Title: Time course of changes in passive properties of the gastrocnemius muscle-tendon unit during 5 min of static stretching.

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Abstract

The minimum time required for Static stretching (SS) to change the passive properties of the muscle–tendon unit (MTU), as well as the association between these passive properties, remains unclear. This study investigated the time course of changes in the passive properties of gastrocnemius MTU during 5 min of SS.

The subjects comprised 20 healthy males (22.0 ± 1.8 years). Passive torque as an index of MTU resistance and myotendinous junction (MTJ) displacement as an index of muscle extensibility were assessed using ultrasonography and dynamometer during 5 min of SS. Significant differences before and every 1 minute during SS were determined using Scheffé’s post hoc test. Relationships between passive torque and MTJ displacement for each subject were determined using Pearson’s product-moment correlation coefficient.

Although gradual changes in both passive torque and MTJ displacement were demonstrated over every minute, these changes became statistically significant after 2, 3, 4, and 5 minutes of SS compared with the values before SS. In addition, passive torque after 5 minutes SS was significantly lower than that after 2 minutes SS. Similarly, MTJ displacement after 5 minutes SS was significantly higher than that after 2 minutes SS. A strong correlation was observed between passive torque and MTJ displacement for each
subject \( r = -0.886 \) to \(-0.991\).

These results suggest that SS for more than 2 minutes effectively increases muscle extensibility, which in turn decreases MTU resistance.
Introduction

Limited joint mobility and decreased muscle flexibility are common problems in clinical situations and athletic settings. Static stretching (SS) is a useful method for preventing joint contracture and improving joint mobility and muscle flexibility. Although recent studies have suggested that SS may not reduce the incidence of injury (Thacker et al., 2004), and that SS may actually decrease muscle strength and performance (Behm & Chaouachi, 2011; Simicet et al., 2012), many previous studies have reported that the maximum range of motion (ROM) increased immediately after SS (Boyce & Brosky, 2008; de Weijer et al., 2003; Depino et al., 2000; Halbertsma et al., 1996; Hubley et al., 1984; O’Sullivan et al., 2009; Ryan et al., 2008b). The effects of SS have been estimated using maximum ROM as an outcome measure in various studies. However, the use of ROM for this purpose has several limitations. For example, maximum ROM measurements are influenced by many factors, such as pain, stretch tolerance, and reflex activation of the agonist muscle (McHugh et al., 1998; Sale et al., 1982). Measuring passive torque and muscle–tendon unit (MTU) stiffness using a dynamometer are effective approach for determining the resistance of MTU during passive movements (Toft et al., 1989a; Magnusson et al., 1996b). Passive torque represents the amount of resistance provided by the MTU at a given joint angle (Toft et
al., 1989a), while MTU stiffness is the shape of the torque-angle curve which demonstrates the relationship between passive torque and joint angle (Magnusson et al., 1996b). Recently, noninvasive methods of measuring the passive properties of muscles and tendons, such as myotendinous junction (MTJ) displacement, have been developed using ultrasonography during passive movement (Kay & Blazevich, 2009a; b; 2010; Mizuno et al., 2011; Morse 2011; Morse et al., 2008; Nakamura et al., 2011; 2012).

Many studies have examined the acute effects of SS on passive torque and MTU stiffness, which is an indicator of MTU resistance, using a dynamometer and have reported that SS of various durations (1–42 min) (Duong et al., 2001; Fowles et al., 2000; Herda et al., 2011; Herda et al., 2009; Magnusson et al., 1996b; Morse et al., 2008; Nordez et al., 2006, Nordez et al., 2008; Ryan et al., 2008a; Ryan et al., 2009) resulted in decreased passive torque or decreased MTU stiffness. On the other hand, Halbertsma et al. (1996) reported that maximum ROM increased immediately after 5 min of SS, but no changes were observed in MTU stiffness. They also suggested that increased stretch tolerance explained the influence of SS on maximum ROM. Similarly, other studies have reported increased stretch tolerance but no decrease in MTU stiffness after SS for 1–2 min (Magnusson et al., 2000; Magnusson et al., 1998; Magnusson et al., 1996a; McNair et al., 2001; Muir et al., 1999). Duration of SS is a key issue for
decreasing MTU resistance, which has not yet reached agreement.

Recently, with the use of ultrasonography, a technique to measure the displacement of MTJ, which could be used as an indicator of muscle extensibility, was enabled. Using this technique, several studies (Kay & Blazevich, 2009 b; Mizuno et al., 2011; Morse et al., 2008; Nakamura et al., 2011) reported that MTU resistance during passive ankle dorsiflexion decreased after SS because of an increase in muscle extensibility. However, the minimum amount of time required for SS to increase muscle extensibility remains undefined. In addition, the influence of muscle extensibility on MTU resistance during SS also remains unclear since these studies did not examine the association between changes in passive torque, which is an indicator of MTU resistance, and MTJ displacement, which is an indicator of muscle extensibility.

This study aimed to determine the minimum time required for SS to decrease MTU resistance and muscle extensibility and to examine the association between the two indicators, i.e., passive torque and MTJ displacement, during 5 min of SS.

Methods

Subjects

Twenty healthy males (age, 22.0 ± 1.8 years; height, 173.4 ± 5.9 cm; body mass, 64.8 ±
6.1 kg; and dorsiflexion ROM, 31.4° ± 4.4°) volunteered for the study. Subjects with a history of neuromuscular disease or musculoskeletal injury involving their lower limbs were excluded from the study. All subjects were fully informed of the procedures and purpose of the study, following which written informed consent was obtained from all of them.

Experimental protocol

The subjects were familiarized with the procedure and instructed to remain relaxed during measurement. They were instructed to lie in the prone position on a dynamometer table, with their hips secured by adjustable lap belts (MYORET RZ-450, Kawasaki Heavy Industries, Kobe, Japan). The knee of the dominant leg was in full extension and the foot of the same leg was attached securely to the footplate of the dynamometer. The ankle was passively dorsiflexed at a constant velocity of 5°/s, starting from 0° to the dorsiflexion ROM, and was held in the dorsiflexion ROM for 5 min. In this study, the dorsiflexion ROM was defined as the angle that the subjects could achieve without discomfort or pain (Nakamura et al., 2011). Passive torque and ultrasound images of the gastrocnemius muscle were obtained every 1 min during 5 min of SS. Passive torque is the resistance of the entire MTU to the direction of
plantarflexion during the passive dorsiflexion (Toft et al., 1989a). Electromyography (EMG) (TeleMyo2400; Noraxon USA, Inc., Scottsdale, AZ, USA) using surface electrodes (Blue Sensor M, Ambu, Denmark) was used to confirm that subjects were relaxed and ensure that there was no high EMG activity in the medial gastrocnemius muscle. For the measurement of changes in the medial head of the gastrocnemius muscle, surface electrodes were placed over the muscle belly. The original low EMG signals processed using a band-pass filter at 20–500 Hz were amplified and collected at a sampling rate of 1500 Hz. EMG activity was calculated using the root mean square (RMS), and full wave rectification was performed using an RMS smoothing algorithm with a window interval of 50 ms. EMG activity within 3 s was calculated during isometric maximum voluntary contraction (MVC) of the ankle plantar flexors with the ankle at 0°. EMG activity recorded during the tests was expressed as a percentage of MVC.

Ultrasound measurements

B-mode ultrasonography (Famio Cube SSA-520A; Toshiba Medical Systems Corporation, Tochigi, Japan) with an 8-MHz linear probe was used to determine the displacement of the distal MTJ of the medial head of the gastrocnemius muscle during
passive ankle dorsiflexion. MTJ was identified as described by Maganaris and Paul (1999). An acoustically reflective marker was placed between the skin and the probe to ensure probe stability during measurements (Morse et al., 2008; Nakamura et al., 2011). A custom-made fixation device was used to secure the probe to the skin. MTJ displacement was defined as the distance between MTJ and the acoustically reflective marker secured to the probe. Ultrasound images of the MTJ were quantified using open-source digital measurement software (Image J, NIH, USA). For accurate measurement, the MTJ was identified at the innermost edges of the fascia surrounding the muscle, where it fuses with the tendon. MTJ displacement was measured between 0° and the dorsiflexion ROM for ankle dorsiflexion (Fig. 1). MTJ displacement is a measure of the degree of muscle extensibility during passive dorsiflexion (Nakamura et al., 2012). In a previous study, high intraclass correlation coefficients demonstrated the reliability of the MTJ displacement measurement procedures used in this study (Nakamura et al., 2011).

Statistical analysis

Significant differences in passive torque and MTJ displacement measurements taken before SS and every 1 min during SS were assessed using one-way repeated analysis of
variance (ANOVA). When a significant effect was found, the differences between measurements taken before SS and those taken every 1 min during SS were determined using Scheffé’s post hoc test.

Relationships between passive torque and MTJ displacement for each subject during 5 min of SS were determined using Pearson’s product-moment correlation coefficient. In addition, the rate of change in passive torque and MTJ displacement was defined using the following formula: rate of change = (value before SS – value 5 min after SS) / (value before SS) × 100. The relationship between rate of change in passive torque and that in MTJ displacement was determined using Pearson’s product-moment correlation coefficient. Differences were considered statistically significant at an alpha level of $P < 0.05$. Descriptive data were determined as means ± SEM with 95% confidence intervals.

Results

EMG activity

Two subjects exhibited obvious EMG activity (>2% MVC) in the medial head of the gastrocnemius muscle during SS. Therefore, results were obtained from the remaining 18 subjects (22.0 ± 1.9 years) with low EMG activity during SS.
Passive torque

ANOVA indicated a significant effect of SS duration on passive torque. The post hoc test indicated no significant difference between passive torque after 1 min of SS and that before SS. However, passive torque after 2, 3, 4, and 5 min of SS was significantly lower than that before SS (Table 1). In addition, passive torque after 4 and 5 min of SS was significantly lower than that after 1 min of SS, and passive torque after 5 min of SS was significantly lower than that after 2 min of SS.

MTJ displacement

ANOVA indicated a significant effect of SS duration on MTJ displacement. The post hoc test indicated no significant difference between MTJ displacement after 1 min of SS and that before SS. However, MTJ displacement after 2, 3, 4, and 5 min of SS was significantly higher than that before SS (Table 1). In addition, MTJ displacement after 4 and 5 min of SS was significantly higher than that after 1 min of SS, and MTJ displacement after 5 min of SS was significantly higher than that after 2 and 3 min of SS.
Relationship between passive torque and MTJ displacement

Pearson’s product-moment correlation coefficient indicated a strong correlation between passive torque and MTJ displacement for each subject ($r = -0.886$ to $-0.991$, $P < 0.05$).

A typical example of the relationship between passive torque and MTJ displacement is shown Figure 2. In addition, Pearson’s product-moment correlation coefficient indicated a significant correlation between rate of change in passive torque and that in MTJ displacement ($r = -0.708$, $P < 0.01$, Fig. 3).

Discussion

Our findings revealed that passive torque was significantly lower after 2 min of SS than before SS, although passive torque decreases gradually over every minute during SS (Table 1). Since passive torque was used to represent the amount of resistance provided by the entire MTU at a given joint angle (Toft et al., 1989a), this result suggests that SS for more than 2 min effectively decreases the resistance of the entire MTU. Other studies have found similar results for passive torque, reporting that SS for periods shorter than 2 min did not decrease passive torque (Magnusson et al., 2000; Magnusson et al., 1998; Magnusson et al., 1996a; McNair et al., 2001). In contrast, Ryan et al. (2009) reported that two 30-s repetitions of SS (i.e., 1 min of SS) decrease the resistance
of the entire MTU. This discrepancy may be due to differences in SS measurement procedures. Ryan et al. (2009) used constant-torque SS, which allowed some joint angle movement, whereas 5-min constant-angle SS was used in our study. Herda et al. (2011) recently suggested that 8-min constant-torque SS may be more effective in decreasing the resistance of the entire MTU compared with the same duration of constant-angle SS. They also suggested that constant-torque SS had more effects on the decreasing the resistance of the entire MTU compared with constant-angle SS for the same duration. Therefore, the constant-torque SS protocol used by Ryan et al. (2009) may have decreased the resistance of the entire MTU in less time compared with the protocol used in our study.

To our knowledge, this is the first report which studied the influence of SS duration on both passive torque as an index of MTU resistance and MTJ displacement as an index of the degree of muscle extensibility. Similar to the results for passive torque, MTJ displacement was significantly greater after 2 min of SS than before SS, although MTJ displacement increases gradually over every minute during SS (Table 1). MTJ displacement is an indicator of degree of muscle extensibility, and an increase in MTJ displacement at the same angle means an increase in muscle extensibility (Nakamura et al., 2012). Therefore, this result suggests that SS for more than 2 min is
effective for increasing muscle extensibility. In addition, gradual changes in both
passive torque and MTJ displacement were observed during 5 min of SS. A significant
difference was observed in the two parameters between 2 min of SS and 5 min of SS.
The results of this study suggest that SS for 5 min may be more effective in decreasing
MTU resistance and increasing muscle extensibility compared with SS for 2 min.

With regard to the relationship between passive torque and MTJ displacement,
there were strong negative correlations between passive torque and MTJ displacement
for each subject ($r = -0.886$ to $-0.991$, $P < 0.05$). In addition, a significant correlation
was found between the rate of change in passive torque and that in MTJ displacement ($r
= -0.708$, $P < 0.01$). This is the first report regarding the relationship between passive
torque and MTJ displacement, and these results suggest that an increase of muscle
extensibility contributes to a decrease of MTU resistance, which is consistent with the
results of previous studies (Kay & Blazevich, 2009 b; Mizuno et al., 2011; Morse et al.,
2008; Nakamura et al., 2011).

Regarding the mechanism of increased muscle extensibility, viscoelastic
materials cause plastic deformation due to external stress. This feature is called stress
relaxation, which refers to the decline in external stress over time when the MTU is
lengthened for a prolonged duration. Viscoelastic stress relaxation of muscles or tendons
has been demonstrated in vitro (Sanjeevi, 1982; Taylor et al., 1982; Taylor et al., 1990) and in vivo (Duong et al., 2001; Fowles et al., 2000; Magnusson et al., 1995; Magnusson et al., 1996c; McHugh et al., 1992; McNair et al., 2001; Toft et al., 1989b) in the MTU of the hamstring and gastrocnemius. In this study, gradual changes in both passive torque and MTJ displacement were observed, and a significant correlation was observed between passive torque and MTJ displacement during 5 min of SS. Therefore, viscoelastic stress relaxation of the gastrocnemius muscle may have contributed to increase muscle extensibility, which may have decreased MTU resistance after SS.

In this study, the time course of changes in passive properties of the gastrocnemius MTU were investigated during 5 min of SS. Recent studies reported that 5 min of SS increased MTU and muscle flexibility, and that this effect was maintained for 10 min after SS (Nakamura et al., 2011). However, the effect disappeared 15 min after SS (Mizuno et al., 2011). The changes in MTU properties observed in this study may also be reversible. In a study examining ROM and muscle fascicle length, O'Sullivan et al. (2012) reported that eccentric training was effective in increasing flexibility. Incorporating other exercise regimens, for example, eccentric, concentric, and isometric contractions with SS may be worthy to study.
Strengths and limitations

This study revealed the minimum amount of time required for SS to decrease MTU resistance and increase muscle extensibility. These data may be effectively used in clinical situations and athletic settings. However, some limitations of this study must be noted. First, the assessor who analyzed MTJ displacement was not blinded to the time of image procurement. Second, the subjects in this study were healthy young men. Similar acute effects of SS cannot always be expected in elderly individuals and patients with limited ROM or contracture. In addition, because differences at 1 min may be statistically significant in a larger sample, future studies should consider including larger samples to confirm this possibility. Third, this study examined the minimum amount of time required for SS to decrease MTU resistance and increase muscle extensibility in only the gastrocnemius muscle; other human muscles needs to be investigated in the future. In addition, further research is required to clarify the minimum amount of time required for SS to increase the extensibility of the gastrocnemius muscle and other muscles in elderly individuals and patients with limited ROM or contracture.

Conclusions
In this study, gradual changes in both passive torque and MTJ displacement reached the significance level after 2 min. Therefore, SS for more than 2 min is recommended to decrease MTU resistance and increase the degree of muscle extensibility.
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Table 1. Passive torque and MTJ displacement during 5 min of static stretching

<table>
<thead>
<tr>
<th>Time</th>
<th>Passive torque (Nm)</th>
<th>MTJ displacement (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>before SS</td>
<td>49.4 ± 2.9 (43.7 to 55.2)</td>
<td>0.90 ± 0.06 (0.79 to 1.01)</td>
</tr>
<tr>
<td>1 minute</td>
<td>42.9 ± 2.6 (37.9 to 47.9)</td>
<td>1.13 ± 0.07 (1.01 to 1.26)</td>
</tr>
<tr>
<td>2 minutes</td>
<td>37.5 ± 2.4 (32.7 to 42.3)**</td>
<td>1.21 ± 0.07 (1.07 to 1.35)*</td>
</tr>
<tr>
<td>3 minutes</td>
<td>36.8 ± 2.4 (32.0 to 41.6)**</td>
<td>1.25 ± 0.07 (1.10 to 1.39)**</td>
</tr>
<tr>
<td>4 minutes</td>
<td>35.9 ± 2.4 (31.1 to 40.7)** ##</td>
<td>1.30 ± 0.07 (1.15 to 1.45)** ##</td>
</tr>
<tr>
<td>5 minutes</td>
<td>33.3 ± 2.3 (28.8 to 37.9)** ## $$</td>
<td>1.35 ± 0.08 (1.20 to 1.50)** ## $$†</td>
</tr>
</tbody>
</table>

Data are means ± SEM (95% confidence intervals). * $P < 0.05$, ** $P < 0.01$; significant difference from values before SS. # $P < 0.05$, ## $P < 0.01$; significant difference from 1-min values. $$ P < 0.01$; significant difference from 2-min values. † $P < 0.05$; significant difference from 3-min values. SS: static stretching; MTJ: myotendinous junction.
Ultrasound image showing measurements taken to determine MTJ displacement from 0° to the dorsiflexion ROM. An acoustically reflective marker (X) is placed between the skin and the ultrasonic probe to ensure probe stability during measurement. The distance between X and MTJ (MTJ displacement) is measured every 1 min during 5 min of SS. MTJ: myotendinous junction; ROM: range of motion; GM: gastrocnemius muscle.

A typical example of the relationship between passive torque and MTJ displacement during 5 min of SS. The line is drawn by linear regression ($r = -0.972, P < 0.01$).

Correlation coefficient between the rate of change in passive torque and that in MTJ displacement. The line is drawn by linear regression ($r = -0.708, P < 0.01$).
Figure 2

![Graph showing the relationship between MTJ displacement (cm) and Passive torque (Nm).]
Figure 3

Change in passive torque (%) vs. Change in MTJ displacement (%)