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Associations between Range of Motion and Tissue Stiffness in Young and Older People

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[1] *Research Fellow of Japan Society for the Promotion of Science, Chiyoda-ku, Tokyo, JAPAN;* [2]*Graduate School of Engineering and Science, Shibaura Institute of Technology, Saitama-shi, Saitama, JAPAN; and* [3]*College of Systems Engineering and Science, Shibaura Institute of Technology, Saitama-shi, Saitama, JAPAN*

ABSTRACT

HIRATA, K., R. YAMADERA, and R. AKAGI. Associations between Range of Motion and Tissue Stiffness in Young and Older People. *Med. Sci. Sports Exerc.*, Vol. 52, No. 10, pp. 2179–2188, 2020. **Purpose:** The purpose of this study was to investigate differences in the associations between passive ankle dorsiflexion range of motion (ROM) and stiffness of the triceps surae, sciatic nerve, and deep fascia located in the posterior leg between young and older people. **Methods:** Twenty young and twenty older males were recruited and were placed in a prone position with their hip and knee fully extended. Passive ankle dorsiflexion ROM was determined based on the onset of pain during passive dorsiflexion at $1°{\cdot}s^{-1}$ using an isokinetic dynamometer. Shear wave speeds (as a stiffness index) of the triceps surae, the sciatic nerve, and the deep fascia in the posterior leg were evaluated by ultrasound shear wave elastography. **Results:** The shear wave speeds of the medial and lateral gastrocnemius measured at 15° dorsiflexion correlated negatively with passive ROM in young but not in older participants. The shear wave speed of the sciatic nerve measured at 15° dorsiflexion correlated negatively with passive ROM only in older participants. No association was observed between passive ROM and shear wave speed of the deep fascia in the posterior leg. For data measured at maximal dorsiflexion angle (as an index of stretch tolerance), shear wave speeds of the triceps surae and passive joint torque correlated positively with passive ROM in both groups. **Conclusion:** These results suggest that the tissues limiting passive ankle dorsiflexion ROM are muscle and nerve for young and older people, respectively, whereas stretch tolerance influences passive ROM for both groups. This implies that the relative contribution of nonmuscular tissues to joint flexibility become stronger than that of muscles with age. **Key Words:** GASTROCNEMIUS, SOLEUS, SCIATIC NERVE, FASCIA, ULTRASOUND SHEAR WAVE ELASTOGRAPHY, AGING

Joint flexibility is well known to decrease with age (1). A decrease in joint range of motion (ROM) is suggested to impair balance and functional ability (2), leading to an increased risk of falls (3) and decrement in the quality of life for older individuals. To maintain and/or improve joint flexibility, it is necessary to clarify the limiting factors of ROM and their age-related changes.

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ROM can be sorted largely into passive and active ROM. Especially in research matter, passive ROM is preferentially used as a represent of joint flexibility because of less complexity, easier experimental settings, and higher reproducibility. Passive ROM is considered to be restricted by tension applied to tissues surrounding a joint (referred to as "mechanical theory") as well as by the perception of such tension (referred to as "sensory theory") (4). Hence, it can be said that compliant tissues and a high tolerance to tissue stretch are necessary for greater joint flexibility. One of the major limiting tissues of passive ROM is the muscle. Indeed, passive ROM was negatively correlated with passive muscle stiffness at angles less than the maximum passive ROM, whereas passive ROM was positively correlated with passive muscle stiffness at the maximum passive ROM angle (5). On the other hand, although passive ROM decreases with age, previous studies (6,7) reported that the muscle of older people is more compliant than that of their younger counterparts. Considering that muscle atrophies with age, the effect of muscle stiffness on passive ROM might become weaker for older people compared with young.

Nonmuscular structures have also been suggested to play a role in limiting passive ROM (8). For instance, a previous study reported that passive ankle dorsiflexion ROM is reduced by cervical flexion and hip flexion (9), implying that nonmuscular tissues, such as nerves and fasciae, can limit passive ROM

because no muscles cross the above-mentioned joints (i.e., from cervical/lumbar vertebrae or hip joint to ankle joint). Fascia is dense connective tissue, and nerve is covered with connective tissue layers (i.e., endoneurium, perineurium, and epineurium). It was suggested that aging increases collagen cross-linking (10) and reduces the diameter of collagen fibril (11). Therefore, the influence of nerve and fascia on passive ROM might be changed by aging due to alteration in tissue mechanical property.

The stiffness of *in vivo* biological tissue can be estimated noninvasively using ultrasound shear wave elastography (SWE). This technique can evaluate localized tissue stiffness from the shear wave propagation speed (SWS) generated by a focused ultrasound beam within the tissue. The validity of ultrasound SWE for stiffness measurement was confirmed using an artificial phantom (12) and a swine brachialis (13). Although the validities for nerve and fascia stiffness measurements have not yet been proven, the feasibility of ultrasound SWE to measure an index of stiffness of nerve (14,15) and fascia (16,17) *in vivo* is ascertained for humans. The purposes of this study were, therefore, to investigate 1) the associations of the passive ankle dorsiflexion ROM with the stiffness of the triceps surae, sciatic nerve, and deep fascia located in the posterior lower limb in older and young people and 2) the differences in stiffness of these tissues between older and young people using ultrasound SWE. We hypothesized that 1) the passive ankle dorsiflexion ROM would be correlated with the muscle stiffness for young people but not for older people, 2) the passive ankle dorsiflexion ROM would be correlated with the nerve and fascia stiffness for both age-groups, and 3) the muscle, nerve, and fascia stiffness would be different between older and young people.

METHODS

Participants

Twenty young males and twenty older males voluntarily participated. *A priori* power analysis was performed to calculate the sample size for the correlation analysis using the G*Power statistical power analysis software. Based on our similar previous study (5) and a pilot study, we assumed a type 1 error of 0.05, a statistical power of 0.80, and an effect size of 0.60. The critical sample size was estimated to be 17. Thus, 20 participants were recruited for each of young (age = 22 ± 1 yr, weight = 67.1 ± 10.6 kg, height = 172.5 ± 6.4 cm, BMI = 22.6 ± 3.4 $kg \cdot m^{-2}$; mean ± SD) and older group (age = 72 ± 5 yr, weight = 68.5 ± 10.2 kg, height = 167.3 ± 6.0 cm, BMI = 24.5 ± 3.4 $kg \cdot m^{-2}$; mean ± SD). Participants were free from neurological or orthopedic disorders as confirmed by self-reporting and were asked to avoid strenuous exercise for 24 h before the measurements. All participants gave informed consent according to the procedures approved by the ethics committee of the Shibaura Institute of Technology. This study was conducted in accordance with the Declaration of Helsinki.

Procedures

The participants were instructed to lay prone on a bed of dynamometer (CON-TREX MJ, PHYSIOMED, Schnaittach, Germany) with their hips and knees fully extended. The right foot was secured with nonelastic straps to the dynamometer foot plate after the tip of the lateral malleolus was visually adjusted to the dynamometer rotational axis. To familiarize the participants with the passive dorsiflexion motion and to avoid a conditioning effect on the tissue stiffness (18), five passive ankle joint rotations were performed between 30° of plantarflexion (PF30) and 15° dorsiflexion (DF15) at $5° \cdot s^{-1}$ (neutral position [NP] was defined as 0°). The ankle joint was then passively dorsiflexed at $1° \cdot s^{-1}$ from PF30 to the angle defined as the onset of pain for each participant. In the present study, this maximal dorsiflexion angle was defined as the passive ROM. The participant was asked to relax as much as possible during the passive ankle rotation. Immediately after the passive ROM measurement, the ankle angle was returned to the plantarflexed position to avoid a stretching effect on the tissue stiffness. The passive ROM measurement was performed once. After the passive ROM measurement, the ankle joint was set at PF30, NP, and DF15 and at the maximal dorsiflexion angle in this order for ultrasound SWE measurements at each joint angle. SWS values measured at submaximal joint angles (i.e., PF30, NP, and DF15) and at the maximal dorsiflexion angle were used as indices of tissue stiffness and stretch tolerance, which has been defined as a willingness of subjects to tolerate greater tension applied to a tissue (4), respectively (5). This series of measurements was repeated five times to obtain the tissue stiffness from multiple sites (see below). To minimize any reduction in tissue stiffness, we attempted to shorten the measurement time in the dorsiflexed position (~30 s). A 2-min rest period was provided between each of the five sets of ultrasound SWE measurements. Lastly, maximal voluntary isometric contraction (MVC) of the plantarflexors was performed for 3 s in the neutral position to normalize EMG signals during ultrasound SWE measurements. The ankle joint angle, passive joint torque, and EMG data were stored simultaneously on a personal computer using a 16-bit analog-to-digital converter (PowerLab 16/35; ADInstrument, Sydney, Australia) with a sampling frequency of 1 kHz.

Ultrasound SWE

An ultrasonic apparatus (ACUSON S2000; Siemens Medical Solutions, Ann Arbor, MI) coupled with a linear transducer array (9 L4 Transducer, 4–9 MHz, Siemens Medical Solutions) was used to quantify SWS as an index of tissue stiffness. The ultrasound probe was longitudinally placed at five sites with water-soluble transmission gel to measure the SWS of the medial gastrocnemius (MG), the lateral gastrocnemius (LG), the soleus, the sciatic nerve, the deep fascia located near MG, and the deep fascia located near the semitendinosus (ST) in a random order. The SWS values were determined at 30% of the lower leg length from the popliteal crease to the lateral malleolus for MG, LG, and the soleus, at slightly proximal to the distal myotendinous junction of MG for the deep fascia located near MG, at 60% of the thigh length from the greater trochanter to the popliteal crease for the sciatic nerve, and at 50% of the thigh length for the deep fascia located near ST (Fig. 1).

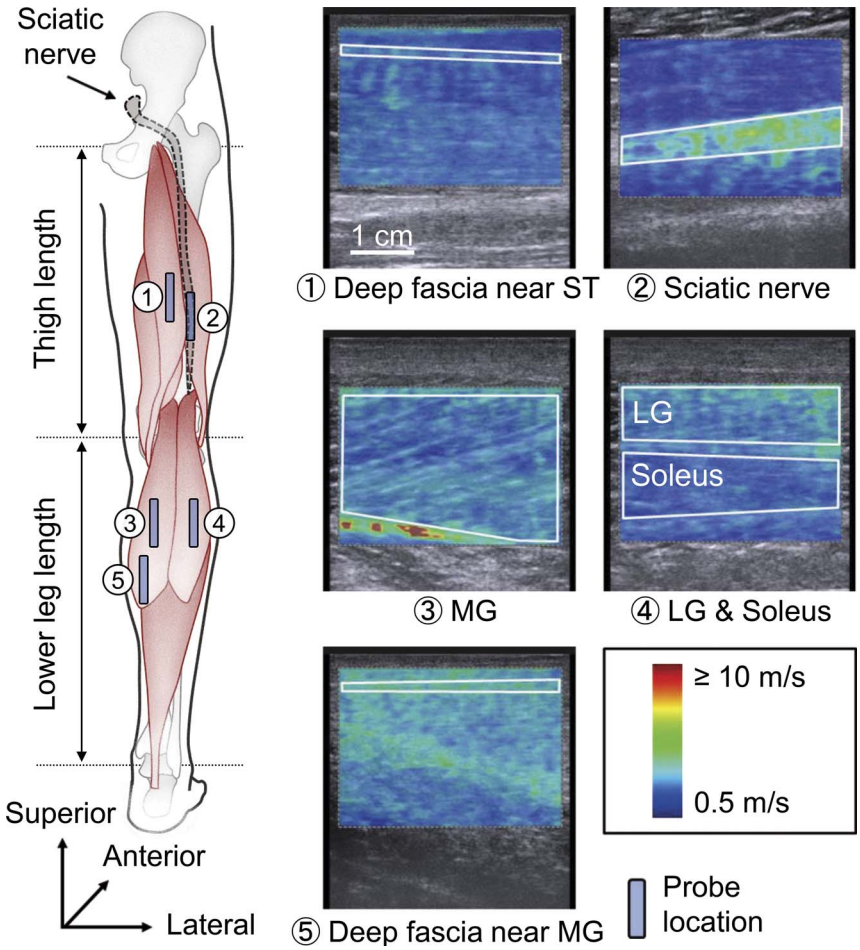

FIGURE 1—Schematic representation of ultrasound probe locations and typical examples of ultrasound shear wave elastographic images. The area surrounded by the white line on each elastographic image represents the region of interest for shear wave speed analysis. LG, lateral gastrocnemius; MG, medial gastrocnemius; ST, semitendinosus.

The probe was basically located at the center of the tissue width. When blood vessels and thick connective tissues within the muscle were obvious in the scanning area at the probe location, the position of the probe was adjusted mediolaterally to accurately evaluate the SWS of the muscles. For accurate assessment of deep fascia SWS, it was ascertained that the ultrasound probe for deep fascia measurements was located at the region free from superficial aponeuroses of MG or ST based on visual inspection and previous findings (e.g., Woodley and Mercer [19]). During the measurement, to avoid influencing the SWS values, the examiner was careful not to press the target tissue. An elastographic image with SWS color map was obtained once from each measurement site at each joint angle. Before storing the image, SWS quality was checked using the system of the ultrasound apparatus, which displays a color-coded image of the measurement area of SWS according to the quality of SWS evaluation: green pixels indicate high quality and orange pixels indicate low quality (Fig. 2 [6]). When yellow-to-orange pixels occupied ≤25% area of a color-coded image within the region of interest (ROI) of an SWS color map, we judged that the SWS quality of the image was acceptable. If not, the ultrasound SWE measurement was repeated until a high-quality image could be acquired.

Electromyography

To ensure that the participants were relaxed during the stiffness measurements, muscle activities of MG, LG, and the soleus were obtained using an EMG system (Bagnoli 8 EMG System; Delsys Inc., Boston, MA). Preamplified bipolar active surface EMG electrodes (electrode shape, parallel bar; size, 1 mm width × 10 mm length; interelectrode distance, 10 mm; DE-2.1, Delsys Inc) with band-pass filtering between 20 and 450 Hz were placed at the bellies of each muscle along the fascicle direction after preparation of the skin by shaving, abrasion with sandpaper, and cleaning with alcohol. The locations of the EMG electrodes were determined after deciding those of the ultrasound probe. The reference electrode was placed on the lateral malleolus of the left foot.

Data analyses. Elastographic images were exported in DICOM format from the ultrasonic apparatus. The ROI on each SWS map was made as large as possible while excluding nontarget tissues (e.g., subcutaneous adipose tissues, aponeuroses, nontarget muscles, etc.) using image processing software (ImageJ; National Institutes of Health, Bethesda, MD). The average value of the SWS over the ROI was calculated for each image using our original analysis software written in MATLAB

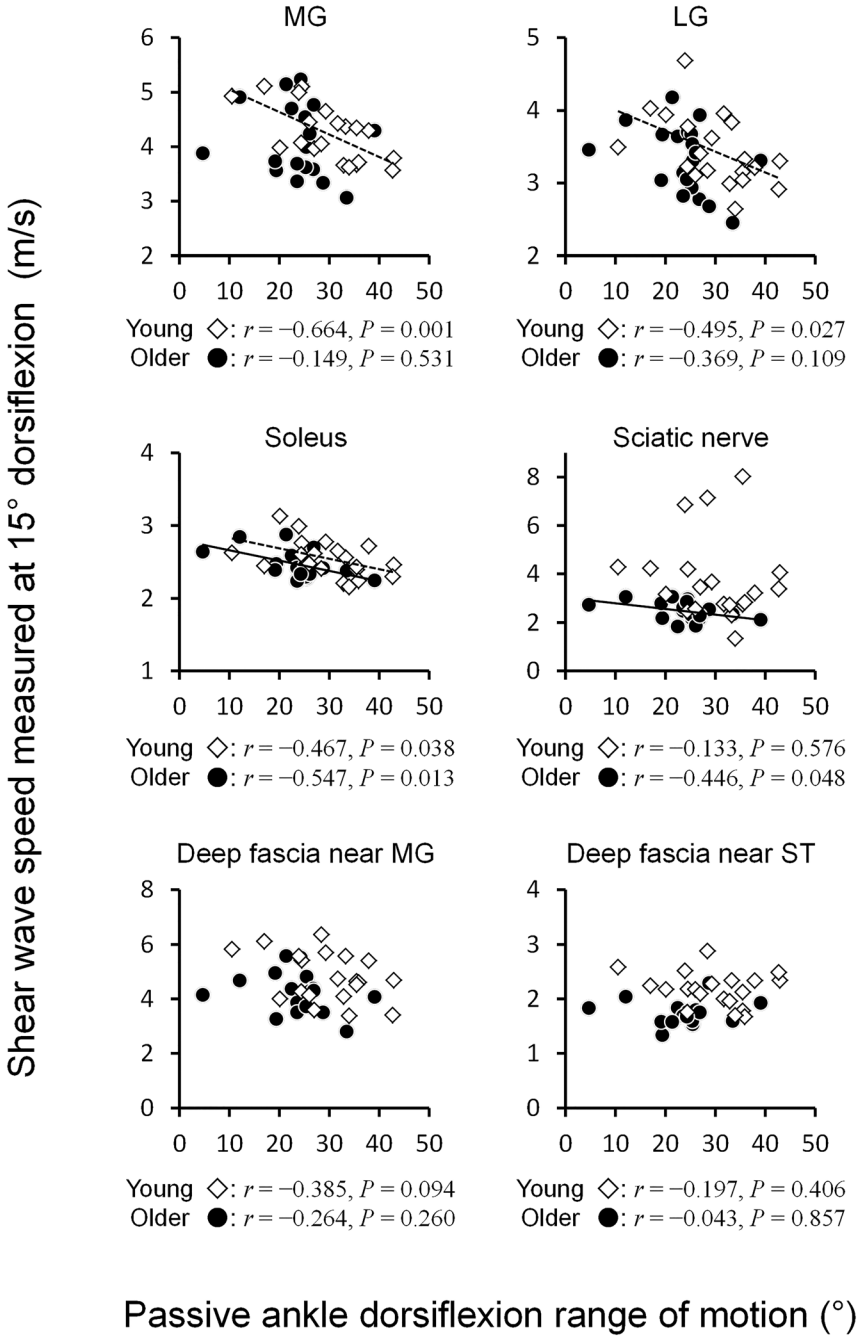

FIGURE 2—Correlations between passive ankle dorsiflexion RoM and shear wave speeds measured at 15° dorsiflexion for young (*open diamond*) and older participants (*closed circle*). Regression lines are shown for young (*broken line*) and older (*solid line*). LG, lateral gastrocnemius; MG, medial gastrocnemius; ST, semitendinosus.

(MATLAB R2018a; MathWorks, Natick, MA), which can convert the RGB values of each pixel within ROI into SWS values according to the color scale of the elastographic image. In the present study, SWS was used as an index of tissue stiffness. When assuming a linear elastic behavior, the shear modulus (μ) can be calculated using SWS (v) as follows:

$$\mu = \rho v^2 \quad [1]$$

where ρ is the tissue density. Furthermore, when a medium is isotropic and sufficiently large, Young's modulus (E) can be calculated as three-times the shear modulus (μ), as follows:

$$E = 3\mu. \quad [2]$$

As these formulae indicate, SWS is directly associated with Young's modulus. However, the mechanical properties of biological tissue show anisotropic behavior. Hence, it is difficult to calculate an accurate value of Young's modulus from SWS alone. On the other hand, the relationship between the shear modulus computed from SWS and Young's modulus was

demonstrated to be linear for muscle (13) and tendon (20). In addition, individual differences in muscle shear modulus calculated from SWS are associated with that in joint stiffness (21,22), and SWS is reported to change with stiffness independently from force (23). Therefore, SWS can be an index of tissue stiffness.

For the passive joint torque and EMG data, the average values and root mean square (EMG_{RMS}) values were calculated over a 500-ms period at each joint angle during the ultrasound SWE measurements, respectively. Because the ultrasound SWE measurements were repeated five times, the five values calculated at each joint angle were averaged to determine the representative values of passive joint torque and EMG_{RMS} at each joint angle. The EMG_{RMS} values were normalized to those for 500 ms during MVC.

Repeatability of the measurements. To ensure repeatability of the ultrasound SWE measurements, an additional acquisition of SWS data was performed for four older and four young participants after the experiment. The coefficient variations of the two measured average values of SWS for each tissue at each joint angle and the intraclass correlation coefficients for each tissue with 95% confidential intervals (CIs) are summarized in Supplemental Digital Content 1 (see Table, Repeatability of measurements for shear wave speeds, http://links.lww.com/MSS/B963).

Statistical analyses. Pearson's product–moment correlation was used to investigate associations of passive ROM with the SWS of each tissue or passive joint torque measured at DF15 and the maximal dorsiflexion angle to clarify the influences of tissue stiffness and stretch tolerance on joint flexibility. Because passive ROM was suggested to be more strongly related with joint or tissue stiffness measured at a given joint angle near the end ROM (5), tissue stiffness can have a larger effect on passive ROM in a stretched position than in a shortened position. Therefore, SWS values and passive joint torque measured at DF15 were used for correlation analyses. The unpaired *t*-tests were performed for passive ROM and maximal passive joint torque to compare between young and older participants. For submaximal passive joint torque, a two-way ANOVA was conducted (between factor: age [young, older]; within factor: joint angle [PF30, NP, DF15]). For SWS, a two-way ANOVA was conducted for maximal dorsiflexion angle data (between factor: age; within factor: tissue [MG, LG, soleus, sciatic nerve, deep fascia near MG, deep fascia near ST]), and a three-way ANOVA was conducted for submaximal joint angle data (between factor: age; within factors: joint angle and tissue). For EMG_{RMS}, a two-way ANOVA was conducted for maximal dorsiflexion angle data (between factor: age; within factor: muscle [MG, LG, soleus]), and a three-way ANOVA was conducted for submaximal joint angle data (between factor: age; within factors: joint angle and muscle). When significant interactions were observed, additional multivariate ANOVA (MANOVA) and/or Bonferroni multiple comparison tests or unpaired *t*-tests were performed. ANOVA was separately conducted for data measured at maximal and submaximal joint angles because submaximal joint angle data were measured at same joint angles among the participants but maximal joint angle data were not. In addition, interpretations of SWS data and passive joint torque in the present study were different between maximal and submaximal as mentioned earlier (i.e., indices of stretch tolerance and tissue stiffness, respectively). These analyses were conducted using statistical software (SPSS Statistics 25; IBM Japan, Tokyo, Japan).

Descriptive data are presented as mean ± SD. The significance level was set at $\alpha = 0.05$. Estimates of effect size were analyzed using Cohen's *d* for unpaired *t*-tests and Bonferroni multiple comparison tests, and partial eta square (η_p^2) for ANOVA and MANOVA. For correlation analyses, the Pearson's product–moment correlation coefficient (*r*) *per se* was regarded as the effect size.

RESULTS

Passive ROM, passive joint torque, and muscle activity. The passive ROM of older participants was less than that of young participants (23.9° ± 7.0° vs 29.7° ± 8.3°, $P = 0.022$, $d = 0.755$). For passive joint torque at submaximal joint angle, a two-way ANOVA (age–joint angle) revealed a significant main effect of joint angle ($P < 0.001$, $\eta_p^2 = 0.928$) without an age–joint angle interaction or a main effect of age. Passive joint torque at maximal dorsiflexion angle of older participants was lower than that of young counterparts (19.8 ± 10.2 N·m vs. 28.6 ± 12.1 N·m, $P = 0.018$, $d = 0.782$). For EMG_{RMS} at submaximal joint angles, a three-way ANOVA (age–joint angle–muscle) revealed a significant simple main effect of age ($P = 0.001$, $\eta_p^2 = 0.257$) without second- or first-order interactions, a main effect of muscle, nor one of joint angle. The averaged value of EMG_{RMS} among muscle in older participants was higher than that in young participants (2.10 ± 0.18 vs 1.18 ± 0.18% MVC, $P = 0.001$, $d = 5.111$). A two-way ANOVA (age–muscle) for EMG_{RMS} at the maximal dorsiflexion angle revealed a significant main effect of muscle ($P = 0.025$, $\eta_p^2 = 0.115$) without significant age–muscle interaction or a main effect of age. However, the *post hoc* Bonferroni multiple comparison test did not show any differences between muscles.

SWS. The results of a three-way ANOVA (age–joint angle–tissue) for SWS at submaximal joint angles are summarized in Supplemental Digital Content 2 (see Table, Statistical results of 3-way ANOVA for shear wave speed at submaximal joint angles, http://links.lww.com/MSS/B964) because of their complexity. Table 1 represents SWS values of young and older participants for each tissue at each joint angle. Briefly, a significant second-order interaction was observed in the three-way ANOVA ($P = 0.037$, $\eta_p^2 = 0.068$). *Post hoc* Bonferroni multiple comparison tests revealed that SWS values for MG and LG in older participants were significantly slower than those in young participants at PF30 and NP ($P \leq 0.046$, $d \geq 0.636$). By contrast, at DF15, SWS values for MG, LG, and the soleus did not vary between the groups. For the sciatic nerve and deep fascia near MG and ST, SWS values were lower in older than in young participants at any joint angle ($P \leq 0.024$, $d \geq 0.748$). For both groups, SWS values became faster as the ankle dorsiflexed

TABLE 1. Shear wave speeds at submaximal joint angle for young and older participants.

		Shear Wave Speed ($m \cdot s^{-1}$)				*P* Value (Cohen's *d*)				
		Mean ± SD	Min	Max	95% CI	vs NP	vs DF15	vs LG	vs Soleus	vs Older
Young (n = 20)										
MG	PF30	2.16 ± 0.18	1.79	2.52	2.08–2.24	$P < 0.001$ ($d = 3.313$)	$P < 0.001$ ($d = 5.439$)	$P < 0.001$ ($d = 1.294$)	$P < 0.001$ ($d = 1.556$)	$P < 0.001$ ($d = 1.885$)
	NP	2.74 ± 0.17	2.42	3.06	2.66–2.82	–	$P < 0.001$ ($d = 3.946$)	$P < 0.001$ ($d = 1.373$)	$P < 0.001$ ($d = 3.541$)	$P = 0.016$ ($d = 0.795$)
	DF15	4.24 ± 0.51	3.57	5.11	4.00–4.48	–	–	$P < 0.001$ ($d = 1.615$)	$P < 0.001$ ($d = 4.208$)	$P = 0.621$ ($d = 0.151$)
LG	PF30	1.90 ± 0.22	1.54	2.59	1.79–2.00	$P < 0.001$ ($d = 2.591$)	$P < 0.001$ ($d = 4.125$)	–	$P = 1.000$ ($d = 0.100$)	$P = 0.034$ ($d = 0.695$)
	NP	2.47 ± 0.22	2.04	2.9	2.37–2.57	–	$P < 0.001$ ($d = 2.598$)	–	$P < 0.001$ ($d = 1.741$)	$P = 0.047$ ($d = 0.636$)
	DF15	3.44 ± 0.48	2.64	4.68	3.22–3.67	–	–	–	$P < 0.001$ ($d = 2.326$)	$P = 0.455$ ($d = 0.234$)
Soleus	PF30	1.88 ± 0.18	1.62	2.3	1.80–1.97	$P < 0.001$ ($d = 1.333$)	$P < 0.001$ ($d = 3.076$)	–	–	$P = 0.074$ ($d = 0.543$)
	NP	2.12 ± 0.18	1.81	2.5	2.03–2.20	–	$P < 0.001$ ($d = 1.974$)	–	–	$P = 0.031$ ($d = 0.764$)
	DF15	2.55 ± 0.25	2.16	3.13	2.43–2.66	–	–	–	–	$P = 0.229$ ($d = 0.405$)
Sciatic nerve	PF30	2.66 ± 0.61	1.32	4.05	2.38–2.95	$P < 0.001$ ($d = 0.477$)	$P < 0.001$ ($d = 0.865$)	–	–	$P < 0.001$ ($d = 1.549$)
	NP	3.09 ± 1.12	1.24	5.87	2.57–3.62	–	$P < 0.001$ ($d = 0.470$)	–	–	$P = 0.001$ ($d = 1.175$)
	DF15	3.77 ± 1.71	1.34	8.02	2.97–4.58	–	–	–	–	$P = 0.002$ ($d = 1.051$)
Deep fascia near MG	PF30	2.19 ± 0.24	1.78	2.87	2.08–2.31	$P < 0.001$ ($d = 2.709$)	$P < 0.001$ ($d = 3.963$)	–	–	$P < 0.001$ ($d = 1.399$)
	NP	3.11 ± 0.41	2.44	3.88	2.92–3.30	–	$P < 0.001$ ($d = 2.417$)	–	–	$P < 0.001$ ($d = 1.496$)
	DF15	4.80 ± 0.90	3.38	6.36	4.38–5.22	–	–	–	–	$P = 0.024$ ($d = 0.748$)
Deep fascia near ST	PF30	2.18 ± 0.33	1.62	2.95	2.02–2.33	$P = 1.000$ ($d = 0.121$)	$P = 1.000$ ($d < 0.001$)	–	–	$P < 0.001$ ($d = 1.815$)
	NP	2.14 ± 0.33	1.62	2.88	1.99–2.30	–	$P = 0.098$ ($d = 0.125$)	–	–	$P < 0.001$ ($d = 1.634$)
	DF15	2.18 ± 0.31	1.67	2.88	2.03–2.33	–	–	–	–	$P < 0.001$ ($d = 1.725$)
Older (n = 20)										
MG	PF30	1.83 ± 0.17	1.39	2.11	1.75–1.91	$P < 0.001$ ($d = 3.462$)	$P < 0.001$ ($d = 4.747$)	$P = 1.000$ ($d = 0.433$)	$P = 1.000$ ($d = 0.250$)	–
	NP	2.57 ± 0.25	2.21	2.9	2.45–2.68	–	$P < 0.001$ ($d = 3.125$)	$P < 0.001$ ($d = 1.019$)	$P < 0.001$ ($d = 2.861$)	–
	DF15	4.15 ± 0.67	3.06	5.23	3.83–4.46	–	–	$P < 0.001$ ($d = 1.427$)	$P < 0.001$ ($d = 3.432$)	–
LG	PF30	1.74 ± 0.24	1.32	2.35	1.62–1.85	$P < 0.001$ ($d = 2.563$)	$P < 0.001$ ($d = 4.334$)	–	$P = 1.000$ ($d = 0.250$)	–
	NP	2.33 ± 0.22	1.9	2.71	2.23–2.43	–	$P < 0.001$ ($d = 2.774$)	–	$P < 0.001$ ($d = 1.826$)	–
	DF15	3.33 ± 0.46	2.45	4.18	3.11–3.54	–	–	–	$P < 0.001$ ($d = 2.472$)	–
Soleus	PF30	1.79 ± 0.15	1.49	2.13	1.71–1.86	$P < 0.001$ ($d = 1.496$)	$P < 0.001$ ($d = 3.914$)	–	–	–
	NP	2.00 ± 0.13	1.81	2.3	1.94–2.07	–	$P < 0.001$ ($d = 2.826$)	–	–	–
	DF15	2.46 ± 0.19	2.24	2.87	2.37–2.55	–	–	–	–	–
Sciatic nerve	PF30	1.92 ± 0.29	1.34	2.3	1.79–2.06	$P = 0.155$ ($d = 0.644$)	$P = 0.027$ ($d = 1.655$)	–	–	–
	NP	2.12 ± 0.33	1.36	2.58	1.97–2.27	–	$P = 0.030$ ($d = 0.998$)	–	–	–
	DF15	2.47 ± 0.37	1.83	3.06	2.30–2.64	–	–	–	–	–
Deep fascia near MG	PF30	1.84 ± 0.26	1.51	2.59	1.72–1.96	$P < 0.001$ ($d = 2.470$)	$P < 0.001$ ($d = 4.526$)	–	–	–
	NP	2.56 ± 0.32	1.93	3.46	2.41–2.71	–	$P < 0.001$ ($d = 3.049$)	–	–	–
	DF15	4.20 ± 0.69	2.79	5.57	3.88–4.53	–	–	–	–	–
Deep fascia near ST	PF30	1.72 ± 0.14	1.49	1.99	1.66–1.79	$P = 1.000$ ($d = 0.120$)	$P = 1.000$ ($d = 0.058$)	–	–	–
	NP	1.70 ± 0.19	1.36	2.27	1.61–1.78	–	$P = 0.098$ ($d = 0.154$)	–	–	–
	DF15	1.73 ± 0.20	1.33	2.29	1.64–1.83	–	–	–	–	–

DF15, 15° dorsiflexion; LG, lateral gastrocnemius; MG, medial gastrocnemius; NP, neutral position; PF30, 30° plantarflexion; ST, semitendinosus.

(main effect [joint angle]: $P \leq 0.031$, $\eta_p^2 \geq 0.172$) except for the SWS of the deep fascia near ST. For both groups, SWS was significantly faster in the order MG, LG, and the soleus at NP and DF15 ($P < 0.001$, $d \geq 1.019$).

For SWS at the maximal dorsiflexion angle, the two-way ANOVA revealed a significant age–tissue interaction ($P = 0.004$, $\eta_p^2 = 0.109$) (Table 2). *Post hoc* unpaired *t*-tests showed that SWS values at the maximal dorsiflexion angle for the soleus, sciatic nerve, deep fascia near MG, and ST in older participants were lower than in young participants ($P \leq 0.033$, $d \geq 0.699$). Intermuscular differences in SWS at the maximal dorsiflexion angle were similar to those at NP and DF15 ($P < 0.001$, $d \geq 0.825$).

Correlation between passive ROM and SWS or passive joint torque. Figure 2 shows scatter plots of the relations between passive ROM and SWS for each tissue at DF15 in young and older participants. In young participants, significant negative correlations were seen between passive ROM and SWS at DF15 for MG, LG, and the soleus ($P \leq 0.038$, $r \leq -0.467$). In older participants, significant negative correlations were seen between passive ROM and SWS at DF15 for the soleus and the sciatic nerve ($P \leq 0.048$, $r \leq -0.446$). Significant negative correlation between passive ROM and passive joint torque at DF15 was observed in young participants ($P = 0.003$, $r = -0.621$) but not in older participants ($P = 0.393$, $r = -0.202$).

In both groups, positive correlations between passive ROM and SWS at the maximal dorsiflexion angle were significant for MG, LG, the soleus, and deep fascia near MG (young: $P \leq 0.007$, $r \geq 0.586$; older: $P \leq 0.001$, $r \geq 0.669$). Scatter plots of the correlation between passive ROM and SWS at the maximal dorsiflexion angle are shown in Figure 3. Significant positive correlation between passive ROM and passive joint torque at maximal dorsiflexion angle was also observed in young participants ($P < 0.001$, $r = 0.749$) and older participants ($P < 0.001$, $r = 0.838$).

Summary of the main findings. The present results revealed significant associations of passive ankle dorsiflexion ROM with the soleus stiffness (i.e., SWS measured at DF15) but not with the gastrocnemii stiffness in older participants. Contrary to this, in young participants, passive ROM was correlated significantly with stiffness of each muscle of the triceps surae. Stiffness of the sciatic nerve associated with passive ROM in older participants but not their younger counterparts. No associations of passive ROM with the stiffness of deep fascia near MG and of ST were detected in both age-groups. In addition, the stiffness of the gastrocnemii in shortened state, the sciatic nerve, and the deep fasciae were lower in older than in young participants.

TABLE 2. Shear wave speeds at maximal joint angle for young and older participants.

	Shear Wave Speed ($m \cdot s^{-1}$)				*P* Value (Cohen's *d*)		
	Mean ± SD	Min	Max	95% CI	vs LG	vs Soleus	vs Older
Young (n = 20)							
MG	6.58 ± 1.17	3.93	9.45	6.02–7.13	$P < 0.001$ ($d = 0.897$)	$P < 0.001$ ($d = 2.988$)	$P = 0.083$ ($d = 0.566$)
LG	5.57 ± 1.08	3.15	7.09	5.07–6.08	–	$P < 0.001$ ($d = 2.052$)	$P = 0.054$ ($d = 0.631$)
Soleus	3.71 ± 0.69	2.52	4.91	3.38–4.03	–	–	$P = 0.015$ ($d = 0.814$)
Sciatic nerve	4.71 ± 1.54	1.42	7.28	3.99–5.43	–	–	$P < 0.001$ ($d = 1.774$)
Deep fascia near MG	7.24 ± 1.45	4.43	9.19	6.56–7.92	–	–	$P = 0.033$ ($d = 0.699$)
Deep fascia near ST	2.27 ± 0.36	1.67	2.96	2.10–2.44	–	–	$P < 0.001$ ($d = 1.786$)
Older (n = 20)							
MG	5.88 ± 1.30	2.94	8.53	5.27–6.49	$P < 0.001$ ($d = 0.825$)	$P < 0.001$ ($d = 2.659$)	–
LG	4.86 ± 1.17	2.78	7.23	4.32–5.41	–	$P < 0.001$ ($d = 1.801$)	–
Soleus	3.18 ± 0.61	2.36	4.35	2.90–3.47	–	–	–
Sciatic nerve	2.69 ± 0.47	1.84	3.54	2.47–2.91	–	–	–
Deep fascia near MG	6.22 ± 1.47	2.64	8.81	5.54–6.91	–	–	–
Deep fascia near ST	1.75 ± 0.20	1.38	2.29	1.65–1.84	–	–	–

LG, lateral gastrocnemius; MG, medial gastrocnemius; ST, semitendinosus.

For both groups, passive ROM was associated with the indices of stretch tolerance (i.e., SWS and passive joint torque measured at the maximal dorsiflexion angle).

DISCUSSION

We aimed to investigate the associations of passive ankle dorsiflexion ROM with SWS for each muscle of the triceps surae, the sciatic nerve, and the deep fascia near MG and ST, and to elucidate the differences in SWS for each tissue between young and older participants. We hypothesized that 1) the passive ankle dorsiflexion ROM would be correlated with the muscle stiffness for young people but not for older people, 2) the passive ankle dorsiflexion ROM would be correlated with the nerve and fascia stiffness for both age-groups, and 3) the muscle, nerve, and fascia stiffness would be different between older and young people. The present results partly support our hypotheses and suggest that 1) the influence of muscle stiffness on passive ROM diminishes with age, and 2) the limiting factors of passive ROM for older people are stiffness of the sciatic nerve and stretch tolerance.

The present results showed that stiffness evaluated from the SWS of the gastrocnemii (MG and LG) measured at a shortened muscle length (i.e., PF30 and NP) was lower in older than in young participants (Table 1), which is consistent with previous studies (6,8,24). Extracellular water content within muscle and intramuscular adipose tissue increase with age (25). These can explain the age-related decrease in muscle stiffness at a shortened muscle length. By contrast, in the present study, the SWS of the lengthened gastrocnemii (i.e., measured at DF15) was not different between older and young participants (Table 1). A previous study similarly found that the SWS of the quadriceps femoris in a lengthened position (90° knee flexion) was comparable between young and older participants (24). Passive muscle stiffness is considerably affected by the connective tissue within the muscle (e.g., perimysium and endomysium) (26). In addition, the intramuscular perimysium and endomysium contents increase with age (27). Because connective tissue is stiffer than myofibrillar protein and fat, especially for muscles in a lengthened state, an older muscle will become stiffer than a young one when stretched. Indeed, Eby et al. (28) reported that the stiffness of the biceps brachii measured using ultrasound SWE in full elbow extension increased with age. Similarly, in the present study, the passive ROM (i.e., maximal dorsiflexion angle) for older was less than that for young participants, despite the SWS of the gastrocnemii at the maximal dorsiflexion angle not differing significantly between groups (Table 2). Collectively, it can be considered that the stiffness of shortened muscles in older people is lower than in young people, and *vice versa*, because of age-related changes in muscle composition, although the older people cannot stretch their gastrocnemii until the muscle become stiff more than young people.

The significant associations of passive ROM with the SWS of the gastrocnemii measured at DF15 were not seen in older participants in contrast with in young participants (Fig. 2). As mentioned above, the stiffness of the gastrocnemii in older participants was not greater than that in young participants within passive ROM determined by the onset of pain. Because muscle tension can be estimated from an area under the muscle stiffness–length (joint angle) curve, it can be assumed that the tension induced in the gastrocnemii is lower for older than for young participants. Nevertheless, passive ROM was narrower for older than for young participants. Considering these findings, the main limiting factor of passive ankle dorsiflexion ROM for older people is tissue other than muscle and/or perception of tension (i.e., sensory theory [4]), rather than muscle tension or stiffness *per se* (i.e., mechanical theory [4]).

Contrary to the SWS for MG and LG, the soleus at DF15 significantly correlated with passive ROM in both groups (Fig. 2). The present study also demonstrated that the SWS for the soleus was comparable at PF30 and DF15 between groups (Table 1). The soleus is a typical slow-twitch muscle (≥80% slow-twitch fibers) (29), and age-related muscle atrophy preferentially occurs in fast-twitch fibers (30). In addition, a previous study reported that the muscle size of the soleus was not different between young and older individuals, in contrast with the gastrocnemii (31). Therefore, it can be predicted that age-related changes in the muscular composition of the soleus are not remarkable. Because of this, a similar significant association of passive ROM with stiffness of the soleus was found in older and young participants. However, it seemed that stiffness of

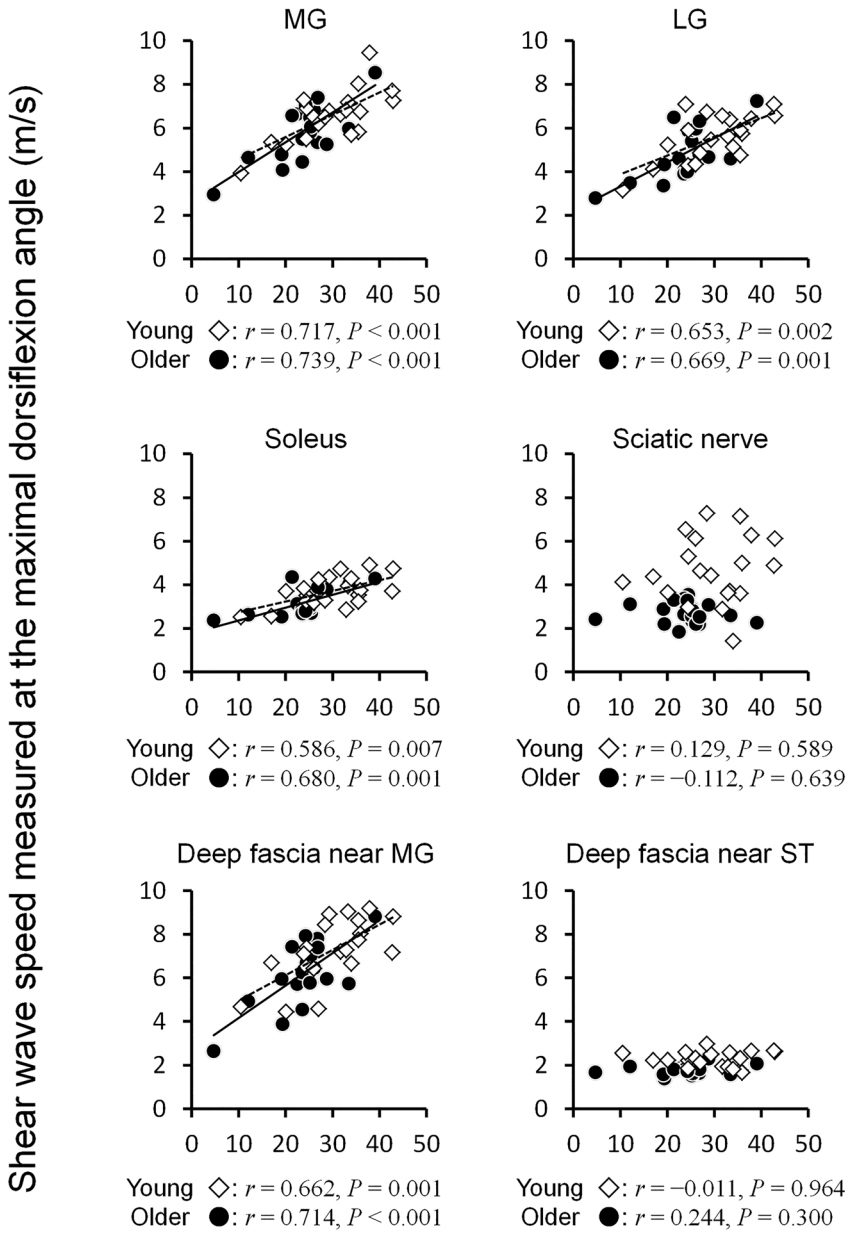

FIGURE 3—Correlations between passive ankle dorsiflexion RoM and shear wave speeds measured at the maximal dorsiflexion angle for young (*open diamond*) and older participants (*closed circle*). Regression lines are shown for young (*broken line*) and older (*solid line*). LG, lateral gastrocnemius; MG, medial gastrocnemius; ST, semitendinosus.

the soleus cannot be a main limiting factor of passive ankle dorsiflexion ROM in the knee-extended position because the soleus was the most compliant of the triceps surae muscles (Table 1). In addition, we previously reported that 5-min static stretching decreased the stiffness of MG but not that of the soleus (32). Hence, tension induced in the soleus in the dorsiflexed position will be low compared with the gastrocnemii. Taking this into account together with the lack of association of passive ROM with gastrocnemii stiffness, the triceps surae may not have a large effect on passive ankle dorsiflexion ROM in older people.

In the present study, the SWS of the sciatic nerve measured at DF15 significantly correlated with passive ROM in older but not in young participants (Fig. 2). It was reported that static ankle dorsiflexion stretching in the long-seated position (90° hip flexion and full knee extension) decreased the sciatic nerve stiffness estimated by ultrasound SWE without any changes in MG stiffness, and reduction of the sciatic nerve stiffness correlated with an increase in passive ankle dorsiflexion ROM (15). Hence, the sciatic nerve can potentially limit the passive ankle dorsiflexion ROM. Based on this, the present results indicate that the influence of the sciatic nerve on passive ROM becomes greater

with age. By contrast, the sciatic nerve SWS was slower in older than in young participants (Table 1). This seems to contradict the difference in the relations between passive ROM and SWS of the sciatic nerve between groups. However, similarly to the present results, a previous study also demonstrated that the SWS values of the median and tibial nerve decrease with age (14). The peripheral nerve has connective tissue sheaths to protect the nerve fibers from mechanical and/or chemical stress. The connective tissue sheaths, especially the perineurium, play an important role in elasticity of the nerve (33). Age-related declines in thickness and collagen fibril content of the perineurium of the sciatic nerve of rat have been suggested (34), and thus the stiffness of the *in vivo* human sciatic nerve may decrease with age. Collectively, stiffening the sciatic nerve impairs ankle joint flexibility in older people, but this may not be due to age-related changes in the sciatic nerve stiffness *per se*. Although the reason for this seeming contradiction was not clarified in the present study, sensitivity to tension applied to the sciatic nerve might increase with age. A future study is warranted to verify this point.

The fascia is suggested to be a limiting factor of passive ROM, along with nerves (8). In addition, a previous study reported a significant negative correlation between the thickness of the deep fascia near the posterior thigh (the biceps femoris) and the sit-and-reach distance in older people (35). However, in the present study, SWS for the deep fascia near MG and ST measured at DF15 did not significantly correlate with passive ROM (Fig. 2). In addition, SWS for the deep fascia near ST did not increase with ankle dorsiflexion (Table 1), although a mechanical interaction, i.e., myofascial force transmission, between ST and plantarflexors was implied (36). Based on these results, it seems that the deep fascia in the posterior leg has less influence on passive ankle dorsiflexion ROM even in older people, at least in the prone position. This discrepancy between previous and present results can be explained by the measurement posture. Because the deep fascia in the posterior leg can be well stretched when the ankle is dorsiflexed with hip flexion, a different relation between deep fascia stiffness and passive ankle dorsiflexion ROM might be observed in the long-seated position.

The present results showed significant positive correlations between passive ROM and SWS of each muscle of the triceps surae (Fig. 3) or passive joint torque measured at the maximal dorsiflexion angle. This suggests that passive ROM is strongly influenced by stretch tolerance, as mentioned earlier (4). In the present study, we failed to observe a significant association of passive ROM with SWS of the gastrocnemii measured at DF15 for older participants. This relation might have been masked by the influence of stretch tolerance on passive ROM. We thus performed partial correlation analyses to control for the effect of stretch tolerance, where SWS of MG measured at the maximal dorsiflexion angle was used as a control variable because the correlation coefficient between passive ROM and SWS for MG ($r = 0.739$) was the highest value among those between passive ROM and SWS for each tissue at the maximal dorsiflexion angle. As a result, significant negative partial correlations were observed between passive ROM and SWS of the gastrocnemii measured at DF15 for older participants (MG: $P < 0.001$, $r = -0.923$; LG: $P < 0.001$, $r = -0.775$). These results indicate that, for older people, passive ankle dorsiflexion ROM is limited mainly by stretch tolerance, but gastrocnemii stiffness can also affect passive ROM.

The present study has a limitation related to tissue density. As represented in equations 1 and 2, the tissue density is needed to calculate the tissue elasticity from the SWS. Therefore, if an age-related change in tissue density is prominent, it is difficult to compare tissue stiffness between age-groups using SWS. Because the densities of muscles and nerves are suggested to decrease with age (37,38), the stiffness of aged muscles and nerves estimated from SWS may be overestimated compared with those of young participants. However, this issue did not affect the present results and interpretations because we used SWS rather than the shear modulus or Young's modulus, the SWS values for older participants were not significantly faster than those for young participants, and the correlation analyses were performed within each age-group.

In conclusion, we investigated the differences in the stiffness of the triceps surae, the sciatic nerve, and the deep fascia in the posterior leg, and the associations of passive ankle dorsiflexion ROM with stiffness of tissues between young and older people using ultrasound SWE. Our results suggest that 1) the influence of muscle stiffness on passive ROM becomes weak with age; 2) in contrast to muscle, the influence of nerve stiffness on passive ROM becomes greater with age; and 3) regardless of age, stretch tolerance has large effect on passive ROM. These findings indicate that the limiting factors of joint flexibility vary between young and older people, and that the relative contribution of nonmuscular tissues to joint flexibility might become greater than that of muscles with age.

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K. H. and R. A. conceived and designed the research. K. H. and R. Y. performed the experiments. K. H. and R. Y. analyzed the data. K. H., R. Y., and R. A. interpreted the data. K. H. drafted the manuscript. R. Y. and R. A. edited and revised the manuscript. All authors approved the final version of the manuscript.

The authors declare no conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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