Title

Age-related changes in muscle thickness and echo intensity of trunk muscles in healthy women: Comparison of 20s to 60s age groups

Author names and affiliations

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Abbreviations

EI; Echo intensity

MT; Muscle thickness

20s; 20–29 years old

30s; 30–39 years old

30s; 40–49 years old

50s; 50–59 years old

60s; 60–69 years old

Abstract

Purpose: The objective of this study was to investigate the age-related changes in muscle thickness and muscle echo intensity of trunk in subjects including wide range of age groups. Methods: The subjects were 112 healthy women (age range, 20-60s). The rectus abdominis, external oblique, internal oblique, transversus abdominis, erector spinae, and lumbar multifidus muscles were examined. To confirm the differences among the age groups, the linear mixed effect models were performed. Results: There were significant decreases in muscle thickness of the rectus abdominis and external oblique muscles in the 50s and 60s age groups compared to those in the 20s age group, and a significant decrease in muscle thickness of the erector spinae muscle in the 60s age group compared to those in the 20s age group. However, there was no significant difference among the age groups in muscle thickness of other trunk muscles. There were significant increases in echo intensity of the abdominal muscles in other age groups compared to those in the 20s age group, and significant increases in echo intensity of the back muscles in the age groups over 40 compared to those in the 20s group. Conclusion: Our study revealed that muscle quality may be more affected by age than muscle quantity and the effects of aging differ among muscles.

Keywords: Aging, Ultrasonography, Muscle thickness, Echo intensity, Sarcopenia

1. Introduction

It is well known that skeletal muscle mass decreases with advancing aging, and the age-related loss of muscle quantity has been labeled sarcopenia (Rosenberg, 1997; Cruz-Jentoft et al., 2010; Rosenberg, 2011). Previous studies using computed tomography (CT), magnetic resonance imaging (MRI), and dual-energy X-ray absorptiometry have provided substantial data on age-related loss of skeletal muscles (Rice et al., 1989; Kehayias et al., 1997; Gallagher et al., 1997, Gallagher and Heymsfield 1998; Janssen et al., 2000). Many recent studies measured the muscle thickness (MT) using ultrasonography as a more convenient and noninvasive method than CT or MRI as an indicator of muscle quantity (Janssen et al., 2000; Miyatani et al., 2003; Kubo et al., 2003; Candow and Chilibeck 2005; Arts et al., 2007, 2010; Ikezoe et al., 2011a, 2011b, 2012; Ota et al., 2012; Fukumoto et al., 2012a, 2015). It has been reported that MT has a high correlation with cadaveric dissection (Kellis et al., 2009; Cartwiright et al., 2013). The muscle cross-sectional area was measured using MRI (Dupont et al.; 2001; Miyatani et al., 2004), and the validity of MT measurements performed using ultrasonography have been verified.

Previous studies demonstrated that the lower limb muscles are more susceptible to aging than the upper limbs (Janssen et al., 2000; Miyatani et al., 2003; Kubo et al., 2003; Candow and Chilibeck 2005; Arts et al., 2007; Fukumoto et al., 2015). In addition, the degree of age-related changes in muscle quantity differs among the lower-limb muscles (Arts et al., 2010; Ikezoe et al., 2011a, 2011b; Fukumoto et al., 2015). Our previous studies (Ikezoe et al., 2011a, 2011b) revealed that MT values of the gluteus maximus, gluteus medius, gluteus minimus, psoas major, rectus femoris, vastus lateralis, vastus medialis, biceps femoris, and gastrocnemius muscles in older subjects were significantly thinner than those in young subjects, whereas no significant intergroup difference was seen in MT of the soleus.

Abdominal and back muscles play an important role in maintaining one’s upright position (Bogduk et al. 1992, Arjmand and Shirazi-Adl 2006) and they also contribute to gait (Marques et al. 2016). A previous study showed that compared to adults who were able to perform activities of daily living involving walking independently, greater atrophy of transversus abdominis and lumbar multifidus muscles was observed in older adults who were not able to maintain a sitting position independently (Ikezoe et al. 2012). Therefore, to maintain a physical activity level that acts against aging, it is important to retain abdominal and back muscle functions. It has been suggested that the degree of age-related changes in muscle quantity differs among trunk muscles as well (Ota et al., 2012; Ikezoe et al., 2012). Our previous study (Ota et al., 2012) investigating age-related changes in MT of abdominal muscles with five age groups (young, adult, middle-aged, young-old, and old-old) showed that the rectus abdominis muscle was significantly thicker in the young group than the other groups, and the external and internal oblique muscles were significantly thicker in the young group than in the middle-aged, young-old, and old-old groups. On the other hand, MT of the transversus abdominis muscle did not differ among the age groups. With regard to age-related changes in MT of abdominal muscles, the findings of our previous study (Ikezoe et al., 2012) indicated no significant difference in MT of the lumbar multifidus muscles between the independent older subjects and the young subjects, although the erector spinae muscles of the older subjects were thinner than those of the young subjects.

Furthermore, there are many recent previous studies on muscle echo intensity (EI) measured using a grayscale analysis of ultrasound images. When non-contractile tissues such as intramuscular fibrous and adipose tissues infiltrate between muscle fibers, the interface of the heterogeneous medium increases, and EI is enhanced (Pillen et al., 2009). It has been confirmed that EI correlates to the amount of intramuscular fibrous and adipose tissue, confirmed by muscle biopsies (Reimers et al., 1993, Pillen et al., 2009). In other words, an enhanced EI represents a decrease in the amount of contractile tissue, and it was concluded that EI is a practical and reproducible method that could be used as an imaging technique for the examination of intramuscular fat. Recent studies have shown that resistance training can elicit significant decreases in EI, which is considered an indication of non-contractile tissues in the muscles (Ikezoe et al.; 2017; Radaelli, et al.; 2014). Furthermore, muscle quality, which is quantified by EI, also changes with aging. With regard to the age-related changes in MT and EI of abdominal muscles, a previous study (Fukumoto et al., 2015) revealed that age-related changes in MT were observed in the rectus abdominis, external oblique, and internal oblique muscles, and not in the transversus abdominis muscle, while changes in EI were observed in all abdominal muscles. This study suggested that deep abdominal muscles such as the transversus abdominis muscle have less age-related muscle atrophy than superficial muscles like the rectus abdominis muscle do, and age-related changes in muscle quality of abdominal muscles may occur earlier than the muscle quantity loss that occurs over a life span. Thus, the age-related changes in MT and EI of abdominal muscles were investigated. However, no study has been performed that focuses on the age-related changes in EI of trunk muscles, including back muscles. Moreover, although previous studies on age-related changes in EI targeted mainly older people, few studies have compared groups classified finely by age. Since the question of age group at which age-related changes occur remains unsettled in a comparative study of young and older subjects only, the appropriate stage of intervention time for preventing sarcopenia remains unclear. If age-related change in muscle quality occurs earlier than advanced age in abdominal and back muscles, all ages including pre middle-aged subjects should be targeted.

Here, this study aimed to investigate (a) the effect of age on MT or EI of abdominal and back muscles, and (b) whether there are age groups in which remarkable changes in MT or EI occur. We hypothesized that (a) age-related increases in the amount of non-contractile tissue such as intramuscular fat would be observed in not only abdominal and also back muscles, and that (b) the increases in the non-contractile tissue due to the promotion of heterotopic adipose differentiation may occur at an earlier age than muscle atrophy does.

2. Material and methods

2.1 Participants

Subjects comprised 112 healthy community-dwelling women (age range, 20–69 years; mean height, 158.0 ± 4.4 cm; mean mass, 52.9 ± 7.3 kg; mean body mass index, 21.2 ± 2.5 kg/m^2). This study received approval from the ethics committee of Kyoto University Graduate School Faculty of Medicine. All subjects were given full written explanations before consenting to participate. The exclusion criterion was a current or past history of severe trauma or surgery and sustained pain. The inclusion criterion was independent daily living that included schoolwork, a job, or housework. Subjects were classified into five age groups: 20–29 years old (20s), 30–39 years old (30s), 40–49 years old (40s), 50–59 years old (50s), and 60–69 years old (60s). The characteristics of each group are shown in Table 1. Height, mass, body mass indices, body fat percentages, and subcutaneous fat thickness on the rectus abdominis were compared among the age groups using the Tukey-Kramer method, and no significant intergroup differences were noted.

2.2 Experimental protocol

The subjects were examined in summer and winter. A Chi-squared test confirmed that values measured in the mornings and afternoons were not significantly different among the age groups. MT and EI were measured using B-mode ultrasonography devices (LOGIQ e; GE Healthcare UK Ltd., Chalfont, Buckinghamshiere, England) with multi-frequency linear probes. Six trunk muscles were examined on the right side, including the rectus abdominis, external oblique, internal oblique, transversus abdominis, erector spinae, and lumbar multifidus muscles. The muscle measurement sites are illustrated in Fig. 1 (Ikezoe et al., 2012). The ultrasonography probe was applied vertically to the skin using the minimum pressure required to achieve a clear image, and cross-sectional images were taken. The subjects were asked to assume the supine position during abdominal muscle measurements and the prone position during abdominal muscle measurements. The subjects were instructed to breathe quietly in a resting supine position during abdominal muscle measurements. All probing techniques during measurements were performed by the same experienced investigator, and the images were taken at expiration by another experienced examiner. Our previous study showed that the test-retest reliability intraclass correlation coefficients (ICC) ranged from 0.87 to 0.99 for MT and EI measurements of lower limb and abdominal muscles, analyzed using two images taken on two separate days from the same healthy subjects (Fukumoto et al., 2012a, 2012b). Thus, the ultrasound measurements were confirmed to have a high reliability. Therefore, each measurement in this study was performed only once. The ultrasound settings including frequency (8 MHz), gain (58 dB), and dynamic focus depth (0 cm) were kept consistent during the measurements.

2.3 Measurement of MT

MT was evaluated using an electric caliper on the ultrasound images as an index of muscle quantity. Each MT was determined as the distance between muscle tissue interfaces.

2.4 Measurement of EI

EI on ultrasound images was evaluated using computer-assisted 8-bit grayscale analysis by the standard histogram function in Image J (U. S. National Institutes of Health, Bethesda, MD, USA) as an index of muscle quality.

A high EI represents the augmented proportion of intramuscular fat and interstitial fibrous tissue; thus, EI indicates muscle quality. A region of interest was selected in each muscle to include as much of the muscle as possible without any bone or surrounding fascia. The mean EI in the region of interest were calculated and expressed as a value of 0 (black) to 255 (white) (Fukumoto et al., 2012a, 2012b).

2.5 Statistical analysis

To investigate the age at which remarkable muscle atrophy and the increases in the non-contractile tissue occur, the differences in MT or EI among the age groups were examined by using the linear mixed effect models. These were performed with MT or EI of each muscle as dependent variables; subjects as random effects; and age groups, muscle parts, and confounding factors between age groups with muscle parts as fixed effects. The level of statistical significance was set at 5%.

3. Results

Tables 2 and 3 summarize MT and EI results. The linear mixed effect models for MT were shown in Table 4, 5 and 6. There were significant differences in MT among age groups and muscle parts, and MTs of total trunk muscle in the 50s and 60s age groups were significantly smaller than those in the 20s age group ($p<0.01$). Moreover, there were significant decreases compared to the 20s age group in MT of the rectus abdominis, external oblique, and erector spinae muscles. It was also revealed that MT values of the rectus abdominis and external oblique muscles were significantly smaller in the 50s and older groups than those in the 20s age group ($p<0.05$), and MT values of the erector spinae muscles were significantly smaller in the 60s age group than those in the 20s age group ($p<0.01$). There was no significant difference in MT of the internal oblique, transversus abdominis, and lumbar multifidus muscles. The linear mixed effect models for EI were shown in Table 7, 8 and 9. There were significant differences in EI among age group and muscle part, and interaction of age group and muscle part was observed. EIs of total trunk muscles in other age groups were significantly larger than those in the 20s age group ($p<0.01$). Moreover, it has revealed that EI values of the abdominal muscles, including the internal oblique and transverse abdominis muscles, were significantly larger in all age groups compared to those in the 20s age group ($p<0.01$), and EI values of the back muscles, including the lumbar multifidus muscles, were significantly larger in the 40s and older groups than those in the 20s age group ($p<0.05$).

4. Discussion

The main findings of this study were that there were significant decreases compared to the 20s age group in MT of the rectus abdominis, external oblique, and erector spinae muscles only, whereas there were no significant differences among the age groups in MT of the internal oblique, transversus abdominis, and lumbar multifidus muscles. On the other hand, there were significant differences among the age groups in EI of all trunk muscles, and greater age-related changes in EI were seen than in MT in the younger groups. To our knowledge, this is the first report to show age-related changes including five age groups covering ages 20–69 years on MT and EI of the trunk muscles.

It is a well-known fact that muscle quantity and quantity decreases with aging. Our results revealed that the degree of age-related changes in muscle quantity and quantity differed among muscles; age-related changes of not only abdominal but also back muscles differed depending on each muscle. One reason for this is the differences in roles among muscles. Our previous study (Ikezoe et al., 2012) found that age-related muscle atrophy in the deep trunk muscles such as the transversus abdominis and lumbar multifidus muscles in older women who were able to independently perform activities of daily living were less than that in the superficial muscles such as the rectus abdominis and erector spinae muscles. On the other hand, MT of the transversus abdominis and the lumbar multifidus muscles in the older women who were impossible to independently maintain a seated position (i.e., chronically bedridden) were significantly thinner than those in young women and independent older women (Ikezoe et al., 2012), which suggests that the muscle quantity of the transversus abdominis and lumbar multifidus muscles among independent older adults may be maintained regardless of the aging process. The deep trunk muscles such as part of the internal oblique, transversus abdominis and lumbar multifidus muscles play an important role in stabilizing the lumbar spine (Bergmark, 1989). Since the subjects of this study were healthy community-dwelling women, muscle contractions during sitting and standing postures against gravity in daily physical activities may have contributed to maintaining muscle quantity of the transversus abdominis and lumbar multifidus muscles. Unlike our previous study (Ikezoe at al. 2012), this study indicated that that there were no differences among the age groups in MT of the internal oblique and erector spinae muscles, in addition to the deep trunk muscles. The subjects targeted were older in our previous study (old-old) (Ikezoe at al. 2012), whereas young-old or younger subjects were included in this study. In this context, another reason may be the composition of muscle fiber type, which is categorized into two main types: slow twitch (type I) and fast twitch (type II). The proportion of muscle fiber type differs depending on the muscle (Johnson et al. 1973; Sirca and Kostevc 1985; Rantanen et al. 1994; Lexell 1995). For example, the gastrocnemius and soleus muscles are the ankle flexor muscles, while the gastrocnemius muscle has a high proportion of type II fibers, whereas the soleus muscle has a high proportion of type I fibers (Johnson et al. 1973). Type II fibers are more affected by aging than are type I fibers. Age-related decreases in type Ⅱ fiber diameters were observed, whereas there was no correlation between type I fiber diameter and age (Sato et al. 1984; Lexell et al. 1988; Nilwik et al. 2013; Kramer et al. 2017). The lumbar multifidus muscles have a high proportion of type I fibers (Sirca and Kostevc 1985; Rantanen et al. 1994), which may also influence the lower age-related atrophy in the lumbar multifidus muscles among healthy independent subjects.

Even though there was no significant difference in MT of the internal oblique, transversus abdominis, and lumbar multifidus muscles, EI of all trunk muscles were significantly different among the age groups. The reason for this is that MT seems to be influenced by non-contractile tissue in addition to the muscle fibers. Even if the muscle areas of the vastus lateralis muscle of the older and young subjects were same, the total fiber area of the late older subjects was smaller than that of the young subjects (Lexell et al. 1988). Moreover, in studies targeting paraspinal muscles, although it is noted that cross-sectional area of the paraspinal muscles was not correlated with age, paraspinal fat fractions correlated directly with age (Dahlqvist et al. 2017). The effects of aging may increase the non-contractile tissue such as intramuscular fat and interstitial fibrous tissue and reduce the amount of substantial muscle fibers. With regard to the physiological mechanism of the age-related changes in MT and EI, sarcopenia, unlike muscle atrophy due to disuse, involves not only muscle fiber atrophy but also a decrease in the number of muscle fibers. It is considered that one of the reasons for this is that the number and proliferation function of muscle satellite cells involved in muscle regeneration decrease with age (Hawke and Garry 1985; Machida and Booth 2004). Furthermore, since muscle cells produced from the muscle satellite cells function in suppressing heterotopic adipose differentiation, muscle atrophy promotes heterotopic adipose differentiation (Uzumi et al. 2010). Promoting heterotopic adipose differentiation due to decreases in proliferation function of muscle satellite cells may occur at an earlier age than muscle fiber atrophy and a decrease in the number of muscle fibers. Therefore, this study potentially found that EI as an index of the proportion of non-contractile tissue such as intramuscular fat may be more susceptible to aging than MT as an index of muscle atrophy. The results of the linear mixed effect models, it was revealed that MTs of total trunk muscles in the age groups over 50 were significantly smaller than those in the 20s age group, and EIs of total trunk muscles in the age groups over 30 were significantly larger than those in the 20s age group. In other words, although age-related decreases in muscle quantity occur after 50 years of age, age-related changes in muscle quality occur after 30 years of age. Although the previous study (Fukumoto et al., 2015) also revealed that age-related changes in muscle quality may occur earlier than age-related changes in muscle quantity, but the previous study did not include subjects 30–40 years of age. Our results showed that age-related changes in abdominal muscle quality occur before middle age. Since muscle strength would be affected by muscle quantity and quality (Fukumoto et al., 2012a; Watanabe et al., 2013; Taniguchi et al., 2017), age-related decreases in muscle strength at an earlier age may be more affected by muscle quality than quantity. Therefore, to prevent age-related muscle weakness, it is appropriate to evaluate MT at EI in subjects over 30 years old. Another feature of this study is that we examined not only abdominal muscles but also abdominal muscles. Similar to the results of previous studies examining lower limbs and abdomen muscles, it was found that age-related changes in back muscles are differ depending on each muscle.

Several limitations to this study should be noted. Because this was a cross-sectional study using the linear mixed effect models, the causes for these changes are not clear, and it is not known whether our results are applicable to longitudinal changes in individual subjects. Another limitation of this study is our inclusion of only women. Reports showed sex-related differences in the degrees of age-related changes in muscle quantity (Janssen et al., 2000). Further longitudinal studies including both men and women would be required to confirm age-related change in muscle quantity and quality. The other limitation is that muscle strength was not measured in this study. Therefore, although MT and EI are related to muscle strength (Fukumoto et al., 2012a; Watanabe et al., 2013; Taniguchi et al., 2017), the influence of changes in MT and EI on performance such as muscle strength remains unknown. Finally, body composition has been reported to be affected by various factors; food and water intake, fitness level, water concentration, physical activity, menstruation, and circadian variations (Chumlea et al., 1996; Deurenberg et al., 1988; Shiose et al., 2017). To generalize our findings, studies that consider these factors are required.

5. Conclusion

We found significant decreases in MT of the rectus abdominis and external oblique muscles in the 50s and 60s age groups compared to those in the 20s age group, and MT of the erector spinae muscle in the 60s age groups compared to those in the 20s age group. There was no significant difference among the age groups in muscle thickness of the internal oblique, transversus abdominis, and lumbar multifidus muscles. Our study indicated that age-related changes in MT were smaller in the deep trunk muscles such as the internal oblique, transversus abdominis, and lumbar multifidus muscles. On the other hand, there were significant differences in EI of all abdominal muscles between the 20s age group and other age groups, and significant differences in EI of all back muscles in the age groups over 40 compared to those in the 20s age group. Our study revealed that muscle quality may be more affected by age than muscle quantity, while age-related changes in muscle quality may occur even in the 30s age group.

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Conflicts

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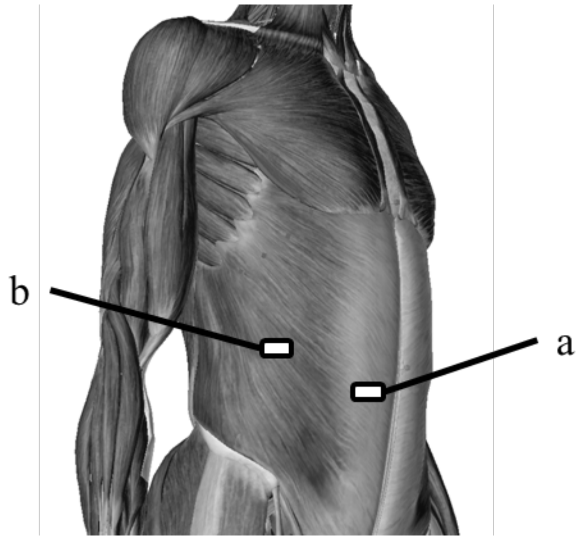
Figure captions

Fig. 1. Measurement site for each muscle: (a) Rectus abdominis, 3 cm lateral to the umbilicus; (b) External oblique, internal oblique, and transversus abdominis, 2.5 cm anterior to the midaxillary line at the midpoint between the inferior rib and the iliac crest; (c) Erector spinae, 4 cm lateral to the Th9 spinous process; and (d) Lumbar multifidus, 2 cm lateral to the L4 spinous process.

These images were provided by Visible Body and modified for our purposes.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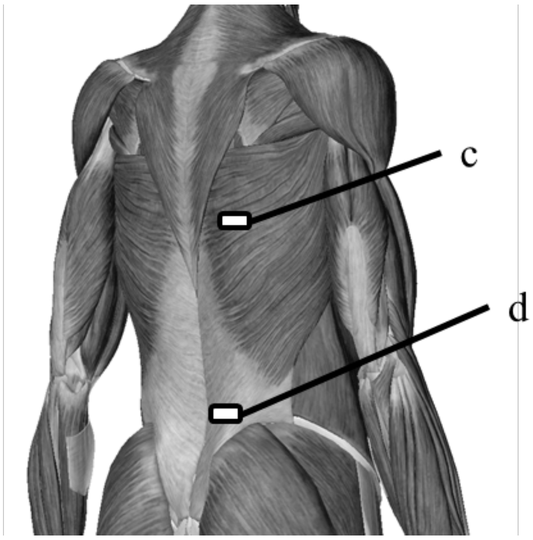
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Table 1. Characteristics of each group

Age group	Number	Age (years old)	Height (cm)	Mass (kg)	BMI (kg/m^2)	Body fat percentage (%)	Subcutaneous fat thickness on the rectus abdominis (mm)
20s	23	24.04 ± 2.64	158.31 ± 5.19	53.77 ± 7.88	21.41 ± 2.61	28.47 ± 5.94	15.93 ± 7.33
30s	18	33.94 ± 2.58	157.67 ± 4.76	52.23 ± 7.10	20.99 ± 2.59	26.48 ± 4.60	12.52 ± 4.59
40s	22	45.09 ± 2.60	159.51 ± 4.16	55.47 ± 7.86	21.78 ± 2.81	27.14 ± 7.48	12.98 ± 7.83
50s	26	54.00 ± 2.55	157.77 ± 3.52	51.69 ± 6.84	20.73 ± 2.30	26.19 ± 6.27	12.70 ± 6.28
60s	23	63.74 ± 2.90	156.53 ± 4.46	51.51 ± 6.37	20.98 ± 2.00	26.80 ± 5.10	12.93 ± 6.08

Abbreviations: BMI, body mass index

Foot note:

Data are expressed as mean ± standard deviation.

Table 2. Data are expressed as mean ± standard deviation of muscle thickness (mm) among the age groups

Muscle	20s (n = 23)	30s (n = 18)	40s (n = 22)	50s (n = 26)	60s (n = 23)
Rectus abdominis	9.59 ± 1.75	8.94 ± 1.86	8.20 ± 1.85	7.72 ± 2.13	7.27 ± 1.39
External oblique	6.15 ± 1.45	5.77 ± 1.49	5.31 ± 1.40	4.39 ± 1.16	4.35 ± 1.11
Internal oblique	7.02 ± 1.72	6.72 ± 1.88	6.57 ± 1.60	6.08 ± 1.70	6.05 ± 1.81
Transversus abdominis	3.72 ± 0.49	3.92 ± 1.18	3.78 ± 1.13	3.28 ± 0.65	3.58 ± 0.82
Erector spinae	19.86 ± 2.63	18.65 ± 3.01	18.85 ± 3.74	18.60 ± 2.88	17.45 ± 4.09
Lumbar multifidus	26.25 ± 3.76	27.18 ± 4.45	26.65 ± 4.53	27.87 ± 4.66	26.85 ± 4.27

Table 3. Data are expressed as mean ± standard deviation of echo intensity (0–255) among the age groups

Muscle	20s (n = 23)	30s (n = 18)	40s (n = 22)	50s (n = 26)	60s (n = 23)
Rectus abdominis	38.93 ± 28.58	60.52 ± 18.68	73.13 ± 18.35	82.24 ± 14.34	87.61 ± 20.98
External oblique	56.77 ± 17.17	73.50 ± 10.82	81.74 ± 13.39	85.18 ± 10.56	88.23 ± 13.49
Internal oblique	31.37 ± 19.43	45.42 ± 10.48	52.49 ± 13.91	66.17 ± 11.95	64.30 ± 17.64
Transversus abdominis	19.42 ± 13.36	32.90 ± 12.84	34.07 ± 15.69	48.68 ± 13.12	46.00 ± 17.61
Erector spinae	36.14 ± 16.71	41.89 ± 12.50	47.91 ± 14.87	54.54 ± 12.72	64.11 ± 15.87
Lumbar multifidus	30.22 ± 13.21	34.23 ± 14.02	41.06 ± 14.91	49.52 ± 10.08	51.36 ± 12.90

Table 4. Fixed effect in the linear mixed effect models for MT

	p value
Intercept	**<0.01**
Age group	**<0.01**
Muscle part	**<0.01**
Age group × Muscle part	0.272

Table 5. The linear mixed effect models for MT of total muscles among the age groups.

Age group	Least squares mean	95% Confidence interval	p value
20s	12.10	11.67－12.52	
30s	11.86	11.38－12.34	0.470
40s	11.56	11.13－11.99	0.082
50s	**11.32**	**10.92－11.72**	**<0.01**
60s	**10.92**	**10.50－11.35**	**<0.01**

Table 6. The linear mixed effect models for MT of each muscle among the age groups.

Muscle	Age group	Least squares mean	95% Confidence interval	p value
Rectus abdominis	20s	9.59	8.55—10.63	
	30s	8.94	7.77—10.12	0.419
	40s	8.20	7.14—9.26	0.067
	50s	**7.72**	**6.74—8.69**	**<0.01**
	60s	**7.27**	**6.23—8.30**	**<0.01**
External oblique	20s	6.15	5.11—7.19	
	30s	5.77	4.59—6.94	0.630
	40s	5.31	4.25—6.37	0266
	50s	**4.39**	**3.41—5.37**	**<0.05**
	60s	**4.35**	**3.31—5.39**	**<0.05**
Internal oblique	20s	7.02	5.98—8.06	
	30s	6.72	5.54—7.89	0.703
	40s	6.57	5.51—7.64	0.553
	50s	6.08	5.11—7.06	0.198
	60s	6.05	5.01—7.09	0.194
Transversus abdominis	20s	3.72	2.68—4.76	
	30s	3.92	2.74—5.09	0.803
	40s	3.78	2.71—4.84	0.937
	50s	3.28	2.31—4.26	0.552
	60s	3.58	2.54—4.62	0.853
Erector spinae	20s	19.86	18.82—20.90	
	30s	18.65	17.47—19.83	0.132
	40s	18.85	17.79—19.91	0.184
	50s	18.60	17.62—19.57	0.083
	60s	**17.45**	**16.41—18.49**	**<0.01**
Lumbar multifidus	20s	26.25	25.21—27.29	
	30s	27.18	26.00—28.35	0.245
	40s	26.65	25.59—27.71	0.596
	50s	**27.87**	**26.89—28.85**	**<0.05**
	60s	26.85	25.81—27.89	0.420

Foot note: the linear mixed effect models were performed by MT or EI of each muscle as dependent variables; subjects as random effects; and age groups, muscle parts, and confounding factors between age groups with muscle parts as fixed effects. Significant difference compared to the 20s age group are shown in bold.

Table 7. Fixed effect in the linear mixed effect models for EI

	p value
Intercept	**<0.01**
Age group	**<0.01**
Muscle part	**<0.01**
Age group × Muscle part	**<0.01**

Table 8. The linear mixed effect models for EI of total muscles among the age groups.

Age group	Least squares mean	95% Confidence interval	p value
20s	35.48	32.89－38.06	
30s	**48.08**	**45.15－51.00**	**<0.01**
40s	**55.07**	**52.42－57.71**	**<0.01**
50s	**64.39**	**61.96－66.82**	**<0.01**
60s	**66.93**	**64.35－69.52**	**<0.01**

Table 9. The linear mixed effect models for EI of each muscle among the age groups.

Muscle	Age group	Least squares mean	95% Confidence interval	p value
Rectus abdominis	20s	38.93	32.60—45.27	
	30s	**60.52**	**53.36—67.68**	**<0.01**
	40s	**73.13**	**66.66—79.61**	**<0.01**
	50s	**82.24**	**76.29—88.20**	**<0.01**
	60s	**87.61**	**81.27—93.94**	**<0.01**
External oblique	20s	56.77	50.44—63.10	
	30s	**73.50**	**66.34—80.66**	**<0.01**
	40s	**81.74**	**75.26—88.21**	**<0.01**
	50s	**85.18**	**79.23—91.14**	**<0.01**
	60s	**88.23**	**81.90—94.56**	**<0.01**
Internal oblique	20s	31.37	25.04—37.70	
	30s	**45.42**	**38.26—52.58**	**<0.01**
	40s	**52.49**	**46.01—58.96**	**<0.01**
	50s	**66.17**	**60.22—72.13**	**<0.01**
	60s	**64.30**	**57.96—70.63**	**<0.01**
Transversus abdominis	20s	19.42	13.08—25.75	
	30s	**32.90**	**25.74—40.05**	**<0.01**
	40s	**34.07**	**27.60—40.55**	**<0.01**
	50s	**48.68**	**42.72—54.64**	**<0.01**
	60s	**46.00**	**39.67—52.33**	**<0.01**
Erector spinae	20s	36.14	29.81—42.47	
	30s	41.89	34.73—49.05	0.238
	40s	**47.91**	**41.43—54.38**	**<0.05**
	50s	**54.54**	**48.58—60.79**	**<0.01**
	60s	**64.11**	**57.78—70.45**	**<0.01**
Lumbar multifidus	20s	30.22	23.89—36.55	
	30s	34.23	27.07—41.39	0.410
	40s	**41.06**	**34.58—47.53**	**<0.05**
	50s	**49.52**	**43.56—55.48**	**<0.01**
	60s	**51.36**	**45.02—57.69**	**<0.01**

Foot note: the linear mixed effect models were performed by MT or EI of each muscle as dependent variables; subjects as random effects; and age groups, muscle parts, and confounding factors between age groups with muscle parts as fixed effects. Significant difference compared to the 20s age group are shown in bold.