

Title page

Intramuscular fat infiltration influences mechanical properties during muscle contraction in older women

Zimin Wang ^{1,2} Masashi Taniguchi ^{1,*} Junya Saeki ^{1,3} Masahide Yagi ¹ Norihiko Murota ¹

Kaede Nakazato ¹ Nanami Niiya ¹ and Noriaki Ichihashi ¹

¹Human Health Sciences, Graduate School of Medicine, Kyoto University, Japan.

²Japan Society for the Promotion of Science, Tokyo, Japan.

³Department of Rehabilitation, Osaka Kawasaki Rehabilitation University, Japan.

***Corresponding author**

Masashi Taniguchi

Human Health Sciences, Graduate School of Medicine, Kyoto University

53 Shogoin-Kawahara-cho, Sakyo-ku,

Kyoto 606-8507, Japan.

E-mail: taniguchi.masashi.7a@kyoto-u.ac.jp

Abstract

Although evidence suggests that intramuscular fat infiltration may influence muscle strength, the precise mechanisms remain unclear. This study aimed to determine whether intramuscular fat infiltration affects muscle mechanical properties during contraction, and whether these mechanical properties mediate the relationship between intramuscular fat infiltration and muscle strength. Seventy-nine healthy older women aged 75.1 ± 6.8 years were included in this study. The echo intensity (EI) of the vastus lateralis (VL) was measured as an intramuscular fat infiltration index using B-mode ultrasonography. Maximum voluntary isometric contraction strength (MVIC) was assessed using a dynamometer. The VL shear elastic modulus (G), a mechanical property index, was measured using ultrasound shear wave elastography under various muscle contraction conditions, at rest and at 15, 30, and 45% MVIC (G0, G15, G30, and G45). To evaluate the degree of increase in the shear elastic modulus with increasing muscle contraction intensity, the slope of the regression line (Gslope) between muscle contraction and shear elastic modulus was calculated for each participant. The results showed that EI was significantly associated with G30 and G45 but not with G0 or G15. The EI can significantly explain the inter-individual differences in Gslope. Mediation analysis revealed that the effect of EI on MVIC through Gslope was significant (indirect effect = -0.31, 95% confidence interval [-0.57, -0.12]). These findings suggest that a greater EI is associated with a lower G during muscle contraction. Furthermore, our results show that the relationship between EI and MVIC is mediated by Gslope.

Keywords: echo intensity, aging, shear elastic modulus, ultrasound imaging, muscle weakness

Introduction

Age-related muscle weakness is attributable to various factors, including impaired neural efferent drive, muscle atrophy, and deterioration of muscle quality, the latter being closely related to intramuscular fat infiltration (Fragala MS et al. 2015; Delmonico MJ et al. 2009). Intramuscular fat infiltration can be assessed by echo intensity (EI); an increase in EI, which indicates the progression of intramuscular fat infiltration, occurs earlier than muscle atrophy (Fukumoto Y et al. 2015; Ota M et al. 2020) and is independently associated with muscle strength (Fukumoto Y et al. 2012; Watanabe Y et al. 2013). Therefore, the age-related decline in muscle quality has attracted attention. Notably, the latest diagnostic criteria for sarcopenia have been revised by the European Working Group on Sarcopenia in Older People (EWGSOP) from low muscle mass to low muscle mass and/or low muscle quality (Cruz-Jentoft AJ et al. 2019). To develop effective physical therapy treatments to prevent and improve age-related muscle weakness, it is necessary to elucidate the mechanisms underlying the decrease in muscle quality, such as intramuscular fat infiltration, which leads to muscle weakness.

During aging, muscle fibers undergo degeneration and atrophy and are replaced with intramuscular fat (Goodpaster BH et al. 2006; Visser M et al. 2005). The degree of intramuscular fat infiltration reflects a decrease in muscle contractile tissue that has been replaced by a given muscle size (Lim JY and Frontera WR 2022; Stock MS and Thompson BJ 2021). Therefore, it is conventionally assumed that the relationship between intramuscular fat infiltration and muscle weakness is related to a decrease in muscle contractile tissue. However, an animal study conducted by Biltz et al. (2020) showed a muscle-intrinsic

association between increased intramuscular adipose tissue volume and decreased contractile tension (muscle strength), which cannot be explained by a reduction in muscle contractile tissue alone. This finding suggests the presence of other mechanisms.

One possible novel mechanism by which intramuscular fat infiltration contributes to muscle weakness is the alteration of the mechanical properties during muscle contraction. Simulation modeling studies have reported that intramuscular fat infiltration impairs the ability of muscle fibers to shorten and bulge transversely during contraction, even when the amount of muscle contractile tissue remains constant (Dick TJM and Wakeling JM 2017; Rahemi H et al. 2015). These changes in the mechanical properties can lead to muscle weakness (Rahemi H et al. 2015). Recently, ultrasound shear wave elastography has been used to quantitatively evaluate the mechanical properties of individual muscles in vivo by measuring the shear elastic modulus (Bravo-Sánchez A et al. 2022; Bachasson D et al. 2018; Miyamoto N et al. 2019). The shear wave velocity propagates faster in stiffer tissues; thus, a higher shear elastic modulus corresponds to a stiffer muscle. Previous studies have examined the changes in shear elastic modulus during muscle contraction with aging. Wang et al. (2017) examined the shear elastic modulus of the vastus intermedius throughout the entire process from rest to maximal muscle strength. They observed that older adults exhibited a lower slope in the curve of the shear elastic modulus with an increasing contraction strength than that for younger adults, suggesting a diminished capacity for the muscle shear elastic modulus to increase during contraction with age (Wang CZ et al., 2017). Similarly, Ateş et al. (2023) found that the active shear elastic modulus of the biceps brachii muscle during 25% maximum voluntary isometric contraction was lower for older adults compared to young

adults. However, to the best of our knowledge, only a few studies have explored age-related changes in shear elastic modulus during muscle contraction (Wang CZ et al., 2017; Setlock CA et al., 2021; Ateş F et al., 2023), and the factors associated with these changes remain unclear. Given that adipose tissue is more compliant than contractile muscle tissue (Chakouch MK et al. 2015), the shear elastic modulus of muscles may decrease due to intramuscular fat infiltration. Additionally, the shear elastic modulus of the vastus lateralis (VL) during contraction is related to the knee extension strength (Soldos P et al. 2021). These results suggest a potential relationship during muscle contraction between intramuscular fat infiltration and shear elastic modulus and between shear elastic modulus and muscle strength. However, it remains unclear whether mechanical properties influence the relationship between intramuscular fat infiltration and muscle strength during muscle contraction in vivo.

We investigated whether intramuscular fat infiltration affects muscle mechanical properties during contraction and whether these mechanical properties mediate the relationship between intramuscular fat infiltration and muscle strength. We hypothesized that 1) the VL EI is associated with the VL shear elastic modulus during muscle contraction and 2) the VL EI correlates with knee extension strength via mechanical properties during muscle contraction.

Materials and methods

Participants

From June 2022 to 2023, 79 local, healthy older women (age: 75.1 ± 6.8 [range, 60–88] years, height: 152.7 ± 5.0 cm, body mass: 50.5 ± 6.7 kg, and body mass index (BMI): 21.9 ± 3.7 kg/m²) were included in the study. The inclusion criteria were the ability to live independently

and walk without assistive devices. Participants with the following conditions were excluded:
1) history of surgery on the right lower extremity; 2) any neuromuscular disorders that may affect the test results; and 3) events that may affect the results during the experiment, such as pain and inability to complete and/or maintain the submaximal voluntary isometric contraction conditions.

All participants were recruited from a previous study in which a physical fitness test was conducted in Kyoto (Fukumoto Y et al. 2018). Those who chose to participate visited the laboratory for data collection. Participants were provided comprehensive information about the study's procedures, objectives, and potential risks. Subsequently, written consent was obtained from each participant after being fully informed. The experimental protocols were approved by the Ethics Committee of Kyoto University (ethics approval number: R1746).

Experimental design

All measurements were obtained from the right thigh of the participants and all ultrasound measurements were specifically focused on the VL. Previous studies indicated a significant alteration in intramuscular fat infiltration in the VL with increasing age (Stock MS et al. 2020; Jacob I et al. 2022). Among the quadriceps femoris muscles, which are crucial for daily activities, the VL contributes the most to the muscle strength (Pal S et al., 2007; Lube J et al., 2017). To investigate the relationship between intramuscular fat infiltration and muscle strength, the VL was chosen as the subject for ultrasound measurement, and the maximum voluntary isometric contraction strength (MVIC) of the knee joint was selected as the measurement parameter for muscle strength. The experimental procedure was as follows.

First, B-mode ultrasound was used to measure the VL muscle thickness (MT) and EI. Subsequently, the shear elastic modulus (G) was measured at rest (G0) using ultrasound shear wave elastography, while the participant sat on a dynamometer. After evaluating the MVIC, 15, 30, and 45% MVIC were calculated, and the shear elastic moduli of the VL under three submaximal voluntary isometric contraction conditions (G15, G30, and G45) were evaluated in random order.

B-mode ultrasound

Before the ultrasound examination, participants lay supine on an examination bed for 3 min to prevent fluid changes caused by muscle contraction (Taniguchi M et al. 2017). The midpoint of the greater trochanter to the lateral knee joint space was marked and ultrasound B-mode (transverse image of the VL) measurements were performed at this site. B-mode ultrasound (Noblus; Hitachi Aloka Medical Systems, Tokyo, Japan) with a 5–18 MHz linear probe was used to measure MT and EI. The equipment settings were unified with a gain of 60 dB, a dynamic range of 70, and a depth of 6 cm.

Participants were instructed to relax completely with the knee joints in full extension and the hip joints in a neutral posture. During the measurement, the probe was placed perpendicular to the long axis of the thigh and controlled to exert as little pressure on the skin as possible to prevent compression of the measured tissue. Additionally, to obtain accurate EI, the ultrasound focus was aligned with the central part of the VL, and the probe was adjusted to display the bone and fascia (Fukumoto Y et al. 2022). Three B-mode ultrasound images were recorded for each participant.

Image processing software (ImageJ, version 1.53e, National Institutes of Health, Bethesda, MD, USA) was used to analyze MT and EI. As a measure of muscle mass index, MT was calculated as the distance between the superior and inferior fasciae by selecting the thickest part in the ultrasound image. For MT analysis, a line perpendicular to the inferior fasciae was led toward the superficial fascia using the ruler feature. As a measure of muscle quality index, EI was determined by grayscale analysis using the standard histogram function, which was expressed in arbitrary units. For EI analysis, the range of interest was taken to be as large as possible by adhering to the fascia using the polygon selection feature. The average value of the three measurements was used as VL's MT and EI for further analyses.

All B-mode ultrasound measurements and analyses were completed by a physical therapist with 6 years of measurement expertise. The intra-class correlation coefficient (ICC1, 3) for MT and EI were 0.99 and 0.98, respectively, obtained from 30 randomly selected participants.

Isometric strength testing

MVIC was measured using a dynamometer (Biodex System 4; Biodex Medical Systems Inc., Shirley, NY, USA), and all data were processed and saved using data analysis software (MyoResearch XP Master Edition, Noraxon Inc., Scottsdale, Arizona, USA). The participants were seated with hip flexion of 85° and knee flexion of 60° on a dynamometer chair, with the center of the knee joint aligned with the center of the dynamometer's power shaft.

After adequate familiarization and warm-up, the participants were instructed to extend the knee joint with maximum force and hold it for 3 s. MVIC testing was conducted twice with a

1 min break between the two attempts. The maximum value performed within 3 s and the average of the two attempts was used for the submaximal voluntary isometric contraction condition settings (15, 30, and 45% MVIC) and data analysis.

Ultrasound shear wave elastography

The shear elastic moduli under different muscle contraction conditions were measured using ultrasound shear wave elastography (Aixplorer version 12.2.0, SuperSonic Imagine, Aix-en-Provence, France) with a 2–10 MHz linear probe (SL10-2, SuperSonic Imagine, Aix-en-Provence, France) (Figure 1). The specific settings were unified as follows: Musculoskeletal/Knee mode, penetration SWE Option, 90% gain, high persistence, 100% opacity, smoothing at 5, frequency 1.5–2.0 Hz, elasticity range set to 0–267 kPa, the depth of region of interest (ROI) during measurement was within the range of 0.5–4 cm. Furthermore, the present ROI size was consistent for all participants (approximately 2.0*2.0 cm).

Ultrasound shear wave elastography measurements were performed at the same VL site (longitudinal image) as the B-mode ultrasound. After confirming the transverse section of the VL, the probe was rotated parallel to the muscle fibers (longitudinal axis of the VL), and the angle of the probe on the skin was marked with an oil marker to maintain consistency throughout the measurement process. During the measurement process, the ROI was manually located at the center of the VL, shear elastic modulus images were captured when the set conditions (15, 30, and 45% MVIC) were met, and color mapping was confirmed to be stable. Three ultrasound images were obtained for each condition.

The analysis of shear elastic modulus was based on ultrasound shear wave elastography Q-

box feature; while placing a circle with a diameter of 1.0 cm Q-box in the center of the ROI, the shear elastic modulus was calculated using the following equation: $G=\rho V^2$, where ρ is the muscle mass density, which was presumed to be 1,000 kg/m³, and V denoted the propagation speed. The average values of three measurements were used for subsequent analysis. Since shear waves propagate faster through stiff tissue, the higher the value of G, the stiffer the muscle during contraction.

Although G is widely used as an index of muscle mechanical properties (Bravo-Sánchez A et al. 2022; Bachasson D et al. 2018; Miyamoto N et al. 2019; Ateş F et al., 2023), the optimal intensity required for precise quantification of mechanical properties during muscle contraction remains unclear. Although each contraction condition exhibits high reproducibility, anomalous values have the potential to cause individual variations in the mechanical properties. To mitigate the influence of these anomalies and comprehensively describe the muscle mechanical properties, we used the linear regression line function of Excel (Microsoft Excel 2016, Microsoft, New Mexico, U.S.) to transform the relationship through G₀, G₁₅, G₃₀, and G₄₅ data points. For each participant, the equation $G = k \cdot \text{submaximal voluntary isometric contraction} + b$, and the single slope value k (G_{slope}) was used as the mechanical property during muscle contractions, which indicates the ability to change the shear elastic modulus with an increase in submaximal voluntary isometric contraction intensity. The higher the G_{slope} value, the greater the muscle's ability to stiffen during contraction (Yoshitake Y et al. 2014; Hug F et al. 2015).

All ultrasound shear wave elastography measurements and analyses were completed by a physical therapist with 6 years of measurement expertise. The ICC 1, 3 for G₀, G₁₅, G₃₀,

and G45 obtained from 30 randomly selected participants demonstrated high repeatability. ICC 1, 3 for G0, G15, G30, and G45 was 0.99, 0.96, 0.97, and 0.96, respectively. The test-retest reliability of the G measurements was examined by measuring the shear elastic modulus on two separate days in 10 randomly selected participants. The ICC 1, 3 for G0, G15, G30, and G45 was 0.78, 0.94, 0.87, and 0.98, respectively, indicating good reliability across test-retest sessions.

Statistical analysis

All data are expressed as the mean $\pm$ standard deviation.

To examine the effect of muscle quality on shear elastic modulus during muscle contraction, we conducted simple regression analyses using G0, G15, G30, G45, and Gslope as dependent variables and EI as an independent variable. Furthermore, to determine whether EI independently affected mechanical properties during muscle contraction, we conducted a multiple regression analysis using Gslope as a dependent variable, EI and MT as independent variables using the stepwise method, and age and BMI as covariates using the forced entry method.

In the mediation analysis, EI was used as the X variable, Gslope as the mediator M, MVIC as the Y variable, and MT as the covariate. These were entered into the mediation model to test whether the mechanical properties during muscle contraction mediate the relationship between muscle quality and strength. Figure 2 illustrates the mediation model used in this study. The c path represents the total impact of X on Y, and this total impact includes the direct impact of X on Y and the indirect impact of X on Y through M. Direct impact, that is, c'

path, represents the impact of X on result Y while keeping M constant. Indirect impacts, including path a (the impact of X on mediating factor M) and path b (the impact of mediating factor M on result Y), describe the impact of X on Y through M.

Before conducting the mediation analysis, regression analysis was used to test the effectiveness of paths c, a, and b. Subsequently, the bootstrap method was used to investigate the mediating effect of mediator M on the relationship between independent variable X and dependent variable Y through bootstrap 95% confidence intervals (CI) of the indirect effect. The bootstrap method does not require assumptions of sampling normality and has the highest power and best Type I error control when compared with the product-of-coefficients approach, which is the most well-known Sobel test (Hayes and Andrew F 2009; Preacher KJ and Hayes AF 2004; Sobel and Michael E 1982). Indirect effects were deemed significant if zero was not included in the bootstrap 95% CI. We report the results of bootstrapping procedures with the resampling of 5,000 bootstrap replicates (95% CI).

Statistical analysis software (SPSS version 22.0; IBM Japan, Inc., Tokyo, Japan) was used to analyze all data. For the mediation analysis, an SPSS macro (PROCESS version 3.0) developed by Hayes was used to analyze the media effect (Preacher KJ and Hayes AF 2004). Statistical significance was defined as $p < 0.05$.

Results

The mean $\pm$ standard deviation and 95% CI of EI, MT (measured by B-mode ultrasound), MVIC, G0, G15, G30, G45, and Gslope (measured by ultrasound shear wave elastography) are summarized in Table 1.

The influence of muscle quality on the muscle mechanical properties-regression analysis

In simple regression analysis, G0 ($\beta=-0.02$, $p=0.835$) and G15 ($\beta=-0.09$, $p=0.438$) were not significantly associated with EI; however, G30 ($\beta=-0.26$, $p=0.021$), G45 ($\beta=-0.40$, $p<0.001$) (Figure 3), and Gslope ($\beta=-0.39$, $p<0.001$) were negatively correlated with EI. The effect sizes (f^2) ranged from 0.06 to 0.17, indicating a small to moderate correlation among G30, G45, Gslope, and EI.

In multiple regression analysis, after adjusting for age and BMI, only EI was identified as the significant independent variable affecting Gslope ($\beta=-0.34$, $p<0.001$). Moreover, age also significantly impacted Gslope ($\beta=-0.25$, $p=0.017$), with EI and age jointly explaining 21.6% of Gslope (adjusted for $R^2=0.216$, Table 2).

The results of mediation analyses

EI was significantly correlated with Gslope (path a; $\beta=-0.39$, $p<0.001$), Gslope with MVIC (path b; $\beta=0.43$, $p<0.001$), and EI with MVIC (path c; $\beta=-0.31$, $p=0.005$) (Figure 4).

The bootstrap method was used to test the mediating effect of Gslope in the relationship between EI and MVIC (Table 3). First, the total effect of EI on MVIC was significant (total effect=- 0.42, $p=0.042$). Within the total effect of EI on MVIC, the direct effect (EI → MVIC) was not significant (direct effect=-0.11, $p=0.585$), but the indirect impact through Gslope (EI → Gslope → MVIC) was significant (indirect effect=-0.31, bootstrap 95% CI [-0.57, -0.12]), that is, the increase in EI leads to a decrease in Gslope, which in turn leads to a decrease in MVIC.

Considering the effects of paths a and b were medium (effect 0.39–0.59), the appropriate sample size for this condition should be 78, referring to a previous study (Fritz MS and Mackinnon DP 2007). Thus, the power of the mediation analysis was sufficient. Additionally, mediation analysis results were obtained by resampling 5,000 bootstrap replicates. Therefore, the bias in the results was corrected to the greatest extent possible.

Discussion

This study focused on the mechanical properties during muscle contraction. It examined the relationship between shear elastic modulus during muscle contraction, EI, and MVIC to explore the potential mechanism of intramuscular fat infiltration leading to muscle weakness. The key findings were as follows: 1) although EI was not correlated with G0 and G15, it was significantly related to G30 and G45; 2) EI could significantly explain the inter-individual differences in Gslope; and 3) EI was linked to MVIC through Gslope. To the best of our knowledge, this is the first study to verify that intramuscular fat infiltration is associated with mechanical properties during muscle contraction in humans and that intramuscular fat infiltration may affect muscle strength through the mechanical properties during muscle contraction.

Single regression analysis revealed that EI was not correlated with the shear elastic modulus at rest or during low-intensity muscle contractions (G0, G15) but was significantly correlated with the shear elastic modulus during moderate-intensity muscle contractions (G30, G45). The finding of G0, that is, the shear elastic modulus of the VL measured at rest, is partly consistent with previous studies, which found no connection between EI and the

shear elastic modulus at rest (Alfuraih AM et al. 2019) or a connection only at specific joint angles (Pinel S et al. 2021; Yoshiko A et al. 2023). During rest or low-intensity contraction conditions, other factors such as the degree of muscle elongation (Yoshiko A et al. 2023; Kobayashi K et al. 2023) and large inter-individual differences in the muscle activity balance between the VL and other muscles (e.g., rectus femoris, vastus intermedius, and vastus medialis) (Hug F et al. 2015; Crouzier M et al. 2019), might affect the shear elastic modulus more than intramuscular fat infiltration, making it challenging to observe the relationship between EI and shear elastic modulus. In contrast, EI was significantly associated with the shear elastic modulus during moderate-intensity contraction conditions. This could be because the muscle activity balance between the VL and other muscles becomes more stable at higher contraction intensities, resulting in few inter-individual differences in VL muscle activity (Hug F et al. 2015). Furthermore, the shear elastic modulus at higher contraction intensities may be more strongly affected by intramuscular fatty infiltration than that at lower contraction intensities. The β value, which indicates the degree of relationship between EI and shear elastic modulus, shows an increasing tendency with the increase in submaximal voluntary isometric contraction intensity. This result indicates that as the contraction intensity increases, the influence of intramuscular fat infiltration on the shear elastic modulus becomes more significant, supporting the above hypothesis.

Multiple regression analysis showed that EI had a negative impact on mechanical characteristics during muscle contraction (Gslope), independent of age and BMI. Mechanical properties during muscle contraction are important indicators of physical function, and previous studies have reported that lower shear elastic modulus values during muscle

contraction are associated with weaker muscle strength and poorer sports performance (Soldos P et al. 2021; Yamazaki K et al. 2022). Nevertheless, the factors that influence the mechanical properties of muscles during submaximal voluntary isometric muscle contraction (%MVIC) remain unknown. A previous study reported that EI negatively correlated with muscle stiffness during contraction, which was measured by pressing the muscle from above the skin using an electronic tissue compliance meter during MVIC exertion (Ikezoe T et al. 2012). Although this method of measuring stiffness is not the same as measuring the shear elastic modulus by ultrasound shear wave elastography, which reflects the long-axis mechanical properties of the muscle, both results suggest that increased intramuscular fat infiltration results in less stiffened muscles during contractions. Previous studies examining age-related changes in shear elastic modulus and EI have shown that with an increase in age, the shear elastic modulus during muscle contraction significantly decreases (Wang CZ et al. 2017; Setlock CA et al. 2021), and the EI significantly increases (Delmonico MJ et al. 2009; Fukumoto Y et al. 2015). Given these results and the findings of this study that intramuscular fat infiltration and age independently affect the mechanical properties of muscle contraction, the decrease in the shear elastic modulus in older adults may be attributed to the effects of aging and/or intramuscular fat infiltration.

Mediation analysis revealed that EI correlates with MVIC through Gslope, supporting the results of a simulation modeling study (Rahemi H et al. 2015). In this study, a significant negative correlation was observed between the EI and muscle strength, confirming the importance of muscle quality, as emphasized in the latest revised EWGSOP guidelines (Cruz-Jentoft AJ et al. 2019). Furthermore, our results suggest that the mechanical properties of

349 muscle contraction may mediate the relationship between EI and muscle strength. These
350 insights deepen our understanding of the association between intramuscular fat infiltration
351 and muscle strength and provide valuable information for the development of effective
352 physical therapy approaches. For instance, interventions aimed at reducing intramuscular fat
353 infiltration and/or improving the mechanical properties of muscle contractions could be
354 beneficial in preventing age-related muscle weakness and sarcopenia. The relationship
355 between intramuscular fat infiltration, mechanical properties during muscle contraction, and
356 muscle strength can be considered from two perspectives: force generation and force
357 transmission. Force generation: the generation of muscle force results from the tilting
358 movement of myosin cross-bridges attached to actin filaments in muscle fibers (Reedy MK et
359 al. 1965). A study using CT to measure intramuscular fat infiltration reported that greater
360 intramuscular fat infiltration was correlated with longer myosin attachment times in myosin-
361 heavy chain I fibers and lower myofilament lattice stiffness. This indicates that intramuscular
362 fat infiltration may slow cross-bridge kinetics and reduce myofilament lattice stiffness
363 (Straight CR et al. 2019). Intramuscular fat infiltration may reduce mechanical properties
364 during muscle contraction because of altered myofilament properties and cross-bridge
365 kinetics; thus, muscles with intramuscular fat infiltration present muscle weakness (Morgan
366 DL 1977; Gerstner GR et al. 2017). For force transmission, as shown in the results of the
367 multiple regression analysis, a higher EI negatively influenced the shear elastic modulus
368 during muscle contraction. Therefore, muscles with intramuscular fat infiltration are softer
369 during contraction than those without fat infiltration. Loss of muscle stiffness due to

intramuscular fat infiltration could limit the force transmission capability, reducing the force transmitted to the joint, resulting in muscle weakness.

This study had some limitations. First, as the measurements were conducted solely in the muscle belly of the VL and the participants were limited to healthy older women, whether the findings can be generalized to other regions within the muscle (Setlock et al 2021) and to other muscles, such as biarticular muscles or muscles with less evident fat infiltration with increasing age, as well as to other populations remains unclear. Further investigations are required to clarify the applicability of these results to other muscle groups and populations. Second, although EI is commonly used as a measure of muscle quality or intramuscular fat infiltration in both research and clinical practice, the correlation coefficient between EI and intramuscular adipose tissue content measured by magnetic resonance imaging, which is considered a robust method for assessing intramuscular fat infiltration, typically ranges from 0.5 to 0.6 (Akima H et al. 2016; Fukumoto Y et al. 2022). This suggests that besides intramuscular fat infiltration, there may also be contributions from other tissues, such as connective tissue, to EI. Therefore, the influence of connective tissue cannot be entirely discounted, which makes distinguishing the effects of intramuscular fat from those of connective tissue on mechanical properties a challenging task. Future studies should consider connective tissue and intramuscular fat separately. Third, because the data used in this study were measured simultaneously, there are limitations in inferring a causal relationship. Prospective longitudinal and interventional studies are required to confirm these findings.

Conclusion

392 The present study investigated the impact of EI on shear elastic modulus during muscle
393 contraction and explored the potential mechanisms underlying the relationship between EI
394 and MVIC. Our findings suggest that a higher EI correlates with a lower shear elastic
395 modulus during muscle contraction. The correlation between EI and shear elastic modulus
396 increases with increased contraction intensity. Furthermore, increased EI is associated with
397 muscle weakness due to a lower shear elastic modulus during muscle contraction.

Acknowledgments

We thank the volunteers who dedicated their valuable time to these studies and Ms. Ibuki and Editage (www.editage.jp) for their assistance with English language editing.

Author contributions

Conceptualization: ZW, MT, JS, NI

Data curation: ZW, NM, KN, NN

Formal analysis: ZW, MT, JS, MY

Funding acquisition: NI

Methodology: ZW, MT, JS, NM, NM, KN, NN

Project administration: ZW, MT, NI

Supervision: MT, JS, MY, NI

Writing – original draft: ZW

Writing – review and editing: MT, JS, MY, KN, NN and NI

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Funding

This work was supported by the Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (JSPS KAKENHI grant number JP21H03303).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

420 **Competing interests**

421 The authors declare there are no competing interests.

422

References

- Akima, H., Hioki, M., Yoshiko, A., Koike, T., Sakakibara, H., Takahashi, H., and Oshida, Y. 2016. Intramuscular adipose tissue determined by T1-weighted MRI at 3T primarily reflects extramyocellular lipids. *Magn. Reson. Imaging.* 34(4), 397–403. doi:10.1016/j.mri.2015.12.038
- Alfuraih, A. M., O'Connor, P., Tan, A. L., Hensor, E. M. A., Ladas, A., Emery, P., et al. 2019. Muscle shear wave elastography in idiopathic inflammatory myopathies: a case-control study with MRI correlation. *Skeletal. Radiol.* 48(8):1209-1219. doi:10.1007/s00256-019-03175-3
- Ateş, F., Marquetand, J., and Zimmer, M. 2023. Detecting age-related changes in skeletal muscle mechanics using ultrasound shear wave elastography. *Sci. Rep.* 13(1), 20062. doi:10.1038/s41598-023-47468-z
- Bachasson, D., Dubois, G. J. R., Allenbach, Y., Benveniste, O., and Hogrel, J. Y. 2018. Muscle Shear Wave Elastography in Inclusion Body Myositis: Feasibility, Reliability and Relationships with Muscle Impairments. *Ultrasound. Med. Biol.* 44(7):1423-1432. doi:10.1016/j.ultrasmedbio.2018.03.026
- Biltz, N. K., Collins, K. H., Shen, K. C., Schwartz, K., Harris, C. A., and Meyer, G. A. 2020. Infiltration of intramuscular adipose tissue impairs skeletal muscle contraction. *J. Physiol.* 598(13):2669-2683. doi:10.1113/JP279595
- Bravo-Sánchez, A., Abián, P., Lucenteforte, G., Jiménez, F., and Abián-Vicén, J. 2022. The Applicability of Shear Wave Elastography to Assess Myotendinous Stiffness of Lower Limbs during an Incremental Isometric Strength Test. *Sensors (Basel).* 22(20):8033. doi:10.3390/s22208033

445 Chakouch, M. K., Charleux, F., and Bensamoun, S. F. 2015. Quantifying the Elastic Property
 446 of Nine Thigh Muscles Using Magnetic Resonance Elastography. *PLoS One*. 10(9):e0138873.
 447 doi:10.1371/journal.pone.0138873
 448 Crouzier, M., Hug, F., Dorel, S., Deschamps, T., Tucker, K., and Lacourpaille, L. 2019. Do
 449 individual differences in the distribution of activation between synergist muscles reflect
 450 individual strategies?. *Exp. Brain. Res.* 237(3):625-635. doi:10.1007/s00221-018-5445-6
 451 Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., et al. 2019.
 452 Sarcopenia: revised European consensus on definition and diagnosis. *Age. Ageing*. 48(4),
 453 601. doi:10.1093/ageing/afz046
 454 Delmonico, M. J., Harris, T. B., Visser, M., Park, S. W., Conroy, M. B., Velasquez-Mieyer,
 455 P., et al. 2009. Longitudinal study of muscle strength, quality, and adipose tissue infiltration.
 456 *Am. J. Clin. Nutr.* 90(6):1579-1585. doi:10.3945/ajcn.2009.28047
 457 Dick, T. J. M., and Wakeling, J. M. 2017. Shifting gears: dynamic muscle shape changes and
 458 force-velocity behavior in the medial gastrocnemius. *J. Appl. Physiol.* 123(6):1433-1442.
 459 doi:10.1152/jappphysiol.01050.2016
 460 Fragala, M. S., Kenny, A. M., and Kuchel, G. A. 2015. Muscle quality in aging: a multi-
 461 dimensional approach to muscle functioning with applications for treatment. *Sports. Med.*
 462 45(5):641-58. doi:10.1007/s40279-015-0305-z
 463 Fritz, M. S., and Mackinnon, D. P. 2007. Required sample size to detect the mediated effect.
 464 *Psychol. Sci.* 18(3):233-239. doi:10.1111/j.1467-9280.2007.01882.x
 465 Fukumoto, Y., Ikezoe, T., Yamada, Y., Tsukagoshi, R., Nakamura, M., Mori, N., et al. 2012.
 466 Skeletal muscle quality assessed from echo intensity is associated with muscle strength of

467 middle-aged and elderly persons. *Eur. J. Appl. Physiol.* 112(4):1519-1525.
 468 doi:10.1007/s00421-011-2099-5
 469 Fukumoto, Y., Ikezoe, T., Yamada, Y., Tsukagoshi, R., Nakamura, M., Takagi, Y., et al.
 470 2015 Age-Related Ultrasound Changes in Muscle Quantity and Quality in Women.
 471 *Ultrasound. Med. Biol.* 41(11):3013-3017. doi:10.1016/j.ultrasmedbio.2015.06.017
 472 Fukumoto, Y., Taniguchi, M., Hirono, T., Yagi, M., Yamagata, M., Nakai, R., et al. 2022.
 473 Influence of ultrasound focus depth on the association between echo intensity and
 474 intramuscular adipose tissue. *Muscle. Nerve.* 66(5):568-575. doi:10.1002/mus.27677
 475 Fukumoto, Y., Yamada, Y., Ikezoe, T., Watanabe, Y., Taniguchi, M., Sawano, S., et al. 2018.
 476 Association of physical activity with age-related changes in muscle echo intensity in older
 477 adults: a 4-year longitudinal study. *J. Appl. Physiol* (1985). 125(5), 1468–1474.
 478 doi:10.1152/jappphysiol.00317.2018
 479 Gerstner, G. R., Giuliani, H. K., Mota, J. A., and Ryan, E. D. 2017. Age-related reductions in
 480 muscle quality influence the relative differences in strength and power. *Exp. Gerontol.* 99:27-
 481 34. doi:10.1016/j.exger.2017.09.009
 482 Goodpaster, B. H., Park, S. W., Harris, T. B., Kritchevsky, S. B., Nevitt, M., Schwartz, A. V., et al. 2006. The loss of skeletal muscle
 483 strength, mass, and quality in older adults: the health, aging and body composition study. *J.*
 484 *Gerontol. A. Biol. Sci. Med. Sci.* 61(10):1059-1064. doi:10.1093/gerona/61.10.1059
 485 Hayes, Andrew F. 2009. Beyond Baron and Kenny: statistical mediation analysis in the new
 486 millennium. *Commun. Monogr.* 76 (4): 408–420. doi: 10.1080/03637750903310360

487 Hug, F., Goupille, C., Baum, D., Raiteri, B. J., Hodges, P. W., and Tucker, K. 2015. Nature
 488 of the coupling between neural drive and force-generating capacity in the human quadriceps
 489 muscle. *Proc. Biol. Sci.* 282(1819):20151908. doi:10.1098/rspb.2015.1908
 490 Hug, F., Tucker, K., Gennisson, J. L., Tanter, M., and Nordez, A. 2015. Elastography for
 491 Muscle Biomechanics: Toward the Estimation of Individual Muscle Force. *Exerc. Sport. Sci.*
 492 *Rev.* 43(3):125-133. doi:10.1249/JES.0000000000000049
 493 Ikezoe, T., Asakawa, Y., Fukumoto, Y., Tsukagoshi, R., and Ichihashi, N. 2012. Associations
 494 of muscle stiffness and thickness with muscle strength and muscle power in elderly women.
 495 *Geriatr. Gerontol. Int.* 12(1):86-92. doi:10.1111/j.1447-0594.2011.00735.x
 496 Jacob, I., Johnson, M. I., Jones, G., Jones, A., and Francis, P. 2022. Age-related differences
 497 of vastus lateralis muscle morphology, contractile properties, upper body grip strength and
 498 lower extremity functional capability in healthy adults aged 18 to 70 years. *BMC. Geriatr.*
 499 22(1):538. doi:10.1186/s12877-022-03183-4
 500 Kobayashi, K., Yagi, M., Tateuchi, H., Ota, M., Umehara, J., Sakata, H., et al. 2023. Effect of
 501 age on shear modulus, muscle thickness, echo intensity of the upper limb, lower limb, and
 502 trunk muscles in healthy women. *Eur. J. Appl. Physiol.* 123(4):797-807. doi:10.1007/s00421-
 503 022-05099-8
 504 Lim, J. Y., and Frontera, W. R. 2022. Single skeletal muscle fiber mechanical properties: a
 505 muscle quality biomarker of human aging. *Eur. J. Appl. Physiol.* 122(6):1383-1395.
 506 doi:10.1007/s00421-022-04924-4
 507 L Lube, J., Flack, N. A. M. S., Cotofana, S., Özkurtul, O., Woodley, S. J., Zachow, S., and
 508 Hammer, N. 2017. Pelvic and lower extremity physiological cross-sectional areas: an MRI

509 study of the living young and comparison to published research literature. *Surg. Radiol. Anat.*
 510 39(8):849-857. doi:10.1007/s00276-016-1807-6
 511 Miyamoto, N., Hirata, K., Inoue, K., and Hashimoto, T. 2019. Muscle Stiffness of the Vastus
 512 Lateralis in Sprinters and Long-Distance Runners. *Med. Sci. Sports. Exerc.* 51(10):2080-
 513 2087. doi:10.1249/MSS.0000000000002024
 514 Morgan D. L. 1977. Separation of active and passive components of short-range stiffness of
 515 muscle. *Am. J. Physiol.* 232(1):C45-C49. doi:10.1152/ajpcell.1977.232.1.C45
 516 Ota, M., Ikezoe, T., Kato, T., Tateuchi, H., and Ichihashi, N. 2020. Age-related changes in
 517 muscle thickness and echo intensity of trunk muscles in healthy women: comparison of 20-
 518 60s age groups. *Eur. J. Appl. Physiol.* 120(8):1805-1814. doi:10.1007/s00421-020-04412-7
 519 Pal, S., Langenderfer, J. E., Stowe, J. Q., Laz, P. J., Petrella, A. J., and Rullkoetter, P. J. 2007.
 520 Probabilistic modeling of knee muscle moment arms: effects of methods, origin-insertion,
 521 and kinematic variability. *Ann. Biomed. Eng.* 35(9):1632-1642. doi:10.1007/s10439-007-
 522 9334-6
 523 Pinel, S., Kelp, N. Y., Bugeja, J. M., Bolsterlee, B., Hug, F., and Dick, T. J. M. 2021.
 524 Quantity versus quality: Age-related differences in muscle volume, intramuscular fat, and
 525 mechanical properties in the triceps surae. *Exp. Gerontol.* 156:111594.
 526 doi:10.1016/j.exger.2021.111594
 527 Preacher, K. J., and Hayes, A. F. 2004. SPSS and SAS procedures for estimating indirect
 528 effects in simple mediation models. *Behav. Res. Methods. Instrum. Comput.* 36(4):717-731.
 529 doi:10.3758/bf03206553

530 Rahemi, H., Nigam, N., and Wakeling, J. M. 2015. The effect of intramuscular fat on skeletal
 531 muscle mechanics: implications for the elderly and obese. *J. R. Soc. Interface.*
 532 12(109):20150365. doi:10.1098/rsif.2015.0365
 533 Reedy, M. K., Holmes, K. C., and Tregear, R. T. 1965. Induced changes in orientation of the
 534 cross-bridges of glycerinated insect flight muscle. *Nature.* 207(5003):1276-1280.
 535 doi:10.1038/2071276a0
 536 Reimers, K., Reimers, C. D., Wagner, S., Paetzke, I., and Pongratz, D. E. 1993. Skeletal
 537 muscle sonography: a correlative study of echogenicity and morphology. *J. Ultrasound. Med.*
 538 12(2):73-77. doi:10.7863/jum.1993.12.2.73
 539 Setlock, C. A., Lulic-Kuryllo, T., Leonardis, J. M., Kulik, M., and Lipps, D. B. 2021. Age
 540 and sex influence the activation-dependent stiffness of the pectoralis major. *J. Anat.*
 541 239(2):479-488. doi:10.1111/joa.13455
 542 Soldos, P., Besenyi, Z., Hideghéty, K., Pávics, L., Hegedűs, Á., Rácz, L., and Kopper, B.
 543 2021. Comparison of Shear Wave Elastography and Dynamometer Test in Muscle Tissue
 544 Characterization for Potential Medical and Sport Application. *Pathol. Oncol. Res.*
 545 27:1609798. doi:10.3389/pore.2021.1609798
 546 Sobel, Michael E. 1982. Asymptotic confidence intervals for indirect effects in structural
 547 equation models. *Sociol. Methodol.* 13: 290–312. doi:10.2307/270723. 10.2307/270723.
 548 Stock, M. S., and Thompson, B. J. 2021. Echo intensity as an indicator of skeletal muscle
 549 quality: applications, methodology, and future directions. *Eur. J. Appl. Physiol.* 121(2):369-
 550 380. doi:10.1007/s00421-020-04556-6

551 Stock, M. S., Oranchuk, D. J., Burton, A. M., and Phan, D. C. 2020. Age-, sex-, and region-
 552 specific differences in skeletal muscle size and quality. *Appl. Physiol. Nutr. Metab.* 45(11),
 553 1253–1260. doi:10.1139/apnm-2020-0114
 554 Straight, C. R., Voigt, T. B., Jala, A. V., Chase, J. D., Ringham, O. R., Ades, P. A., et al.
 555 2019. Quadriceps Lipid Content Has Sex-Specific Associations With Whole-Muscle, Cellular,
 556 and Molecular Contractile Function in Older Adults. *J. Gerontol. A. Biol. Sci. Med. Sci.*
 557 74(12):1879-1886. doi:10.1093/gerona/gly235
 558 Taniguchi, M., Yamada, Y., Fukumoto, Y., Sawano, S., Minami, S., Ikezoe, T., et al. 2017.
 559 Increase in echo intensity and extracellular-to-intracellular water ratio is independently
 560 associated with muscle weakness in elderly women. *Eur. J. Appl. Physiol.* 117(10):2001-
 561 2007. doi:10.1007/s00421-017-3686-x
 562 Visser, M., Goodpaster, B. H., Kritchevsky, S. B., Newman, A. B., Nevitt, M., Rubin, S. M.,
 563 et al. 2005. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident
 564 mobility limitations in well-functioning older persons. *J. Gerontol. A. Biol. Sci. Med. Sci.*
 565 60(3):324-333. doi:10.1093/gerona/60.3.324
 566 Wang, C. Z., Guo, J. Y., Li, T. J., Zhou, Y., Shi, W., and Zheng, Y. P. 2017. Age and Sex
 567 Effects on the Active Stiffness of Vastus Intermedius under Isometric Contraction. *Biomed.*
 568 *Res. Int.* 2017:9469548. doi:10.1155/2017/9469548
 569 Watanabe, Y., Yamada, Y., Fukumoto, Y., Ishihara, T., Yokoyama, K., Yoshida, T., et al.
 570 2013. Echo intensity obtained from ultrasonography images reflecting muscle strength in
 571 elderly men. *Clin. Interv. Aging.* 8:993-998. doi:10.2147/CIA.S47263

572 Yamazaki, K., Inoue, K., and Miyamoto, N. 2022. Passive and active muscle elasticity of
573 medial gastrocnemius is related to performance in sprinters. *Eur. J. Appl. Physiol.*
574 122(2):447-457. doi:10.1007/s00421-021-04848-5

575 Yoshiko, A., Ando, R., and Akima, H. 2023. Passive muscle stiffness is correlated with the
576 intramuscular adipose tissue in young individuals. *Eur. J. Appl. Physiol.* 123(5):1081-1090.
577 doi:10.1007/s00421-023-05137-z

578 Yoshitake, Y., Takai, Y., Kanehisa, H., and Shinohara, M. 2014. Muscle shear modulus
579 measured with ultrasound shear-wave elastography across a wide range of contraction
580 intensity. *Muscle. Nerve.* 50(1):103-113. doi:10.1002/mus.24104

Table

Table 1. Vastus lateralis echo intensity (EI), muscle thickness (MT), maximal voluntary isometric contraction strength (MVIC) of knee extension, and vastus lateralis shear elastic modulus.

	Mean $\pm$ SD (N=79)	95% CI
EI (a.u.)	78.24 $\pm$ 11.30	[75.71, 80.77]
MT (cm)	1.62 $\pm$ 0.37	[1.54, 1.70]
MVIC (Nm)	100.81 $\pm$ 21.36	[97.03, 105.60]
G0 (kPa)	6.17 $\pm$ 2.70	[5.56, 6.77]
G15 (kPa)	24.80 $\pm$ 7.62	[23.09, 26.50]
G30 (kPa)	38.33 $\pm$ 10.75	[35.92, 40.74]
G45 (kPa)	49.71 $\pm$ 13.04	[46.79, 52.63]
Gslope	0.96 $\pm$ 0.30	[0.90, 1.03]

Notes: G0 = shear elastic modulus at rest; G15 = shear elastic modulus at 15% MVIC; G30 = shear elastic modulus at 30% MVIC; G45 = shear elastic modulus at 45% MVIC; Gslope = slope of G increase during submaximal voluntary isometric contraction.

589 Table 2. Multiple regression models for predicting for the slope of shear elastic modulus
590 increases during submaximal voluntary isometric contraction (Gslope).

Adjusted R ²	Independent variables	Coefficient (B)	Standardized coefficient(β)	t-value	p-value	95% CI	
						lower	upper
0.216	(Constant)	2.76		7.04	<0.001	1.976	3.535
	EI	-0.01	-0.34	-3.37	<0.001	-0.014	-0.004
	Age	-0.01	-0.25	-2.44	0.017	-0.020	-0.002
	BMI	-0.01	-0.16	-1.51	0.136	-0.029	0.004

591 *Notes:* EI = echo intensity; BMI = body mass index.

592

593 Table 3. Total, direct, and indirect effect of vastus lateralis echo intensity (EI) on maximal
594 voluntary isometric contraction strength (MVIC) of knee extension.

Mediator	Total effect		Direct effect		Indirect effect
	Effect		Effect		Effect
	[Bootstrap 95%CI]	p	[Bootstrap 95%CI]	p	[Bootstrap 95%CI]
Gslope	-0.42 [-0.83, -0.01]	0.042	-0.11 [-0.52, 0.29]	0.585	-0.31 [-0.57, -0.12]

595 *Notes:* Gslope the slope of shear elastic modulus increases during submaximal voluntary
596 isometric contraction.

597 Indirect effects were deemed significant if zero was not included in the bootstrap 95% CI.

598

Figure legends

Figure 1. Vastus lateralis shear elastic modulus under different muscle contraction conditions.

G0: shear elastic modulus at rest; G15: shear elastic modulus at 15% MVIC; G30: shear elastic modulus at 30% MVIC; G45: shear elastic modulus at 45% MVIC.

Within the region of interest, blue indicates a low shear elastic modulus, whereas yellow and red indicate high shear elastic moduli.

Figure 2. Conceptual model of mediation analysis.

Path a = effect of X on M;

Path b = effect of M on Y;

Path c' = direct effect of X on Y controlling for M;

Path ab = indirect effect of X on Y through M;

Path c = total effect of X on Y (direct and indirect).

EI, echo intensity; MT, muscle thickness; MVIC, maximal voluntary isometric contraction strength of knee extension; Gslope, slope of the shear elastic modulus increases during submaximal voluntary isometric contraction.

Figure 3. Relationship between vastus lateralis echo intensity (EI) and shear elastic modulus at different submaximal voluntary isometric contractions.

G0: shear elastic modulus at rest; G15: shear elastic modulus at 15% maximal voluntary isometric contraction; G30: shear elastic modulus at 30% maximal voluntary isometric contraction; G45: shear elastic modulus at 45% maximal voluntary isometric contraction.

621

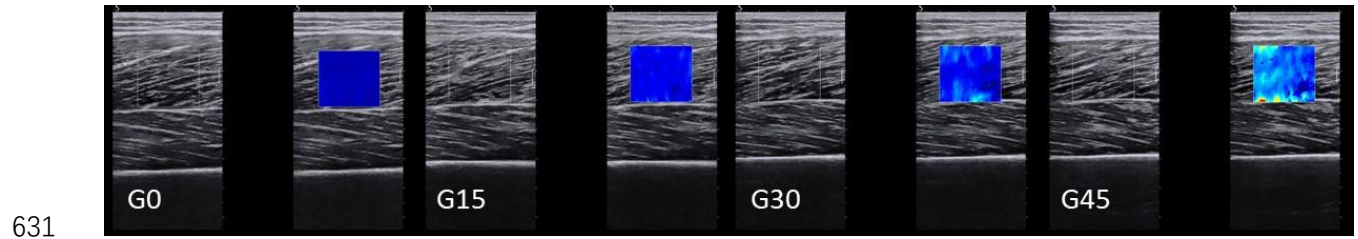
622 Figure 4. Mediation model of the association between vastus lateralis echo intensity (EI) and
623 maximal voluntary isometric contraction (MVIC) strength of knee extension with the slope of
624 shear elastic modulus increases during submaximal voluntary isometric contraction as
625 mediators, controlling for vastus lateralis muscle thickness (MT).

626 Path a, b, c, and c' are expressed as the regression standardized coefficient (β).

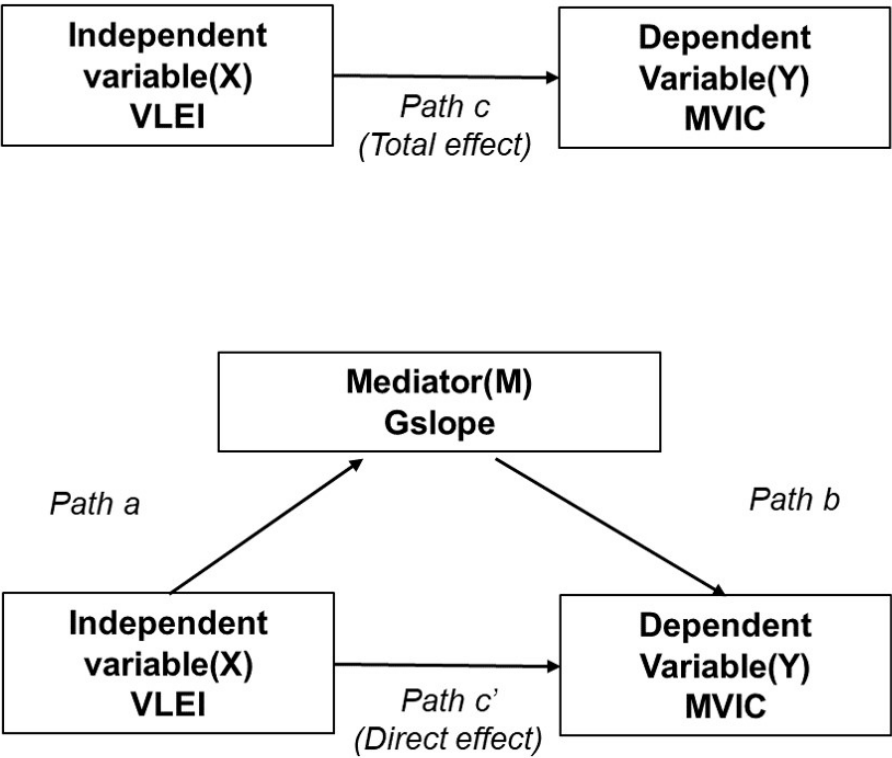
627 Gslope the slope of shear elastic modulus increases during submaximal voluntary isometric
628 contraction.

629

630 Figure 1.



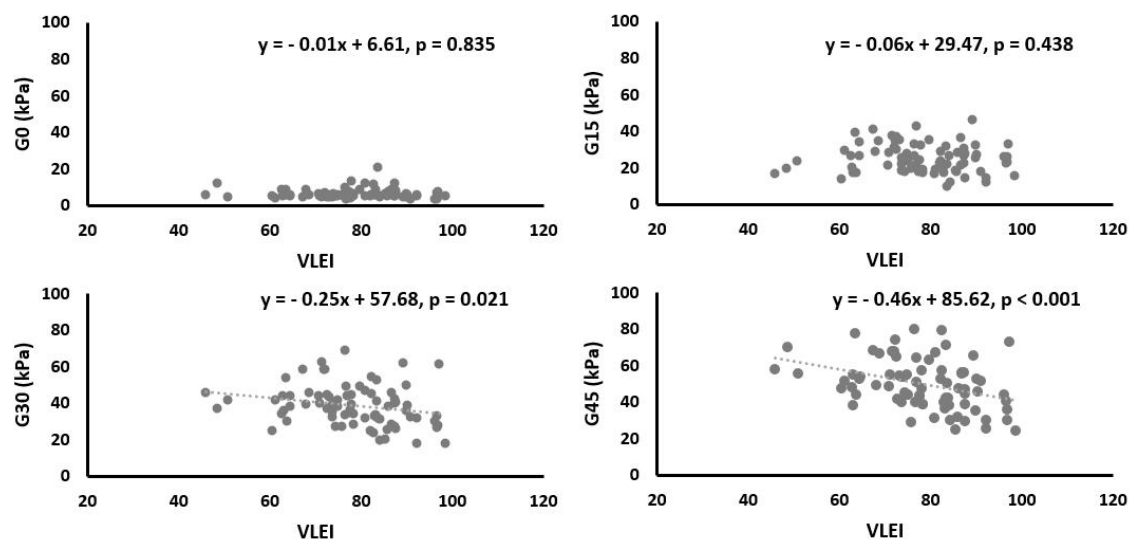
633 Figure 2.



634

635

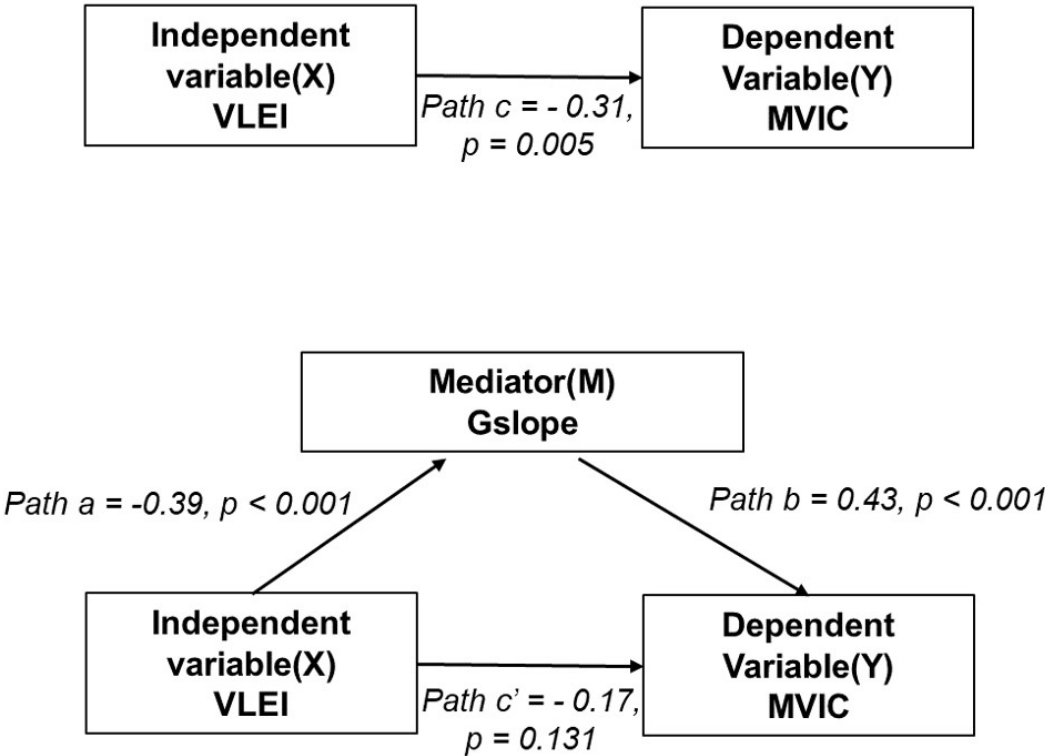
636 Figure 3.



637

638

639 Figure 4.



640