The Time Course of Muscle-Tendon Unit Function and Structure Following Three Minutes of Static Stretching

Andreas Konrad and Markus Tilp
Graz University, Institute of Sport Science, Graz, Austria

Abstract
While the time course of muscle-tendon unit (MTU) function and structure following short (1 min) and long (5 min) durations of static stretching has already been investigated, less is known about medium stretch durations. Therefore, the purpose of this study was to investigate the time course of these parameters following 3 min of static stretching of the plantar flexor muscles. Fourteen healthy volunteers were tested on two separate days in a random order with two different rest times (0 min, 5 min) after 3 \times 60 \text{s} of stretching. During each test, the dorsiflexion range of motion (RoM), passive resistive torque (PRT), and maximum voluntary contraction (MVC) were measured with a dynamometer. Ultrasonography of the gastrocnemius medialis (GM) muscle-tendon junction displacement and motion capture allowed us to determine the length changes in the tendon and muscle, respectively, and hence to calculate their stiffness. We observed an increase in RoM and a decrease in PRT and muscle-tendon stiffness at 0 min and 5 min post-stretching. This can be attributed to a decrease in muscle stiffness, as a decrease in PRT and muscle stiffness was observed up to 5 min after the stretching. No changes were detected in MVC torque and tendon stiffness. Static stretching for 3 \times 60 \text{s} changes the muscle-tendon functions (RoM, PRT) for at least 5 min. These changes are related to mechano-morphological changes of the muscle, but not the tendon structure.

Key words: Stiffness, ultrasound, passive resistive torque, maximum voluntary contraction, range of motion.

Introduction
Static stretching is widely used in sports practice during warm-up, with the goal being to acutely increase the range of motion (RoM) of a joint. The consistently observed increased RoM is accompanied with an increased tolerance to stretch and mechano-morphological changes in the muscle-tendon unit (MTU). The mechano-morphological changes following a single stretching exercise are often related to a decrease in overall muscle-tendon stiffness, which is sometimes also referred to as “joint stiffness” (Kay et al., 2015; Konrad et al., 2017a; 2017b) or “passive resistive torque” (PRT) (Konrad et al., 2017a; 2017b; Nakamura et al., 2013). Both measures describe the resistance to stretch of the MTU. Most of the studies that distinguished between the muscular and tendinous tissue of the whole MTU have reported a decrease in the muscle stiffness (Kay and Blazevich, 2009; Kay et al., 2015; Konrad et al.2017a; 2017b; 2019a), while only some have reported a decrease in tendon stiffness (without changes in the muscular component) (Kubo et al., 2001; Kato et al., 2010). These controversial results can be explained by the different stretching durations or intensities applied. While shorter stretch durations and lower stretch intensities are related to changes in the muscle structure, longer stretch durations (> 10 min) and/or greater stretch intensities (e.g. including maximum active contractions) seem to predominantly affect the tendon structure.

Static stretching might also affect muscle performance with a clear “dose-response relationship”, as analyzed by several reviews (Behm and Chaouachi, 2011; Kay and Blazevich, 2012; Behm et al., 2016). These authors pointed out that stretches that last longer than 60 s probably have a detrimental effect on muscle performance, while this is not expected for stretches of less than 60 s.

Although several studies have investigated the acute effect of stretching on RoM, muscle-tendon structure, and performance, less is known about the time course of these measures following stretching. Depending on the stretch duration, some authors have reported an increased RoM, which lasts between 10 min (Ryan et al., 2008a, Konrad and Tilp, 2019; Konrad et al., 2019) and up to 120 min after stretching (Power et al., 2004).

Following 5 min of static stretching, changes in muscle-tendon stiffness have been observed both immediately (Mizuno et al., 2012; Konrad et al., 2019) and up to 5 min after stretching (Mizuno et al., 2012), but these changes recovered within 10 min. The changes are associated with a decreased PRT, muscle stiffness, or increased muscle elongation for up to 5 min (Mizuno et al., 2012; Konrad et al., 2019). All the structural changes that occurred in the muscle had recovered within 10 min. The effects of shorter stretching durations (3 min) were analyzed by Kay and Blazevich (2009), who reported a decrease in muscle stiffness immediately after stretching, which recovered after 30 min. However, the development of the responses up to 30 min after the stretching were not investigated. A shorter stretching duration of 1 min of static stretching, however, did not lead to changes in muscle-tendon stiffness and muscle stiffness immediately, 20 min, and 40 min after the stretching exercise, respectively (Konrad and Tilp, 2019).

A decrease in muscle performance (measured as maximum voluntary contraction (MVC)) was observed after 5 min of static stretching, for at least 10 min (Konrad et al., 2019), but not after 1 min of static stretching (Konrad and Tilp, 2019).

Hence, for the full picture of the time course of the dose-response relationship of stretching, there is still a knowledge gap with regard to the precise time course of the response of muscle and tendon properties (e.g. passive muscle and tendon stiffness, active tendon stiffness) and...
function responses (e.g. RoM, MVC) within the first minutes after a static stretching exercise of medium (between 1 and 5 min) duration.

Therefore, the objective of this study was to analyze the time course (immediately, 5 min) of the properties and functional responses of the plantar flexor muscle-tendon system following a 3-min stretching exercise. Based on previous results, we hypothesized an increase in RoM and a decrease in PRT and MVC, both immediately and 5 min after the stretching. We further assumed that these changes would be associated with a decrease in muscle stiffness, and that these changes would last up to 5 min after the stretching.

Methods

Experimental design

On the first day, subjects were familiarized with the laboratory equipment, the assessments (RoM, passive, active), and the stretching regime. Moreover, participants visited the laboratory for a further two sessions on different days (with a 2- to 7-day break in between) at the same time of day to assess the effects of stretching immediately (0min-post) and 5 min (5min-post) after the stretching, in a randomized order. Before and after the two conditions (0 min_post, 5 min_post), we determined the RoM, PRT, MVC torque, muscle-tendon stiffness, muscle stiffness, and passive and active tendon stiffness of the gastrocnemius medialis (GM) muscle.

Subjects

Three healthy female (mean ± SD; 24.6 ± 2.3 years, 1.71 ± 0.02 m, 64.2 ± 5.7 kg) and 11 healthy male (mean ± SD; 24.8 ± 3.8 years, 1.83 ± 0.05 m, 75.6 ± 9.0 kg) physically active volunteers with no history of lower leg injuries participated in this study. Subjects were informed about the testing procedure, but were not informed about the study’s aim and hypotheses. According to a sample size calculation (primary outcome variable muscle stiffness) for a univariate linear model based on the literature and on our own data (mean change = 4% (i.e. Kay and Blazevich, 2009); SD = 5%, alpha = 0.05, beta = 0.9) suggests a necessary group size of 14 subjects.

The study was approved by the local research ethics board (GZ. 39/77/63 ex 2013/14), and written informed consent was obtained from all volunteers before the onset of the experimental procedures.

Measures

The temperature in the laboratory was kept constant at around 20.5 °C. Measurements were performed without any warm-up and in the following order: pre-tests: RoM (1-min rest), PRT (1-min rest), MVC (1-min rest); intervention: stretching for 3 × 60 s; post-tests: immediately following stretching, or following 5 min of rest in the same order (RoM (1-min rest), PRT (1-min rest), MVC).

RoM measurement: RoM was determined with an isokinetic dynamometer (CON-TREX MJ, CMV AG, Duebendorf, Switzerland) in a seated position with a hip joint angle of 110°, with the foot resting on the dynamometer foot plate and the knee fully extended. Two oblique straps on the upper body and one strap around the thigh were used to secure the participant to the dynamometer and exclude any evasive movement. The estimated ankle joint center was carefully aligned with the axis of the dynamometer and the foot was fixed barefooted with a strap to the dynamometer foot plate to avoid any heel displacement. Participants were moved to the neutral ankle joint position in the dynamometer (90° between foot sole and tibia), and were subsequently asked to regulate the motor of the dynamometer with a remote control to get into a dorsiflexion (stretching) position until they reached their individual maximum tolerable stretch. The angular velocity of the dynamometer during this procedure was set to 5°/s. The difference between the neutral ankle position and the maximum dorsiflexion was defined as the dorsiflexion RoM.

Maximum voluntary contraction (MVC) measurement: MVC measurement was performed with the dynamometer at an ankle position of 10° of plantar flexion. Participants were instructed to perform two isometric MVCs of the plantar flexors for 5 s, with rest periods of at least 1 min between the measurements, to avoid any fatigue. The attempt with the highest MVC torque value was taken for further analysis.

Electromyography (EMG): Muscular activity was monitored by electromyography (EMG) (myon 320, myon AG, Zurich, Switzerland) during PRT and MVC measurements. After standard skin preparation, surface electrodes (Blue Sensor N, Ambu A/S, Ballerup, Denmark) were placed on the muscle bellies of the GM and the tibialis anterior (TA), according to SENIAM recommendations (Hermens et al., 1999). In the RoM and PRT measurements, the raw EMG was monitored online to ensure that the subject was relaxed. In the case of an observed increase in the EMG of the GM or the TA, the RoM or PRT measurements were repeated.

Measurement of elongation of the muscle-tendon structures: A real-time ultrasound apparatus (mylab 60, Esaote S.p.A., Genova, Italy) with a 10-cm B-mode linear-array probe (LA 923, Esaote S.p.A., Genova, Italy) was used to obtain longitudinal ultrasound images of the GM.

During the PRT and MVC measurements, the ultra-
sound probe was placed on the distal end of the GM (as described in a previous study, Konrad et al. (2014), see Figure 1), where the muscle merges into the Achilles tendon, i.e., the muscle-tendon junction (Kato et al., 2010). The ultrasound probe was attached to the lower leg with a custom-built styrofoam block and secured with elastic bands to prevent any displacement of the probe. During previous studies (Konrad et al., 2017a; Stafilidis and Tilp, 2015), we confirmed that this kind of fixation of the ultrasound probe did not lead to any unwanted shifts of the probe during the measurement. To determine the muscle displacement during PRT and MVC measurements, the echoes of the muscle-tendon junction in the ultrasound videos were manually tracked (Kato et al., 2010).

The ultrasound images were recorded at 25 Hz. During PRT and MVC measurements, the videos were synchronized with the rest of the data using a custom-built manual trigger. The videos were cut and digitized in VirtualDub open-source software (version 1.6.19, www.virtualdub.org) and analyzed in ImageJ open-source software (version 1.44p, National Institutes of Health, U.S.). Each video was analyzed by two investigators, and the mean values of the measurements were used for further analysis of the muscle-tendon structure. Only the principal investigator, and not the supporting investigator, was informed about the hypotheses of the study and the group allocation of the subjects. During the analysis of the PRT measurement, every fifth frame was analyzed by the investigators, corresponding to a time resolution of 0.2 s. Moreover, during the analysis of the MVC measurement, every second frame was analyzed, corresponding to a time resolution of 0.08 s.

**Tendon and muscle lengths:** Tendon and muscle lengths were analyzed during the PRT and MVC assessments, using a combination of ultrasound and 3D kinematics. Reflective markers were placed on the calcaneus (Marker A, see Fig. 1), on the ultrasound probe (Marker B), and on the medial epicondyle of the femur (Marker C), and captured with a four-camera near-infrared VICON® motion capture system (V612, Oxford Metrics Ltd, UK). The tendon length was calculated as the distance between Marker A (insertion of Achilles tendon) and Marker B plus the distance from Marker B to the muscle-tendon junction (measured with ultrasound). Moreover, muscle length was calculated as the distance between Marker C (= origin of GM) and Marker B minus the distance from Marker B to the muscle-tendon junction.

**Calculation of muscle/tendon force, passive muscle/tendon stiffness, active tendon stiffness, and muscle-tendon stiffness:** The muscle force of the GM was estimated by multiplying the measured torque by the relative contribution of the physiological cross-sectional area (18%) of the GM within the plantar flexor muscles (Kubo et al., 2002; Mahieu et al., 2009), and dividing by the moment arm of the triceps surae muscle, which was individually measured by tape measure as the distance between the malleolus lateralis and the Achilles tendon at rest at neutral ankle position (Konrad and Tilp, 2014). The mean value of the moment arm was 4.5 cm, with a range of 4–5.5 cm.

Active tendon stiffness was calculated as the change in the active force divided by the change of the related tendon length during the MVC measurements over a range of force of 50–90% of MVC (Kay et al., 2015) at 10° plantar flexion. Passive tendon stiffness, muscle stiffness, and muscle-tendon stiffness were calculated as the change in passive force produced at the last 10° up to maximum dorsiflexion, divided by the change of the related tendon length, muscle length, and joint angle, respectively. In accordance with Magnusson et al. (1997), the stretching maximum of the pre-test was also taken in the post-test, to allow a comparison.

**Stretching exercise**

The stretching exercise was undertaken within the dynamometer. Starting at neutral ankle position (90°), the subjects were asked to regulate the motor of the dynamometer with a remote control and a maximum angular velocity of 5°/s to get into a dorsiflexion (stretching) position corresponding to the previously determined end RoM. This position was held for 60 s and the procedure was repeated two more times, resulting in a total stretch period of 180 s. Between the stretches, the dynamometer moved the ankle into neutral position and back again into the stretching position at 5°/s. The breaks in between the stretches lasted around 20 s. Subjects were asked to be fully relaxed during the stretching exercise.

**Statistical analyses**

SPSS (version 25.0, SPSS Inc., Chicago, Illinois) was used for all the statistical analyses. To determine the inter-rater reliability of the muscle-tendon displacement measurements, intraclass correlation coefficients (ICCs; (3,k)) were used. A Shapiro-Wilk test was used to test the normal distribution of all the variables. Subsequently, if the data were normally distributed, we performed a two-way repeated measures ANOVA test (factors: time [pre vs. post] and rest time [0 min vs. 5 min]). Otherwise, we performed a Friedman test to test the effects of the stretching protocols (0 min and 5 min). If the ANOVA test with repeated measures or the Friedman test was significant, we performed a t-test or a Wilcoxon test, respectively. An alpha level of $P = 0.05$ was defined for the statistical significance of all the tests.
Results

Measurement quality
The mean ICCs of the inter-rater tests of the ultrasound video analysis were 0.97 and 0.97 for the muscle-tendon junction displacement during the PRT and MVC measurements, respectively.

Range of motion (RoM)
A significant time effect (p < 0.0001, F\(1,13\) = 15.937) revealed that the overall RoM increases immediately (13.86%) and in 5 min after the stretching (11.39%) with a large effect (\(d_{\text{immediately}} = 0.73\); \(d_{\text{5min}} = 1.30\)). The pairwise comparison showed a significant increase in RoM, both immediately and 5 min after the stretching (see Table 1). There was no significant difference in RoM between the changes in RoM (post minus pre) in the 0 min and 5 min conditions.

Passive resistive torque (PRT) and the related structural muscle-tendon parameters
A significant time effect (p = 0.002, F\(1,13\) = 15.937) revealed that the overall PRT decreases immediately (10.69%) and 5 min after the stretching (8.06%) with a large and a moderate effect immediately (\(d = 0.97\)) and 5 min (\(d = 0.67\)) after the stretching, respectively. Moreover, PRT showed no group effect (p = 0.32, F\(1,13\) = 1.08). The pairwise comparison revealed a significant increase in RoM, both immediately and 5 min after the stretching (see Table 1). There was no significant difference in RoM between the changes in RoM (post minus pre) in the 0 min and 5 min conditions.

<table>
<thead>
<tr>
<th>Rest Interval</th>
<th>RoM (°)#</th>
<th>PRT (Nm)#</th>
<th>MTS (Nm/°)#</th>
<th>MVC (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>28.08 ± 8.56</td>
<td>21.67 ± 10.65</td>
<td>1.16 ± 0.43</td>
<td>91.31 ± 29.24</td>
</tr>
<tr>
<td>post</td>
<td>31.97 ± 9.23*</td>
<td>19.36 ± 9.43*</td>
<td>1.01 ± 0.40*</td>
<td>87.33 ± 30.31</td>
</tr>
<tr>
<td>5 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>27.40 ± 10.09</td>
<td>22.49 ± 14.31</td>
<td>1.20 ± 0.61</td>
<td>87.50 ± 34.22</td>
</tr>
<tr>
<td>post</td>
<td>30.52 ± 10.12*</td>
<td>20.68 ± 12.28*</td>
<td>0.97 ± 0.49*</td>
<td>88.60 ± 39.21</td>
</tr>
</tbody>
</table>

Mean ± SD. # = significant time (pre vs. post) effect (ANOVA). * = significant difference between the pre- and post-measurements within the condition (0 min/5 min).

Discussion
The purpose of this study was to investigate the time course (immediately after stretching = 0min-post and 5 min after stretching = 5min-post) of possible changes in function and mechanical properties of the plantar flexor MTU following a 3 × 60 s stretching exercise. As expected, we found an increase in RoM and a decrease in PRT, both immediately and 5 min after the stretching. This was accompanied by a decrease in muscle stiffness, both immediately and 5 min after the stretching. Against our hypothesis, no changes were detected in MVC at any time. Furthermore, no effects were found in the tendon tissue properties at any instant following the stretching. There was no significant difference in the changes (post minus pre) in any measured parameter between the 0 min and 5 min conditions.

The increase in RoM was in accordance with previous studies applying similar stretching durations (Kato et al., 2010; 2017a; 2017b; 2019). As in the previous studies (Power et al., 2004; Ryan et al., 2008a; Mizuno et al., 2013;
Konrad et al., 2019), the increase in RoM persisted until at least 5 min after the stretching exercise. Ryan et al. (2008a) reported an increase in RoM following 2-, 4-, and 8-min static stretches, which lasted between 10 and 20 min. Mizuno and co-workers reported an increase in RoM following a 5-min static stretch, which lasted between 30 and 60 min (Mizuno et al., 2013). Power et al. (2004) showed an increase in RoM after a 4.5 min stretch until at least 120 min. This is in accordance with the findings of Konrad et al. (2019), who reported that RoM was increased for at least 10 min (> 10 min was not measured by the authors) following a 5-min static stretch. Moreover, a shorter stretch duration (1 min) was found to be enough to increase the RoM for at least 40 min (Konrad and Tilp, 2019). A possible explanation for the faster recovery of RoM in the study of Ryan et al. (2008a) compared to others (Mizuno et al., 2013, Konrad et al., 2019, Konrad and Tilp, 2019) might be the duration of the single stretching bouts. While Ryan et al. (2008a) stretched in 30 s bouts (i.e. 4 ×, 8 ×, and 16 × for 30 s for the 2-, 4-, and 8-min protocols, respectively), subjects in the other studies (Power et al., 2004; Mizuno et al., 2013; Konrad et al., 2019; Konrad and Tilp, 2019) stretched for 45 s or 60 s per stretching bout. Although the overall stretching time was the same in the different studies, the increased number of breaks between the single stretching bouts could have led to a decrease in stretching intensity (i.e. Freitas et al. 2015). Since we reported an increased RoM following a 1-min static stretching exercise for at least 40 min in a comparable study (Konrad and Tilp, 2019), one could assume that the 3-min stretch in the present study would also have led to an increased RoM for at least 40 min.

In our experiment, muscle-tendon (joint) stiffness and PRT were found to be decreased, both immediately and 5 min after the stretching. Ryan et al. (2008b) determined a decrease in muscle-tendon stiffness after 10 min, following 4- and 8-min static stretching, which, however, returned to baseline after 20 min. In the same study, they reported a decrease in muscle-tendon stiffness after a 2-min static stretching exercise only immediately after the stretch. Our group found that subjects that stretched for 5 min decreased their muscle-tendon stiffness immediately after the stretch (Konrad et al., 2019). Muscle-tendon stiffness showed the tendency to decrease both 5 min (p = 0.06) and 10 min (p = 0.07) after the stretch (Konrad et al., 2019). Bringing the previous and present findings together, one could assume that 3 min (or more) of static stretching might be stimulus enough to induce changes in muscle-tendon stiffness over a time range of at least 5 min.

Several studies have reported a decreased performance, measured as MVC torque, following a single static stretching exercise (Herda et al., 2008; Marek et al., 2005; Kay and Blazevich, 2008 (at 60 s stretching duration); Konrad et al., 2019), while others did not observe such detrimental effects (Kubo et al., 2001; Kay and Blazevich, 2008 (at 5 s to 20 s stretching durations); Konrad et al., 2017a; 2017b; Kubo et al., 2001; Stafilidis and Tilp, 2015). These controversial results could possibly be explained by the differences in overall stretch duration, as suggested in the reviews by Kay and Blazevich (2012) and Behm et al. (2016), who pointed out that it was only stretching for a minimum of 60 s or longer that might have a detrimental effect on maximum performance. However, in the present study, we stretched the plantar flexors for 3 min and did not observe a significant decrease in MVC. Previous studies in our laboratory showed similar results with a stretching stimulus of 2 min (Konrad et al., 2017a; 2017b), while the decrease in MVC lasted for at least 10 min after a 5-min static stretching exercise (Konrad et al., 2019). According to the measurements taken in our laboratory, we assume that the detrimental effect of a single static stretching exercise can be expected after a stretch for more than 3 min. The discrepancy in stretching duration and force loss between the findings of Kay and Blazevich (2012) and the studies in our laboratory (i.e. Konrad et al. 2017a and the present results) might be explained by the different stretching intensities used. While several studies in the review of Kay and Blazevich (2012) stretched at a constant torque, we applied a constant-angle stretch, which has been shown to be less efficient with regard to RoM changes (Cabido et al., 2014), but also less detrimental with regard to performance.

In addition to the parameters of the muscle-tendon function (RoM, PRT, MVC, and muscle-tendon stiffness), we also investigated the effect on the muscle and tendon structure separately. The parameters assessed were muscle stiffness, passive tendon stiffness (measured when the MTU is passively stretched), and active tendon stiffness (measured during MVC). As also reported in previous studies (Kay and Blazevich, 2009; Kay et al., 2015; Konrad et al., 2017a; 2017b; 2019), we observed a decrease in muscle stiffness, but not in tendon stiffness (neither passive nor active), following the single static stretching exercise. However, others have reported a decrease in tendon stiffness (Kato et al., 2010 (passive); Kubo et al., 2001 (active)), with no changes in muscle stiffness (Kato et al., 2010), following a single static stretch. Possible reasons for these controversial results might be found in the different stretch durations (10 min in Kubo et al. (2001); 20 min in Kato et al. (2010)), which we previously discussed in Konrad et al. (2017a). Concerning the time course, we observed a decrease in muscle stiffness, both immediately and 5 min after the 3-min stretching exercise. In a previous study (Konrad et al., 2019), we observed a decrease in muscle stiffness following a 5-min static stretching exercise, which recovered between 5–10 min. Therefore, one could assume that, due to the lower stretching duration (3 min) in the present study, muscle stiffness will return to baseline between 5–10 min. These results are also in accordance with the findings of Mizuno et al. (2012), who reported an increased displacement of the muscle-tendon junction (an indication of increased muscle belly length) up to 5 min after 5 min of stretching at 15° of dorsiflexion only (not at 5° or 10°).

**Conclusion**

We conclude that a single static stretching exercise of 3 × 60 s increases the RoM and decreases PRT for at least 5 min. These changes can be explained by more compliant muscle tissue within the first 5 min after the stretching.
There were no significant changes in performance (MVC) at any time point after the stretching. Hence, increased RoM and decreased PRT can be associated with more compliant muscle tissue for at least 5 min after a 3-min static stretching exercise.

Acknowledgements

This study was supported by a grant (Project 27665) from the Austrian Science Fund (FWF). The study complied with the laws of the country of the authors’ affiliation. The authors have no conflict of interest to declare.

References


Key points

- Three minutes of static stretching led to an increase in range of motion and a decrease in passive resistive torque for at least 5 minutes.
- The changes in the muscle tendon function can be explained by more compliant muscle tissue.
- Maximum voluntary contraction torque values and tendon stiffness did not change following the single static stretching exercise.

AUTHOR BIOGRAPHY

Andreas KONRAD
Employment
Mag. Dr. at Institute of Sports Science, University of Graz
Degree
PhD
Research interests
Biomechanics, training science, muscle-tendon-unit, soccer science
E-mail: andreas.konrad@uni-graz.at

Markus TILP
Employment
Univ. Prof. Mag. Dr. at Institute of Sports Science, University of Graz
Degree
PhD
Research interests
Biomechanics, training science, muscle-tendon-unit, sports game analysis
E-mail: markus.tilp@uni-graz.at

Mag. Dr. Andreas Konrad
Institute of Sports Science, University of Graz, Mozartgasse 14, A-8010 Graz, Austria