

Alterations in leg flexibility, joint stiffness, and muscle architecture following a single bout of stretching and eccentric exercise in female older adults

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Abstract

Poor joint flexibility can increase frailty and mortality rates in older adults. However, the evidence of the benefit in the response to flexibility exercises is still less attention especially in female older adults. This study examined the immediate effects of dynamic closed-chain stretching (DCS), eccentric exercise (ECC), and static stretching (ST) on muscle architecture, flexibility, and extensibility. We recruited 48 female elderly adults with a mean age of 66.2 ± 3.6 years and randomly divided them into three groups. DCS included dynamic hamstring stretching for 48 repetitions, while ECC comprised 12 repetitions of hamstring eccentric exercise followed by calf down for 12 repetitions. Static hamstring stretching was done eight times in the ST group. The measurements for hamstrings and plantar flexor musculotendinous stiffness, straight leg raise (SLR), passive ankle dorsiflexion (PDF), and muscle ultrasound imaging of biceps femoris (BF) and medial gastrocnemius (MG) muscles were performed before and immediately after exercise intervention. There was no change in hamstrings and plantar flexor passive stiffness in response to a single bout intervention. Moreover, SLR improvement was observed after all three interventions ($p < 0.01$) without significant differences between groups with increasing resting BF fascicle length ($p < 0.01$). Following ECC intervention, a similar change in PDF angle and MG fascicle length was also noticed. Our findings suggested that hamstrings and plantar flexor flexibilities were significantly improved with a contributable change in fascicle length rather than musculotendinous mechanical properties. Moreover, apart from the static stretching-related changes in leg flexibility, DCS or ECC could also be considered somewhat beneficial for the subsequent performance.

Key Words: extensibility, active stretching, dynamic stretching, musculotendinous stiffness, elderly

Introduction

The soft tissue stiffness changed by aging is caused by the increase in collagen cross-linking, extracellular water content within the muscle, and intramuscular adipose tissue (Marcus et al., 2010), and less in the diameter of collagen fibril compared to younger age (Hirata et al., 2020b). Stretching exercise usually aims for joint flexibility improvement in the aging population (Hirata et al., 2020a; Lim et al., 2019). An increase in ROM after a few stretches or stretching training can be explained by changes in musculotendinous unit (MTU) stiffness, muscle spindles, and Golgi tendon organ, or increasing stretch tolerance (Apostolopoulos et al., 2015). Moreover, static stretching has been performed for flexibility due to its effectiveness and ease of conducting (Kyranoudis et al., 2019; Takeuchi & Nakamura, 2020). However, previous evidence has reported that this prolonged exercise negatively affects or decreases muscle strength for the subsequent performance (Behm et al., 2021).

Dynamic stretching is usually selected as an alternative exercise for ROM and performance preparation purposes (Iwata et al., 2019; Popelka & Pivovarniček, 2018). There are still few experiments on the effect of dynamic stretching in older adults. Zhou et al. (2019) found that ROM was improved by 7% and remained to increase by 8% after 60 min of dynamic hamstrings stretching without load for 50 repetitions with 30-sec rest, three sets in total. Dalleck (2012) also reported that dynamic stretching performed before balance exercise did not compromise the balance ability. Moreover, 45% improvement in hamstrings flexibility, 10% in knee strength torque increase, and 14.3% decreased time up and go were reported in the results of seven repetitions of active hamstrings stretching (1 min with 30-sec rest, twice a week for four weeks) in older women (Batista et al., 2009).

At the same time, some studies observed that eccentric exercise also leads to ROM improvement (Abdel-Aziem et al., 2018; Delvaux et al., 2020), which could be due to sarcomerogenesis and changing stretch reflex after training for six weeks long (Delvaux et al., 2020). Generally, the eccentric exercise aims to strengthen muscle or muscle function improvement. This exercise has been recommended for older adults to

perform with benefits in less metabolic cost and effort uses (Quinlan et al., 2019) in terms of tissue extensibility together with muscle strengthening enhancements. For example, the improvements of leg strength, mobility, and postural stability after eccentric exercise were greater than concentric exercise in 17 healthy older adults (Katsura et al., 2019). The similar result was found for improving power, jump and stair climbing performance in the response to plyometric exercise (Van Roie et al., 2020). The review of Kulkarni and colleagues (2022) also suggested that eccentric exercise could be an effective intervention for geriatric function improvement. To the best of our knowledge, there is still remain less finding on flexibility as a result of eccentric exercise in older adult.

Muscle ultrasound imaging to assess muscle thickness has been widely used to detect morphology or any adaptation in response to intervention. The muscle thickness was related to muscle mass measured by the bioimpedance assay and grip strength in older adults (Madden et al. 2021). Moreover, change in fascicle length (FL) has also been indicated in the alteration of longer fascicles adaptation following a such period of flexibility training (Donti et al., 2019) or a single stretching intervention (Sá et al., 2016; Santos et al., 2020) or eccentric exercise (Cuthbert et al., 2020; Marzilger et al., 2020). These architectures provide information for muscular tissue changing in response to given exercises. However, there was rare evidence to reveal the change in FL following dynamic stretching. Passive musculotendinous unit (MTU) stiffness has long been investigated as the contribution of mechanical property changes in the results of tissue extensibility exercises, and the results were controversial after a single bout of static stretching (Hirata et al., 2017; Muanjai et al., 2017a; Nakamura et al., 2011). Not changing the mechanical properties of MTU could not explain the increase in ankle ROM as a response to the dynamic stretching (Mizuno, 2017). A similar non-alteration in MTU stiffness following eccentric exercise also was seen and suggested to be due to a decrease in muscle stiffness whereas an increase in tendon stiffness (Blazevich, 2019).

Despite widespread use, a few studies about the beneficial effect associated with a single session of these interventions were documented, especially in older adults for the subsequent performance rather than ROM improvement alone. Therefore, we speculated that the results from this study would suggest the exercise direction in older adults apart in forms of resistance exercise, balance training, and aerobic exercise, as well as the safety while performing the exercise. Moreover, there is also less attention to investigating female participants. The possible gender differences are mentioned to consider the sensation or viscoelastic property changes in response to stretching exercise (Hoge et al., 2010). Thus, this study aimed to examine the changes in muscle architecture, flexibility, and passive stiffness after the response of a single bout of dynamic stretching, eccentric exercise, or static stretching in female older adults. We hypothesized that any changes in FL or passive MTU stiffness would be explained by leg ROM improvements following those exercises in older adults.

Material & methods

Participants

Forty-eight female elderly from Chonburi Province, Thailand, with a mean age of 66.2 ± 3.6 years and a mean BMI of 22.2 ± 2.5 $\text{Kg}\cdot\text{m}^{-2}$ were included in this study. The inclusion criteria included those aged 60–75 who agreed with the physical activity readiness questionnaire and could walk independently. Exclusion criteria were the involvement in neuromuscular or skeletal injury associated with lower extremities or lower back in the previous six months, regular stretching or strengthening training regimen, the inability to follow the instruction during the assessment, having moderate back or lower extremities pain (visual analog scale $> 3/10$), and obesity ($\text{BMI} > 24.9$ $\text{kg}\cdot\text{m}^{-2}$). The detail of the characteristic of the participants for each group is presented in **Table 1**. Participants acknowledged the testing and training procedures and provided written informed consent to the study. This study was approved by the Research and Innovation Administration Division of Burapha University Ethics Committee (IRB1-007/2565) following the Declaration of Helsinki.

Study design

In this randomized clinical trial, all participants took part in a familiarization session at Physical Therapy Department, Allied Health Science Faculty, Burapha University, a few days before the experiment. They were randomly assigned to three intervention groups (dynamic closed-chain stretching (DCS), eccentric exercise (ECC), and static stretching (ST)) by blocks of six randomizations. On the testing day, measurements including muscle ultrasound imaging (USI) of the long head of bicep femoris (BF) and medial gastrocnemius (MG) muscles, range of motion (ROM), and passive MTU stiffness were taken in the same order for all individuals. Participants did a standardized warm-up for 7 min on a cycle ergometer just after USI testing. Measures were made right before and immediately after the intervention. The same investigators made all tests, and the participants' right leg was only measured and exercised. The room temperature of testing was kept at 25°C throughout the study.

Measurements

Muscle ultrasound imaging

The use of B-mode ultrasonography (M5 series, Shenzhen, Mindray Bio-Medical, China) with linear 4 cm, 7.5 MHz probe (MSK preset) was conducted to capture the muscle images of the right leg. For BF muscles, the US probe was located 15 cm above the popliteal line in a relaxed prone position. Then, the images of MG

Table 1 The characteristic of participants in three groups' intervention

	DCS group (N=16)	ECC group (N=16)	ST group (N=16)
General			
Age (years)	66.9 ± 3.5	65.0 ± 3.6	66.7 ± 3.8
Weight (Kg)	52.2 ± 8.0	55.8 ± 5.1	52.4 ± 7.0
Height (cm)	153.5 ± 5.6	156.1 ± 5.2	156.0 ± 4.4
BMI (Kg·m ⁻²)	22.1 ± 2.7	22.9 ± 1.7	21.5 ± 2.7
Exercise volume (min·wk ⁻¹)	117 ± 88	158 ± 111	143 ± 132
Joint ROM			
SLR (°)	86 ± 12	85 ± 8	86 ± 11
Passive DF (°)	20 ± 4	17 ± 3	17 ± 4
Passive MTU stiffness			
Hamstrings (Nm/°)	0.78 ± 0.16	0.74 ± 0.19	0.68 ± 0.24
Calves (Nm/°)	0.71 ± 0.35	0.68 ± 0.22	0.72 ± 0.19
Muscle architecture			
Resting BF FL (cm)	8.13 ± 2.68	8.84 ± 3.10	8.02 ± 2.90
Resting BF MT (cm)	1.37 ± 0.38	1.44 ± 0.42	1.43 ± 0.43
Resting BF PA (°)	9.9 ± 2.7	10.1 ± 3.4	11.4 ± 3.5
Resting MG FL (cm)	4.33 ± 1.20	4.49 ± 1.21	4.46 ± 1.21
Resting MG MT (cm)	1.37 ± 0.39	1.49 ± 0.40	1.41 ± 0.39
Resting MG PA (°)	19.8 ± 2.9	19.9 ± 2.3	19.3 ± 2.8

Data are mean and SD. DCS, dynamic stretching; ECC, eccentric exercise; ST, static stretching; BMI, body mass index; SLR, straight leg raise; DF, dorsiflexion; MTU, musculotendinous unit; BF, biceps femoris; FL, fascicle length; MG, medial gastrocnemius. No significant difference in these characteristics between groups was observed in this study.

Muscle were taken 10 cm below the popliteal line, where the examined foot was relaxed in a neutral position (90°). Moreover, a transparent pen marked manually these locations on their skin for reproducibility. Each muscle was captured for two images (resting FL) and at the endpoint during passive SLR and DF procedures in the longitudinal view with some US gel and minimal pressure for the US probe. The strain elongated FL during passive motion was calculated by FL measured at the endpoint subtracted with the resting FL (elongated or strain FL) for the maximal passive angle trial image. The ultrasound setting of 10 MHz, gain 54 dB, and dynamic range 60 with the depth of 5 cm were kept consistent across participants.

All muscle thickness (MT) and pennation angle (PA) were analyzed with Tracker 6.0.10 software, in which MT indicates the distance between superficial and deep aponeurosis. In addition, PA was indicated by the angle between the visible FL and deep aponeurosis. Lastly, FL was predicted by drawing the extrapolated line of visible fascicles between the superficial and deep aponeurosis (Muanjai et al., 2020). All the averages of two images for MT, PA, and FL were further used for the analysis. The intraclass correlation coefficient (ICC) and the coefficient of variation (CV) for BF FL and FL of MG muscle in the present study were 0.860 (CI: 0.753, 0.911), CV of 5.9%, and 0.936 (0.898, 0.988), CV of 4.3%, respectively.

Range of motion

Hamstring flexibility evaluation was assessed by passive SLR. The contralateral thigh was fixed by a belted strain system in the bed at the pelvis to prevent the initiation of the posterior pelvic tilt. The tester positioned the inclinometer (bubble inclinometer, Baseline, Fabrication Enterprises, Elmsford, NY, USA) using a foam fixation holder at the distal end of the tibia. At the same time, one hand of the examiner grasped at the back of the calcaneus, and another was placed over the knee to keep it straight. The endpoint of passive motion of the hip flexion was indicated by the perception of firm resistance or onset of pelvic rotation while participants were lying in a relaxed position (Ayala et al., 2011). At this point, the maximum angle read from the inclinometer was noted, with the US capturing the longitudinal image of BF muscle and repeatedly triplicating with 15 s in between. The average of the two most closed angles and SLR images was further analyzed. The ICC for SLR measurement was 0.932 (0.902, 0.979), and the CV of the mean angle was 2.9%.

Passive ankle dorsiflexion (PDF) was evaluated for calf ROM assessment, with the participants lying prone in a relaxed position and tested foot out of the bed's edge. The examiner firstly positioned the participant's foot in a neutral position (90°) using a standard goniometer (Mahidol University, Bangkok, Thailand), where the stationary arm was parallel with the fibular head, and the movable arm parallel with the lateral side of the foot below the fifth metatarsal and the longitudinal images of MG muscle were also captured for FL at this initial position. Hence, the examiner passively ankles dorsiflexion without rotation or deviations of the participant's foot while the knee was kept straight (Abdel-aziem & Mohammad, 2012; Konrad et al., 2020). The maximum angle read at this point was noted together with capturing the longitudinal image of MG muscle and repeatedly done triplicate with 15 s intervals. The average of the two most closed angles and passive DF images was further analyzed. In this study, the ICC for PDF measurement was 0.889 (0.810, 0.920), and the CV of the mean angle was 4.1%.

Passive joint stiffness

A Biodex System 4 isokinetic dynamometer (Biodex Medical Systems, Inc., Shipley, New York, USA) was used for passive MTU testing. For knee flexors, the participant was seated on the chair with no shoes, the hip flexion at approximately 120°, and the shank at 20° below the horizontal. The pelvis and both thighs were stabilized with Velcro straps. The axis of rotation was fixed at the right knee joint, and the lever arm pad was attached proximally to the malleolus. In this position, the dynamometer passively extended the knee at 5°/s to the end point of maximum discomfort without pain. However, during familiarization, difficulty in informing a similar endpoint was observed in participants. Hence, the trial of passive hamstring immediately after exercise with maximal knee extension angle was used to analyze the MTU stiffness until the same maximal endpoint was measured before exercise intervention at the same ROM. At the same time, ankle plantar flexors stiffness was investigated during supine semi-fowler 40° without sock and shoe. The examined thigh was secured on the chair. The axis of ankle rotation was fixed at the level of the lateral malleolus with the straight knee (Andrade et al., 2016). During the test, the dynamometer passively toward the ankle at 2°/s from the 40° plantar-flexed position to the endpoint of 30° ankle dorsiflexion. Both passive stiffnesses were determined from the slope of the passive torque-angle relationship, with gravity correction for the weight of the lower leg, as the increase in passive torque between 50%–80% of maximum ROM using a least-squares method (Cabido et al., 2014; Muanjai et al., 2017a). The surface electromyography (EMG) was used to ensure no muscle activities for rectus femoris, biceps femoris, tibialis anterior, and gastrocnemius muscles. Three passive maximum ROM, peak torque, and stiffness measures were measured for further analysis. In this study, the ICC for passive knee extension stiffness measurement was 0.777 (0.720, 0.789) and the coefficient of variation was 6.7% and passive ankle dorsiflexion (0.897 (0.854, 0.967) with CV 3.6%).

Intervention

Dynamic stretching

After the baseline investigation, the closed kinetic chain hamstrings stretching exercise was used to perform DCS intervention, as mentioned in Chen et al. (2018). The participants stood on the involved leg with the knee slightly flexed 10°–15° and were then asked to slowly lean forward until reaching the end of the hip flexion ROM. At the same time, the contralateral leg was lifted into the hip extension to maintain spine straightness and provide body balance. The participant bent their knee slowly for 1 s down, alternately extending to stretch the hamstring muscles for 1 s. The stretch intensity was set at maximum discomfort without pain, and less tension was adjusted by decreasing the trunk-leaning forward angle. This DCS procedure was continuously done for eight repetitions per set in six sets with 15-s rest between sets and performed with supervised physical therapist guidance throughout the experiment. The chair's back or the wall was used to secure the stability for participants in this procedure while the stance was on one leg and leaning forward.

Eccentric exercise

This exercise program consisted of two different positions. First, a single leg Roman deadlift T-drop was performed for hamstrings intervention (Delvaux et al., 2020). The participants stood on the exercised leg with the knee slightly flexed 10°–15° and were then asked to slowly lean forward until the end of the hip flexion ROM. At the same time, the contralateral leg was slowly lifted into the hip extension to maintain a neutral spine. Hence, the participants slowly returned to the starting position (i.e., stood on both legs). Calf down exercise was designed for the calves intervention (Katsura et al., 2019). Again, the participants stood on the exercised leg with the knee contralateral flexed at 90°, and then they lifted the body up to maximum plantarflexion while the hand could support this manner. From this position, they asked to slow down the body to the floor regardless of knee bending. For the safety procedure, the chair's back or the wall was used to secure the stability for participants in this procedure while performing this exercise regimen. Each exercise was done six times per set, two sets in total, with rest for 1 min between sets and 2 min between exercises.

Static stretching control

Participants were relaxed in the supine position while using the towel wrapped under the plantar of stretched leg. During the procedure, they lifted their leg with the towel while their knees were straight, and ankle dorsiflexion was kept. The hip flexion motion was ended by the most tension feeling along the posterior of the thigh or hamstrings without pain, and this stretched perception was held for 30 sec by slightly adjusting the hip angle (Muanjai et al., 2017a). This exercise was repeatedly done eight times with 15-sec rest.

Statistical analysis

The sample size of the present study was determined from the study of Opplert & Babault (2019), who investigated the changes in FL and yielded a value of 0.11 ± 0.03 cm before stretching and 0.15 ± 0.05 cm after performing static stretching exercises. The G power software was used to calculate the sample size with the error type of 0.05, the statistical power of 0.80, and the minimal sample size of 13. For the dropout potential, the sample size was increased by 20%, i.e., 16 in each group.

The mean and standard deviation (SD) were computed for all descriptive variables. Data were initially tested for normality using the Shapiro–Wilk test. The absolute difference was given as mean and 95% confidence interval for comparing exercise protocols. All baseline measurements and characteristics between groups were compared by one-way ANOVA. Time and interaction effects were determined by repeated-

measures analysis of variance (ANOVA) [time (baseline, post) $\times$ intervention (DS, ECC, and ST)], and post hoc multiple comparisons were made using Bonferroni correction for significant interactions. The effect size (ES) was reported as the partial eta-squared for repeated measure (magnitude of effect; small = 0.02, moderate = 0.13, large = 0.26) (Bakeman, 2005) and as Cohen's d where the pairwise comparison was made (small ($d = 0.2$), moderate ($d = 0.5$), and large ($d = 0.8$)) (Cohen, 2013). The ICC for each measure was calculated from the familiarization sessions. An alpha level of 0.05 was used to determine the statistical significance. All statistical analysis was performed using the Statistic Package for the Social Sciences (SPSS for Windows, version 24.0, Chicago, IL, USA).

Results

No adverse effect was reported following a single bout of intervention. The demographic characteristics, including joint flexibility, muscle architecture, and MTU stiffness for a baseline for each group of non-obese older adults aged 60–75 years, are shown in **Table 1**. One-way ANOVA results showed similar baseline characteristics, ROM, passive mechanical properties, and leg muscle states between those three groups ($p > 0.05$).

Joint flexibility

Immediately after all interventions, passive SLR angle or hamstrings flexibility was increased ($F(1,45) = 53.4$, $p < 0.001$, $\eta_p^2 = 0.54$) by a mean of 4.9° (95% CI; 2.2 , 7.7° , $p = 0.001$, $d = 0.40$) for DCS group, 6.1° (3.3 , 8.8° , $p < 0.001$, $d = 0.75$) for ECC group, and by 6.4° (3.7 , 9.2° , $p < 0.001$, $d = 0.58$) for ST group. There were no significant differences between groups ($F(2,45) = 0.10$, $p = 0.90$) or increasing by 5.9%, 7.3%, and 7.5%, respectively (**Fig.1**). There were a higher improvement in PDF angle investigating calf flexibility ($F(1,45) = 15.6$, $p < 0.001$, $\eta_p^2 = 0.26$) after a single bout of ECC performing with calf down on average 2.5° (1.1 , 3.9° , $p = 0.001$, $d = 0.57$) or 15.7% increasing (**Fig.1**). However, there was no any significant change in PDF angle for DCS and ST groups ($p > 0.05$).

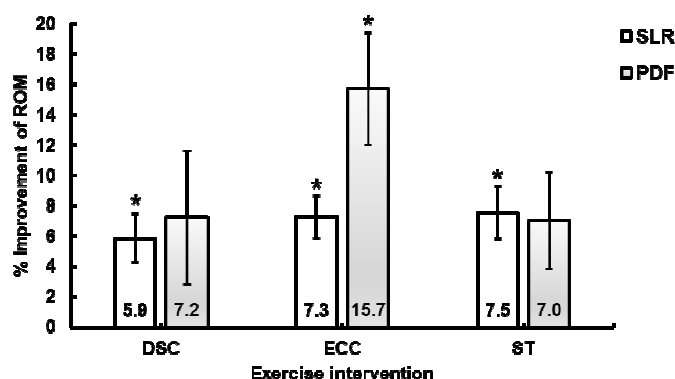


Fig. 1 Changes in ROM after exercise interventions. The blank bar is data for SLR measurement, and the shaded bar is for the PDF test. Data are given as mean and SEM (N = 16). * Significant difference after exercise intervention ($p < 0.05$)

Leg muscle architecture

Leg muscle architecture changes in response to those three exercises are shown in **Table 2**. MT was changed after exercise intervention by two-way ANOVA analysis ($F(1,44) = 15.8$, $p < 0.001$, $\eta_p^2 = 0.27$) without group differences ($p > 0.05$). With the post hoc test, BF muscle thicker was immediately seen after dynamic closed chain stretching by 0.11 cm (0.04 , 0.18 cm, $p = 0.002$, $d = 0.28$), but no changes were seen for ECC and ST groups. There was, however, no change in MT of MG following all those three exercises ($F(1,45) = 2.3$, $p = 0.14$), likewise for PA of BF muscle. While a smaller PA for MG was seen immediately after exercise interventions ($F(1,45) = 11.4$, $p = 0.002$, $\eta_p^2 = 0.20$). There were a lessor angle of MG muscle at resting of 1.4° (0.13 , 2.58° , $p = 0.031$, $d = 0.74$) for ECC and 1.3° (0.01 , 2.48° , $p = 0.048$, $d = 0.47$) for ST immediately after exercise by post hoc test. A longer resting BF FL was significantly seen for immediate following all those three exercises ($F(1,44) = 44.1$, $p < 0.001$, $\eta_p^2 = 0.50$) without significant between groups ($F(2,44) = 0.40$, $p = 0.68$). Changes of 0.81 cm (0.31 , 1.31 cm, $p = 0.002$, $d = 0.28$), 0.97 cm (0.45 , 1.48 cm, $p < 0.001$, $d = 0.29$) and 1.12 cm (0.62 , 1.62 cm, $p < 0.001$, $d = 0.38$) in resting BF FL were seen immediately after DCS, ECC, and ST, respectively or on average 10–15% as shown in **Table 2**. Interestingly, A higher strain in FL of BF muscle was substantially noticed ($F(1,44) = 12.7$, $p = 0.001$, $\eta_p^2 = 0.22$) where post hoc showed BF significantly stretched longer by 2.0 cm (0.6 , 3.4 cm, $p = 0.007$, $d = 0.41$) immediately after DCS intervention. After a single session of ECC, a resting MG FL was longer ($F(1,45) = 9.6$, $p = 0.003$, $\eta_p^2 = 0.18$) by on average 0.4 cm (0.2 , 0.6 cm, $p = 0.001$, $d = 0.33$). There was no difference between times and groups for strained MG FL.

Table 2. Muscle architectures to represent the changes following a single bout of exercise interventions (N = 16 per group)

USI	Group	Pre	Post	%change
BF MT (cm)	DCS	1.37 ± 0.38	1.48 ± 0.40	↑ 8.3%*
	ECC	1.44 ± 0.42	1.50 ± 0.42	-
	ST	1.43 ± 0.43	1.49 ± 0.44	-
MG MT (cm)	DCS	1.37 ± 0.39	1.36 ± 0.39	-
	ECC	1.49 ± 0.40	1.49 ± 0.39	-
	ST	1.41 ± 0.39	1.36 ± 0.39	-
BF PA (°)	DCS	9.9 ± 2.7	9.7 ± 2.8	-
	ECC	10.1 ± 3.4	9.3 ± 2.7	-
	ST	11.4 ± 3.5	10.4 ± 3.8	-
MG PA (°)	DCS	19.8 ± 2.9	18.8 ± 2.5	-
	ECC	19.9 ± 2.3	18.5 ± 1.4	↓ 5.8%*
	ST	19.3 ± 2.8	18.0 ± 2.7	↓ 5.9%*
BF FL _R (cm)	DCS	8.13 ± 2.68	8.93 ± 3.06	↑ 10.4%*
	ECC	8.84 ± 3.10	9.80 ± 3.46	↑ 11.2%*
	ST	8.02 ± 2.90	9.14 ± 3.00	↑ 15.6%*
BF FL _S (cm)	DCS	8.39 ± 4.03	10.24 ± 5.00	↑ 26.9%*
	ECC	8.13 ± 3.45	9.38 ± 4.53	-
	ST	8.78 ± 4.85	9.74 ± 5.32	-
MG FL _R (cm)	DCS	4.33 ± 1.20	4.42 ± 1.21	-
	ECC	4.49 ± 1.21	4.90 ± 1.26	↑ 9.8%*
	ST	4.46 ± 1.21	4.56 ± 1.21	-
MG FL _S (cm)	DCS	1.05 ± 0.53	1.01 ± 0.53	-
	ECC	0.96 ± 0.50	0.79 ± 0.42	-
	ST	0.93 ± 0.60	1.17 ± 0.62	-

Data are mean and SD. USI, ultrasound imaging; DCS, dynamic stretching; ECC, eccentric exercise; ST, static stretching; BF, biceps femoris; MT, muscle thickness; MG, medial gastrocnemius; PA, pennation angle; FL_R, resting fascicle length; FL_S, fascicle length strain. *Significant change from pre-exercise ($p < 0.05$).

Passive MTU stiffness

The passive mechanical properties of hamstrings and ankle plantar flexors MTU are shown in **Fig. 2**. When considering the angle-torque relationship at the actual same endpoint ROM, the stiffness calculated from 50-80% of maximum ROM of the curve was not altered following all three exercises for either knee flexors MTU ($F(1,45) = 0.2$, $p = 0.68$, **Fig. 2 A-C**) or ankle plantar flexors MTU ($F(1,43) = 0.6$, $p = 0.46$, **Fig. 2 D-F**). A similar finding, no alteration in peak passive resistive torque detected at the end point of the same ROM, was also presented in the present study for both either knee flexors MTU ($F(1,45) = 2.5$, $p = 0.12$) or ankle plantar flexors MTU ($F(1,43) = 0.1$, $p = 0.94$) following immediately after exercise interventions.

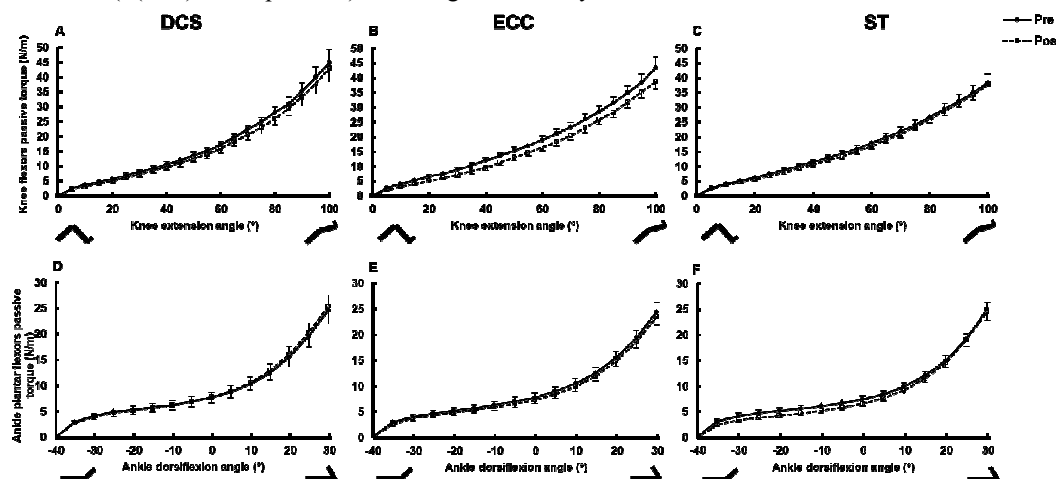


Fig. 2 MTU compliance assessed by passive knee extension and ankle dorsiflexion. The angle-torque relationship was determined before intervention (pre, black line with filled circle) and immediately after intervention (post, dashed line with blank square). DCS, dynamic stretching; ECC, eccentric exercise; ST, static stretching. Data are given as mean and SEM (N=16). No significant change in MTU stiffness after exercise intervention was seen for this investigation ($p > 0.05$).

Discussion

This study investigated the changes in muscle architecture, flexibility, and passive stiffness after the response to a single bout of dynamic stretching, eccentric exercise, or static stretching in female older adults. The results showed that all these three interventions significantly improved hamstrings flexibility without different improvements among the groups, while PDF angle increased because of a single session of ECC. This ROM improvement might be due to increased stretch tolerance or resting fascicle elongation rather than changes in its MTU stiffness. Therefore, dynamic stretching and eccentric exercise were beneficial in tissue extensibility and safe to perform in healthy older adult participants. These selective interventions could be performed in their daily life for flexibility purposes or other aspects of performance enhancements.

Muscle stiffness plays a vital role in muscle performance, function, and injury prevention (Freitas et al., 2022). Hence, dynamic stretching can be considered for these benefits. Interestingly, one-way to perform dynamic stretching with a closed-chain position was suggested to have several beneficial effects on the substantial performance of increased eccentric peak torque, shift angle of peak torque to the longer muscle lengths, and unimpaired joint proprioception in young male subjects (Chen et al., 2018a). Our results showed that acute increases in ROM, assessed by SLR test, were seen in the DCS group by 5.9% regardless of any change in ankle plantar flexors flexibility in female older adults, which is in agreement with that study increasing considerably higher by 27.9% (Chen et al., 2018a). The hamstrings flexibility improvement seen here might occur because of a longer resting FL of BF muscle and a higher fascicle strain when compared to pre-exercise or even stretch tolerance suggesting by Magnusson et al. (2007); Blazevich (2019). Despite the substantially decreased hamstrings, MTU stiffness involved increased ROM following 8×6 dynamic closed chain stretching was not seen in this study (**Fig. 2A**) which was similar to Chen et al. (2018) study when muscle stiffness was indicated by myotonometer. Moreover, our finding on the increase in FL after stretching is contrary to the previous study, which investigated MG muscle and documented that no acute change in FL measured at rest and stretching position were seen immediately after ballistic exercise (Konrad et al., 2017) as well as the study of Opplert and Babault (2019) for dynamic stretching. Hence, the change in FL or its compliance needs to be repeated to confirm this investigation which might be evaluated clearly by the extended field-of-view method (Marušič et al. 2020).

Eccentric exercise has also been suggested to be a procedure for joint ROM improvement after a few weeks of training (Blazevich, 2019; Delvaux et al., 2020). Our findings confirm that a single session of 6×2 hamstrings ECC could moderately increase SLR angle by 7.3%. In comparison, a 15.7% improvement of PDF angle was also seen after 12 calves down exercises in this study. Based on this finding, it was in agreement with the previous study, which suggested an improvement of ROM by a mean of 10° when performing knee flexors ECC twice a week for four weeks (Batista et al., 2009). Moreover, these two joint ROM improvements are not entirely explained by the mechanical property changes (**Fig. 2 B&E**) as discussed in the recent review (Blazevich, 2019) but contribute to a longer FL. The resting FL of the BF was revealed to increase by 34% following eight weeks of ECC training (Potier et al., 2009) while increasing on average by 11.2% for the present study where performing only one visit. On the other hand, the suggestion of the addition of sarcomere in series within the muscle advocated this change (Potier et al., 2009). However, this acute adaptation should be further confirmed this finding as a result of ECC exercise not training for both investigated muscles. There was also no change in PA in that study, whereas MT was not thoroughly investigated. Marušič et al. (2020) also revealed that the decreased PA after ECC training concurred with this study. The possible mechanism of eccentric exercise training improving flexibility is also suggested to be due to sarcomerogenesis (Lynn & Morgan, 1994), which leads to the force production at longer muscle length and increases muscle FL (Delvaux et al., 2020). Additionally, the adaptation of viscoelasticity of the connective tissue also has an important role in ROM improvement in shortened muscles of older women (Batista et al., 2009).

This finding of a moderate improvement of ROM by a mean of 6.4° coincides with another study (Muanjai et al., 2017b) that reported that passive SLR was increased by a median of 6° in young females performing a similar stretching dosage. Static stretching has been widely performed for tissue extensibility improvement in older adults and emphasized its effectiveness (Hirata et al., 2020a; Nakamura et al., 2021). Despite the similar results in a response of a single session of ST in this study, the changes in the angle-passive torque relationship seemed unlikely to explain an acute joint ROM improvement, as has been widely documented (Muanjai et al., 2017b; Santos et al., 2020). While hip flexion angle increases could be due to a longer FL as a response to ST exercise, as also seen in the previous study (Sá et al., 2016), which speculated that this change might be linked to more compliant structures after three sets of 30 s hold stretching, but disagreement with the previous findings (Santos et al., 2020; Yahata et al., 2021). Stretching the posterior thigh with the straight knee was not produced any change in ankle joint flexibility. This could be due to a lack of substantial stress on the posterior leg muscle in this stretching position. A long duration of stretching was believed to be a better improvement in ROM. In this regard, the previous study supported the claim that muscle stiffness changed (Feland et al., 2001). However, prolonged hamstrings stretching for triplicate 60 s long has been suggested to place increased stress and strain on the nerve root and later on affected the neural function in older adults with tight hamstrings compared to 30 s hold (Moustafa et al., 2021). It should be noted that this

negative effect on the subsequent performance could occur in this study where 8×30 s of ST was performed for these participants. Based on the previous finding, they have also suggested that performance required balance, precision, and coordination should not be performed after static stretching, especially in senior participants (Smajla et al., 2019).

A decrease in joint ROM or flexibility is due to several impairments in balance and poor functional ability in older adults with an increased risk of falls, leading to low quality of life (Hirata et al., 2020b). Dynamic stretching or eccentric exercise may be chosen to perform safely in the older adult when considering the exercise's benefit for tissue extensibility. Dynamic stretching in a closed-chain position may enhance balance ability. This was done with a single leg and perhaps without effect on the subsequent performance or muscle force production (Chen et al., 2018b). Moreover, eccentric contractions generally contribute to many daily activities (Sañudo et al., 2022). Hence functional ability maintenance or improvement could be a crucial procedure for quality of life at this age via eccentric exercise based. According to the previous findings, the benefits of ECC included the effect on the balance, mobility, power, endurance, and quality of life in older adults (Kulkarni et al., 2022; Van Roie et al., 2020).

Limitation

There are several limitations to this study. One of the limitations is stretch volume, which could present a superior effect on ROM improvement between exercise interventions. Likewise, a magnitude of change in ROM and stiffness was found to be correlated with stretching intensity (Hatano et al., 2022). There was, however, the maximal discomfort end feel should be sufficient for exercise protocol in the older adults' environment setting. The acute effect of an exercise intervention on peak torque or muscle function was not thoroughly investigated in this study to confirm its benefits on performance rather than ROM improvement. Furthermore, a chronic adaptation should be determined for flexibility, architecture, and MTU stiffness in response to these interventions. Better understanding, the localized soft tissue stiffness should be further investigated using elastography since an acutely decreased passive stiffness (shear modulus) were seen after a single bout of leg ECC (Kawama et al., 2022) or after piriformis syndrome treatment (Hisashi et al., 2022). The other muscle contributions in the same muscle group or hip muscle should also be further considered.

Conclusion

In summary, this study demonstrated that hamstrings and plantar flexor flexibilities were significantly improved immediately after exercise interventions regardless the different between exercises with a contributable change in FL rather than musculotendinous mechanical properties. Moreover, apart from the static stretching-related in leg flexibility changes, DCS or ECC could also be considered somewhat beneficial concerning its effect on the subsequent performance. It is likely that DCS or ECC training may be considered as a part of a clinical intervention or program for geriatric flexibility enhancement.

Conflicts of interest: The authors declare that they have no conflict of interest.

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