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Original Article

Short-term Effects of Static Stretching on Hamstring Passive Stiffness in Young and Older Women

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Abstract

Objectives: The objectives of this study were to assess the acute effects of static stretching on hamstring passive stiffness in young and older women. A secondary objective was to compare hamstring muscle size and quality measurements (cross-sectional area and echo intensity) between the two groups and to determine if these characteristics are related to passive stiffness at baseline. **Methods**: Fifteen young (23±4 years) and 15 older (73±5 years) women underwent two randomized conditions that included a control treatment and an experimental treatment of four, 15-s static stretches of the hamstrings. Passive stiffness was calculated before (pre-test) and after (post-test) each treatment using a passive knee extension test. Ultrasound imaging was used to measure hamstring muscle cross-sectional area and echo intensity. **Results**: Passive stiffness collapsed across group decreased from pre- to post-test for the stretching treatment (P=0.001) but not for the control (P=0.467). The older women had lower cross-sectional area (P=0.033) and greater baseline (pre-test) passive stiffness (P=0.042-0.049) and echo intensity (P=0.022) than the young women. Moreover, baseline passive stiffness was significantly related to echo intensity (r=0.430, P=0.018) but not cross-sectional area (r=-0.014, P=0.943). **Conclusion**: An acute bout of static stretching decreased passive stiffness in both young and older women.

Keywords: Cross-sectional Area, Echo Intensity, Hamstrings, Passive Knee Extension, Ultrasound

Introduction

Static stretching treatments designed to alleviate muscle tightness in the hamstrings are of clinical interest to physical therapists who work with older adults. A decrease in muscle tightness after stretching may help improve performance and reduce the risk of injury[1]. Measurements of passive stiffness are often used to evaluate muscle tightness[2]. Passive stiffness is typically calculated as the slope of the angle-torque curve recorded during passive stretch[3]. Research suggests that passive stiffness of the hamstrings may be relevant to postural balance performance[4]. Indeed, higher hamstring passive stiffness values have been associated with a decrease in postural balance in older adults[5]. Such a decrease may impair muscle function and increase one's risk of falls during physical activity[6]. If passive stiffness is detrimental to postural balance performance, then it may be important to identify pre-activity static stretching treatments that are effective at reducing the passive stiffness characteristics of muscles. The reductions in passive stiffness that occur after acute bouts of static stretching have been well documented in the literature. Numerous studies have shown significant stretch-induced decreases in passive stiffness for the plantar flexors and hamstrings in both young and older adults[4,7-9]. However, the majority of these studies used long-duration static stretching treatments of five minutes or greater. These treatments are not representative of the stretching durations used in clinical settings[5]. Current recommendations suggest one minute of static stretching[10]; therefore, it may be of great value to elucidate the effects of static stretching for this duration.

Despite numerous studies investigating the acute effects of static stretching on passive stiffness in young and older adults, little research has compared the stretch-

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Table 1. Mean (SD) values for demographic characteristics and hamstring muscle cross-sectional area and echo intensity.

Variable	Young (*n*=15)	Older (*n*=15)
Demographics		
Age (years)	23.0 (3.9)	72.6 (5.3)
Height (cm)	161 (7)	160 (5)
Body Mass (kg)	59.4 (13.6)	63.5 (9.4)
BMI ($kg \cdot m^{-2}$)	22.6 (3.9)	24.8 (3.5)
Physical Activity ($h \cdot wk^{-1}$)	7.80 (3.49)	5.37 (4.65)
Hamstring		
Cross-sectional Area (cm^2)	18.8 (4.2)	15.5 (3.9)*
Echo Intensity (AU)	111 (15)	125 (17)*

**Significantly lower cross-sectional area and higher echo intensity for the older compared to the young women ($P \leq 0.050$). BMI = body mass index.*

induced changes in passive stiffness between these groups. Palmer[4] compared the passive stiffness responses between young and older men after static stretching of the posterior hip and thigh muscles. Results showed a greater decrease in passive stiffness for the older men than the young men from pre- to post-stretching[4]. Although these findings support the notion that static stretching may be more effective at altering passive stiffness in older compared to young men, it remains unclear whether age plays a role in the efficacy of static stretching at reducing passive stiffness in women. Moreover, the study by Palmer[4] used an eight-minute static stretching duration, which is far longer than the stretching durations used in a typical warm-up (i.e., the total stretching duration per muscle group in a typical warm-up is 30 to 120 s)[11]. Previous research has demonstrated that four 15-s static stretches were effective at decreasing passive stiffness of the hamstrings in older men[5]. However, to our knowledge, no previous research has examined the effects of four 15-s static stretches on hamstring passive stiffness in older women, nor have there been any studies that have compared these effects to a younger group of participants.

Greater passive stiffness at baseline (i.e., before stretching) has been reported in older adults than young adults[4]. This difference between age groups may influence the magnitude of the stretch-induced change in passive stiffness; for example, a stiffer muscle has been reported to experience a greater decrease in passive stiffness than a more compliant one[12]. Therefore, if older adults have greater baseline passive stiffness before stretching, they may experience a greater decrease in passive stiffness after stretching; however, further research is needed to test this hypothesis. Age-related decreases in muscle cross-sectional area have been shown to be accompanied by increases in baseline passive stiffness[4]. Consequently, it has been suggested that muscle cross-sectional area is relevant to the changes in passive stiffness with aging[13]. Muscle quality, which is reflected by echo intensity, is indicative of a muscle's fat[14] and fibrous tissue content[15]. It too may contribute to the age-related increases in baseline passive stiffness.

Previous research[13] has shown a significant positive relationship between hamstring echo intensity and passive stiffness variables. However, the passive stiffness data used in this research was limited to a straight-leg raise test[13]. It remains to be determined if hamstring echo intensity is related to the stiffness measured from a passive knee extension. The passive knee extension has been reported to be a safe and reliable test for evaluating passive stiffness of the hamstrings[16]. As mentioned above, the passive stiffness response to stretching is negatively associated with the stiffness of the muscle at baseline[4,12]. Consequently, baseline passive stiffness and its relationship with muscle morphology characteristics (i.e., cross-sectional area and echo intensity) are important and thus, warrant further research. Moreover, previous studies investigating the passive stiffness responses to hamstring stretching have used manually-applied passive movements (performed by the investigator) to lengthen the muscles[5,10]. Additional research investigating the effects of computer-controlled stretches using an isokinetic dynamometer is needed. The isokinetic dynamometer provides an effective means for lengthening the muscles at a slow, constant velocity during the dynamic phase of a static stretch[17]. Using such a device may yield consistent results that can be better generalized to other researchers and practitioners. Thus, the purpose of the present study was to assess the acute effects of static stretching using an isokinetic dynamometer on passive stiffness of the hamstrings in healthy young and older women. A secondary aim was to compare hamstring muscle cross-sectional area and echo intensity between the two groups and to determine if these characteristics are related to passive stiffness at baseline.

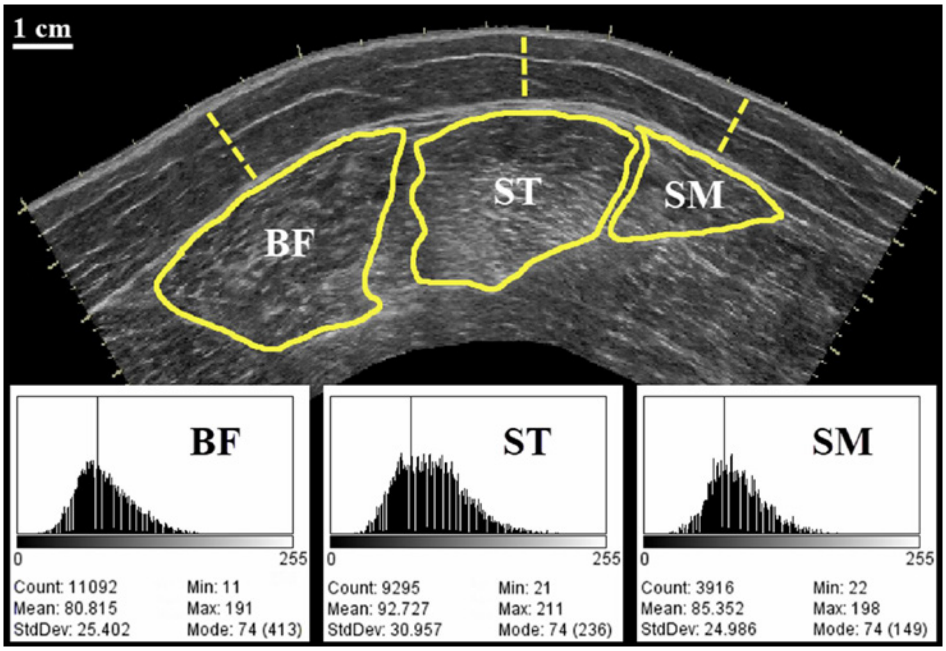

Figure 1. Panoramic ultrasound image of the biceps femoris (BF), semitendinosus (ST), and semimembranosus (SM) muscles. Hamstring muscle cross-sectional area and echo intensity were determined by taking the sum of the cross-sectional areas and the mean of the echo intensities of the BF, ST, and SM, respectively. The solid lines represent the borders of the muscles. The dashed lines indicate the locations where subcutaneous fat thickness was measured. Examples of the corresponding gray-scale histogram values from each muscle are provided.

Materials and Methods

Participants

An a priori power analysis was performed for a between-subjects, repeated-measures design. Using G*Power software (version 3.1.9.2; Heinrich Heine University, Düsseldorf, Germany) and effect sizes from relevant research[4], it was determined that a minimum of 14 participants in each group was needed to achieve a statistical power of 0.80 at an alpha level of 0.05. Thus, 15 young and 15 older healthy women were recruited to participate in the present study (all demographics including body mass index [BMI] are presented in Table 1). Young participants were recruited from the university and older participants were recruited from the community. All participants were free of any neuromuscular diseases or musculoskeletal injuries specific to the hip, knee, or ankle joints. None of the participants were competitive athletes; however, given their reported levels of physical activity (Table 1), they might be best categorized as healthy, recreationally-trained individuals. Each participant signed and completed a written informed consent document and health history questionnaire.

Procedures

Each participant visited the laboratory on two occasions separated by 2-7 days at approximately the same time of day (±2 hours). Participants underwent a non-stretching (control) and stretching treatment that were randomly assigned, such that those who were stretched during the first visit did the control treatment in the second visit and vice versa[18]. The stretching treatment consisted of four, 15-s passive static stretches[5]. Each 15-s stretch was separated by 15 s of rest. The control treatment consisted of quiet resting in the seated position for two minutes, which was equivalent to the total duration of the stretching treatment including rest between stretches. During each visit, participants underwent two passive knee extension assessments before (pre-test) and after (post-test) the treatment intervention.

Panoramic Ultrasound Imaging

Prior to the passive knee extension assessments (during the first visit only), panoramic ultrasound images of the hamstrings (long head of the biceps femoris [BF], semitendinosus [ST], and semimembranosus [SM] muscles) were obtained on the right thigh using a portable B-mode ultrasound imaging device (GE Logiq e BT12, GE Healthcare, Milwaukee, WI) and linear-array probe (12 L-RS, 5-13 MHz, 38.4 mm field-of-view). Ultrasound settings were optimized for image quality, including gain (50 dB), depth (5 cm), and frequency (12 MHz). These settings were set prior to testing and held constant across participants. All ultrasound images were scanned with the probe oriented in the transverse plane at the midpoint between the greater trochanter and the lateral joint line of the knee[2]. For each scan, participants laid on a padded table in the prone position with the lower limbs extended and relaxed while the primary investigator manually moved the probe at a slow and continuous rate along the surface of the skin from the lateral to the medial sides of the hamstring musculature. An adjustable, custom-made apparatus that was fitted over each participant's right thigh was used during each assessment to assist with keeping the probe perpendicular to the skin. A generous amount of water-soluble transmission gel was applied to both the probe and the skin to reduce possible near-field artifacts and enhance acoustic coupling. Two panoramic ultrasound images were taken for each participant, and the mean of the two images was reported for each variable.

All ultrasound images were analyzed using ImageJ software (version 1.50i, National Institutes of Health, Bethesda, MD) and were scaled from pixels to cm before analysis (Figure 1). Muscle cross-sectional area values of the BF, ST, and SM were determined using the polygon function by selecting a region of interest within each muscle that included as much of the muscle as possible without any surrounding bone or fascia. Muscle quality was determined from the echo intensity values assessed by gray-scale analysis using the standard histogram function of the same pre-selected regions of interest used to calculate cross-sectional area for each muscle. Echo intensity values were corrected for subcutaneous fat thickness, which was calculated at the midline of the BF, ST, and SM using the method described by Young et al.[19]. Hamstring muscle cross-sectional area and echo intensity were determined by taking the sum of the cross-sectional areas and the mean of the echo intensities of the BF, ST, and SM, respectively[13].

Passive Knee Extension

Hamstring passive stiffness was quantified from the passive knee extension assessments using an isokinetic dynamometer (System 3, Biodex Medical Systems, Shirley NY) programmed in passive mode to extend the leg at $5°\cdot s^{-1}$. For each passive knee extension, participants sat in an upright position with restraining straps placed over the shoulders and right thigh. During the passive knee extension, the right hip was flexed with a 60° angle between the thigh and torso and the input axis of the dynamometer was aligned with the lateral condyle of the femur. All passive knee extensions were performed on the right leg to the point of discomfort but not pain, as verbally acknowledged by the participant. Once this point was reached, the leg was then immediately returned to the baseline position, which was a knee joint angle of 80° below full extension[20].

Signal Processing

During each passive knee extension assessment, torque (Nm) and joint angle position (°) signals were sampled simultaneously at 1000 Hz with a Biopac data acquisition system (MP150WSW, Biopac System Inc., Santa Barbara, CA) and processed off-line using custom-written software (LabVIEW 11.0, National Instruments, Austin, TX). Torque and position signals were low-pass filtered with a zero-phase lag, fourth-order Butterworth filter at a cutoff frequency of 10 Hz[4]. All subsequent analyses were conducted on the filtered signals.

For passive stiffness, gravity correction was performed during each passive knee extension assessment using a cosine function in which the limb mass was subtracted from the torque signal across the range of motion. The gravity-corrected torque and joint angle signals were plotted as passive angle-torque curves and fitted with a fourth-order polynomial regression model based on the procedures described by Nordez et al.[7]. Passive stiffness was quantified as the tangential slope ($Nm\cdot°^{-1}$) of the angle-torque curve at the second to last common joint angle (θ) for all passive knee extensions performed on each participant[20]. Consequently, the same absolute joint angle could be used to calculate passive stiffness for each assessment. Passive stiffness was calculated with the following equation[7], where θ represents the joint angle, and *m, n, o*, and *p* are coefficients in the fourth-order polynomial regression model that was fitted accordingly with the passive angle-torque curve:

$$\textit{Passive stiffness } (\theta) = 4m\theta^3 + 3n\theta^2 + 2o\theta + p$$

Static Stretching

Repeated static stretching of the right hamstring muscles was performed on the isokinetic dynamometer using a passive knee extension. The dynamometer passively extended the leg at $5°\cdot s^{-1}$ until the participant verbally acknowledged discomfort but not pain by saying "stop." Stretches were held at this position for 15-s bouts with a 15-s rest period between bouts, in which the leg was returned to the baseline position. Each participant underwent four 15-s bouts of static stretching totaling one minute of time under stretch and lasting approximately two total minutes[5].

Statistical Analyses

We inspected data for normality using the Shapiro-Wilk test. Independent samples *t*-tests were used for age-related comparisons of normally distributed variables, whereas Mann-Whitney *U*-tests were used for non-normally distributed variables[21]. A three-way mixed factorial ANOVA (group [young vs. older] × treatment [stretching vs. control] × time [pre-test vs. post-test]) was used to analyze the passive stiffness data. When a significant interaction occurred, follow-up analyses included post-hoc *t*-tests. Pearson correlation coefficients *(r)* were used to examine the relationships between baseline (pre-test) passive stiffness collapsed across treatment and

Table 2. Mean (SD) values for passive stiffness at each time point (pre- and post-test) for the control and stretching treatments in the young and older age groups.

Variable		Control		Stretching		2-Way Interaction (*P*)		
	Group	Pre	Post	Pre	Post	G × Tr	G × Ti	Tr × Ti
Stiffness ($Nm^{\cdot o-1}$)	Young	0.65 (0.20)	0.68 (0.30)	0.66 (0.18)	0.61 (0.18)*	0.422	0.343	0.004
	Older	0.78 (0.14)‡	0.81 (0.25)	0.78 (0.16)‡	0.65 (0.18)*			

G × Tr = group × treatment; G × Ti = group × time; Tr × Ti = treatment × time.
**Significant decrease from pre- to post-test for the stretching treatment when collapsed across group (P = 0.001).*
‡There was a significant group effect (P = 0.027). The older women had greater pre-test values than the younger women for the control and stretching treatments.
There were no significant main effects for treatment (P = 0.092) or time (P = 0.222).

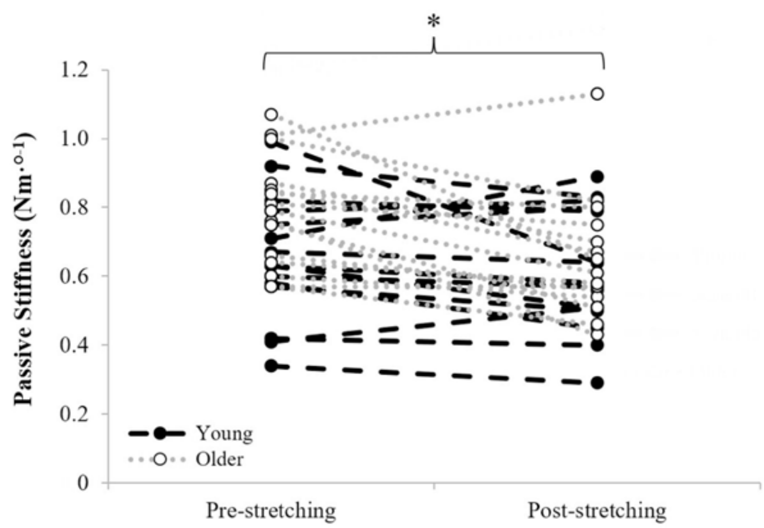

Figure 2. Passive stiffness values at pre- and post-stretching for each participant. *Indicates a significant stretch-induced decrease (*P*=0.001) in passive stiffness collapsed across group.

hamstring muscle cross-sectional area and echo intensity. Separate correlation coefficients were calculated to examine the relationships between BMI and echo intensity as well as age and the percent change in passive stiffness from pre- to post-stretching. Statistical analyses were performed using SPSS software (version 26; IBM Corp, Armonk, NY), and an alpha level of $P \leq 0.050$ was used to determine statistical significance.

Results

All dependent variables were normally distributed except for age in the young women and physical activity in the older women. Mean and standard deviation (SD) values for demographic characteristics and hamstring muscle cross-sectional area and echo intensity are presented in Table 1. There were no significant differences between the young and older women for height (P=0.525), body mass (P=0.348), BMI (P=0.115), or volume of physical activity (P=0.070). The older women had lower cross-sectional area (P=0.033) and higher echo intensity values (P=0.022) than the young women.

Table 2 shows the means and SDs for passive stiffness at each time point for the control and stretching treatments. For passive stiffness, there was no significant three-way interaction (P=0.226) and no significant two-way interactions for group × treatment (P=0.422) or group × time (P=0.343); however, there was a significant interaction for treatment × time (P=0.004). Follow-up analyses indicated that the marginal mean for passive stiffness collapsed across group decreased from pre- to post-test (P=0.001) for the stretching treatment (Figure 2). There was no significant change in

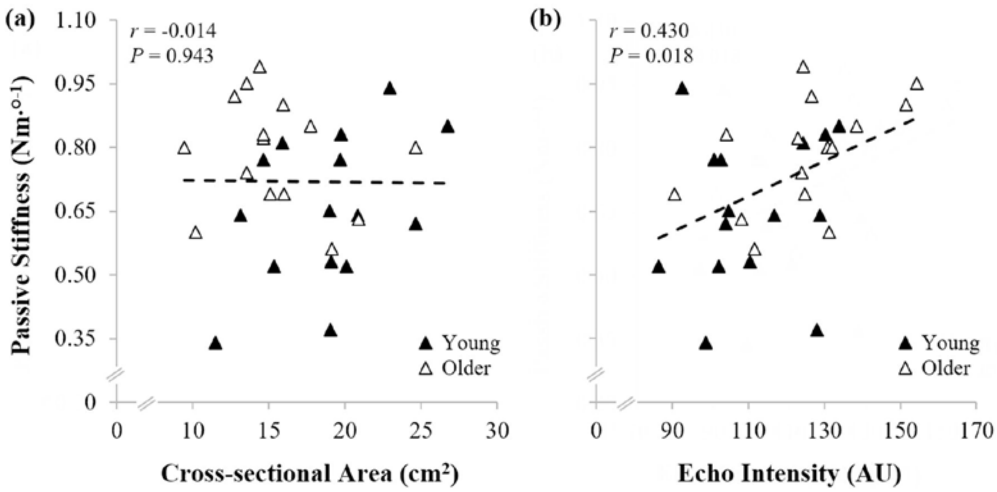

Figure 3. Relationships between baseline passive stiffness (collapsed across treatment) and hamstring muscle (a) cross-sectional area and (b) echo intensity.

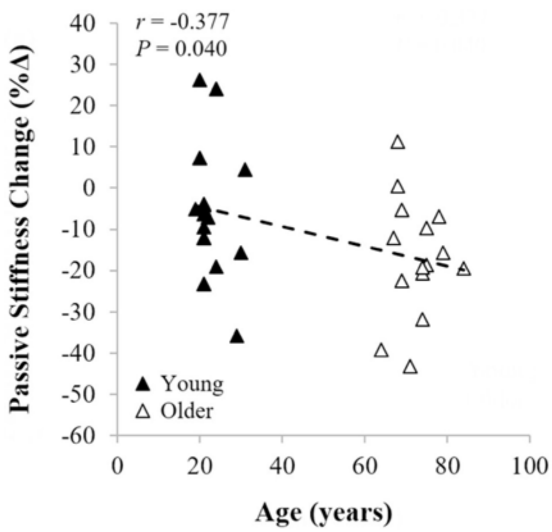

Figure 4. Relationship between age and the percent change in passive stiffness from pre- to post-stretching.

passive stiffness (collapsed across group) from pre- to post-test (P=0.467) for the control.

The older women exhibited greater baseline (pre-test) passive stiffness values than the young women for the control (P=0.042) and stretching (P=0.049) treatments (Table 2). The P value for this group effect (collapsed across treatment) was 0.027. There was no significant difference in passive stiffness between the groups after stretching (P=0.572). A significant positive relationship was observed between baseline passive stiffness (collapsed across treatment) and echo intensity (r=0.430, P=0.018); however, no significant relationship was observed between baseline passive stiffness and cross-sectional area (r= -0.014, P=0.943) (Figure 3). A significant negative relationship was observed between age

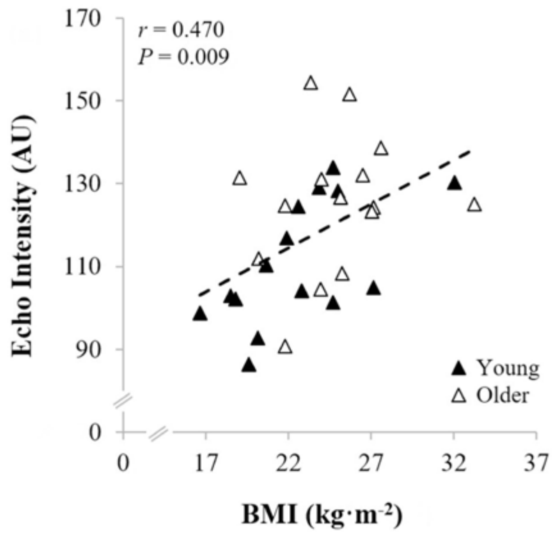

Figure 5. Relationship between body mass index (BMI) and hamstring echo intensity.

and the percent change in passive stiffness from pre- to post-stretching (r= -0.377, P=0.040) (Figure 4). A significant positive relationship was observed between BMI and echo intensity (r=0.470, P=0.009) (Figure 5).

Discussion

In this study, static stretching decreased hamstring passive stiffness in both the young and older women (Table 2). The older women had lower cross-sectional area and greater baseline (pre-test) passive stiffness and echo intensity than the young women (Tables 1 and 2). Moreover, baseline passive stiffness collapsed across treatment was significantly related to hamstring echo intensity but not cross-sectional area (Figure 3).

Our findings revealed that hamstring passive stiffness collapsed across group decreased from pre- to post-test as a result of the stretching treatment (Table 2). Numerous studies have reported significant stretch-induced decreases in passive stiffness for the plantar flexors[8,9] and hamstrings[4,7]. However, the majority of these studies used long-duration static stretching treatments of five minutes or greater. In contrast, our study used a short, practical bout of four, 15-s static stretches. We found that the cumulative effects of these stretches were capable of reducing hamstring passive stiffness in healthy young and older women. There are several mechanisms that have been proposed to explain the stretch-induced decreases in passive stiffness. These mechanisms include increases in tendon compliance[22], changes in muscle fascicle length[23], and deformation of the noncontractile proteins of the endosarcomeric and exosarcomeric cytoskeletons (i.e., titin and desmin)[24]. Another mechanism may be alterations in the intramuscular connective tissues[5]. Evidence suggests that the connective tissues are a major contributor to passive stiffness[25]. Because increases in the length of the connective tissues have been reported to occur as a result of stretching[24], such changes may decrease passive tissue resistance, which could cause a reduction in the stiffness characteristics of the muscle. Further research using ultrasound imaging combined with passive angle-torque data is needed to determine the mechanisms responsible for the decreases in stiffness observed after stretching.

Greater hamstring passive stiffness has been reported in older compared to young men[4], and in the present study, we found greater hamstring passive stiffness at baseline (pre-test) in the older compared to the young women (Table 2). These differences in passive stiffness between young and older adults may be due to age-related changes in muscle quality. The quality of the hamstrings was evaluated in this study using echo intensity as an index of the amount of fat and connective tissue within the muscle. Our study revealed that the older women (as a group) had a significantly higher echo intensity than the young women (Table 1). This finding supports the notion that muscle quality is adversely influenced by age. Previous authors have suggested a possible link between greater passive stiffness and lower muscle quality in older adults[4,24]. In the present study, a significant positive relationship (r=0.470) was observed between BMI and hamstring echo intensity (Figure 5). We also found a significant positive relationship (r=0.430) between hamstring echo intensity and passive knee extension stiffness at baseline (Figure 3). Previous research has reported a significant positive relationship in older men between hamstring echo intensity and passive stiffness as assessed from a straight-leg raise[13]. Taking these findings together, it is possible that the greater baseline passive stiffness observed for older adults may be due to their muscles having a greater amount of fat and connective tissue[13,26].

Baseline passive stiffness was not significantly related (r= -0.014) to cross-sectional area in the present study (Figure 3). This finding is consistent with that of previous research[27] and suggests that muscle size may not be relevant to the passive stiffness of the hamstrings. Alternatively, Magnusson et al.[2] showed that lateral hamstring cross-sectional area was significantly related to mid-range passive stiffness. Nevertheless, this study tested elite-level athletes with varying levels of flexibility[2]. Testing such a diverse sample of athletes may have contributed to a stronger correlation between passive stiffness and cross-sectional area compared to that observed in the present study, which tested groups of young and older non-athletes. In this study, muscle cross-sectional area of the hamstrings was significantly lower in the older compared to the young women (Table 1). Previous studies have reported similar findings in older compared to young men[4,13]. Such findings indicate that aging may have a deleterious effect on hamstring muscle size.

We found a significant negative relationship (r= -0.377) between age and the percent change in passive stiffness from pre- to post-stretching (Figure 4). Palmer[4] demonstrated a greater stretch-induced decrease in passive stiffness for older compared to younger adults. Collectively, these findings suggest that age may play a role in the stretch-induced decreases in passive stiffness. Additional research with larger sample sizes is needed to further examine the importance of age as it relates to the changes in stiffness observed after stretching. From a functional standpoint, the significant decrease in passive stiffness from pre- to post-test may have a positive effect on the postural stability of older adults. Previous research[5] showed that four 15-s static stretches of the hamstrings elicited significant decreases (10-15%) in passive stiffness that were associated with improvements in postural balance in older men. It was suggested that these decreases in passive stiffness may reduce the risk of falls that occur during physical activity[5]. Because similar declines in passive stiffness were observed for the hamstrings in the present study, such changes may also be beneficial for improving postural balance and reducing the risk of falls in older women. Future research investigating the influence of static stretching on passive stiffness and the prevalence of falls in older women is needed to further examine these findings.

This study was designed to investigate the acute effects of four, 15-s static stretches on passive stiffness of the hamstrings in healthy young and older women. We also aimed to examine the differences in hamstring muscle cross-sectional area and echo intensity between the two groups and to determine if these characteristics are related to passive stiffness at baseline. Our findings revealed that the stretching treatment produced a significant decrease in passive stiffness collapsed across group (Table 2). These findings indicate that a short, practical bout of static stretching may be effective at reducing hamstring passive stiffness in both young and older adults. The older women in this study had lower cross-sectional area and greater baseline (pre-test) passive stiffness and echo intensity than the young women (Tables 1 and 2). Moreover, we found that baseline passive stiffness was significantly related to echo intensity but not cross-sectional area (Figure 3). This finding suggests that hamstring muscle quality rather than size may be relevant to the stiffness measured during a passive knee extension test. The results of our study may have important implications for creating stretching interventions that can be used in clinical practice to help attenuate the negative effects of stiffness on muscle function. Such interventions may be beneficial for improving postural balance and reducing the risk of falls, fall-related injuries, and other adverse events that are common debilitating occurrences in older populations.

Ethics approval

This study was approved by the Texas Tech University institutional review board for human subject research.

Consent to participate

Each participant signed and completed a written informed consent document and health history questionnaire.

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