sample size calculation was performed. However, post hoc power analysis demonstrated excellent statistical power for detecting differences between measurement sites across both age groups (power > 0.99), indicating that the study was well powered despite the lack of an *a priori* sample size calculation. Although most older participants were community-dwelling and generally robust with preserved mobility, other health-related factors may have influenced the observed differences between age groups and could not be fully controlled when comparing populations across a wide age range. Furthermore, the monocentric study design may limit the generalizability of the findings. Future studies with larger, multicenter, and more representative cohorts are warranted to validate and extend these findings.

A major strength of this study is the inclusion of both younger and older participants, with ages ranging from 20 to 95 years. This wide age range allows for a more comprehensive understanding of muscle stiffness across the lifespan. Furthermore, the same muscle was examined at multiple anatomical locations using a consistent and standardized protocol. This approach enabled a systematic comparison of stiffness differences by anatomical location, an aspect that is often difficult to address in the literature due to methodological variability across studies[4,5].

Conclusion

The regional distribution of muscle stiffness along the longitudinal axis of the rectus femoris differed between younger and older adults, highlighting the importance of considering both anatomical location and age when interpreting SWE. These findings support the need for standardized SWE measurement protocols to improve comparability across studies. Such standardization is an important step toward establishing reference values and investigating whether SWE may have a future role in monitoring muscle health. Future research should evaluate the reproducibility these protocols and determine whether these patterns are consistent across different populations and clinical settings.

Ethics approval

Ethical approval was granted by the Ethics Committee of Ziekenhuis aan de Stroom (ZAS) Antwerpen (Commissie voor Medische Ethiek ZAS Middelheim, Cadix, Palfijn, Hoge Beuken, Joostens; approval number 5249).

Consent to participate

Written informed consent was obtained from all participants.

Authors' contributions

SP, BH, and SB contributed to participant recruitment and conceptualization of the study and analysis. BH collected and analyzed the data. UB supervised the

statistical analysis.SB, AMC, MG, KS and SP did a critical revision of the manuscript for important intellectual content. All authors critically reviewed and approved the final manuscript.

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Supplementary Material

Supplementary Material 1. Multilevel mixed-effects model

To identify factors influencing muscle stiffness, a multilevel mixed-effects model was constructed using repeated measurements from the rectus femoris (RF) at its three anatomical sites (proximal, midpoint, distal). The dependent variable (muscle stiffness) was log-transformed to improve normality. The hierarchical structure of the data, with repeated measurements nested within individuals, was accounted for by including a random intercept for participant ID.

Fixed effects included age group (<60 vs ≥60 years), sex, anatomical site, fat mass (FM), and appendicular skeletal muscle mass (ASM). Interaction terms between age and anatomical site and between sex and anatomical site were explored to account for potential physiological differences; only statistically significant interactions were retained in the final model.

Diagnostic residual plots, including a residual-versus-fitted plot and a normal Q–Q plot (Figures S2 and S3, respectively), showed no evidence of violations of the model assumption.

Model equation:

$$log(Y_{ij}) = \beta_0 + \beta_1\, Age_i + \beta_2\, Site1_{ij} + \beta_3\, Site2_{ij} + \beta_4\, Sex_i + \beta_5\, FM_i + \beta_6\, ASM_i + \beta_7\, (Age_i \times Site1_{ij}) + \beta_8\, (Age_i \times Site2_{ij}) + u_i + \epsilon_{ij}$$

where

Y_{ij} = RF stiffness at site jfor subject *i*

u_i = subject-specific random intercept

ϵ_{ij} = residual error

The variance of the random intercept was 0.026 and the residual variance was 0.085. The resulting intraclass correlation coefficient (ICC) was 0.23, indicating that approximately 23% of the unexplained variance in muscle stiffness occurred at the individual level. The analysis included 191 observations from 64 participants, corresponding to an average of m=2.98 observations per person. This yields a design effect of DEFF=1+(m−1)·ICC=1.46 , resulting in an effective sample size of neff=n/DEFF=130.5. This provides a reasonable basis for estimating the nine parameters included in the mixed-effects model following recommendations on model complexity and sample size considerations (Peter Diggle et al., 2002; Frank E. Harrell Jr., 2015, Chapter 4).

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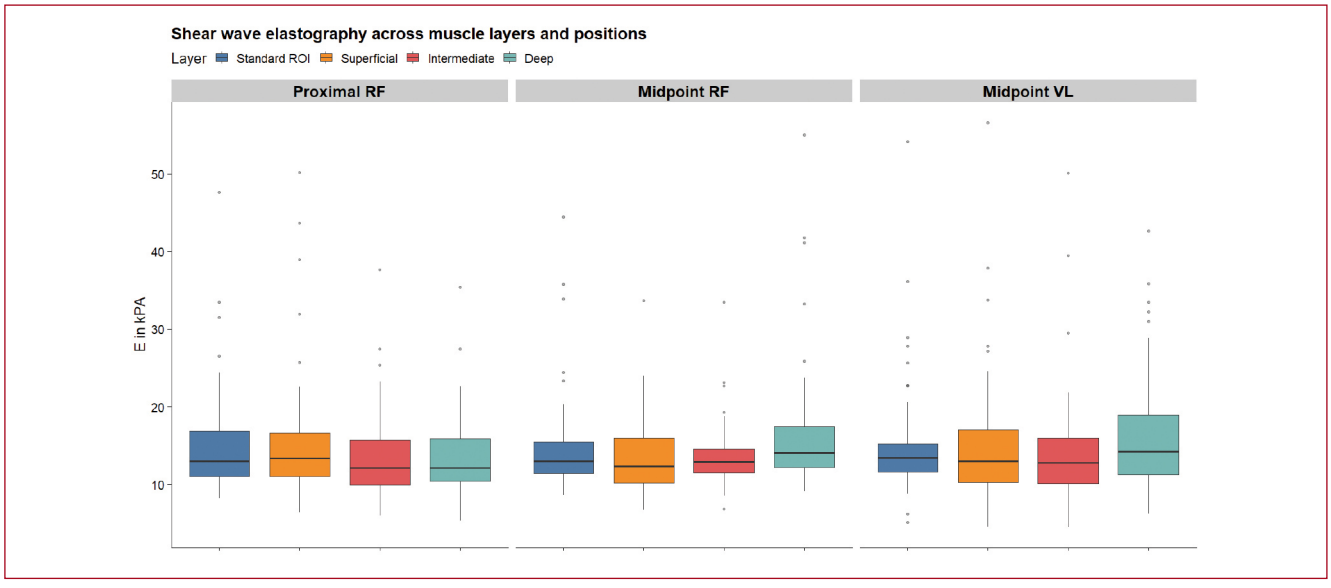

Figure S1. Variability of Muscle Stiffness measured by SWE Across Muscle Layer Depths and Thigh Locations.

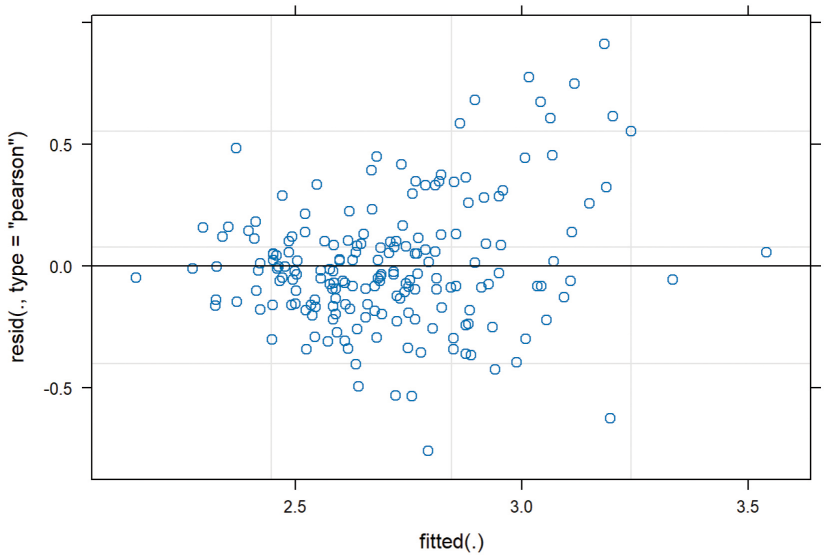

Figure S2. Model diagnostics - residual-versus-fitt.

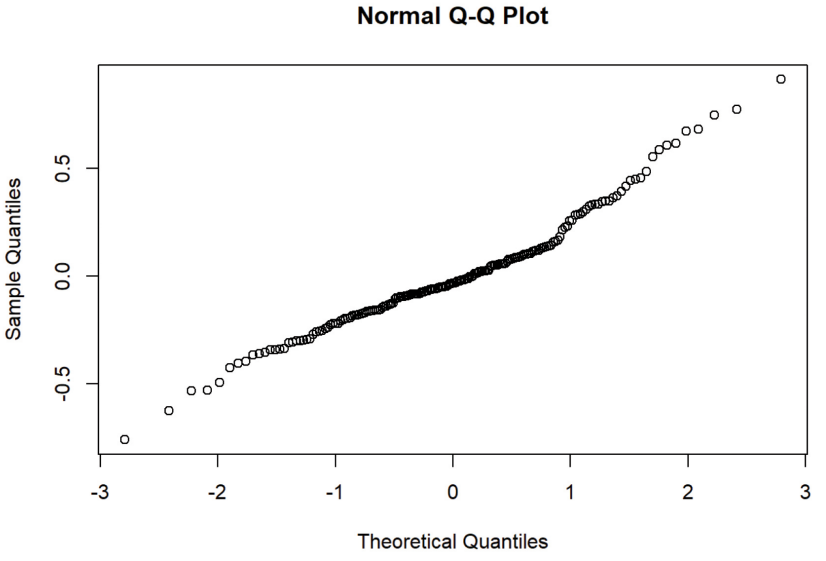

Figure S3. Model diagnostics - Q–Q plots.

	Female		Male	
	<60 (N=12)	60+ (N=18)	<60 (N=14)	60+ (N=24)
Age (years)				
Mean (SD)	30.7 (10.8)	80.6 (8.04)	26.0 (5.26)	80.6 (9.22)
Median [Q1, Q3]	27.5 [24.8, 31.3]	82.0 [79.3, 86.5]	25.0 [21.3, 29.5]	82.5 [76.8, 86.5]
HGS (kg)				
Mean (SD)	30.7 (5.57)	16.9 (5.58)	43.8 (7.07)	28.3 (8.86)
Median [Q1, Q3]	30.0 [28.0, 33.3]	15.0 [12.5, 20.0]	41.5 [38.5, 50.8]	28.5 [24.8, 31.0]
CST (seconds)				
Mean (SD)	7.51 (0.908)	16.3 (4.76)	7.19 (1.20)	18.2 (8.59)
Median [Q1, Q3]	7.40 [6.90, 8.03]	14.7 [13.1, 18.0]	7.10 [6.63, 7.93]	16.2 [12.8, 20.0]
Not possible	0 (0%)	2 (11.1%)	0 (0%)	4 (16.7%)
ASM (kg)				
Mean (SD)	22.1 (2.69)	15.8 (3.28)	29.9 (4.14)	23.0 (3.11)
Median [Q1, Q3]	21.8 [20.2, 23.9]	15.3 [13.4, 17.9]	29.7 [26.8, 32.1]	23.7 [20.8, 25.0]
Missing	0 (0%)	1 (5.6%)	0 (0%)	3 (12.5%)
ASMI (kg)				
Mean (SD)	7.79 (0.943)	6.34 (0.881)	9.35 (0.649)	7.73 (0.958)
Median [Q1, Q3]	7.56 [7.10, 8.01]	6.25 [5.83, 7.08]	9.26 [8.94, 9.66]	7.98 [7.02, 8.27]
Missing	0 (0%)	1 (5.6%)	0 (0%)	3 (12.5%)
Leg SMM (kg)				
Mean (SD)	8.70 (0.941)	6.11 (1.37)	11.6 (1.64)	8.70 (1.18)
Median [Q1, Q3]	8.73 [7.88, 9.32]	5.93 [5.01, 6.91]	11.7 [10.6, 12.4]	8.86 [7.82, 9.38]
Missing	0 (0%)	1 (5.6%)	0 (0%)	3 (12.5%)
FM (kg)				
Mean (SD)	20.0 (10.7)	27.5 (9.47)	8.30 (5.39)	22.2 (8.63)
Median [Q1, Q3]	20.2 [12.1, 23.4]	24.4 [22.7, 36.0]	7.20 [4.88, 9.55]	20.4 [17.1, 29.0]
Missing	0 (0%)	1 (5.6%)	0 (0%)	3 (12.5%)
Phase angle (degree)				
Mean (SD)	6.04 (0.557)	4.20 (0.684)	7.14 (0.506)	4.49 (1.01)
Median [Q1, Q3]	5.95 [5.65, 6.38]	4.30 [3.70, 4.80]	7.00 [6.90, 7.58]	4.10 [3.70, 5.20]
Missing	0 (0%)	1 (5.6%)	0 (0%)	3 (12.5%)

HGS – Maximum Handgrip Strength, CST – Chair Stand Test, ASM – Appendicular Skeletal Muscle Mass, ASMI – (ASM/height^2) Appendicular Skeletal Muscle Mass Index (normalized by height squared), Leg SMM – Dominant Leg .Segmental Muscle Mass, FM – Fat Mass.

Table S1. Sample characteristics stratified by age category and sex.

	Females (N=30)	Males (N=38)	p-value	Effect size
Proximal RF E (kPa)				
Mean (SD)	16.3 (8.14)	14.0 (4.74)	0.241 [a]	0.14
Median [Q1, Q3]	13.6 [12.0, 18.4]	12.3 [11.0, 15.7]		
Mid RF E (kPa)				
Mean (SD)	16.9 (8.14)	12.6 (2.23)	**0.0057** [a]	**0.34**
Median [Q1, Q3]	14.1 [12.3, 17.4]	12.2 [11.1, 14.2]		
Distal RF E (kPa)				
Mean (SD)	21.7 (11.9)	16.7 (7.23)	**0.0514** [a]	**0.24**
Median [Q1, Q3]	16.9 [13.5, 25.3]	14.7 [12.3, 19.1]		
Missing	0 (0%)	1 (2.6%)		
Mid VL E (kPa)				
Mean (SD)	17.3 (9.58)	13.1 (3.13)	**0.027** [a]	**0.27**
Median [Q1, Q3]	14.6 [12.5, 18.0]	13.0 [11.4, 14.4]		

Proximal RF E – Proximal Rectus Femoris Stiffness, Mid RF E – Midpoint Rectus Femoris Stiffness, Distal RF E – Distal Rectus Femoris Stiffness, Mid VL E – Midpoint Vastus Lateralis Stiffness. [a] Mann–Whitney U test; effect size reported as Wilcoxon effect size (r): 0.10–<0.30 = small, 0.30–<0.50 = moderate, ≥0.50 = large. Bold values indicate statistical significance (p < 0.05).

Table S2. Muscle stiffness stratified by sex at different measurement sites.

	Superficial third E	Intermediate third E	Deep third E
Proximal RF E	0.70 (0.51 – 0.80); p < 0.01	0.84 (0.72 – 0.90); p < 0.01	0.62 (0.45 – 0.75); p <0.01
Mid RF E	0.66 (0.51 – 0.80); p < 0.01	0.64 (0.48 – 0.77); p < 0.01	0.64 (0.46 – 0.79); p < 0.01
Mid VL E	0.75 (0.60 – 0.84); p < 0.01	0.69 (0.51 – 0.82); p < 0.01	0.64 (0.47 – 0.76); p < 0.01

Table S3. Spearman Correlation Between Standard ROI and Depth-Layer Measurements of Muscle Stiffness.