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Title

Effect of age on shear modulus, muscle thickness, echo intensity of the upper limb, lower limb, and trunk muscles in healthy women

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Abstract

Purpose

This study aimed to examine the effect of age on the mechanical properties, muscle size, and muscle quality in the upper and lower limb and trunk muscles.

Methods

We evaluated the shear modulus (G), muscle thickness (MT), and echo intensity (EI) of the upper and lower limb and trunk muscles of 83 healthy women (21-83-year-old). The G values of some limb muscles were measured in relaxed and stretched positions.

Results

Regarding the effect of age on G at the distinct positions, the G of the upper limb muscles were not significantly correlated with age in the relaxed and stretched positions. In contrast, the G of the iliacus showed a significant negative correlation in both positions. Additionally, the G of the rectus femoris had a significant negative correlation only in the relaxed position. Regarding differences among body parts, the G of the lower limb and oblique abdominal muscles showed a significant negative correlation, but no correlation in the upper limb, rectus abdominis, and back muscles. Moreover, MT showed a significant negative correlation with age in the lower limb, abdominal, and erector spinae muscles, but no correlation was detected in the upper limb and lumbar multifidus muscles. EI had a significant positive correlation in all the muscles.

Conclusion

The effect of age on G depended on body parts, and the G of the lower limb and oblique abdominal muscles negatively associated with age. Additionally, G in the relaxed position may be more susceptible to aging than G in the stretched position.

Keywords

ultrasound, elastography, shear modulus, muscle thickness, echo intensity, aging

Declarations

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Conflicts of interest/Competing interests

None

Availability of data and material

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Code availability

Not applicable

Author's Contributions:

All authors have made substantial contributions to the conception and design of the study, collection, analysis, or interpretation of data. Moreover, all authors revised the manuscript critically for important intellectual content and read and approved the final manuscript.
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Study conception and design: Kobayashi, Yagi, Tateuchi, Umehara, Ichihashi
Acquisition of data: Kobayashi, Yagi, Sakata, Okada
Analysis and interpretation of data: Kobayashi, Yagi, Tateuchi, Ota, Umehara, Ichihashi

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Ethics approval (include appropriate approvals or waivers)

All procedures were approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine (R1674).

Consent to participate and Consent for publication

Prior to this study, the procedures and goals of the study were explained to all participants, who then provided written informed consent.

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Abbreviations

G: shear modulus
MT: muscle thickness
EI: echo intensity
SSP: the supraspinatus
ISP: infraspinatus
DT: middle part of the deltoid
BB: long head of the biceps brachii
UT: upper trapezius
PM: clavicular region of the pectoralis major
Pm: pectoralis minor
IL: iliacus
RF: rectus femoris
GM: gluteus maximus
BF: biceps femoris
RA: the rectus abdominis
EO: external oblique
IO: internal oblique
TrA: transverse abdominis
ThEL: thoracic erector spinae
LEL: lumbar erector spinae
MF: lumbar multifidus
ROI: region of interest
V: shear wave velocity
ρ : muscle density
ICC: intraclass correlation coefficient

1. Introduction

Muscle performance declines with age, which causes adverse health events, mortality, and increased healthcare costs (Miller et al. 2021). One explanation for the decline in muscle performance with age is the loss of muscle mass and quality. It has been shown that muscle thickness (MT), as the indicator of muscle size, decreases, and echo intensity (EI), as the indicator of muscle quality, increases with age (Fukumoto et al. 2015; Ota et al. 2020). In recent years, shear wave elastography (SWE) has been recognized as a reliable method to evaluate the shear modulus (G) as the indicator of the mechanical properties of muscle (Eby et al. 2013). SWE generates shear waves in the tissue by using the acoustic radiation force from a push pulse. Then, SWE automatically provides a two-dimensional map of G calculated from the shear wave velocity on a B-mode image of the target tissue (Lacourpaille et al. 2012). Several previous studies have reported that in addition to MT and EI, G is also affected by age (Akagi et al. 2015; Alfuraih et al. 2019).

Although studies have been conducted to determine the effect of age on G in various skeletal muscles, the results are contradictory. For example, G has been reported to be positively correlated (Eby et al. 2015; Baumer et al. 2018; Chodock et al. 2020; Liu et al. 2021), negatively correlated (Akagi et al. 2015; Alfuraih et al. 2019; Hirata et al. 2020), or not correlated with age (Akagi et al. 2015; Chodock et al. 2020; Hirata et al. 2020; Lindemann et al. 2020; Şendur et al. 2020). These conflicting results may be due to variance in the measurement positions and/or differences in a part of the body. First, the variance in the measurement positions could change G because it is highly affected by muscle length (Lacourpaille et al. 2013; Chodock et al. 2020). However, most previous studies have evaluated G in only one measurement position (Stenroth et al. 2012; Akagi et al. 2015; Baumer et al. 2018; Chodock et al. 2020; Lindemann et al. 2020; Şendur et al. 2020). As recent studies have shown that the effect of age on G vary, depending on the measurement positions for the gastrocnemius, quadriceps femoris, and biceps brachii muscles (Eby et al. 2015; Alfuraih et al. 2019; Hirata et al. 2020; Liu et al. 2021; Xu et al. 2021), it is critical to measure G in the relaxed and stretched positions to determine the original effect of age on G. Second, although the change in G could vary, depending on the body part, few studies have examined the effect of age on G for the upper and lower limb muscles in one participant. Previous studies have reported G were positively correlated with age in the upper limb muscles (Chodock et al. 2020), such as the supraspinatus (Baumer et al. 2018) and biceps brachii (Eby et al. 2015), and G were negatively correlated with age in the lower muscles, such as the quadriceps (Akagi et al. 2015; Alfuraih et al. 2019) and hamstrings (Alfuraih et al. 2019), with age. However, most of these studies have examined effect of age on G of only one or a few muscles. Therefore, no conclusion has been reached whether age-related changes in G vary with body part. Additionally, to our knowledge, there are no reports on how the G of trunk muscles is correlated with age. Therefore, it is necessary to evaluate the G values of the upper limb, lower limb, and trunk muscles in one participant.

This study aimed to examine the effect of age on G, MT, and EI of the upper limb, lower limb, and trunk muscles using an ultrasound imaging system. Additionally, we focused on whether the age-related change in G differed between the relaxed and stretched positions and whether there were differences among body parts. We predicted that G in the relaxed position would be negatively correlated with age because fatty tissue in the muscle increases with age (Pinel et al. 2021), and it is softer than the muscle tissue (Chakouch et al. 2015). In contrast, G in the stretched position may reflect shortening of muscle length. Muscle length decreases with age (Gajdosik et al. 1999), and a previous study suggested that age-related shortening of muscles may result from increased amounts of collagen, cross-linked collagen fibers, and proportion of slow twitch muscle fibers (Gajdosik et al. 2005). Thus, we expect G in the stretched position to be positively correlated with age. We also expected MT to be negatively correlated and EI to be positively correlated with age, as shown in previous studies (Fukumoto et al. 2015; Ota et al. 2020).

2. Methods

2.1 Participants

Eighty-three women (age, 46.4 ± 16.0 years, 21-82 years; height, 158.1 ± 5.2 cm; mass, 52.5 ± 7.5 kg) participated in this study. The sample size was calculated using the G*Power software (version 3.1; Heinrich Heine University, Dusseldorf, Germany) for a correlation analysis model (effect size = 0.3, α error = 0.05, and power = 0.8). The required sample size was 82 participants. Thus, 83 participants were recruited in this study, considering the absence of data. All the participants were fully informed of the study procedures and purpose, and they signed a participation agreement before the experiment. This study was approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine (approval number: R1674). The exclusion criteria were current or previous history of orthopedic or nervous system abnormalities, surgery, trauma, or sustained pain. The inclusion criterion was independent daily living, which included schoolwork, jobs, or housework.

2.2 Experimental protocol

This study was a cross-sectional observational design. Ultrasound imaging systems and the linear probe SL 10-2 (Aixplorer version 12.2.0; SuperSonic Imagine, Aix-en Provence, France) were used to capture the B-mode and elastographic images. The B-mode and elastographic images were captured twice on the right side. The measured muscles were as follows; 1) upper limb muscles; the supraspinatus (SSP), infraspinatus (ISP), middle part of the deltoid (DT), long head of the biceps brachii (BB), upper trapezius (UT), and clavicular region of the pectoralis major (PM) and pectoralis minor (Pm); 2) lower limb muscles; the iliacus (IL), rectus femoris (RF), gluteus maximus (GM), and biceps femoris (BF); 3) abdominal muscles: the rectus abdominis (RA), external oblique (EO), internal oblique (IO), and transverse abdominis (TrA); 4) back muscles; the thoracic erector spinae (ThEL), lumbar erector spinae (LEL), and lumbar multifidus (MF). Images were captured in the following five positions (Figure 1): 1) lowering position: sitting, right shoulder 0° flexion, rotation at neutral, and elbow 0° flexion; 2) raising position: sitting, right shoulder 90° flexion, 90° internal rotation, and elbow 90° flexion; 3) supine position: right hip rotation at neutral; 4) prone position: right shoulder 90° abduction and cervical spine 90° left rotation; and 5) Thomas position: right knee 90° flexion, left hip, and knee flexion. First, B-mode and elastographic images of SSP, ISP, DT, BB, UT, PM, and Pm were taken at the lowering position. Moreover, elastographic images of SSP, ISP, DT, and BB were captured at the raising position to compare the effect of age on G in the relaxed position with G in the stretched position. Since SSP and DT have a moment arm of shoulder flexion (Ackland et al. 2008) and BB has a moment arm of elbow flexion (Hale et al. 2011), we expected that the muscles would be more relaxed in the raising position than in the lower position. Additionally, since the ISP has a moment arm of shoulder external rotation (Ackland and Pandy 2011), we predicted that the muscle would be more stretched in the raising position than in the lowering position. Elastographic images of the UT were also taken in the prone position, and those of the PM or Pm were taken in the supine position to exclude the effect of gravity and variance in the scapula position. Next, the B-mode and elastographic images of the IL, RF, RA, EO, IO, and TrA were taken in the supine position, and those of the GM, BF, ThEL, LEL, and MF were taken in the prone position. Moreover, the elastographic images of IL and RF were also captured at the Thomas position to compare the effect of age on G in the relaxed position with G in the stretched position. Since the IL and RF have a moment arm of hip flexion (Dostal et al. 1986) and RF has a moment arm of knee extension (Buford et al. 1997), we expected that the muscles would be more stretched in the Thomas position than in the supine position. The ultrasound measurement sites for each muscle are shown in Table 1. The measurement site for each muscle is marked. Based on the marks at the measurement sites, the muscle belly was identified on the ultrasound image, and the images were captured. In an additional test, we tested the day-to-day repeatability since the measurements were taken over a period of seven days. The intraclass correlation coefficient (ICC) (1, 1) was 0.622–0.992 for all muscles, confirming moderate to excellent reliability. In addition, one examiner performed the measurements on all 83 individuals.

2.3 Measurements of G

We used elastography mode to measure G in longitudinal images of the muscle or parallel to the muscle fibers (Figure 2). The measurement modes were custom musculoskeletal preset penetration modes with high persistence, smoothing level of 5, opacity of 100%, and gain of 90%. For image analysis using the Aixplorer software, the region of interest (ROI) was set to 1 × 1 cm at the center of the muscle belly. Although we used a constant ROI size, we manually traced to avoid including non-muscle tissue (i.e., bone and fascia) and artifacts and calculated G using the largest ROI that would include only muscle. Using an ROI with a size of 2.5 cm (length) × 1 cm (width), EO-G, IO-G, or TrA-G were calculated from a single image.

Shear modulus was calculated from the shear wave velocity (V) and muscle density (ρ) as follows (Bercoff et al. 2004):

$$G = \rho V^2$$

Muscle density was assumed to be 1 g/cm^3 (Nordez et al. 2008). The Aixplorer system can automatically compute the average Young's modulus in the ROI by using the above equation. G was calculated by dividing the value computed by the Aixplorer system by three (Lacourpaille et al. 2012). We measured the shear modulus of each muscle twice because a pilot study showed that these measurements had high repeatability with ICC (1, 2) of 0.923–0.998.

An average of two measurements was used for the analysis. The ICC (1, 2) for the two measurements was >0.916 for all the muscles, which confirms high reproducibility.

2.4 Measurements of MT

Muscle thickness was measured from the transverse image of the muscle using B-mode (Figure 2). The measurement modes were musculoskeletal preset penetration modes with a frequency of 36 MHz, gain of 70%, and a dynamic focus depth of 0 cm. We imported the B-mode images in a DICOM format into OsiriX MD (version 9.0; OsiriX, Geneva, Switzerland) and measured the distance between the fascia to determine the MT. An average of two measurements was used for the analysis. The ICC (1, 2) for the two measurements was >0.986 for all the muscles, confirming high reproducibility.

2.5 Measurements of EI

Echo intensity was evaluated from the same transverse B-mode images, as well as the measurement of MT (Figure 2). The measured location and device were the same as for the MT measurements. We traced the largest ROI without the bone or fascia using Osirix MD. The average EI within the ROI was calculated as a value scaled from 0 (black) to 255 (white) using an 8-bit grayscale image of the standard histogram. An increase in EI indicates an increase in the percentage of intramuscular fat and interstitial fibrous tissue, indicating poor muscle quality (Fukumoto et al. 2012, 2015). The ICC (1, 2) for the two measurements was >0.946 for all the muscles, confirming high reproducibility.

2.6 Statistical analysis

Statistical analyses were performed using SPSS software (version 22; IBM Japan, Tokyo, Japan). First, the Shapiro-Wilk test was performed to confirm data normality; however, the normality was not confirmed for several variables. Next, the Wilcoxon's rank sum test was used to compare SSP-G, ISP-G, DT-G, and BB-G in the lowering and raising positions, and IL-G and RF-G in the supine and Thomas positions to determine the relaxed and stretched positions for each muscle. The effect of age was determined based on the relationship between age and the G, MT, and EI of each muscle using Spearman's rank correlation coefficient. Finally, for muscles in which G in both relaxed and stretched positions were significantly correlated with age, Z-scores were used to determine the positions in which G was more sensitive to the influence of age. Data from participants aged 20–29 (20s) (age, 23.4 ± 2.1 years; height, 158.7 ± 6.3 cm; mass, 51.6 ± 5.9 kg) and participants aged 60–82 (over 60s) (age, 66.5 ± 5.0 years; height, 155.5 ± 4.6 cm; mass, 49.7 ± 6.4 kg) were used to calculate Z-scores. Z-scores with individual data expressed as the number of standard deviations (SDs) from the mean for the 20s, were calculated as:

$$\mathrm{Z} = \frac{\chi - \mu}{\sigma}$$

χ is the value for an individual, and μ and σ are the mean and SD, respectively, of the corresponding 20s (Maden-Wilkinson et al. 2014; Hioki et al. 2020). We compared Z-scores obtained from participants in 20s and over 60s using the paired-sample t-test. The level of statistical significance was set at 5%.

3. Results

3.1 Comparison between G measured at the distinct positions

The SSP-G, DT-G, and BB-G were significantly higher in the lowering position than in the raising position, and IL-G and RF-G were higher in the Thomas position than in the supine position, but there was no significant difference in ISP-G between the lowering and raising positions (Figure 3). Therefore, we defined SSP-G, DT-G, BB-G at the raising position, IL-G, and RF-G in the supine position as the relaxed position (SSP-RelaxedG, DT-RelaxedG, BB-RelaxedG, IL-RelaxedG, and RF-RelaxedG, respectively), and SSP-G, DT-G, BB-G at the lowering position, IL-G, and RF-G at the Thomas position as the stretched position (SSP-StretchedG, DT-StretchedG, BB-StretchedG, IL-StretchedG, and RF-StretchedG, respectively).

3.2 The effect of age on G

Among the muscles whose G was measured in the relaxed and stretched positions, the SSP-G, DT-G, and BB-G were not correlated with age in either the relaxed or stretched positions (Table 2 and Online Figure 1). In contrast, the IL-RelaxedG and IL-StretchedG were negatively correlated with age (Table 2 and Online Figure 2). Moreover, the RF-RelaxedG was significantly negatively correlated with age, while the RF-StretchedG was not significantly correlated with age (Table 2 and Online Figure 2). For the other muscles, there were significant positive correlations for UT-G (lowering position) and significant negative correlations for Pm-G (lowering position), GM-G, BF-G, EO-G, IO-G, and TrA-G, but no significant correlations for the other muscles (Table 2 and Online Figures 1, 2, and 3).

3.3 The effect of age on MT

The IL-MT, RF-MT, GM-MT, BF-MT, RA-MT, EO-MT, IO-MT, TrA-MT, ThEL-MT, and LEL-MT showed a significant negative correlation with age, but no significant correlation in other muscles (Table 2, and Online Figures 1, 2, and 3).

3.4 The effect of age on EI

The EI of all the muscles had a significant positive correlation with age (Table 2, and Online Figures 1, 2, and 3).

3.5 Z-scores of IL-RelaxedG and IL-StretchedG in 20s and over 60s groups

Both IL-RelaxedG and IL-StretchedG were significantly correlated with age; thus, we calculated Z-scores for these parameters. Z-scores in the over 60s group were significantly lower than those in the 20s group for both the IL-RelaxedG and IL-StretchedG. The effect sizes of IL-RelaxedG and IL-StretchedG were 1.717 and 1.098, respectively, indicating that IL-RelaxedG was larger (Figure 4).

4. Discussion

This study evaluated G, MT, and EI of the upper limb, lower limb, and trunk muscles in healthy women using an ultrasound system and also examined how these parameters change with age. The G values of some muscles were examined to determine whether the effect of age differed in the relaxed and stretched positions. As a result, the G of some muscles were affected by age, and the effect of age on G depended on the measurement position and body parts. Additionally, the MT of the lower limb, abdominal, and erector spinae muscles were significantly negatively correlated with age, and the EI of all the muscles were significantly positively correlated. These results partially support our prediction that G in the relaxed position has a negative correlation and G in the stretched position has a positive correlation with age, and MT has a negative correlation and EI has a positive correlation with age. This is the first study to comprehensively assess G, MT, and EI of the upper limb, lower limb, and trunk muscles, and to determine the effect of age on these parameters.

4.1 Differences of the effect of age on G in the relaxed and stretched positions

The SSP-G, DT-G, and BB-G measured in the relaxed and stretched positions were found to be unassociated with age. In contrast, both IL-RelaxedG and IL-StretchedG had a significantly negative correlation with age. Additionally, the RF-RelaxedG had a significantly negative correlation, although the RF-StretchedG were not unassociated with age. The IL-RelaxedG was more sensitive to the influence of age than the IL-StretchedG based on Z-scores.

Several studies (Eby et al. 2015; Alfuraih et al. 2019; Xu et al. 2021) have also explored the effect of age on the BB-G and RF-G measured in multiple positions. Alfuraih et al. (Alfuraih et al. 2019) reported RF-RelaxedG (supine position) in the older group (75-94 years) was significant lower compared with the young (20-35 years) and middle-aged (40-55 years) groups, but no difference in RF-StretchedG (seated with the hips and knees flexed at 90°) among the three groups. These results are consistent with those reported by Alfuraih et al. In contrast, Eby et al. (Eby et al. 2015) reported that BB-RelaxedG (90° elbow flexion) were not unassociated with age, but BB-StretchedG (full extension) had a significantly positive correlation in the older age group (60 years and older). Additionally, Xu et al. (Xu et al. 2021) reported that compared to a younger group (20-32 years old), there was no difference in RF-RelaxedG (knee flexed at 30°) in an older group (50-70 years), but RF-StretchedG (knee flexed at 60°, 90°, and 105°) was higher. This finding was inconsistent with the results of their study. Although MT and EI were not evaluated in these previous studies, differences in participant age (Eby et al. 2015), physical activity level (Bastijns et al. 2020), and intramuscular fat (Pinel et al. 2021) may have affected the discrepancy in the results for G.

Interestingly, only the RF-stretchedG were not unassociated with age, although RF-relaxedG had a significantly negative correlation with age. A previous study presumed that G in the relaxed position (muscle length below the slack angle) reflects the elasticity of the muscle, while G in the stretched position (muscle length above the slack angle) includes the effect of passive tension in addition to elasticity (Koo et al. 2013). For three reasons, the RF could be stretched more than the IL in the Thomas position: the hip flexion moment arm of RF is larger than that of IL (Dostal et al. 1986), G was measured at 90° knee flexion in the Thomas position, and RF became shorter with age (Nonaka et al. 2002). Actually, the RF-StretchedG was 3.37

times higher than the RF-RelaxedG, whereas the IL-StretchedG was 1.94 times higher than the IL-RelaxedG in this study. Therefore, we consider that the RF-RelaxedG had a negative correlation with age, but the effect of age on RF-StretchedG may have been canceled because the shortened RF generates greater passive tension in older groups. In contrast, the IL-StretchedG had a negative correlation with age, like the IL-RelaxedG, because the IL was not as short as the RF with age, and the IL was not as stretched at the stretched position as the RF. Therefore, the IL may generate less passive tension.

4.2 Differences in the effect of age on G between body parts

It was found that the UT-G (lowered position) had a positive correlation and Pm-G (lowered position) had a negative correlation with age. In contrast, the UT-G (prone position) and Pm-G (supine position) measured in the position without gravity or the scapula position effect, were not significantly correlated with age. Therefore, it is likely that the increased UT-G and decreased Pm-G at the lowering position may have been caused by the effects of gravity and scapula position. The UT-G (lowering position) seemed to be correlated positively with age because the UT worked to prevent shoulder girdle depression at the lowering position. Moreover, Pm-G (lowering position) may be correlated negatively with age because the increased scapular anterior tilt with age (Endo et al. 2004), and may shorten the Pm length in the lowering position.

The G of the upper limb muscles, excluding UT-G (lowering position) and Pm-G (lowering position), were not significantly correlated with age. Baumer et al. (Baumer et al. 2018) showed that SSP-G had a positive correlation with age, which is inconsistent with the findings of this study that SSP-G did not change with age. Due to the small sample size (19 participants) in their study (Baumer et al. 2018), their results may not reveal genuine effect of age on G in the entire population. For the lower limb muscles, we found that IL-G, RF-G, GM-

G, and BF-G had a negative correlation with age. Previous studies have also shown that RF-G (Akagi et al. 2015) and BF-G (Alfuraih et al. 2019) had a negative correlation with age, which is consistent with the results of this study.

The UT-G and Pm-G were affected not only by age but also by gravity and scapula position. Therefore, we could postulate that the G of the upper limb muscles, back muscles, and RA were not correlated with age. In contrast, the G values of the lower limb muscles or abdominal oblique muscle groups had a negative correlation with age. The negative correlation with age on G is presumably caused by the age-dependent loss of resistance properties in the elastic fiber system of the muscle extracellular matrix, myosteatosis, myofiber size variation, or connective tissue disorders (Rodrigues and Rodrigues 2000; Vandervoort 2002; Kragstrup et al. 2011). Previous studies have suggested that mechanical loading stress on the muscles in daily life may affect G (Akagi et al. 2015). Since an age-related decline in physical activity can affect the lower limb muscles, whose strength is highly responsible for general physical activity (walking and stepping the stairs), the effects of age are more apparent in the lower limb muscles than in the upper limb muscles (Janssen et al. 2000), which could have induced the effect of age on G in the lower limb muscles but not in the upper limb muscles. Additionally, the mechanical load on the back muscle groups in daily life can increase with age because a flexed posture of the trunk occurs with age (Balzini et al. 2003). Therefore, we considered that the effect of age on G did not occur in the back muscle group.

4.3 The effect of age on G, MT, and EI

The MT of the upper limb muscles or lumbar multifidus were not correlated, while the MT of the lower limb muscles, abdominal muscle groups, or erector spinae had a negative correlation with age. Our results are supported by previous studies that reported the upper limb muscles have less atrophy than the lower limb muscles (Kubo et al. 2003), and that the lumbar multifidus and deeper muscle of the trunk have less atrophy among the back muscles (Stokes et al. 2005). In contrast, EI had a positive correlation with age in all the muscles. Similar to previous studies (Fukumoto et al. 2015; Ota et al. 2020), our study indicated that EI is affected by age in all muscles, even in participants without generalized pain.

4.4 Limitation

This study had some limitations. First, we could not examine all muscles in different positions. In the future, it will be necessary to measure the G of other muscles in multiple positions to examine the effect of age on G. Second, the participants were limited to women, as previous studies have shown that there are sex differences in G, MT, and EI (Palmer et al. 2015; Saeki et al. 2019). The effect of age on these parameters in men needs to be further investigated. Third, we used a linear model in this analysis, due to sample size and normality issues. With an increased sample size, we may be able to use ANOVA to make comparisons by age group and obtain more detailed findings. Fourth, we expressed the mechanical properties of the muscle using “shear modulus” not “shear wave velocity”. It should be noted that SWE does not directly measure the shear modulus but calculates the shear modulus from the shear wave velocity. In addition, the shear modulus value could depend on the machine manufacturer, because some machines may perform concise calculations. Fifth, we calculated G using a constant ROI size for all muscles. In the future, changes in ROI size for each muscle should be explored to determine whether this impacts the results.

5. Conclusion

We evaluated the G, MT, and EI of the upper limb, lower limb, and trunk muscles in healthy women of various ages using an ultrasound system, and examined the effect of age on these parameters. As a result, the G of the lower limb muscles and oblique abdominal muscles had a negative correlation with age, but G of the upper limb, rectus abdominis, and back muscles were not correlated with age. Additionally, we suggest that G in the relaxed position may be more susceptible to aging than G in the stretched position. The results of this study may help improve rehabilitation against age-related physical performance decline.

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7. Disclosure statement

The authors declare no conflict of interest.

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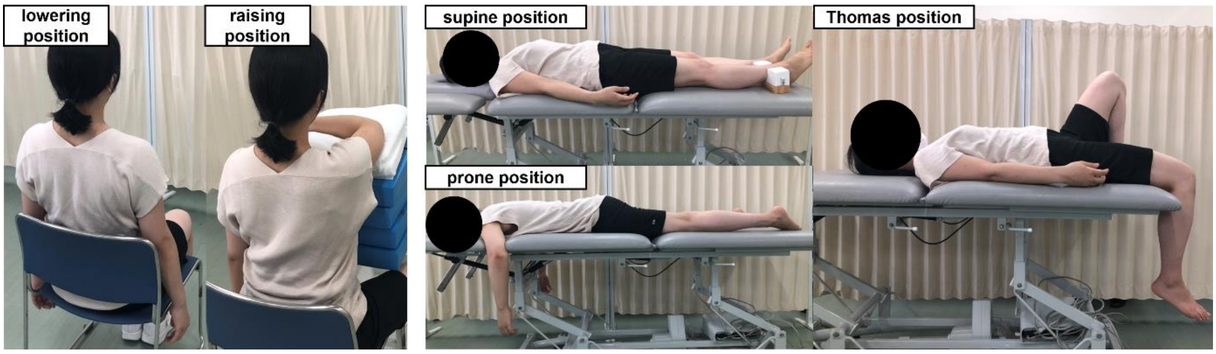

Figure 1. Measurement positions

Lowering position: sitting, right shoulder 0° flexion, rotation at neutral, and elbow 0° flexion; raising position: sitting, right shoulder 90° flexion, 90° internal rotation, and elbow 90° flexion; supine position: right hip rotation at neutral; prone position: right shoulder 90° abduction and cervical spine 90° left rotation; Thomas position: right knee 90° flexion, left hip, and knee flexion.

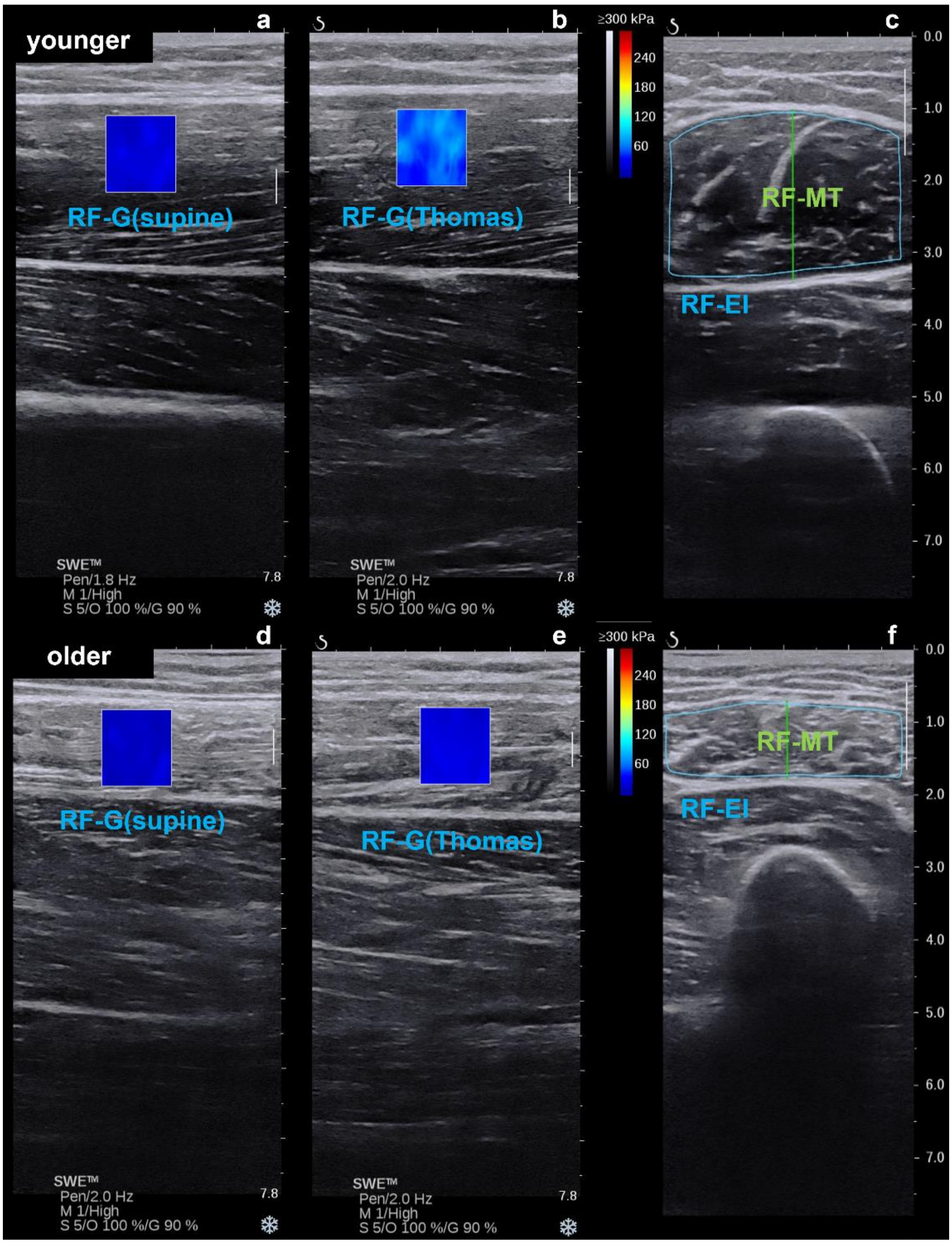

Figure 2. Example ultrasound images

2a-c and 2d-f represent ultrasound images of the RF from younger (22 years old) and an older (60 years old) participant, respectively. 2a and 2d are longitudinal ultrasound images of RF with a color map of Young's modulus in the supine position, while 2b and 2e are those at Thomas positions. 2c and 2f are transverse images of RF. The green line indicates the RF-MT, and the RF-EI is calculated within the light blue box.

RF: rectus femoris.

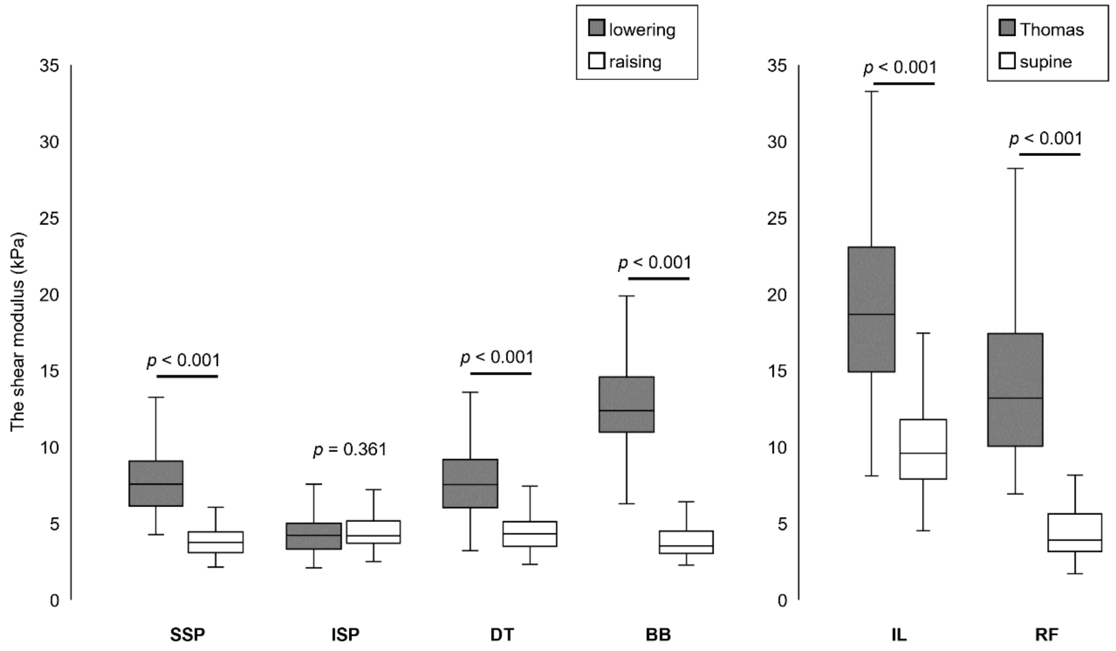

Figure 3. Comparison between the shear moduli measured at the distinct positions

Horizontal bars indicate significant differences, and a significant value is indicated.

SSP: supraspinatus; ISP: infraspinatus; DT: middle part of deltoid; BB: biceps brachii; IL: iliacus; RF: rectus femoris.

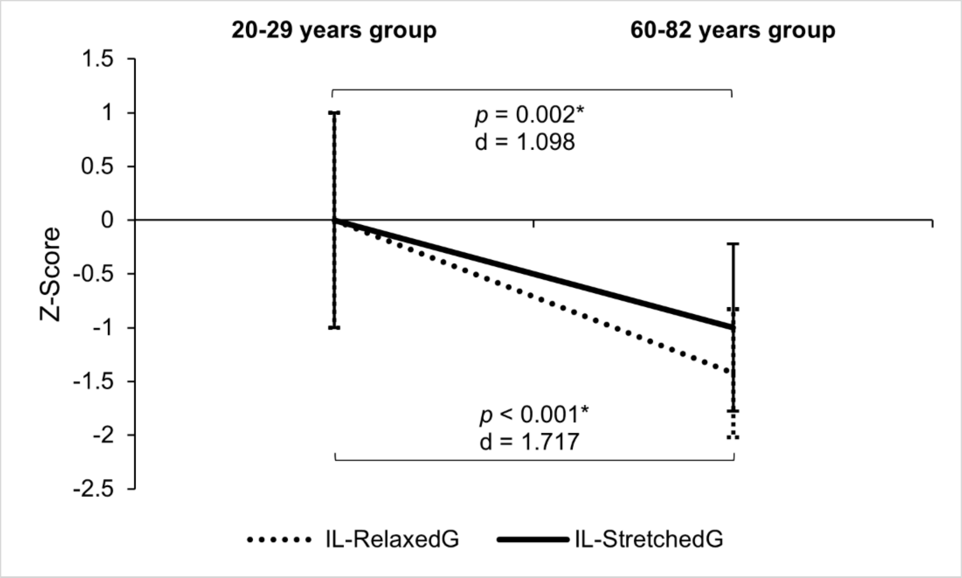

Figure 4. Comparison between Z-Scores (20-29 years group vs 60-82 years group) of IL-Relaxed G and IL-StretchedG

IL, iliacus; RelaxedG, shear modulus at a relaxed position; StretchedG, shear modulus at a stretched position.

Table 1. Ultrasound measurement sites of each muscle

Muscle	Measurement site	Reference
Supraspinatus	The midpoint of the line connecting the midpoint of the clavicle and the midpoint of the spine of scapula	(Itoigawa et al. 2015)
Infraspinatus	Intersection of the line that connects the greater tubercle to the midpoint between trigonum scapulae and the inferior angle, and the line that connects the inferior angle to the midpoint between trigonum scapulae and the acromial angle	(Umehara et al. 2017a)
Middle part of deltoid	The midpoint of the lines joining the lateral part of the acromion and the deltoid tuberosity	(Hatta et al. 2016)
Biceps brachii	Two-thirds of the way between the acromion and the antecubital crease	(Fukumoto et al. 2015)
Upper trapezius	The midpoint of the lines joining the acromion and the C7 spinous process	(Yanase et al. 2021)
Clavicular region of pectoralis major	The midpoint between the greater tubercle of the humerus and sternoclavicular joint	(Umehara et al. 2021)
Pectoralis minor	The midpoint between the coracoid process and the fourth rib–sternum junction	(Umehara et al. 2017b)
Iliacus	4 cm distal from anterior superior iliac spines	(Nojiri et al. 2021)
Rectus femoris	The midway between the anterior superior iliac spine and the proximal end of the patella	(Ikezoe et al. 2011)
Gluteus maximus	30% proximal between posterior superior iliac spine and the greater trochanter	(Ikezoe et al. 2011)
Biceps femoris	The midway between the ischial tuberosity and the lateral condyle of the tibia	(Ikezoe et al. 2011)
Rectus abdominis	3 cm lateral to the umbilicus	(Ota et al. 2020)
External oblique	2.5 cm anterior to the midaxillary line at the midpoint between the inferior rib and the iliac crest	(Ota et al. 2020)
Internal oblique		
Transversus abdominis		
Thoracic erector spinae	4 cm lateral to the Th9 spinous process	(Ota et al. 2020)
Lumbar erector spinae	7 cm lateral to the L3 spinous process	(Masaki et al. 2015)
Lumbar multifidus	2 cm lateral to the L4 spinous process	(Ota et al. 2020)

Based on previous studies, the ultrasound measurement sites for each muscle were determined. We used elastography mode to measure the shear modulus in longitudinal images of the muscle or parallel to the muscle fibers. Muscle thickness and Echo intensity were measured from the transverse image of the muscle using B-mode.

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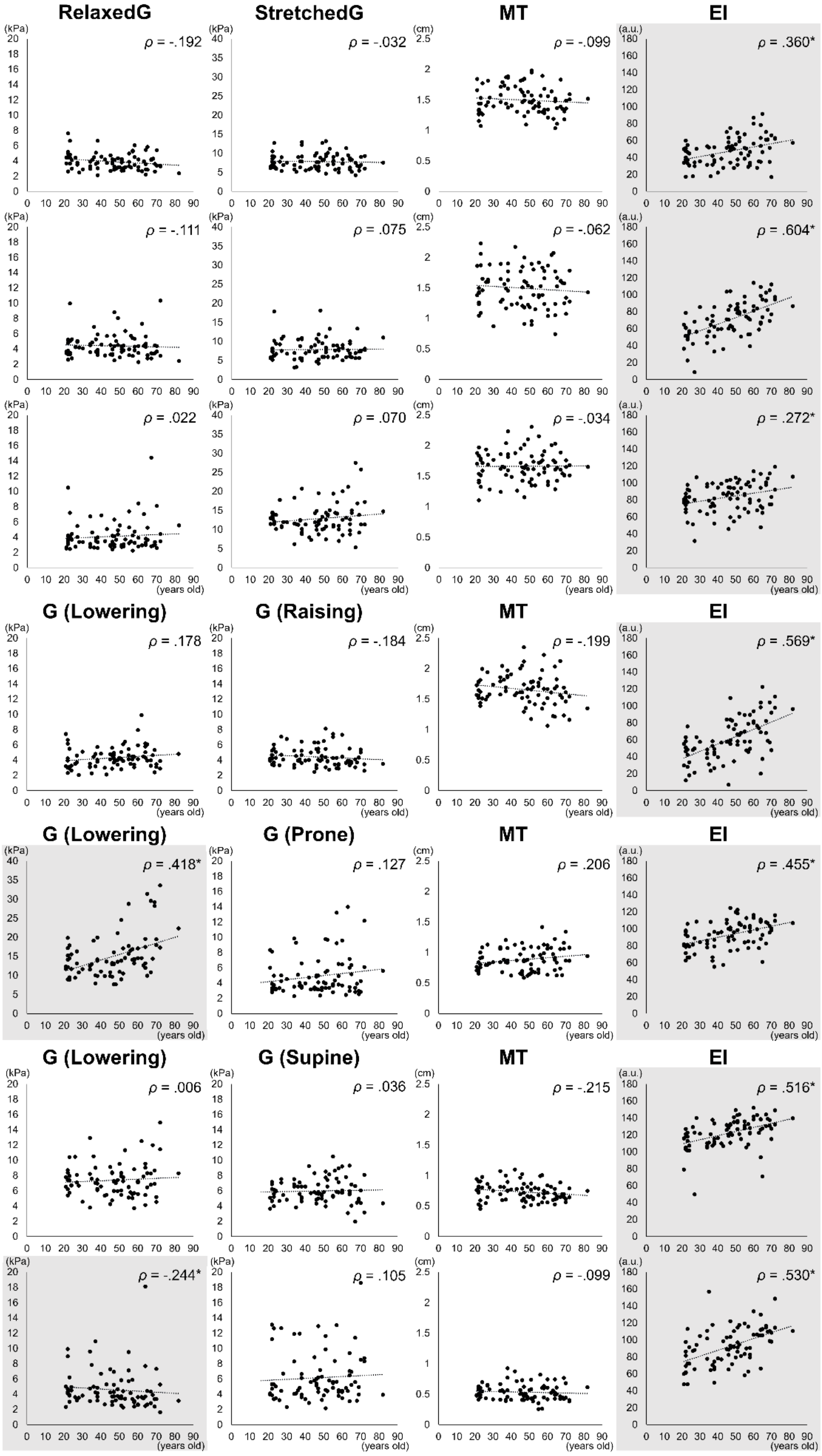
Table 2. Correlation of the shear modulus, muscle thickness, and echo intensity with age

	ρ	*p* value	ρ	*p* value	ρ	*p* value	ρ	*p* value
	RelaxedG (kPa)		StretchedG (kPa)		MT (cm)		EI (a.u.)	
SSP	-0.192	0.083	-0.032	0.775	-0.099	0.373	**0.360***	**0.001**
DT	-0.111	0.319	0.075	0.501	-0.062	0.578	**0.604***	**<0.001**
BB	0.022	0.844	0.070	0.527	-0.034	0.761	**0.272***	**0.013**
IL	**-0.465***	**<0.001**	**-0.359***	**0.001**	**-0.486***	**<0.001**	**0.565***	**<0.001**
RF	**-0.324***	**0.003**	-0.092	0.410	**-0.448***	**<0.001**	**0.525***	**<0.001**
	G (lowering) (kPa)		G (raising) (kPa)		MT (cm)		EI (a.u.)	
ISP	0.178	0.107	-0.184	0.096	-0.199	0.072	**0.569***	**<0.001**
	G (lowering) (kPa)		G (prone) (kPa)		MT (cm)		EI (a.u.)	
UT	**0.418***	**<0.001**	0.127	0.254	0.206	0.062	**0.455***	**<0.001**
	G (lowering) (kPa)		G (supine) (kPa)		MT (cm)		EI (a.u.)	
PM	0.006	0.957	0.036	0.746	-0.215	0.051	**0.516***	**<0.001**
Pm	**-0.244***	**0.026**	0.105	0.347	-0.099	0.374	**0.530***	**<0.001**
	ρ		*p value*		ρ	*p* value	ρ	*p* value
	G (kPa)				MT (cm)		EI (a.u.)	
GM	**-0.250***		**0.023**		**-0.262***	**0.017**	**0.434***	**<0.001**
BF	**-0.221***		**0.045**		**-0.433***	**<0.001**	**0.519***	**<0.001**
RA	0.084		0.451		**-0.560***	**<0.001**	**0.769***	**<0.001**
EO	**-0.384***		**<0.001**		**-0.263***	**0.016**	**0.649***	**<0.001**
IO	**-0.463***		**<0.001**		**-0.313***	**0.004**	**0.632***	**<0.001**
TrA	**-0.430***		**<0.001**		**-0.261***	**0.017**	**0.633***	**<0.001**
ThEL	0.047		0.672		**-0.310***	**0.004**	**0.452***	**<0.001**
LEL	0.081		0.469		**-0.300***	**0.006**	**0.375***	**<0.001**
MF	0.193		0.080		-0.178	0.108	**0.484***	**<0.001**

where ρ denotes the correlation coefficient. * and bold indicate a significant correlation with age.

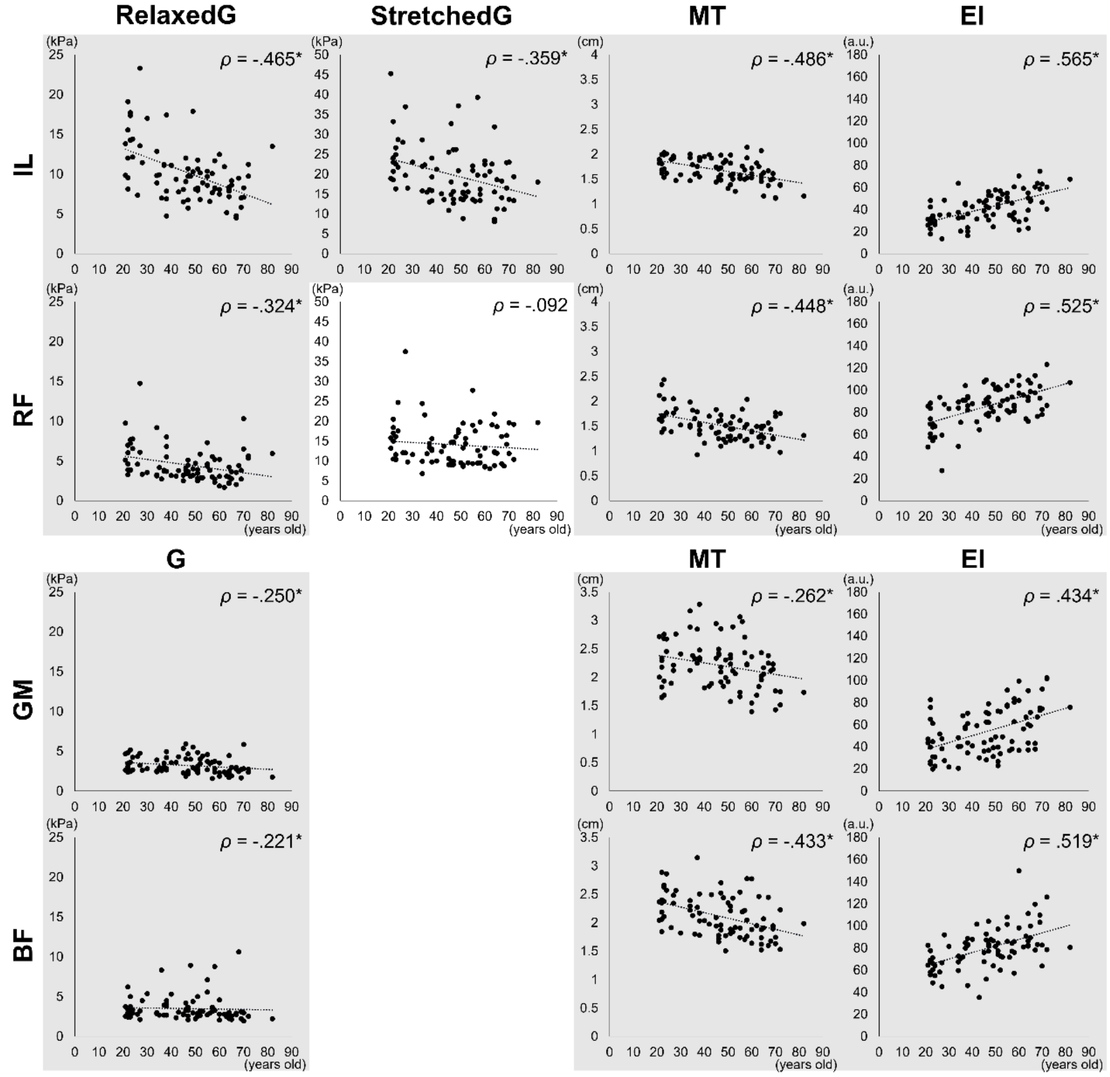
SSP, supraspinatus; DT, middle part of deltoid; BB, biceps brachii; IL, iliacus; RF, rectus femoris; ISP, infraspinatus; UT, upper trapezius; PM, clavicular region of pectoralis major; Pm, pectoralis minor; GM, gluteus maximus; BF, biceps femoris; RA, rectus abdominis; EO, external oblique; IO, internal oblique; TrA, transversus abdominis; ThEL, thoracic erector spinae; LEL, lumbar erector spinae; MF, lumbar multifidus; G, shear modulus; MT, muscle thickness; EI, echo intensity; RelaxedG, shear modulus at a relaxed position; StretchedG, shear modulus at a stretched position

Online Figure 1. Correlation of the shear modulus, muscle thickness, and echo intensity with age in the upper limb muscles



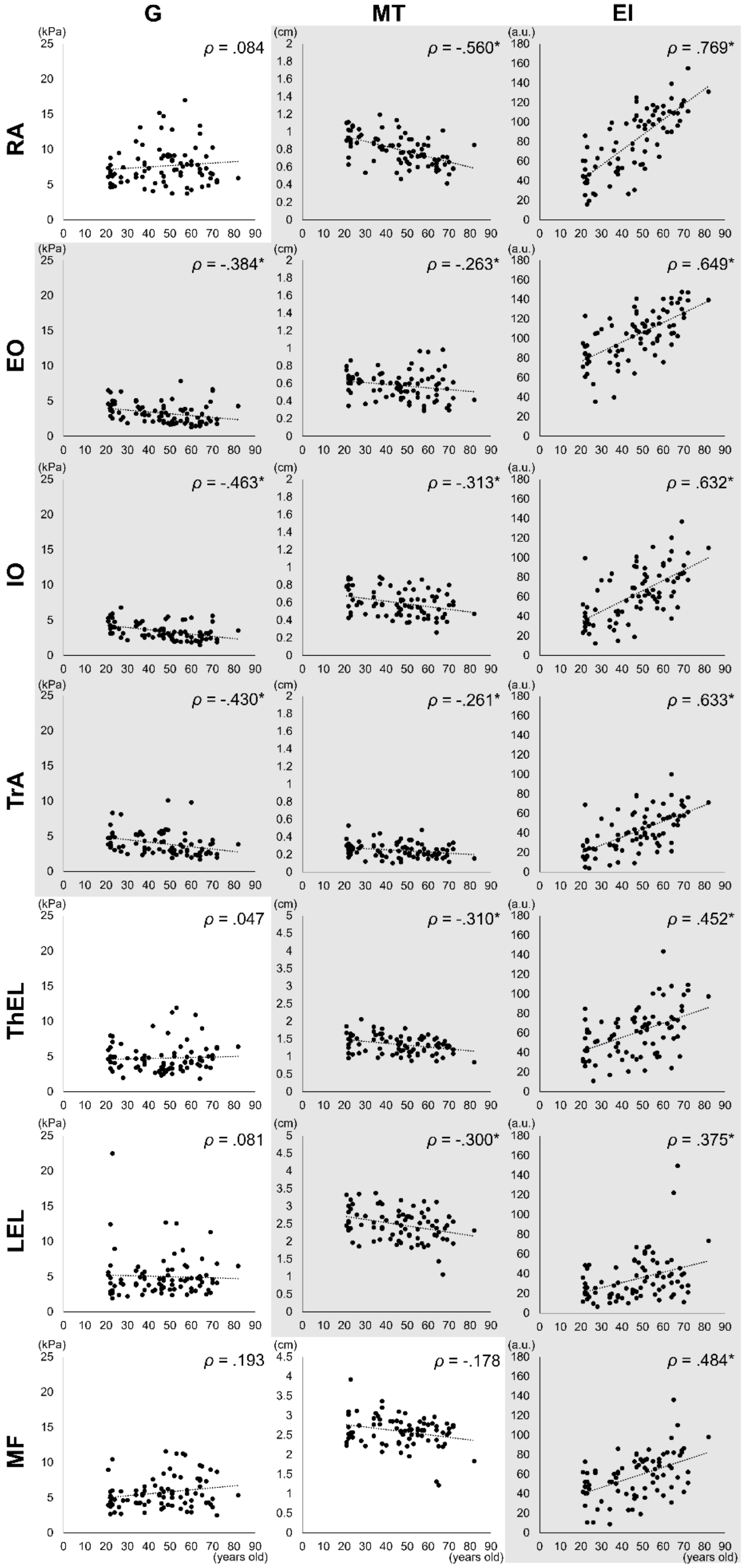
This figure shows correlations between age and muscle properties in the upper limb muscles. SSP, supraspinatus; DT, middle part of the deltoid; BB, biceps brachii; ISP, infraspinatus; UT, upper trapezius; PM, clavicular region of the pectoralis major; Pm, pectoralis minor; G, shear modulus; MT, muscle thickness; EI, echo intensity; RelaxedG, shear modulus at a relaxed position; StretchedG, shear modulus at a stretched position. The transverse axes of all graphs indicate age (years), the vertical axis of the G graphs indicates the shear modulus (kPa), the vertical axis of the MT graphs indicates muscle thickness (cm), and the vertical axis of EI graphs indicates echo intensity (0-255). ρ indicates the correlation coefficient and * indicates that there is a significant correlation with age. Those with a significant correlation with age are colored gray.

Online Figure 2. Correlation of the shear modulus, muscle thickness, and echo intensity with age in the lower limb muscles



This figure shows correlations between age and muscle properties in the lower limb muscles. IL, iliacus; RF, rectus femoris; GM, gluteus maximus; BF, biceps femoris; G, shear modulus; MT, muscle thickness; EI, echo intensity; RelaxedG, shear modulus at a relaxed position; StretchedG, shear modulus at a stretched position. The transverse axes of all graphs indicate age (years), the vertical axis of the G graphs indicates the shear modulus (kPa), the vertical axis of the MT graphs indicates muscle thickness (cm), and the vertical axis of EI graphs indicates echo intensity (0-255). ρ indicates the correlation coefficient and * indicates that there is a significant correlation with age. Those with a significant correlation with age are colored gray.

Online Figure 3. Correlation of the shear modulus, muscle thickness, and echo intensity with age in the trunk muscles



This figure shows correlations between age and muscle properties in the trunk muscles. RA: rectus abdominis, EO: external oblique, IO: internal oblique, TrA: transversus abdominis, ThEL: thoracic erector spinae, LEL: lumbar erector spinae, MF: lumbar multifidus, G: shear modulus, MT: muscle thickness, EI: echo intensity. The transverse axes of all graphs indicate the age (years), the vertical axis of the G graphs indicates the shear modulus (kPa), the vertical axis of the MT graphs indicates the muscle thickness (cm), and the vertical axis of the EI graphs indicates the echo intensity (0-255). ρ indicates the correlation coefficient and * indicates that there is a significant correlation with age. Those with a significant correlation with age are colored gray.