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# Association between TMG-derived contractile muscle parameters and MRI-based muscle structure in sarcopenia

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Title: Association between TMG-derived contractile muscle parameters and MRI-based muscle structure in sarcopenia

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## ABSTRACT

**Background:** Muscle fat fraction (MFF) obtained through magnetic resonance imaging (MRI) is the gold standard for assessing muscle quality, but it is expensive and time consuming. Portable methods to examine muscles such as tensiomyography (TMG) are emerging and could enable broader screening. This study aims to examine associations between TMG-derived muscle contractile parameters and MFF in older adults with and without sarcopenia.

**Methods:** A sample of 51 Slovenian older adults (53% females) were scanned with Dixon MRIs to evaluate muscle MFF and contractile parameters were assessed with TMG estimating delay time (Td), maximal displacement (Dm) and radial contraction velocity (Vc). Right leg vastus lateralis (VL) and biceps femoris (BF) were analyzed. Sarcopenia was defined using both European Working Group on Sarcopenia in Older People (EWGSOP2) and Sarcopenia Definition and Outcomes Consortium (SDOC) criteria. Regression models adjusted for age and sex were used to assess associations between TMG-derived contractile parameters and MFF.

**Results:** Age- and sex-adjusted models revealed associations between increased MFF and reduced Dm ( $R^2 = .29$ ,  $p = .003$ ) and Vc ( $R^2 = .32$ ,  $p = .002$ ) for the VL. SDOC-classified sarcopenic individuals showed increased VL MFF (27.2% vs 22.5%,  $p = .019$ ), while EWGSOP2 classified sarcopenia displayed no differences.

**Discussion:** The study reveals that increased MFF is associated with reduced muscle contractility in VL. MFF differs between sarcopenic and non-sarcopenic groups using only SDOC criteria. Since the TMG Dm increase is regularly found in atrophic muscles after bed rest, in sarcopenic muscle MFF explains lowering of the Dm, highlighting the TMG potential for early detection of changes in aging muscle.

Keywords: sarcopenia, contractile parameters, tensiomyography, muscle fat fraction, muscle function, older adults

## Background

Muscle composition changes with increasing age and pathology that leads to muscle wasting; one of them being sarcopenia which is defined as the age-related decrease in muscle mass and muscle function [1]. The description of sarcopenia and its diagnostic classification have undergone multiple definition adjustments, however most include components of reduced muscle strength and physical performance [1,2]. While the revised European Working Group on Sarcopenia in Older People (EWGSOP2) classification includes low muscle strength alongside reduced muscle mass and low physical performance, Sarcopenia Definition and Outcomes Consortium (SDOC) proposed a definition based solely on combination of low muscle strength and poor physical performance [1,2]. Aging *per se* is characterized with increased adipose tissue in the human body, including within skeletal muscle, leading to impaired mobility and function [3,4]. Myosteatorsis refers to the abnormal buildup of fat within skeletal muscle (i.e. muscle fat infiltration), which negatively impacts both metabolic and musculoskeletal health [5]. Myosteatorsis plays a critical role in mobility impairments, that range from minor to complete loss of independent movement. This is seen in various musculoskeletal, neurological and neuromuscular conditions, including sarcopenia - which ultimately reduces quality of life [6,7]. Maintaining skeletal muscle health (both size and quality) is essential for sustaining mobility and independence in older years. Deterioration of muscle quality negatively impacts muscle function by reducing strength and physical performance [8]. Myosteatorsis can be quantified as muscle fat fraction (MFF), which has been shown to increase 3-5 % in older adults as compared to younger adults [9].

Higher myosteatorsis is known as a muscle quality marker, associated with lower contraction power and force production [10]. It has been associated with aging, injuries, reduced sex steroid hormones, reduced capillary blood flow, physical inactivity/bed rest, obesity, insulin resistance and low-grade inflammation and mitochondrial abnormalities [10,11]. Magnetic resonance imaging (MRI) is the gold standard for assessing skeletal muscle health as it provides detailed, non-invasive data on muscle size and composition [12]. Additionally, age-related changes are not uniform across muscles. Postural distal muscles have been shown to decline faster than non-postural in atrophy studies [13] and myosteatorsis appears to be muscle-specific and more pronounced in muscles with a higher proportion of type II fibers [14].

Research has indicated that muscle characteristics undergo significant changes with aging including reduction in size and architecture (including pennation angle and fascicle length), as well as alterations in contractile parameters [15]. Tensiomyography (TMG) is a mechanomyography method that enables the measurement of single muscle contractile parameters being assessed directly at the skin surface, just above the muscle belly. TMG evaluates the radial displacement of the muscle belly which occurs due to the muscle bulk

movement, thickening and oscillation of the muscle fibers during electrically evoked contraction to compensate for constant muscle volume. The method is relatively simple to acquire, portable, and able to capture multiple contractile parameters, including delay time (Td), contraction time (Tc), half-relaxation time (Tr), maximal displacement (Dm) and contractile velocity (Vc). Previous research has established strong negative correlations between TMG-derived parameters (Td, Tc and Tr) and the proportion of myosin heavy chain type I in the vastus lateralis (VL) muscle [16,17]. Also, Dm has been negatively correlated with muscle thickness loss due to atrophy following a 35-day bed rest [18]. Notably, a study demonstrated that Dm increases after only a few days of bed rest, even with muscle thickness remaining unchanged, being an early hallmark of muscle atrophy [17] which is of great importance for older people undergoing period of physical inactivity as they atrophy quicker with lower recovery capacity [19]. However, it is unknown about the Dm changes in sarcopenic muscle.

Despite evidence that aging alters both muscle composition and contractile properties, few studies have examined their direct association [20,21]. Clarifying this link could improve our understanding of the aging muscle and provide a method for early detection of muscle quality decline affording earlier interventions. The aims of the current study are 1) to examine the association between contractile parameters (through TMG-derived parameters) of the muscle and MFF in older adults and 2) to determine whether MFF differs in people with and without sarcopenia classified using two different diagnostic criteria. We hypothesized that TMG-derived parameters will differ between sarcopenic and non-sarcopenic participants and will be associated with MFF, specifically we assumed that lower Dm will be associated with increased MFF.

## Methods

### *Study design and participants*

Participants were recruited from different settings: nursing homes, community dwelling groups and retirement groups across Slovenia. Participants were included in the study if they were a) older than 65 years, b) had body mass index lower than 30 kg/m<sup>2</sup> at initial screening (to eliminate those with sarcopenic obesity); c) were able to walk 4 m independently or with a walking aid, and d) were able to understand and follow instructions. Participants with history of serious lower limb musculoskeletal injuries in the last 12 months or with presence of a neurological disorder were excluded from the study. An a priori power analysis was conducted using G\*Power (version 3.1). Assuming an anticipated R<sup>2</sup> of .30, with  $\alpha = .05$ , statistical power = .80, and three predictors, the required minimum sample size was  $n = 38$ .

### *Tensiomyography*

We evaluated contractile properties with TMG (TMG-BMC ltd., Ljubljana, Slovenia) on two muscles of the right leg: VL and biceps femoris long head (BF). Participants were positioned supine with 30 degrees knee flexion (supported by foam pad), while BF assessment required a prone position of the participant with 5 degrees knee flexion and neutral ankle position (also foam pad-supported). Electrode placement (cathode distal, anode proximal) and sensor positioning (the thickest region of the muscle belly) followed a standardized protocol that did not require any skin preparation [22] which recorded values for four parameters: Tc, Dm, Td and Vc (calculated as  $0.8 \cdot Dm/Tc$ ) [23].

### *MRI Protocol*

MRI was performed supine on a 3.0 T Siemens Vida Fit scanner (Siemens Healthineers, Erlangen, Germany) with a dedicated phased array-coil (18-channel body coil, 32-channel spine coil). Participants were placed in a supine position and the base protocol included a T1 weighted (T1w) turbo spin echo sequence for high-resolution anatomical reference imaging (sequence parameters: image resolution 1.1x1.1x4.0 mm<sup>3</sup>; slices: 40; no spacing between slices; matrix size: 400x225; repetition time (TR): 550 ms; echo time (TE): 9.80 ms; Bandwidth: 500 Hz/Px; acquisition time: 2:05 min). The protocol included an additional gradient echo Volumetric Interpolated Breath hold Examination 6pt Dixon sequence (sequence parameters: image resolution: 1.3x1.3x3.0 mm<sup>3</sup>; slices: 60, 20% spacing between slices; matrix size: 352x352; TR: 14.00 ms; Tes: 1.90, 3.73, 5.56, 7.39, 9.22 and 11.05 ms; Bandwidth: 710 Hz/Px; acquisition time: 1:49 min). Minimum possible TE values were selected at the given image resolution. Additional image contrasts (in-phase contrast, opposed phase contrast, water contrast and fat contrast images) were obtained from the Dixon sequence [24,25].

### *Physical performance*

Physical performance was evaluated using gait speed (time in seconds) over 4 meters length as well as the timed up-and-go (TUG) test. For the gait speed assessment, two time measuring gates (Beam trainer timing system, Seedgrov d.o.o., Ljubljana, Slovenia) were set over the course of 4 metres. Participants began one meter behind the starting line and walked for 4 meters at their habitual speed. The test was performed twice, and gait speed was calculated for both attempts, with the average being used for analysis [1]. TUG test was employed to assess dynamic balance and functional mobility along the 3-meter course [26]. Time for test completion (in seconds) was recorded as participants stood up from a chair, walked 3 meters, pivoted around a cone, returned and sat down on a chair again. The test was conducted twice, with the average time used in the analysis.

#### *Sarcopenia screening and classification*

The handgrip strength of the dominant hand was measured with a hydraulic dynamometer (Jamar Smart, Sammons Preston, USA) in a seated position, elbow flexed at 90°. The participants were instructed to squeeze the dynamometer three times with maximal effort. The average of the three attempts was used for the analysis. For the five sit-to-stand test (5STS), participants were instructed to stand up from the chair to a fully extended position five times as fast as possible with their arms on their chest. Total time was measured with a stopwatch in seconds. For safety reasons, the chair was placed in front of a wall. Body composition was obtained using dual energy X-ray absorptiometry (Lunar Prodigy, GE Medicals, Madison, WI, USA) during whole-body scan with the participant lying supine with legs and arms fully extended. Appendicular lean mass was computed as the sum of arms and legs lean mass, which was normalized to squared body height in meters.

The presence of sarcopenia was assigned using two different classifications: with the European Working Group on Sarcopenia in Older People (EWGSOP2) classification [1] and with the Sarcopenia Definition and Outcome Consortium (SDOC) classification [2]. For both classifications, we used the modified cut-off points, recently proposed by Westbury et al. [27], which lead to higher prevalence rates of sarcopenia while preserving the capacity to predict key health outcomes. The cut-off points used for EWGSOP2 were: handgrip strength < 35.5kg for males and < 20kg for females and appendicular lean mass < 7.0 kg/m<sup>2</sup> for males and < 5.5 kg/m<sup>2</sup> for females. In SDOC, the same handgrip strength cut-off points were used and gait speed < 1.0 m/s.

#### *Data analysis*

Out-of-phase scans were processed using deep-learning muscle MRI annotation software to segment the individual muscles of the thigh (Daphne, version 1.7-alpha, Basel, Switzerland). The data were then imported into 3D Slicer (3D Slicer, version 5.7.0) for manual correction by two researchers (K.P. and D.A.M.F.) blinded to the sarcopenia

status of participants. The slice with the largest cross-sectional area along with two adjacent slices above and below were selected for analysis, resulting in a total of 5 segmented slices. BF and VL of the right leg were included to pair with the available TMG data. MFF was calculated from fat signal intensity (IF) and water signal intensity (IW):

$$\text{MFF}(\%) = \frac{\text{IF}}{\text{IF} + \text{IW}} * 100.$$

#### *Statistical analyses*

Statistical analyses were conducted in SPSS (v 29.0.2.0, IBM, Chicago, IL, USA). Normality was checked by visual inspection of the distribution and quantil-quantil plot, and analytically with kurtosis, asymmetry and Shapiro-Wilk test. Results revealed that Td and Dm were non-normally distributed in both muscles ( $p < .05$ ), therefore, a natural log transformation was applied. We performed unadjusted and adjusted (for age and sex) multiple linear regressions where Dm, Td and Vc, derived from TMG (for both VL and BF) served as dependent variables and MFF as the independent variables. Statistical significance was set to  $p < .05$ . When we reached significance an effect size was presented with Cohen's d.

## Results

The sample consisted of 51 participants, aged  $82.3 \pm 8.2$  years (53% females) and sarcopenia was present in 31% ( $n = 16$ ) when classified with EWGSOP2 and 55% ( $n = 28$ ) when using SDOC classifications. The characteristics of the sample are presented in **Table 1**.

**Table 1: Descriptive statistics and sex differences**

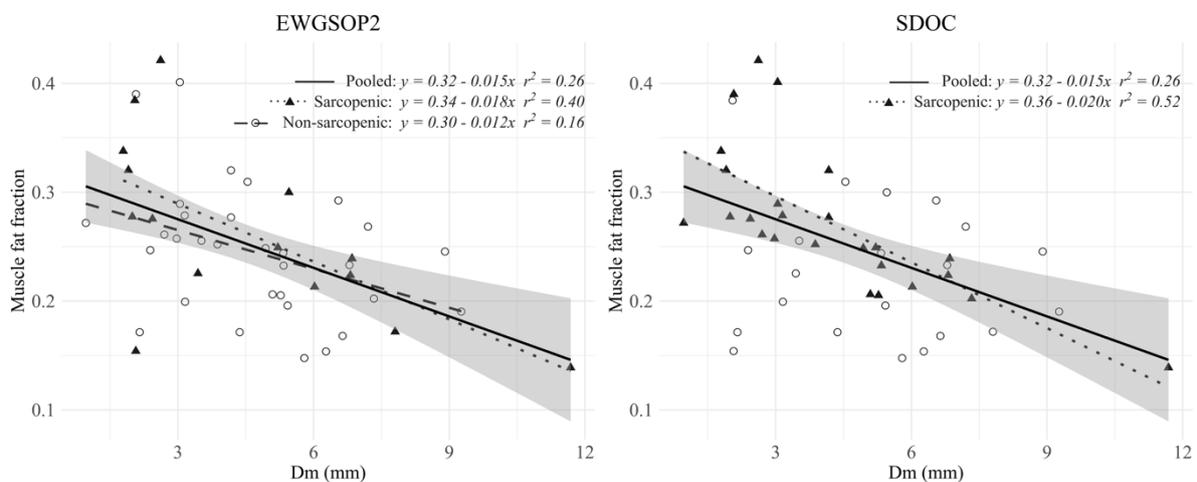
	<b>Pooled data</b>	<b>Males</b>	<b>Females</b>	<b>p-value (Cohen's d)</b>
<b>Age</b>	$82.3 \pm 8.2$ ( $n = 51$ )	$85.0 \pm 7.70$ ( $n = 24$ )	$79.2 \pm 7.87$ ( $n = 27$ )	.010
<b>Height (cm)</b>	$164.8 \pm 10.6$ ( $n = 51$ )	$157.4 \pm 6.87$ ( $n = 24$ )	$173.1 \pm 7.45$ ( $n = 27$ )	<.001#
<b>Body mass (kg)</b>	$67.6 \pm 13.1$ ( $n = 51$ )	$60.1 \pm 10.7$ ( $n = 24$ )	$76.1 \pm 9.99$ ( $n = 27$ )	<.001 (-1.54)
<b>BMI</b>	$24.8 \pm 3.23$ ( $n = 51$ )	$24.2 \pm 3.38$ ( $n = 24$ )	$25.4 \pm 2.99$ ( $n = 27$ )	.174
<b>ALM (kg)</b>	$18.1 \pm 4.7$ ( $n = 51$ )	$14.6 \pm 2.11$ ( $n = 24$ )	$22.1 \pm 3.56$ ( $n = 27$ )	<.001#
<b>ALM/ht<sup>2</sup> (kg/cm<sup>2</sup>)</b>	$6.58 \pm 1.08$ ( $n = 51$ )	$5.91 \pm .69$ ( $n = 24$ )	$7.34 \pm .93$ ( $n = 27$ )	<.001#
<b>HGS (kg)</b>	$23.0 \pm 9.25$ ( $n = 51$ )	$17.4 \pm 4.84$ ( $n = 24$ )	$29.2 \pm 9.04$ ( $n = 27$ )	<.001#
<b>TUG (s)</b>	$14.4 \pm 9.57$ ( $n = 51$ )	$17.2 \pm 11.5$ ( $n = 24$ )	$11.2 \pm 5.39$ ( $n = 27$ )	.038#
<b>5STS (s)</b>	$16.3 \pm 6.69$ ( $n = 49$ )	$18.0 \pm 7.38$ ( $n = 24$ )	$14.4 \pm 5.44$ ( $n = 25$ )	.097#
<b>Gait speed (m/s)</b>	$.92 \pm .37$ ( $n = 50$ )	$.81 \pm .35$ ( $n = 24$ )	$1.04 \pm .36$ ( $n = 26$ )	.027 (-.65)
<b>Volume BF (cm<sup>3</sup>)</b>	$153.2 \pm 93.8$ ( $n = 46$ )	$115.2 \pm 45.7$ ( $n = 21$ )	$198.5 \pm 115.5$ ( $n = 25$ )	.002#
<b>Volume VL (cm<sup>3</sup>)</b>	$370.7 \pm 177.1$ ( $n = 46$ )	$281.1 \pm 89.5$ ( $n = 21$ )	$477.4 \pm 197.4$ ( $n = 25$ )	<.001#
<b>MFF BF (%)</b>	$39.8 \pm 10.2$ ( $n = 46$ )	$41.8 \pm 11.3$ ( $n = 21$ )	$37.4 \pm 8.33$ ( $n = 25$ )	.134
<b>MFF VL (%)</b>	$25.0 \pm 6.76$	$27.0 \pm .07$	$22.6 \pm 6.0$	.025

	( <i>n</i> = 46)	( <i>n</i> = 21)	( <i>n</i> = 25)	(.44)
<b>Dm BF (mm)</b>	5.97 ± 3.78	5.13 ± 3.88	6.84 ± 3.53	.057#
	( <i>n</i> = 49)	( <i>n</i> = 24)	( <i>n</i> = 25)	
<b>Dm VL (mm)</b>	4.92 ± 2.50	4.24 ± 2.42	5.68 ± 2.40	.134#
	( <i>n</i> = 51)	( <i>n</i> = 24)	( <i>n</i> = 27)	
<b>Vc BF (mm/ms)</b>	.12 ± .08	.11 ± .08	.14 ± .08	.022#
	( <i>n</i> = 49)	( <i>n</i> = 24)	( <i>n</i> = 25)	
<b>Vc VL (mm/ms)</b>	.15 ± .08	.13 ± .07	.18 ± .07	.029#
	( <i>n</i> = 51)	( <i>n</i> = 24)	( <i>n</i> = 27)	

# non parametric Mann-Whitney test; BMI: body mass index; ALM: appendicular lean mass; ALM/ht<sup>2</sup>: appendicular lean mass standardized to squared height; HGS: hand grip strength; TUG: timed up-and-go; 5STS: five sit to stand; BF: biceps femoris long head, VL: vastus lateralis; MFF: muscle fat fraction; Dm: radial displacement; Vc: contraction velocity;

**Figure 1** shows the association between MFF and Dm of VL, highlighting a negative linear association across both EWGSOP2 and SDOC classifications. The pooled dataset is identical and is represented by a solid black regression line. To highlight differences in sarcopenia classification, non-sarcopenic individuals are shown with a dashed line (where significant) and sarcopenic individuals are shown with a dotted line, and all of the presented lines have been significant ( $p < .05$ ).

**Figure 1: Associations between muscle fat fraction and tensiomyographic maximal displacement (Dm) of vastus lateralis according to both classifications: revised European Working group of Sarcopenia in Older People (EWGSOP2) and Sarcopenia definitions and outcomes consortium (SDOC)**



A series of multiple regression analyses examined the association between MFF and Dm of BF and VL, both before and after adjusting for age and sex (**Table 2**). For the BF, MFF demonstrated a negative association with Dm in the unadjusted model ( $R^2 = .120$ ,  $p = .021$ ). However, after adjusting for age and sex, the association attenuated and was no longer significant ( $R^2 = .155$ ,  $p = .088$ ). The overall regression model was not significant:  $F(3, 40) = 2.449$ ,  $p = .078$ , explaining 15.5% of the variance in Dm in BF ( $R^2 = .155$ ). Similarly, MFF showed a negative association with VL Dm in the unadjusted model ( $R^2 = .240$ ,  $p < .001$ ). This association persisted after the adjustment for age and sex ( $R^2 = .293$ ,  $p = .003$ ) with effect size being slightly reduced. The overall regression model was significant with  $F(3, 42) = 5.684$ ,  $p = .002$ , with 28.9% of the variance in VL Dm ( $R^2 = .289$ ) explained by the model.

**Table 2: Results of single and multiple regression models predicting Dm in BF and VL**

Model		B (95% CI)	Standard error	Beta	t-value	p-value
<b>Biceps femoris</b>						
1	MFF BF	-1.89 [-3.47, -.292]	.786	-.346	-2.39	.021*
	MFF BF	-1.51 [-3.26, .233]	.863	-.278	-1.75	.088
2	Age	.005 [-.028, .019]	.012	-.068	-.425	.673
	Sex	.19 [-.155, .535]	.171	.17	1.11	.273
<b>Vastus lateralis</b>						
1	MFF VL	-3.82 [-5.88, -1.75]	1.02	-.490	-3.73	<.001*
2	MFF VL	-3.11 [-5.31, -.917]	1.09	-.399	-2.86	.003*
	Age	-.010 [-.29, .008]	.009	-.156	-1.12	.269
	Sex	.156 [-.144, .455]	.148	.149	1.05	.300

1: unadjusted model, 2: adjusted model, CI: confidence interval, MFF BF: muscle fat fraction in biceps femoris long head, MFF VL: muscle fat fraction in vastus lateralis

Two multiple regressions were conducted to predict Vc from MFF, in both, BF and VL before and after adjusting for age and sex (**Table 3**). For BF, the unadjusted model revealed a trend to association between MFF and Vc BF ( $R^2 = .087$ ,  $p = .051$ ). When age and sex were included as covariates in the model, the association between MFF and Vc remained non-significant ( $R^2 = .092$ ,  $p = .109$ ). The overall regression model was not significant with  $F(3, 40) = 1.352$ ,  $p = .271$ . For VL, the unadjusted regression demonstrated a negative association between MFF and Vc in the unadjusted model ( $R^2 = .284$ ,  $p < .001$ ). After adjusting for age and sex, the association remained ( $R^2 = .315$ ,  $p = .002$ ). The overall regression model was significant with  $F(3, 42) = 6.425$ ,  $p = .001$ , with the model explaining 31.5% of the variance in Vc in VL ( $R^2 = .315$ ).

**Table 3: Results of single and multiple regression models predicting Vc in BF and VL**

Model		B (95% CI)	Standard error	Beta	t-value	p-value
<b>Biceps femoris</b>						
1	MFF BF	-.219 [-.44, .001]	.109	-.296	-2.01	.051
	MFF BF	-.2 [-.447, .047]	.122	-.27	-1.64	.109
2	Age	0 [-.004, .003]	.002	-.028	-.166	.869
	Sex	.009 [-.04, .058]	.024	.061	.383	.704
<b>Vastus lateralis</b>						
1	MFF VL	-.543 [-.805, -.281]	.13	-.533	-4.18	<.001*
	MFF VL	-.473 [-.755, -.19]	.14	-.464	-3.38	.002*
2	Age	-.001 [-.003, .002]	.001	-.087	-.636	.528
	Sex	.019 [-.02, .058]	.019	.139	.997	.324

1: unadjusted model, 2: adjusted model, SE: standard error, CI: confidence interval, MFF BF: muscle fat fraction in biceps femoris long head, MFF VL: muscle fat fraction in vastus lateralis

A series of multiple regression analyses were conducted to predict Td in BF from MFF, both with and without adjustments for age and sex (**Table 4**). In BF, the unadjusted model showed that MFF is associated to Td ( $R^2 = .123$ ,  $p = .02$ ). After adjusting for age and sex (Model 2), the association slightly strengthened ( $R^2 = .192$ ,  $p = .015$ ). The overall regression model was significant with  $F(3, 40) = 3.167$ ,  $p = .035$ , with the model explaining 19.2% of the variance in Td in BF ( $R^2 = .192$ ). Results for VL showed no association between MFF and Td in either the unadjusted model VL ( $R^2 = .028$ ,  $p = .27$ ) or the age and sex adjusted model ( $R^2 = .131$ ,  $p = .602$ ). The overall regression model was not significant with  $F(3, 42) = 2.115$ ,  $p = .113$ .

**Table 4: Results of single and multiple regression models predicting Td in BF and VL**

Model		B (95% CI)	Standard error	Beta	t-value	p-value
<b>Biceps femoris</b>						
1	MFF BF	.454 [.077, .831]	.187	.351	2.43	.020*
	MFF BF	.512 [.106, .919]	.201	.396	2.55	.015*
2	Age	-.004 [-.01, .001]	.003	-.246	-1.57	.124
	Sex	-.051 [-.131, .03]	.04	-.19	-1.271	.211
<b>Vastus lateralis</b>						

1	MFF VL	.264 [-.213, .741]	.237	.166	1.116	.270
	MFF VL	.129 [-.368, .627]	.247	.081	.525	.602
2	Age	-.001 [-.006, .003]	.002	-.11	-.715	.478
	Sex	-.075 [-.143, -.007]	.034	-.351	-2.23	.031

1: unadjusted model, 2: adjusted model, SE: standard error, CI: confidence interval, MFF BF: muscle fat fraction in biceps femoris long head, MFF VL: muscle fat fraction in vastus lateralis

MFF in BF and VL were compared among the groups of sarcopenic and non-sarcopenic participants, classified with EWGSOP2 and SDOC, presented in **Table 5**. While no differences were observed in BF using EWGSOP2, a trend towards elevated MFF in the sarcopenic group presented using SDOC classification ( $p = .059$ ). VL showed higher MFF values in the sarcopenic group classified with SDOC ( $p = .019$ , Cohen's  $d = .723$ ) but not when classified with EWGSOP2 ( $p = .419$ ).

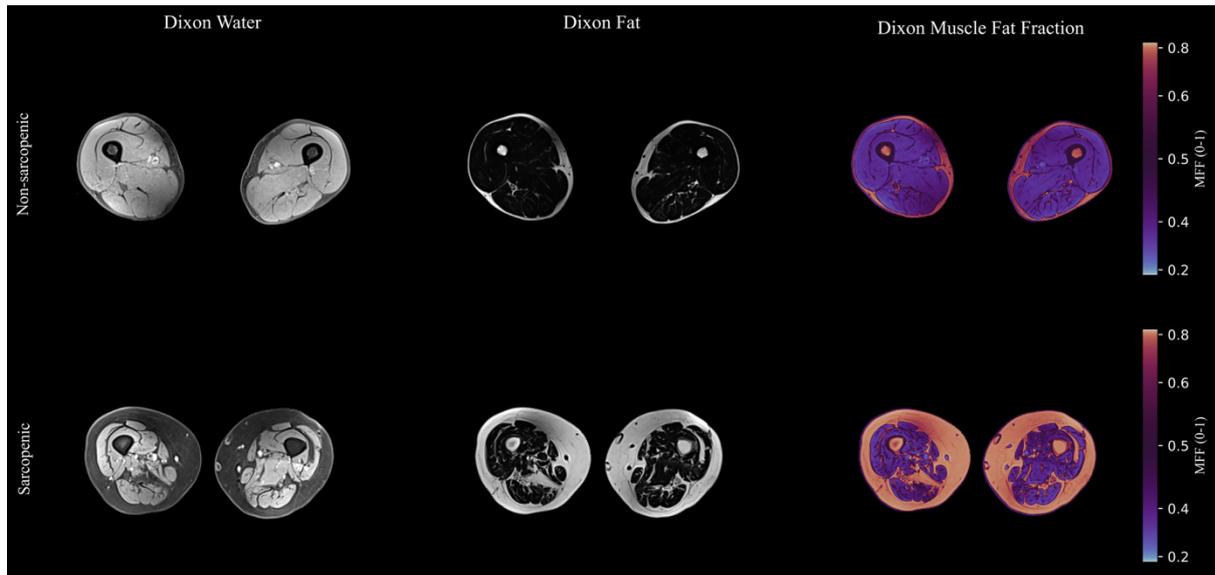
**Table 5: Differences in MFF between sarcopenic and non-sarcopenic groups classified with EWGSOP2 and SDOC**

	Sarcopenic	Non-sarcopenic	p-value (d)
<b>Biceps femoris</b>			
EWGSOP2	40.6 ± 12.9 ( $n = 15$ )	39.4 ± 8.82 ( $n = 31$ )	.776
SDOC	42.4 ± 11.2 ( $n = 25$ )	36.7 ± 8.02 ( $n = 21$ )	.059
<b>Vastus lateralis</b>			
EWGSOP2	26.2 ± 8.11 ( $n = 15$ )	24.5 ± 6.07 ( $n = 31$ )	.419
SDOC	27.2 ± 6.58 ( $n = 25$ )	22.5 ± 6.22 ( $n = 21$ )	.019* (-.723)

EWGSOP2: European working group on sarcopenia in older people; SDOC: Sarcopenia definition and outcomes consortium; d: Cohen's d value.

**Figure 2** represents Dixon-based MRI images comparing muscle composition of non-sarcopenic and sarcopenic participant classified with both EWGSOP2 and SDOC. MFF reveals markedly higher infiltration in the sarcopenic participant, showing reduced muscle quality.

**Figure 2: Dixon-based MFF map comparing muscle composition of non-sarcopenic and sarcopenic participants**



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## Discussion

The main results of this study are: i) Dm is negatively associated with MFF in VL after age and sex adjustment and ii) differences exist among MFF between sarcopenic and non-sarcopenic individuals, classified with SDOC in VL. Additionally, we found that increased MFF is negatively associated with Vc in VL and positively associated with Td in BF.

Between ages 20–70 years, the MFF of the thigh increases annually by 1.3% in males, reflecting progressive myosteatosis with aging [24]. In our study, higher MFF was associated with lower Dm, indicating reduced muscle contractile ability when sarcopenia was present in VL. These findings align with previous research showing that myosteatosis is closely linked to reduced muscle force production, quality and physical performance [8,28]. Studies utilizing MRI-based MFF quantification have reported that MFF content rises with age, and higher MFF correlates with functional deficits and reduced mobility - which are also used in sarcopenia classification [1,29]. Our results reveal that Dm is not only sensitive to muscle mass loss, but is also associated to muscle quality deterioration through myosteatosis.

### *Associations between Dm and Vc with MFF*

We found that Dm of both BF ( $R^2 = .120$ ,  $p = .012$ ) and VL ( $R^2 = .240$ ,  $p < .001$ ) are negatively associated with MFF, indicating that increased MFF corresponds to reduced Dm. After adjusting for age and sex, association in VL remained significant ( $R^2 = .293$ ,  $p = .003$ ), while in BF the association was no longer significant but presented a trend ( $R^2 = .155$ ,  $p = .088$ ). Dm refers to radial displacement of the muscle belly during isometric twitch contraction and has been previously shown to be negatively correlated to muscle stiffness in atrophy studies [17,18,20], elasticity of the muscle [30], lower protein density and remodeling of muscle fibers towards type I [16]. We have previously reported lower Dm values in sarcopenic older adults [31] compared to those without sarcopenia, and the results of the current study confirm lower Dm values (higher stiffness) in individuals with both - those with decreased physical performance and those with higher MFF. When comparing to young, older participants have lower stiffness (measured on shear modulus) and higher MFF in gastrocnemius medialis [9]. Increased myosteatosis occurs in both aging and unloading, yet the direction of Dm differs. Bed rest studies consistently report increased Dm in lower limb muscles, interpreted as passive stiffness due to fluid shifts, decreased muscle tone and connective tissue remodeling [20,32]. Even short-term unloading (3-10 days) reduces muscle stiffness and tone and increases Dm, likely reflecting acute viscoelastic adaptations. In contrast, our findings show reduced Dm with higher MFF in older adults, suggesting that the dominant mechanism in sarcopenia differs from atrophy [33,34]. One of the potential mechanisms could be changes in viscoelastic properties (tone, stiffness and elasticity), which have been shown to change in disuse studies. Specifically, the decreases in rectus femoris stiffness (7.3%), tone

(10.2%) and elasticity (31.5%) were reported after three days of dry immersion [35]. Longer periods of inactivity further decreased muscle tone for ~60% and stiffness for ~9% [36] which was paralleled with Dm increase, found in bed rest studies [17,18,20]. However, in sarcopenic muscle, active muscle stiffness drops more than in normally aged muscle [15], while passive stiffness (in vivo) has been thought to be related to the properties of sarcolemma [37], surrounding connective tissues [38], fat infiltration [39] and titin filaments within the sarcomere structures [40].

BF and VL present different contractile properties which change differently with aging [41], disuse models [17,18] and pathologies. The muscles' primary function and their habitual load could be the main reason for these distinctions. The stronger association in VL may reflect its role in weight-bearing activities [41], making it more vulnerable to age-related fat infiltration compared to the BF, which primarily aids hip extension/knee flexion [42]. VL's metabolic demands and slower fiber type composition could amplify sensitivity to MFF related stiffness loss.

Similar to Dm results, we also show a negative association between MFF and Vc in VL ( $R^2 = .315$ ,  $p = .001$ ), where reduced Vc responds to increased MFF; however, this association was not significant in BF after adjustment for age and sex ( $R^2 = .092$ ,  $p = .271$ ). In comparison to younger adults, older adults reach lower Vc values, which makes our results expected [43]. On the other hand, muscle atrophy showed an increase of TMG-derived Vc after 35-day bed rest [17], being confirmed also with higher unloaded contractile velocity of single fibres after 17 days of bed rest [44] which further showcases the differences in mechanical properties between atrophic and sarcopenic muscle.

Clinically, increased MFF has been linked to impaired muscle quality, metabolic abnormalities and higher mortality in kidney disease patients [10]. This study revealed that fat infiltration leads to several changes in muscle properties, including fiber orientation, reduced elasticity, increased passive tension and reduced force generation, which are associated with mechanical properties of skeletal muscle [45]. Our findings contribute to this understanding by highlighting the mechanical consequences of MFF as seen in decreased Dm and Vc. Although myosteatosis negatively impacts muscle function, the precise mechanisms remain unclear.

#### *Associations between Td and MFF*

We found that Td of BF ( $R^2 = .123$ ,  $p = .020$ ) is positively associated with MFF, indicating that increased MFF corresponds with higher Td and the association slightly strengthens after adjustment for age and sex ( $R^2 = .192$ ,  $p = .015$ ). In contrast, there was no association between Td in VL and MFF. Td quantifies the interval between the electrical stimulus initiation and mechanical contraction onset and reflects complex neuromuscular

processes, including action potential generation, calcium release and binding and cross-bridges coupling, which degrade with aging [46,47]. Delay time consists of two phases – electrochemical (which does not alter significantly with aging) and mechanical processes (which are more affected in aged [48]). The positive association found in this study could imply that MFF disrupts neuromuscular efficiency.

Electromechanical delay is shaped by electrochemical and mechanical processes. Electrochemical processes include the propagation of the action potential along the muscle membrane and the excitation-contraction coupling that leads to calcium release and cross-bridge cycling. This phase lasts for about 3-5 ms and is relatively stable across different joint angles and muscle lengths [48-50]. In contrast, the mechanical phase involves the transmission of force through a series of elastic components of the muscle-tendon unit before the force is transmitted to the skeleton. This phase is more variable and can be affected by age, gender and pathology [51,52] and is easily detected by TMG-derived Td. Our previous findings have shown that Td is increased in older and sarcopenic adults, and the current study provides further evidence by linking prolonged Td to elevated MFF, thereby providing a more comprehensive understanding of the mechanisms underlying reduced muscle function in these populations.

#### *Differences between sarcopenic and non-sarcopenic groups*

We found differences in MFF between sarcopenic and non-sarcopenic individuals, classified with SDOC in VL ( $p = .019$ , Cohen's  $d = .723$ ) and a non-significant trend in BF ( $p = .059$ ). In contrast, no differences were found between the groups when classified with EWGSOP2. MRI and computed tomography based studies have previously demonstrated that increased MFF is associated with lower muscle strength, slower gait speed and impaired physical function, even in individuals with preserved muscle mass [3,28,53]. These discrepancies could stem from fundamental distinctions between the classifications as EWGSOP2 sarcopenia prevalence is lower in comparison to other classifications and its rigorous criteria can therefore exclude some individuals with sarcopenia [54-56]. SDOC defines sarcopenia based on functional tests (reduced hand grip strength and decreased gait speed), which are directly tied to muscle function and quality [2,57]. Additionally, unlike EWGSOP2, SDOC excludes muscle mass measurements from its diagnostic criteria; therefore, muscle function is the main classifying parameter, even in individuals with preserved muscle mass. MFF's impact on muscle function is therefore more prominent as MFF is not solely dependent on muscle mass.

Another difference is in the muscle function and muscle-specific fat infiltration patterns – BF is a non-postural muscle, used for deceleration and hip-driven motions, while VL is

a postural muscle, used in weight-bearing activities (such as walking and standing). A longitudinal study has shown that over the course of 5 years VL declines faster than BF, compounded by MFF [58]. Additionally, type II muscle fibers are more prone to fat accumulation [14] and the accumulation of fat within muscle triggers inflammatory processes which promote further accumulation of adipocytes and causes progressive deterioration of architecture and functional capacity [59]. In summary, differences in MFF between sarcopenic and non-sarcopenic individuals could stem from differences in diagnostic criteria and muscle-specific fat infiltration patterns.

This study has several limitations. The cross-sectional design precludes causal interference, therefore we cannot determine whether increased MFF leads to altered contractile properties or they both arise concurrently as a part of sarcopenic process. Additionally, muscle composition was evaluated using a set of five MRI slices centered at the largest cross-sectional area rather than the full muscle volume. Because fat infiltration is heterogeneously distributed along the muscle length, regional variation may not be fully captured by this approach, and the positioning of the region of interest can meaningfully influence fat fraction estimates. Although single-slice and Dixon-based fat fraction measurements have been shown to provide reliable and reproducible estimates [60], restricted sampling may not entirely reflect whole-muscle composition and should therefore be considered when interpreting the findings.

## **Conclusion**

This study demonstrates that increased MFF in older adults is linked to worse muscle contractile properties, particularly in weight-bearing VL, as shown by sensitive TMG-derived parameters. Importantly, our findings reveal that increased MFF partially explains the reduction of Dm, which is a valuable insight for rapid sarcopenia classification. In addition, results from this study highlight the potential of TMG as a practical and non-invasive method for evaluating muscle quality, that can complement existing compositional MRI analyses. TMG offers the potential to improve access to early detection and monitoring of sarcopenia in both- clinical and community settings. By revealing nuanced muscle changes that are linked to functional decline, TMG enables promotion of personalized and timely interventions for those at risk.

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#### Clinical Trial Number

Not applicable

#### Conflict of interest

While unrelated to the current project, JRM has a financial interest in *JET Worldwide Enterprises Inc.*, a startup company spun out of research from Albert Einstein College of Medicine. The remaining authors have no conflict of interest.

#### Author Contribution

All authors contributed to the conceptualization and execution of the study. KP, DAMF, AAW and BŠ contributed to data curation and formal analysis. KP was involved in the investigation and project administration. KP, JRM, and BŠ contributed to the development of the methodology. BŠ was responsible for funding acquisition and project supervision. DAMF and MK prepared the figures. KP drafted the initial version of the manuscript, and DAMF, AAW, MK, JRM, and BŠ contributed to review and editing. All authors reviewed and approved the final manuscript.

#### Ethics and Consent to participate

All participants provided informed written consent to this study, which was approved by Slovenian National Medical Ethics Committee (ID: 0120-76/2021/6). All research was performed in accordance with the *Declaration of Helsinki*.

#### Availability of Data and Materials

Data is available at the Repository of University of Ljubljana upon request.

#### Consent for publication

Written informed consent for publication was obtained from the participant(s). No identifying images, personal information, or clinical details that could compromise anonymity are included in this manuscript.

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