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Effect of PNF stretching training on the properties of human muscle and tendon structures

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The purpose of this study was to investigate the influence of a 6-week proprioceptive neuromuscular facilitation (PNF) stretching training program on the various parameters of the human gastrocnemius medialis muscle and the Achilles tendon. Therefore, 49 volunteers were randomly assigned into PNF stretching and control groups. Before and after the stretching intervention, we determined the maximum dorsiflexion range of motion (RoM) with the corresponding fascicle length and pennation angle. Passive resistive torque (PRT) and maximum voluntary contraction (MVC) of the musculo-articular complex were measured with a dynamometer. Muscle-tendon junction (MTJ) displacement allowed us to determine the length changes in tendon and muscle, and hence to calculate stiffness. Mean RoM increased from 31.1 ± 7.2° to 33.1 ± 7.2° ($P = 0.02$), stiffness of the tendon decreased significantly in both active (from 21.1 ± 8.0 to 18.1 ± 5.5 N/mm) and passive (from 12.1 ± 4.9 to 9.6 ± 3.2 N/mm) conditions, and the pennation angle increased from 18.5 ± 1.8° to 19.5 ± 2.1° ($P = 0.01$) at the neutral ankle position (90°), only in the intervention group, whereas MVC and PRT values remained unchanged. We conclude that a 6-week PNF stretching training program increases RoM and decreases tendon stiffness, despite no change in PRT.

The three most common stretching methods are static, ballistic, and proprioceptive neuromuscular facilitation (PNF) stretching (Magnusson et al., 1996a; Feland et al., 2001; Sharman et al., 2006; Miyahara et al., 2013). All methods are used for both acute (a single stretching training) and short-term (repeated stretching training for 3–8 weeks) stretching and are able to increase the range of motion (RoM) (Magnusson, 1998; Mahieu et al., 2007, 2009; Nakamura et al., 2012). Regarding short-term stretching training, the literature suggests that PNF stretching increases RoM the most (Sady et al., 1982; Wallin et al., 1985; Etnyre & Lee, 1988). This stretching method can furthermore be subdivided into passive and active techniques. In the passive techniques ("contract-relax" or "hold-relax"), the target muscle is placed into a position of stretch followed by a static contraction. The muscle is then passively moved into a greater position of stretch (Cornelius, 1983; Etnyre & Abraham, 1986; Hanten & Chandler, 1994; Ferber et al., 2002). In the active technique ("contract-relax-antagonist-contract"), the final passive stretch is exchanged by an active contraction of the antagonist, which stretches the target muscle (Cornelius, 1983; Rowlands et al., 2003). Both PNF methods cause an increase in RoM (Etnyre & Abraham, 1986; Sharman et al., 2006). On the one hand, it is believed that autogenic inhibitory input occurs from the Golgi tendon organs and is due to the elongation of the agonist (stretched) muscle (Etnyre & Abraham, 1986; Sharman et al., 2006). On the other hand, reciprocal inhibition of the antagonist contributes to the increase in RoM in the agonist muscle (Sharman et al., 2006). However, Chalmers (2004) and Sharman et al. (2006) noted the lack of experimental evidence for these hypotheses and referred to Magnusson et al. (1996a) who concluded that contract/relax stretching induces gains in RoM due to modified stretch perception.

Besides RoM, several other functional [maximal isometric torque, passive resistive torque (PRT)] or structural parameters (muscle stiffness, tendon stiffness, fascicle length, pennation angle) were measured in this study. "Functional" parameters all involve assessment of the entire musculo-articular complex (Nordez et al., 2008), whereas the "structural" parameters involve assessment of specific components of the musculo-articular complex. Various authors have reported that repeated static stretching does not affect the torque angle curve at the same angle (Halbertsma & Göeken, 1994; Magnusson et al., 1996b; Reid & McNair, 2004; Gajdosik et al., 2005; Weppler & Magnusson, 2010) and standardized torque (Folpp et al., 2006; Law et al., 2009; Ben & Harvey, 2010; Weppler & Magnusson, 2010) in the pre- and post-intervention. A handful of investigators

determined decreasing PRT and therefore changes in the torque angle curve after a short-term static stretching regime (Kubo et al., 2002; Guissard & Duchateau, 2004; Mahieu et al., 2007; Nakamura et al., 2012). Furthermore, static stretching does not alter maximal isometric torque (Kubo et al., 2002), or tendon stiffness (defined as force-length relationship during an isometric ramp contraction with maximal voluntary effort; Kubo et al., 2002; Mahieu et al., 2007), after a 3- to 6-week training period. Studies that investigate the effects of PNF (Mahieu et al., 2009) or ballistic (Mahieu et al., 2007) stretching training on structural parameters are scarce. To the best of our knowledge, so far only, Mahieu et al. (2009) have analyzed the effect of a 6-week PNF stretching program on functional parameters and structural tendon properties. Mahieu et al. (2009) reported an increase in RoM but no changes in passive torque or in Achilles tendon stiffness. Thus, they concluded that the increased RoM can be explained by an increase in stretch tolerance rather than structural changes. However, several structural parameters, which might affect and explain RoM changes, such as muscle and tendon stiffness during passive movements (Kato, 2009), as well as fascicle length and pennation angle (Morse et al., 2008), were not analyzed by Mahieu et al. (2009). Therefore, the objective of this study was to analyze the effect of a PNF stretching program on the functional and structural parameters of the ankle joint.

Due to the findings in the literature, we hypothesized that adaptational changes would occur in the functional parameters of ROM and PRT but not in MVC, and that changes would occur in all of the structural parameters (muscle stiffness, tendon stiffness, fascicle length, pennation angle) following a short-term PNF stretching training program.

Methods

Experimental design

A total of 49 police cadets participated in the study. They were randomly assigned to a PNF stretching group ($N = 25$) and a control group ($N = 24$) by picking cards in a blinded manner. All subjects were asked to maintain their normal physical activities during the study. Teachers of the police school were informed about the study and were asked to maintain intensity and extent of physical activities during their lessons (2/week). The PNF stretching group executed a collective PNF stretching training program five times a week for 6 weeks, in the morning before education in police school started. Investigators controlled the stretching training at least once a week. Furthermore, subjects had to keep a diary of the stretching performance, which was collected at the end of the study. Before and after the 6-week intervention, the RoM, PRT, maximum voluntary contraction (MVC), and several parameters of the muscle and tendon structure of the gastrocnemius medialis (GM) were determined.

Subjects

Thirty-one healthy male (mean ± SD; 23.6 ± 2.5 years, 180.3 ± 5.0 cm, 77.6 ± 8.0 kg) and 18 healthy female (mean ± SD; 23.3 ± 3.1 years, 170.0 ± 4.2 cm, 62.7 ± 5.4 kg) police cadets participated in this study. The baseline characteristics of both PNF stretching group and control group are shown in Table 1. Each subject was informed about the testing procedure but not about our hypotheses, and they each gave written consent to participate in the study. Competitive athletes and participants with a history of lower leg injuries were excluded. The Ethical Committee of the University of Graz approved the study. Sample size was determined by power analysis based on a pilot study.

Table 1. Baseline characteristics of the proprioceptive neuromuscular facilitation (PNF) stretching group and the control group, mean ± SD

	PNF	Control	*P*
Range of motion (°)	31.1 ± 7.2	32.1 ± 7.7	0.67
Fascicle length at rest (cm)	6.3 ± 0.9	6.1 ± 0.8	0.52
Fascicle length in stretching position (cm)	7.1 ± 0.8	7.4 ± 0.9	0.30
Pennation angle at rest (°)	18.5 ± 1.8	17.8 ± 1.9	0.26
Pennation angle in stretching position (°)	16.8 ± 1.2	15.4 ± 1.8	0.01
Passive resistive torque (N·m)	22.4 ± 7.4	22.2 ± 7.5	0.95
Passive tendon stiffness (N/mm)	12.1 ± 4.9	13.9 ± 3.7	0.24
Muscle stiffness (N/mm)	7.0 ± 2.4	6.8 ± 2.1	0.81
Muscle-tendon stiffness (N·m/°)	0.79 ± 0.18	0.78 ± 0.22	0.87
MVC torque (N·m)	104.9 ± 47.4	92.7 ± 29.3	0.34
Active tendon stiffness (N/mm)	21.1 ± 8.0	20.2 ± 5.8	0.69

Measures

To ensure a high scientific standard, all measurements were undertaken by the same investigator. In addition to a written introduction, subjects were personally informed about the procedure. Pre- and post-training tests were executed at the same time of day, and 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: (a) RoM (10 min break), (b) PRT (1 min break), (c) MVC (see Fig. 1).

Measurements of end-range stretch to estimate RoM

End-range stretch was measured with an electronic goniometer (Biovision, Wehrheim, Germany) fixed on the ankle joint with Leukotape® (BSN medical S.A.S., Vibraye, France). Participants were first instructed to stay upright in a neutral position with the ankle joint angle at 90°. They were then asked to step back with one leg and bring the ankle joint to maximum dorsiflexion, keeping their heel on the ground. The knee of the testing leg had to remain fully extended and the knee of the opposite leg flexed. Both feet were in a parallel position, and hands could be placed on a wall to ensure balance. Special attention was laid on the appropriate position of the stretched leg during the measurement to avoid any pronation of the foot. If some pronation was observed, the measurement was repeated. The difference between the maximum dorsiflexion and the position in rest (neutral position) was defined as dorsiflexion RoM.

PRT measurements

To investigate PRT, an isokinetic dynamometer (CON-TREX MJ, CMV AG, Duebendorf, Switzerland) was used, and the standard

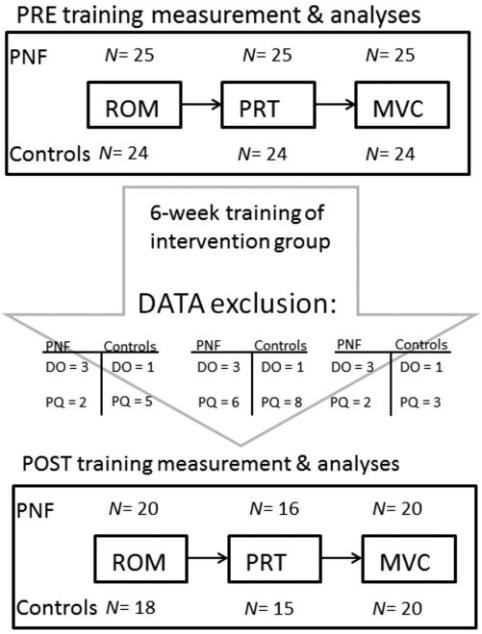

Fig. 1. Schematic presentation of the study and subject flow: DO = dropouts, PQ = poor quality of the ultrasound videos.

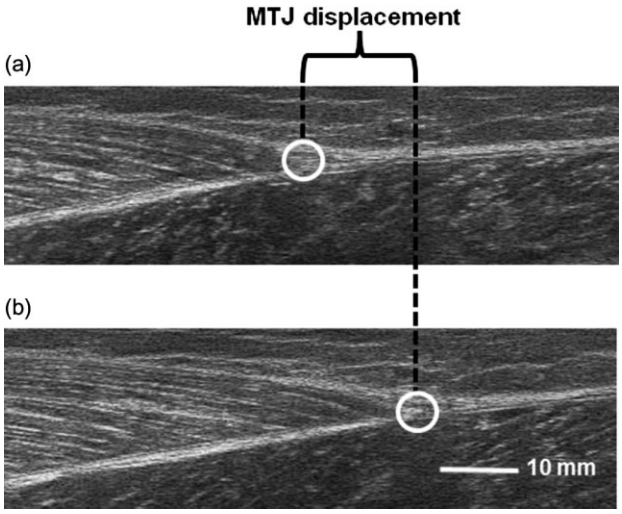

Fig. 2. Images showing the displacement of the muscle-tendon junction (MTJ) during a passive movement from neutral position (a) of the ankle joint to maximum dorsiflexion (b).

setup for ankle joint movement of the dynamometer was adjusted. Subjects lay prone with the knee fully extended on a bench and were secured with a strap on the upper body to exclude any evasive movement. The foot was fixed barefooted with a strap to the foot plate of the dynamometer. The ankle joint was carefully aligned with the axis of the dynamometer to avoid heel displacement. The dynamometer moved the ankle joint from a 10° plantar flexion to a dorsiflexion position, which corresponded to 95% of the individual maximum dorsiflexion RoM previously measured in the RoM measurement. The ankle joint was moved passively for three cycles. During pilot measurements, we recognized a conditioning effect during the first two passive movements, similar to the active conditioning reported by Maganaris (2003). Therefore, measurements were taken during the third cycle to avoid the conditioning effect. Similar to the studies by Kubo et al. (2002) and Mahieu et al. (2009), the velocity of the dynamometer was set at 5°/s to exclude any reflexive muscle activity. Participants were asked to relax during the measurements.

MVC measurements

MVC measurements were performed with the dynamometer at a neutral ankle position (90°). Participants were instructed to perform three 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 value was taken for further analysis.

Electromyography (EMG)

Muscular activity was monitored by EMG (myon 320, myon AG, Zurich, Switzerland) during PRT and MVC measurements. Surface electrodes (Blue Sensor N, Ambu A/S, Ballerup, Denmark) were placed on the muscle bellies of the GM and the tibialis anterior. In PRT measurements, the EMG (normalized to plantarflexor MVC) was monitored post-hoc to ensure that the subject was relaxed, i.e., did not show any EMG activity. Sample rate was 2000 Hz. EMG signals were high-pass filtered (10 Hz, Butterworth), and root mean square (50 ms window) values were calculated.

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.) was used to obtain a longitudinal ultrasound image of the GM.

During the PRT and MVC measurement, the ultrasound probe was placed on the distal end of the GM (Fig. 2), where the muscle is connected to the Achilles tendon, i.e., the muscle-tendon junction (MTJ; Kato et al., 2010). The ultrasound probe was secured with a standard orthopedic stocking to prevent displacement of the probe. To determine the muscle displacement during PRT measurement, the echoes of the MTJ in the ultrasound videos were manually tracked (Kato et al., 2005, 2010). To determine the tendon displacement during MVC measurements, the echoes of a fascicle insertion at the deep aponeurosis near the MTJ were manually tracked (Kubo et al., 2002). Thus, tendon displacements during MVC measurement represent tendon and parts of the distal aponeurosis.

During RoM measurements, the length of the GM fascicle and its pennation angle with the deep aponeurosis was determined from ultrasound videos. The ultrasound probe was placed at 50% of the GM muscle length (Morse et al., 2008). The fascicle length and the pennation angle were measured at a neutral position of the ankle joint (90°) and at maximum dorsiflexion.

Ultrasound images were recorded at 25 Hz with a depth image resolution of 74 mm. During PRT and MVC measurement, the videos were synchronized with the rest of the data via the signals of a function generator (Voltkraft®, Hirschau, Germany). Videos were cut and digitized by VirtualDub open-source software (version 1.6.19, http://www.virtualdub.org) and were analyzed in ImageJ open-source software (version 1.44p, National Institutes of Health, Bethesda, Maryland, USA).

Each video was measured by two investigators, and the mean value of both measurements was used for further analyses of the muscle-tendon structure. Except for the principal investigator, the investigators were blinded to the hypotheses of the study, however, not to the group allocation and subjects' names. During the analyses of PRT measurement, every fifth frame, and for MVC

measurement, every second frame, were measured by the investigators, corresponding to a time resolution of 0.2 and 0.08 s, respectively.

Similar to the approach used by other authors (Morse et al., 2008, 2013; Kato et al., 2010; Maïsetti et al., 2012), the cadaveric regression model of Grieve et al. (1978) was used to obtain the length changes of the muscle-tendon unit (MTU) of the GM during passive movements. The difference between the MTU length change and the displacement of the muscle was defined as the tendon displacement.

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 with 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., 2007, 2009), and dividing by the moment arm of the triceps surae muscle (MA), which was measured individually as the distance between the malleolus lateralis and the Achilles tendon in rest (neutral position) with a measuring tape. The mean value of the moment arm was 4.71 cm, with a range of 4.0–6.0 cm.

Active tendon stiffness was calculated by linear regression between active force and related tendon length changes during MVC measurements over the whole range of force (0–100% MVC). Passive tendon stiffness, muscle stiffness, and muscle-tendon stiffness were calculated by linear regression between passive force (~ 0–25% MVC) produced from neutral ankle position (90°) to maximum dorsiflexion and related tendon length, muscle length, and joint angle changes, respectively. Please note that the term "passive tendon stiffness" was used for the force-length relationship during a MVC measurement in previous studies (Mahieu et al., 2007, 2009). To distinguish between the force-length relationships from passive measurements we performed in our study, we have defined this parameter as "active tendon stiffness" throughout the text. The quality of the linear regressions was assessed with the Pearson correlation coefficient.

PNF stretching program

Subjects of the PNF stretching group were asked to undertake a "contract-relax-antagonist-contract" PNF stretching program (Sharman et al., 2006) for the plantar flexor muscles. The stretching was performed five times a week for a 6-week period. To ensure an efficient stretching intervention in the class, the police cadets were asked to perform the PNF stretching training independently, so that no assistance of another person was needed. Each subject was informed about the stretching procedure. Subjects were instructed to undertake the stretching of the plantar flexors in a standing wall push position and to stretch until a point of discomfort was reached. One stretching intervention consisted of a 15-s static stretch of the lower leg followed by an isometric contraction of the stretched muscle for 6 s. Afterward, the subjects were instructed to contract the antagonistic dorsi flexor muscle for another 15 s (Mahieu et al., 2009) to induce another stretch for the plantar flexors. This procedure was repeated four times during each stretching session, alternating both legs, with no rest in between, resulting in a total stretch period of 144 s for each muscle.

Statistical analyses

SPSS (version 20.0, SPSS Inc., Chicago, Illinois, USA) was used for all the statistical analyses. To determine inter-rater reliability of the muscle-tendon displacement measurements, an intraclass correlation coefficient (ICC) was used. Kolmogorov-Smirnov test was used to verify normal distribution on all parameters. To prove homogeneity between the baseline characteristic of both groups, *t*-tests were performed. To assess the validity of our methods, paired *t*-tests were performed to test if mean values of pre and post measurements of the control group were equal. Subsequently, we performed paired *t*-tests to test the effect of the stretching protocol in the intervention group. Tendon, muscle, and muscle-tendon stiffness calculations were controlled with a Pearson correlation coefficient. An alpha level of $P = 0.05$ was defined for the statistical significance for all the tests.

Results

Data exclusion and measurement quality

Baseline characteristics of subjects are presented in Table 1. There was no significant difference between the groups except in the parameter "pennation angle in stretching position." Due to subject drop-out and poor quality of the ultrasound videos, 5 (6) subjects of the RoM measurement, 9 (9) subjects of the PRT measurement, and 5(4) subjects of the MVC measurement of the PNF stretching (control) group, respectively, had to be excluded from the study (Fig. 1). Drop outs of subjects were all due to injuries. In ultrasound videos with poor quality, fascicle insertion points at the deep aponeurosis (MVC measurement) or the MTJ (PRT measurement) were not identifiable with necessary precision. Drop outs and data exclusion did not change homogeneity of groups.

The mean (range) ICC of the ultrasound video analysis of both investigators were 0.99 (0.985–0.998), 0.96 (0.812–0.999), and 0.95 (0.801–1.000) for the RoM, PRT, and MVC measurements, respectively. Values above 0.80 were classified as acceptable (Vincent, 1999; Tilp et al., 2011).

The mean values of the Pearson correlation coefficients at the linear regression were 0.98, 0.96, 0.89, and 0.96, with ranges of 0.88–0.99, 0.65–0.99, 0.82–0.97, and 0.91–0.98, with all $P < 0.05$, for passive tendon stiffness, active tendon stiffness, muscle stiffness, and muscle-tendon stiffness, respectively.

RoM and the related structural muscle parameters

Following the 6-week stretching intervention, the PNF stretching group had a significantly increased dorsiflexion RoM ($P = 0.02$; see Table 2A). Furthermore, the pennation angle increased significantly in the PNF stretching group in a neutral position ($P = 0.01$) but not in the maximum dorsiflexion position. Fascicle length did not change in either position. No parameter changes were observed in the control group.

PRT and related structural muscle-tendon parameters

There was no significant effect on PRT at the same maximum ankle joint angle for the pre- and post-session data (Table 2B). Figure 3 shows the relationship

Table 2. Results of maximum dorsiflexion range of motion (RoM) as well as changes in fascicle length and pennation angle during RoM measurement (A). Results of passive resistive torque (PRT), passive tendon stiffness, muscle stiffness, and muscle-tendon stiffness during passive measurements (B). Results of maximum voluntary contraction (MVC) torque and active tendon stiffness during MVC measurements (C)

A	PNF ($N = 20$)				Control ($N = 18$)			
	Pre	Post	Post-Pre	P	Pre	Post	Post-Pre	P
RoM (°)	31.1 ± 7.2	33.1 ± 7.2*	2 ± 3.7	0.02	32.1 ± 7.7	31.8 ± 7	−0.3 ± 2.6	0.61
Fascicle length at rest (cm)	6.3 ± 0.9	6.2 ± 0.7	−0.1 ± 0.5	0.27	6.1 ± 0.8	6.2 ± 0.9	0.1 ± 0.5	0.50
Fascicle length in stretching position (cm)	7.1 ± 0.8	7.2 ± 0.7	0.1 ± 0.4	0.15	7.4 ± 0.9	7.4 ± 0.9	0 ± 0.6	0.86
Pennation angle at rest (°)	18.5 ± 1.8	19.5 ± 2.1*	1.1 ± 1.6	0.01	17.8 ± 1.9	18.2 ± 2.3	0.4 ± 2	0.45
Pennation angle in stretching position (°)	16.8 ± 1.2	16.7 ± 1.3	0 ± 1.2	0.93	15.4 ± 1.8	15.6 ± 1.6	0.1 ± 1.6	0.76

B	PNF ($N = 16$)				Control ($N = 15$)			
	Pre	Post	Post-Pre	P	Pre	Post	Post-Pre	P
PRT (N·m)	22.4 ± 7.4	21.2 ± 8.9	−1.1 ± 4.4	0.32	22.2 ± 7.5	21.8 ± 8.3	−0.4 ± 4	0.68
Passive tendon stiffness (N/mm)	12.1 ± 4.9	9.6 ± 3.2*	−2.4 ± 3.6	0.01	13.9 ± 3.7	12.4 ± 4.9	−1.5 ± 5	0.25
Muscle stiffness (N/mm)	7 ± 2.4	7.1 ± 2.6	0.1 ± 3.5	0.94	6.8 ± 2.1	6.8 ± 2.7	0 ± 1.9	0.94
Muscle-tendon stiffness (N·m/°)	0.79 ± 0.18	0.75 ± 0.23	−0.04 ± 0.18	0.43	0.78 ± 0.22	0.71 ± 0.21	−0.1 ± 0.14	0.12

C	PNF ($N = 20$)				Control ($N = 20$)			
	Pre	Post	Post-Pre	P	Pre	Post	Post-Pre	P
MVC torque (N·m)	104.9 ± 47.4	104.2 ± 41.0	−0.6 ± 20.8	0.89	92.7 ± 29.3	90.1 ± 33.2	−2.6 ± 24.1	0.63
Active tendon stiffness (N/mm)	21.1 ± 8.0	18.1 ± 5.5*	−3 ± 6.1	0.04	20.2 ± 5.8	19.3 ± 5.1	−0.9 ± 4.8	0.40

*Significant difference between pre and post data, mean ± SD.

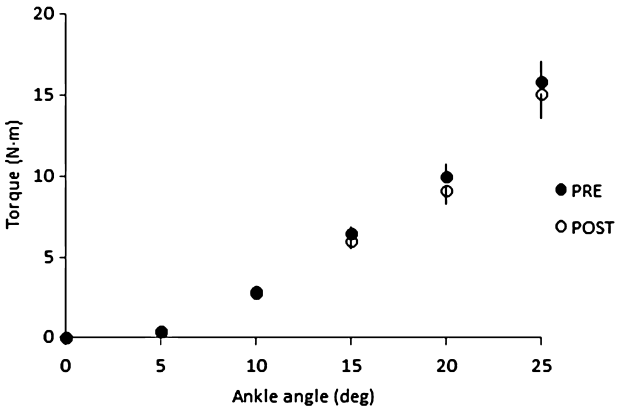

Fig. 3. Relationship between passive resistive torque and ankle joint angle before and after the proprioceptive neuromuscular facilitation stretching intervention ($N = 16$), mean ± SEM. Data from control group are omitted because there is no statistical or visible difference between the measurements.

between ankle joint angle and the corresponding PRT of the PNF stretching group. No significant differences were observed in any joint angle. Moreover, passive muscle-tendon and muscle stiffness did not change. However, passive tendon stiffness did significantly decrease after the PNF stretching intervention ($P = 0.01$). No parameter changes could be found in the control group.

In Fig. 4(a) and (b), the elongation of muscle and tendon in relation to the PRT data is shown in steps of 5° from 0° to 25°. Moreover, Fig. 4(c) and (d) shows the elongation of the tendon and muscle as a function of the ankle angle. During passive movements from neutral ankle position to 95% of dorsiflexion ROM, the MTU was elongated from 43.7 (±2.9 cm) to 45.8 (±3.0 cm). Before the stretching training this elongation (2.1 cm) was divided into a tendon and muscle elongation of 0.8 and 1.3 cm, respectively. After the training period, this distribution changed non-significantly to 0.9 and 1.2 cm for tendon and muscle, respectively. Please note that no significant changes were observed between the values before and after the training.

MVC and tendon stiffness

Plantarflexor MVC was the same after the short-term stretching intervention. However, (active) tendon stiffness calculated from the MVC measurements decreased significantly in the PNF stretching group when comparing pre- and post-session data ($P = 0.04$; see Table 2C). No parameter changes were detected in the control group.

Discussion

Similar to other studies (Mahieu et al., 2009; Maddigan et al., 2012; Miyahara et al., 2013), the present study

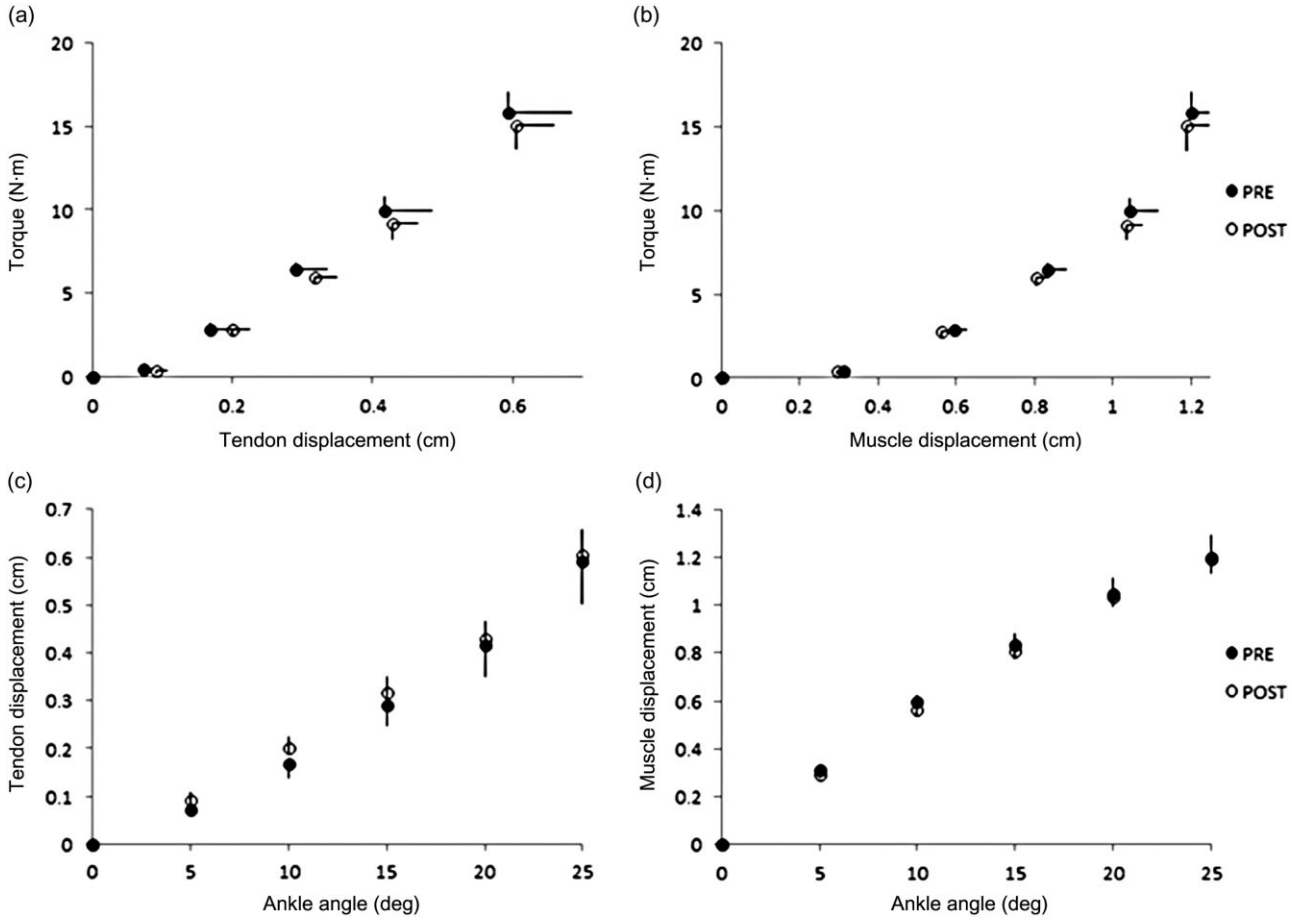

Fig. 4. Relationship between passive resistive torque and tendon (a) and muscle displacement (b) during passive movement before and after the proprioceptive neuromuscular facilitation (PNF) stretching intervention. Displacement of the tendon (c) and the muscle (d) during passive dorsiflexion in relation to ankle angle before and after the PNF stretching intervention ($N = 16$), mean ± SEM. Data from the control group are omitted because there is no statistical or visible difference between the measurements.

reveals that a PNF stretching program increases RoM. However, although the literature indicates that PNF stretching results in the highest yield of RoM compared with other stretching methods (Sady et al., 1982; Wallin et al., 1985; Etnyre & Lee, 1988; Feland et al., 2001; Funk et al., 2003; O'Hora et al., 2011); RoM increases in the present study are smaller than previously reported by Mahieu et al. (2009), with 2.0° compared to 6.0°, respectively. An explanation for this discrepancy could be the difference in the mean pre-training dorsiflexion RoM of 28.3° compared to 31.1° between Mahieu et al. (2009) and the present study. According to Moseley et al. (2001), our subjects were already very flexible before the training period, which might have led to the smaller increases in RoM. Another explanation could be the amount of stretching. Subjects in the study by Mahieu et al. (2009) stretched every day, and each stretching session was repeated five times. This resulted in a total stretching time of approximately 6300 s, in contrast to 4320 s in the present study. The results of PRT showed no significant changes when comparing the pre- and post-intervention data. This is in accordance with the major part of research on short-term static stretching, which reported unchanged torque angle curves (Halbertsma & Göeken, 1994; Magnusson et al., 1996b; Reid & McNair, 2004; Gajdosik et al., 2005; Folpp et al., 2006; Law et al., 2009; Ben & Harvey, 2010). However, Kubo et al. (2002), Nakamura et al. (2012), and Mahieu et al. (2007) reported decreasing PRT and therefore an adaptation in torque angle curves in their studies. Mahieu et al. (2007) hypothesized that such a decrease in PRT might be due to an increase in the number of sarcomeres based on findings in animal studies (Coutinho et al., 2004). Furthermore, no changes in PRT are reported in studies on ballistic (Mahieu et al., 2007) and PNF stretching (Mahieu et al., 2009). MVC did not change due to the stretching training. This is in accordance with the results of other authors who reported constant maximum isometric torque values in short-term PNF stretching training (Higgs & Winter, 2009), as well as in short-term static stretching training (Kubo et al., 2002). Therefore, our results confirm that short-term stretching does not impair maximum muscle force.

It has been reported that Achilles tendon stiffness decreases after ballistic stretching training (Mahieu et al., 2007) but remains constant after static (Kubo

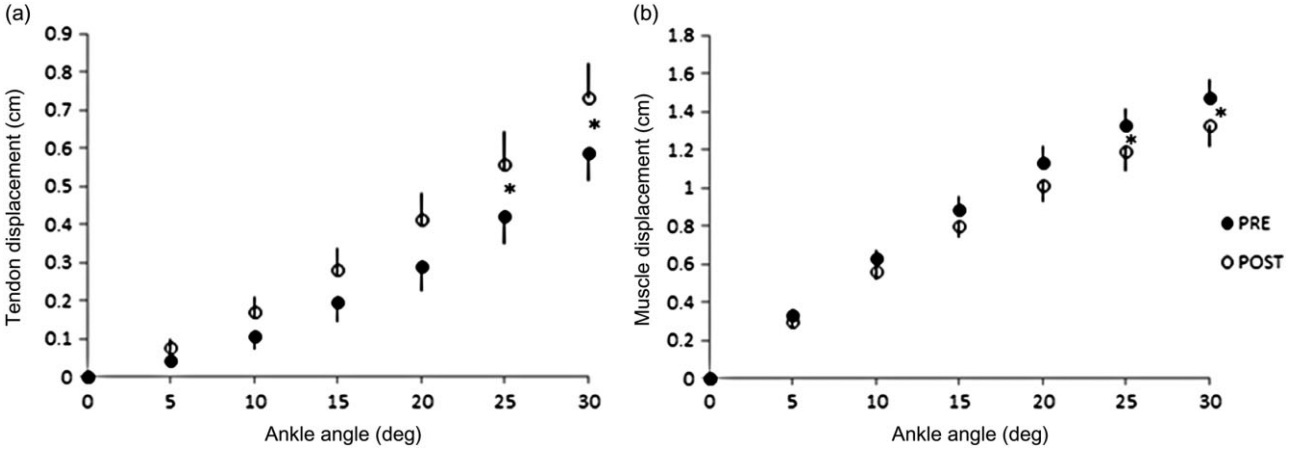

Fig. 5. Ankle angle for tendon (a) and muscle (b) displacement of the seven most flexible subjects. *Significant difference between pre and post data ($N = 7$), mean ± SEM.

et al., 2002; Mahieu et al., 2007) or PNF stretching training (Mahieu et al., 2009). However, in the present study, both passive tendon stiffness (from 12.1 ± 4.9 to 9.6 ± 3.2 N/mm) and active tendon stiffness (from 21.1 ± 8.0 to 18.1 ± 5.5 N/mm) decreased significantly due to short-term PNF stretching. A decrease in tendon stiffness has also been reported by Kato et al. (2010) after acute static stretching for 20 min. A possible explanation for such a decrease after acute stretching could be wave-like course of collagen fibers in unstressed tendon that gets straightened when stretched (Stromberg & Wiederhielm, 1969). One could speculate that this process of fiber straightening gets facilitated due to habitual stretching. Another explanation was formulated by McNair et al. (2001) when studying cyclic motions. They speculated that polysaccharides and water are redistributed within the collagen framework, which leads to decreases in stiffness.

(Passive) Muscle stiffness and muscle-tendon stiffness remained unchanged in the passive trial. These results are therefore in contrast with the results of Mahieu et al. (2009) and provide, for the first time, evidence for structural changes in the tendon after PNF stretching, similar to ballistic stretching. Tendon stiffness values in the present study were smaller (20 N/mm vs 46 N/mm) compared with the results of Mahieu et al. (2009), who conducted a similar study. One possible explanation for this could be the moment arm of the Achilles tendon, which was individually measured in the present study, while Mahieu et al. used the same moment arm for all subjects. Further differences are the warm-up routine and shoed subjects in the Mahieu et al. (2009, see their fig. 1) study. This could have led to higher MVC values and thus to higher stiffness values assuming an increase of stiffness with higher force. However, changes rather than absolute stiffness values are of importance in both studies.

Summarizing the results of human studies, there is some evidence that short-term stretching programs have different effects on tendon stiffness, depending upon the type of stretch training involved. The short-term static stretching programs investigated so far have not found altered tendon stiffness. One study involving a short-term ballistic stretching program did provide evidence of decreased tendon stiffness (Mahieu et al., 2007). Short-term PNF stretching programs have shown mixed results. Mahieu et al. (2009) found no change in tendon stiffness, whereas the present study found significant decreases in both passive and active tendon stiffness. Similar to the ballistic stretching group in the study of Mahieu et al. (2007), decreased tendon stiffness found in the present study did not result in decreased stiffness at the functional level as PRT did not change and was therefore not the direct cause of increase in RoM.

One would expect that decreased tendon stiffness should also lead to a decrease in PRT or to increased muscle stiffness if PRT remains constant. However, neither PRT nor muscle stiffness changed significantly. Therefore, an altered perception of stretch and pain or stretch tolerance (Halbertsma & Göeken, 1994; Halbertsma et al., 1996; Magnusson et al., 1996a) seems a likely explanation for RoM increase. Since there was a trend toward reduced PRT and increased muscle stiffness (see Figs 3, 4(b), and 5(b)) we would speculate, although without statistical evidence, that the PNF training induced a stiffening of the muscle structure. The reason could be repeated active contractions during PNF stretching training.

Alterations in tendon properties by reducing its stiffness have only been reported after short-term ballistic or PNF stretching, but not after static stretching programs studied so far. The main difference between ballistic and PNF stretching compared to static stretching is the amount of developed tendon force. It is fair to assume that due to ballistic movements or active muscle contraction, tendon force and therefore tendon strain is greater compared to a purely static stretch. Arampatzis et al.

(2007) concluded that the alteration of tendon properties is strain dependent. They reported that only high strains due to active contraction lead to changes in tendon properties. They argued that there is a strain threshold that has to be exceeded to induce adaptations. Since tendon stiffness increased in the study by Arampatzis et al. (2007) and decreased in the present study, and considering the reports about ballistic stretching (Mahieu et al., 2007), it seems that strains due to purely active contraction and strains due to a stretch or a combination of active contraction and stretch are different stimuli for tendon tissue.

The decreased tendon stiffness combined with an increased RoM found in the present study would necessarily lead to an increased elongation of the tendon structure during passive movements or active contractions. However, this could not be confirmed by the presented results (Fig. 4(c)). Tendon length changes remained unchanged after passive movements or active contractions. This apparent contradiction can be explained by the heterogeneity in the flexibility of our subjects. During passive movements, we stretched the MTU of our subjects by up to 95% of their maximum dorsiflexion position. This position varied from a 17.5° to 40.0° ankle angle. The presented figures are limited to a dorsiflexion angle of 25° to include the majority (12/16) of our subjects in the results, while all the subjects were included in the calculation of tendon stiffness. However, seven subjects were able to stretch their ankle joint to at least 30 °. In Fig. 5, we present the tendon and muscle elongations from these subjects up to a dorsiflexion angle of 30 °. The statistical analysis reveals significantly greater tendon elongations and significantly smaller muscle elongations at 25° and 30°, respectively. Further statistical analysis also revealed that these subjects seem primarily responsible for significant changes in tendon stiffness (from 14.2 to 10.9 N/mm, $P < 0.01$) while there was only a tendency of decrease in the group with less flexible subjects (from 10.4 to 8.6 N/mm, $P = 0.28$). However, PRT did not change in either of these groups. Thus, it appears that the more flexible subjects responded stronger to the training compared to less flexible subjects.

An implication for sports practice can be related to tendon stiffness. Arampatzis et al. (2007) reported that endurance runners have more compliant tendons than sprinters. Together with the results from Oda et al. (2013), who reported that high-performance 5000 m runners have more compliant tendons than medium-performance runners, this suggests the advantage of having compliant tendons in endurance sports, probably due to increased efficiency. Hence, according to the findings in the present study, PNF stretching could be an interesting training method to increase the performance of endurance runners.

The decreased stiffness of the tendon is reflected by a significant increase in the pennation angle in a neutral position (from $18.5 \pm 1.8°$ to $19.5 \pm 2.1°$). Assuming the same tendon force and the same MTU length in the neutral position in the pre- and post-intervention data, the decreased tendon stiffness would result in a longer tendon, which has to be compensated for by a shorter muscle complex. In the present study, this could be explained by a greater angle of pennation rather than shorter muscle fascicles. However, these assumptions are based on a simple two-dimensional MTU model not including the complexity on the cellular level of MTU structure. It is also difficult to estimate the functional significance of such a small change in pennation angle. However, there was no adaptation of the pennation angle in stretching position. This could be due to the nonlinear change of pennation angles with high forces at the end of the stretch, which do not lead to significant results or due to the heterogeneity of the subjects in this parameter.

There are some limitations of this study. First, the persons taking measurements were not all blinded to the intervention. Therefore, a bias in the results cannot be completely excluded although the inter-rater reliability was excellent (mean ICC: 0.95–0.99). Second, the method of measuring the moment arm of the ankle joint *in vivo* was quite simple. However, values obtained in this study were very similar to others using magnetic resonance imaging data (Rugg et al., 1990) or ultrasound (Lee & Piazza, 2009). Further limitations of the measurement technique, such as estimated muscle cross-sectional area and movements during isometric contractions, are discussed in Kubo et al. (2002).

Perspectives

This study showed that a 6-week PNF stretching training program of the lower leg muscle increases dorsiflexion RoM and affects tendon structure by decreasing its stiffness, while the muscular structure and PRT of the musculo-articular complex observed in this study is not altered. An additional finding was the different adaptive responses to stretch in different subject groups (flexible and less flexible). Since this might be from clinical relevance, we suggest performing further studies on this topic to identify possible causes for this result. Further studies including all the structural muscle and tendon parameters that might affect MTU function should investigate the effects of other (static, ballistic) acute and short-term stretching programs. Furthermore, future studies should include follow-up measurements to estimate the decline in the changes, to determine if and when there is a retrogression of MTU structure.

Key words: Stiffness, ultrasound, proprioceptive neuromuscular facilitation, passive resistive torque, MVC, range of motion.

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