

Original Article

Investigation of Varying Durations of Dynamic Stretches on Muscle Stiffness of the Ankle Plantar Flexors Using Shear Wave Ultrasound Elastography

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Objectives: This study investigated the acute effects of dynamic stretch (DS) duration on the muscle stiffness of the ankle plantar flexor using shear wave ultrasound elastography. **Methods:** Eighteen healthy young participants were enrolled in this study. DS with one set (DS1) or four sets (DS4) of 30 s each was performed randomly. Shear wave velocity in the medial gastrocnemius (MG) was measured before and after DS to assess muscle stiffness of the MG. **Results:** Two-way repeated-measures analysis of variance (condition \times time) showed a significant interaction with the shear wave velocity ($p = 0.02$). Shear wave velocity significantly decreased after the DS4 than before (before: 3.09 ± 0.59 m/s; after: 2.86 ± 0.43 m/s). However, no significant differences were observed in shear wave velocity between before and after DS1 (before: 2.96 ± 0.56 m/s; after: 3.19 ± 0.56 m/s). There were no significant differences in shear wave velocity at baseline condition. After the intervention, significantly lower shear wave velocity was observed in DS4 than in DS1. **Conclusions:** The results of this study demonstrate that DS with four sets of 30 s effectively decreased the muscle stiffness of the MG.

Keywords: Dynamic Stretching, Exercise Effects, Shear Wave Ultrasound Elastography, Stiffness

Introduction

Stretching exercise is a common part of warm-up routines for athletic performance enhancement, gaining joint flexibility, and injury prevention. Dynamic stretching (DS) involves repeated movements throughout the full range of motion (ROM) of a joint by contracting antagonist muscles¹. DS elicits an increase in power, agility, balance, and jump performance²⁻⁶. Therefore, DS is recommended for the optimization of athletic performance.

Static stretching (SS) is commonly performed by holding

the target muscle-tendon tissue in a stretched position for a certain period of time. Rhythmic joint movement with DS has been reported an increase in muscle temperature as well as a decrease in muscle-tendon tissue viscosity, which are expected to change the muscle and tendon tissue properties⁷⁻⁹. The DS effects on muscle-tendon tissue properties have been assessed with a change of joint stiffness determined by passive joint torque and joint angles^{8,10,11}. As for DS protocols, 1, 4, and 7 sets for 30 s did not change the joint stiffness¹⁰, and another study also revealed that both 30s - and 60s- DS did not result in changes in joint stiffness¹¹. Hence, 10 sets of DS for 30 s decreased joint stiffness⁸. These results demonstrated that a longer DS duration is needed to decrease joint stiffness. Joint stiffness may interact with skin, fascia, ligament, articular structures, and connective tissues across joints other than muscles and tendons¹². However, it is still uncertain joint stiffness measurement remains in question which tissues were affected with DS.

Shear wave elastography (SWE) has been used to assess muscle stiffness¹³⁻¹⁵. Shear waves within tissue propagate through the tissues and the shear wave velocity is directly

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related to tissue stiffness¹⁶. With SWE, muscle stiffness has been reported to decrease after SS^{13–15}. Only one previous study using SWE demonstrated that stiffness of medial gastrocnemius (MG) was increased by DS¹⁷. In the previous study¹⁷, a heel-raise exercise withstanding on the edge of a step at a frequency of 100 beats/min was conducted as DS. The ankle plantar flexors contracted eccentrically and concentrically, and the stretch loading for the target muscle may be excessive. DS was conducted to concentrically contract the antagonist muscles, leading to reciprocal inhibition¹. In contrast, a previous study of Pamboris et al.¹⁷ utilized both concentric and eccentric contractions of the targeting muscles, which may refer to autonomic inhibition. Increased muscle stiffness is associated with a higher risk of muscle strain injury^{18,19}. A better understanding of the mechanism by which DS reduces muscle stiffness is required to provide valuable insights into its role in the prevention of muscle strain injuries. However, it is unclear whether DS can reduce muscle stiffness as SS. Furthermore, the effects of DS with a short stretch duration that has been used in sports settings should also be demonstrated. SS for at least 2 min was needed to increase the extensibility of MG²⁰. Therefore, it is necessary to clarify how DS at short stretch durations, such as those performed in sports settings, and DS at moderate stretch durations as recommended in the SS, affect muscle stiffness. This study aimed to investigate the effect of short and moderate DS duration (1 and 4 sets) for ankle plantar flexors on the stiffness of medial gastrocnemius by SWE. Based on a previous study²⁰, we hypothesized that DS with four sets of 30 s would reduce MG stiffness, whereas DS with one set would not change MG stiffness.

Materials and Methods

Experimental design

This study employed a randomized crossover design to compare the effects of DS duration on MG stiffness as determined by SWE. All participants performed DS with one set (DS1) and four sets (DS4). The two dynamic stretching conditions were applied on the same day, with each intervention applied to the left and right legs separately. The intervention side and allocation order were randomized using the envelope method. The participants were instructed to sit and rest for 10 min to adapt the laboratory environment. As a warm-up exercise, walking for 5 min at a comfortable speed was conducted. MG stiffness was measured using SWE before stretching and immediately after the DS intervention. The temperature and humidity in the laboratory room were maintained at 22–24°C and the humidity was controlled with 45–60%, respectively.

Participants

The sample size was estimated using G*Power 3.1 software (Heinrich Heine University Düsseldorf, Düsseldorf, Germany) with the following parameters: effect size: 0.25; α error: 0.05; power: 0.80. Estimated minimum number of



Figure 1. Dynamic stretching of ankle plantar flexors.

participants was set 17 and 18 young, healthy participants (9 men and 9 women; age: 23.7 ± 0.6 years; height: 165.7 ± 8.6 cm; mass: 61.1 ± 14.2 kg) volunteered for this study. None of the participants had any current orthopedic diseases involving the lower limbs or a history of neuromuscular diseases. None of the participants engaged in regular stretching training. They were asked to refrain from intensive exercise, strength training, or alcohol consumption for 24 h before each session.

DS protocols

The participants stood with their right leg elevated from the floor, maintaining fully extended knees and hips flexed at a 30° (Figure 1). The participants were instructed to place their hands against the wall to maintain balance and reduce the impact of fatigue on the lower extremity on the standing side. The participants were instructed rhythmical active maximal dorsiflexion and plantar flexion at ankle joint under the supervision of the examiner. Before DS, the participants were ensured that the ankle plantar flexors were sufficiently stretched in the maximal active dorsiflexion position. DS rhythm was set with an electrical metronome at 60 beats/min. A total of 15 maximal dorsiflexion and plantarflexion were performed. For the DS protocol, one and four sets for 30 s each were performed¹⁰. The rest interval between sets was for 30 s, while the rest was allowed in a standing position.



Figure 2. SWE set-up for measuring the shear wave velocity.

Verbal instruction was provided to move the ankle joint to maximum active range of motion during the DS protocols.

SWE

An ultrasound (Aplio i900 device, Canon Medical Systems, Japan) and a PLI-705BX probe (central frequency, 8.5 MHz; range, 7.2–11.5 MHz) were used to quantify the shear wave velocity as an index of muscle stiffness. Each participant lay in a prone position on a bed with the hip joint at 0° flexion and the knee joint in full extension. The ankle joint was fixated at 0° of dorsiflexion (Figure 2). The ultrasound probe was longitudinally placed on the skin surface at 30% of the lower leg, from the popliteal crease to the lateral malleolus, to measure the shear wave velocity of the MG²¹. The probe was positioned at the location of the MG where the largest muscle thickness was. When blood vessels and thick connective tissues within the MG were evident in the scanned area, the probe placement was readjusted to ensure precise evaluation of the shear wave velocity. A registered medical sonographer with 8 years of clinical experience (M.M.) performed ultrasound measurements, and was blinded to the present study protocol. The examiner exercised with caution throughout the measurement to prevent any influence on the shear wave velocity and refrained from exerting any pressure on the target tissues. A rectangular region of interest (ROI) was determined at the maximum width (35 mm) and depth between the superficial and deep fascia of the MG. The ultrasound probe was maintained for 10 s during the multi-shot mode, and color-coded elastography and propagation view images of the MG were obtained (Figure 3). A circular ROI (diameter: 6 mm) was placed to avoid the superficial and deep fascia on MG, and the SWE quantification area was determined as follows: (i) the arrangement of shear waves was not disturbed in the propagation view image (right

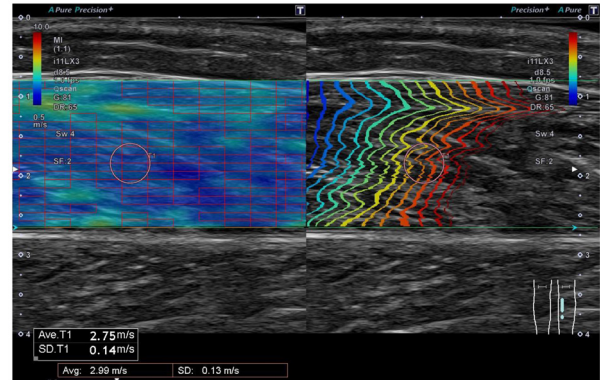


Figure 3. A typical example of color-coded elastography images and propagation view images of the medial gastrocnemius. A circular region of interest with a diameter of 6 mm was positioned within the designated region for shear wave velocity quantification. This location was determined based on two criteria: the shear wave arrangement in the propagation view image (right panel) remained significantly undisturbed; areas with a shear wave velocity standard deviation below the predefined threshold of 0.2 m/s, as determined by the SWE measurement area detection in the color-coded elastography image, were included.

picture in Figure 3), and (ii) the areas where the standard deviation (SD) of the shear wave velocity was less than 0.2 m/s when a circular ROI measurement was located (left picture in the Figure 3)^{21,22}. Ultrasound SWE measurement was repeated until a high-quality image was acquired and the measurement for the shear wave velocity were conducted at five times. The mean value from the shear wave velocity measurements (m/s) was calculated as SWE. The stiffer the tissue, the faster the shear waves propagate through it; therefore, faster shear wave velocities indicate greater muscle stiffness²³. Shear wave velocity is considered reliable if the ratio of the interquartile range to the median (IQR/med) is ≤ 0.3 for five measurements²⁴. A pilot study was conducted ($n = 11$), and intraclass correlation coefficients ($ICC_{(1,5)}$) were calculated to ensure the test-retest reliability of the shear wave velocity before and after standing for 210 s, with same DS and rest duration. The pilot results revealed that $ICC_{(1,5)}$ was 0.90 [95%CI: 0.67 – 0.97; SEM: 0.15] for shear wave velocity.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (ver. 27.0; IBM Japan Co., Japan). The Shapiro–Wilk test was used to evaluate the normality of all parameters. Two-way repeated-measures analysis of variance (condition [DS1 and DS4] \times time [pre and

post]) was used to determine significant differences. Multiple comparisons were performed when significant effects were found when comparing DS conditions and pre- and post-stretching. If a significant change in the shear wave velocity of the MG was observed, Pearson product-moment correlation analyses were conducted between the shear wave velocity of the MG at pre-stretching and the percent change in shear wave velocity of the MG by DS to determine the association between baseline muscle stiffness and the stretching effect on muscle stiffness. The effect size was calculated using partial eta-squared values (η_p^2), and Cohen's *d* was obtained for repeated measures and between pre- and post-stretching comparisons, respectively. The significance level was set at $p < 0.05$; all data were presented as the mean \pm SD.

Data availability

The datasets are available upon reasonable request from the corresponding author.

Results

SWE reliability

The ratio of the IQR/med was less than 0.30 with DS1 (pre: 0.01–0.21; post: 0.01–0.23) and with DS4 (pre: 0.03–0.25; post: 0.02–0.29).

Shear wave velocity

A significant two-way interaction (condition \times time) ($p = 0.02$; $\eta_p^2 = 0.30$), but no significant main effect of condition ($p = 0.38$; $\eta_p^2 = 0.05$) and time ($p = 0.99$; $\eta_p^2 < 0.01$) were found for shear wave velocity (Figure 4). A post-hoc test indicated that DS4 significantly decreased shear wave velocity from pre- to post- measurement (3.09 ± 0.59 to 2.86 ± 0.43 m/s; $p = 0.04$; $d = 0.45$). However, no significant differences were observed in shear wave velocity between pre- and post-measurement for DS1 (2.96 ± 0.56 to 3.19 ± 0.56 m/s; $p = 0.09$; $d = 0.41$). When comparing the conditions, a significantly lower shear wave velocity was found with DS4 than with DS1 at post-measurement ($p = 0.03$; $d = 0.23$), although no significant difference was observed at pre-measurement ($p = 0.37$; $d = 0.66$). For DS4, Pearson product-moment correlation analyses revealed that the shear wave velocity of the MG at baseline was significantly negatively correlated with the percentage change in the shear wave velocity of the MG after DS (Figure 5).

Discussion

This study demonstrated the acute effects of short (DS1) and moderate (DS4) stretch durations in DS on the shear wave velocity of the MG. Our findings indicated that DS4 significantly decreased the shear wave velocity in the MG, whereas DS1 did not. These results suggest that longer DS duration effectively decreased muscle stiffness.

The primary finding in this study is that four sets of 30-s

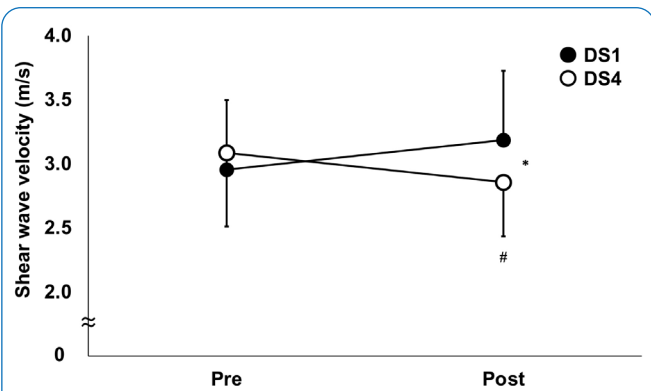


Figure 4. Shear wave velocity pre- and post- stretching. Data are presented as mean \pm SD. # Significant difference between pre- and post-intervention measurements. * Significant difference between DS1 and DS4 conditions.

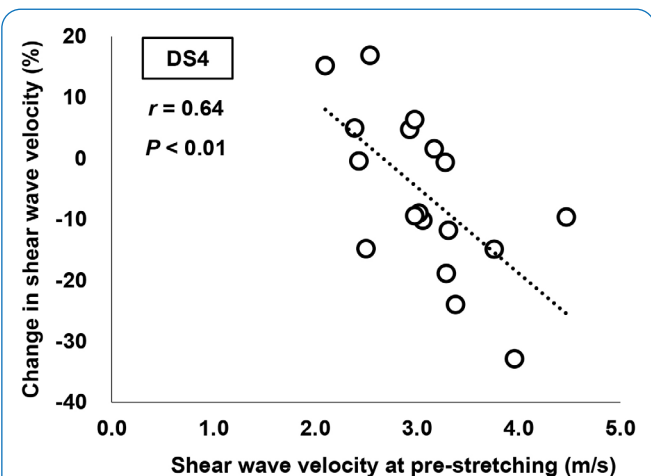


Figure 5. Correlations between percent change in shear wave velocity at DS4 and the shear wave velocity at baseline.

DS decreased MG stiffness. Theoretically, DS, characterized by rhythmic muscle contractions, can effectively decrease muscle viscosity and increase muscle temperature. These physiological responses are expected to contribute to changes in muscle stiffness^{7–9}. However, a previous study utilizing SWE demonstrated that DS at 100 beats/min with three sets for 12 s increased the shear wave velocity of the MG¹⁷. In the study, DS was performed in a weight-bearing position (like heel-raise exercise on the edge of a step), and the ankle plantar flexors eccentrically contracted. In contrast, the DS used in this study was conducted to contract the

tibialis anterior in a non-weight-bearing position. Moreover, participants were encouraged to move the ankle joint to its maximum ROM to ensure a sufficient stretch sensation in the ankle plantarflexors throughout the DS protocol. Therefore, DS in a non-weight-bearing position while being felt stretched may effectively reduce the muscle stiffness of the MG.

The present findings indicated a dose-response relationship between stretch duration in DS and a decrease in MG stiffness. Hence, there were no changes in joint stiffness with DS for 30–210 s^{10,11}, whereas decreases in joint stiffness have been observed with a total DS duration of 120–300 s^{8,25}. A long stretch duration is considered to decrease the joint stiffness^{8,25}. Our study demonstrated that four sets of 30-s DS resulted in a significant decrease in muscle stiffness in the MG; however, one set of 30-s DS did not. Therefore, muscle stiffness was found to require a certain amount of longer stretch duration. Our data showed that a total DS duration of 120 s, but not 30 s, effectively reduced muscle stiffness in the MG and could possibly contribute to the prevention of muscle strain injuries.

In this study, the baseline muscle stiffness was significantly negatively correlated with a stretch-induced decrease in muscle stiffness after DS. The stiffer muscles in the stretched position showed a greater decrease in muscle stiffness after SS, as shown by the SWE^{26,27}. SS with a higher stretch intensity results in greater reductions in muscle stiffness^{14,28}. A similar relationship between stretch intensity and stretch-induced decrease in muscle stiffness may be observed with DS. However, the present study did not estimate the shear wave velocity at the maximal angle of dorsiflexion during DS. Future studies should consider the shear wave velocity during maximal dorsiflexion as a baseline to determine tensile stress during DS. In contrast, DS of the ankle joint stretches the ankle plantar flexors, including the lateral head of the gastrocnemius and soleus. However, our study only evaluated muscle stiffness in the MG muscle. Consequently, we were unable to address the effects of stretching on the lateral head of the gastrocnemius and soleus muscles. Future studies should investigate how DS affects the stiffness of each muscle within the ankle plantar flexors in order to optimize treatment and develop injury prevention strategies that specifically focus on stiffer muscles.

This study has several limitations. First, the ankle joint angle was not measured during the DS. It is unclear whether participants were able to see the maximum ankle dorsiflexion angle and appropriately stretch their ankle plantar flexors by contracting the tibialis anterior muscle. If dorsiflexor strength is insufficient, the stretch protocol would not facilitate stretching of the plantar flexors. Therefore, stretching in the weight-bearing position described by Pamboris et al.¹⁷ may be more effective in stretching the ankle plantar flexors. Second, the control condition (no stretching) was not implemented, thus it cannot be ruled out that the difference between DS1 and DS4 could potentially be caused not only by the stretch duration of DS but also holding standing position for a certain period of time. The pilot study, however, showed that ICC_{1,5} was 0.90 for the shear wave velocity of MG and there were no

significant changes (3.14 ± 0.51 m/s to 3.10 ± 0.41 m/s; $p = 0.51$) before and after standing for 210 seconds, indicating that the impact of standing posture on the shear wave velocity of the MG would be negligible. Third, electromyography was not used to confirm the muscle activity of the MG during the SWE measurement. The examiner constantly checked the shear wave propagation image throughout the SWE measurement to ensure that no muscle contraction was occurred. Shear wave velocity was derived from five images, and the ratio of IQR/med was ensured to be less than 0.30. Based on these findings, we conclude that the effect of muscle activity is minimal. Fourth, physiological outcomes, such as muscle temperature and blood flow, were not assessed. The relationship between physiological outcomes and changes in muscle stiffness remains unclear. Fifth, the present study compared the effects of one set and four sets of 30-second DS for ankle plantar flexors on muscle stiffness. Nonetheless, two and three sets of DS were not demonstrated. Therefore, future studies should clarify time course changes in muscle stiffness following repeated DS to determine the minimum number of stretch duration required for muscle stiffness reduction.

In conclusion, this study investigated the acute effects of short and moderate DS durations on MG stiffness using ultrasound with SWE. The present results showed that four sets of DS resulted in a significant reduction in shear wave velocity, whereas one set of DS did not. DS for a total duration of 120 s was effective in reducing muscle stiffness in the MG.

Ethics Approval

The institutional review board of Hokkaido university approved the present study (approval number: 22-75). All the study procedures were conducted according to the principles of the Declaration of Helsinki.

Consent to Participate

The participants were informed of the details of this study and provided written informed consent for participation.

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Authors' contributions

Kensuke Oba: Conceptualization, Methodology, Data curation, Formal analysis, Writing- Original draft preparation, Visualization. Michito Murayama: Conceptualization, Methodology, Data curation, Formal analysis. Sanae Kaga: Conceptualization, Methodology, and Writing - Review & Editing. Mina Samukawa: Conceptualization, Methodology, Writing - Review & Editing, Project administration, and Supervision. All authors read and approved the final version of the manuscript.

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