Effects of Flexibility Training on Eccentric Exercise–Induced Muscle Damage

CHE-HSIU CHEN[1,2], KAZUNORI NOSAKA[3], HSIN-LIAN CHEN[1], MING-JU LIN[4], KUO-WEI TSENG[5], and TREVOR C. CHEN[1]

[1]*Department of Physical Education, National Chiayi University, Chiayi County, TAIWAN;* [2]*School and Graduate Institute of Physical Therapy, National Taiwan University, Taipei City, TAIWAN;* [3]*School of Exercise, Biomedical and Health Sciences, Edith Cowan University, Western Australia, AUSTRALIA;* [4]*Graduate Institute of Sport Coaching Science, Chinese Culture University, Taipei City, TAIWAN; and* [5]*Department of Physical Education and Health, Taipei Physical Education College, Taipei City, TAIWAN*

ABSTRACT

CHEN, C-H., K. NOSAKA, H-L. CHEN, M-J. LIN, K-W. TSENG, and T. C. CHEN. Effects of Flexibility Training on Eccentric Exercise–Induced Muscle Damage. *Med. Sci. Sports Exerc.*, Vol. 43, No. 3, pp. 491–500, 2011. **Purpose**: This study investigated whether flexibility training would attenuate muscle damage induced by maximal eccentric exercise. **Methods**: Thirty untrained young men were allocated to static stretching (SS), proprioceptive neuromuscular facilitation (PNF), or control group ($n = 10$ per group). The SS consisted of 30 sets of a 30-s standard SS with a 30-s rest between sets, and the PNF included 5 sets of the 30-s standard SS followed by 3 sets of three "contract–relax–agonist–contract" procedures. These were performed three times a week for 8 wk, and all subjects performed six sets of 10 maximal isokinetic ($30°\cdot s^{-1}$) lengthening contractions of the knee flexors after the 8-wk training or 8 wk after the baseline measures (control). Changes in indirect markers of muscle damage before and for 5 d after the eccentric exercise were compared among the groups. **Results**: The range of motion (ROM) of the hip joint increased by 25°, and the optimum angle of the knee flexors shifted ($P < 0.05$) to a longer muscle length by 10° after training, without significant differences between SS and PNF. No significant changes in these variables were evident for the control group. Compared with the control group, the SS and PNF groups showed significantly ($P < 0.05$) smaller decreases and faster recovery of knee flexor muscle strength and smaller changes in optimum angle, ROM, muscle soreness, and plasma creatine kinase activity and myoglobin concentration without significant differences between the groups. The preeccentric exercise ROM or optimum angle was significantly ($P < 0.05$) correlated with the changes in the muscle damage markers. **Conclusions**: These results suggest that both SS and PNF training are effective in attenuating eccentric exercise–induced muscle damage and that flexible muscles are less susceptible to the damage. **Key Words:** PROPRIOCEPTIVE NEUROMUSCULAR FACILITATION, STATIC STRETCHING, MUSCLE STRENGTH, OPTIMUM ANGLE, MUSCLE SORENESS, PASSIVE RANGE OF MOTION

Muscle damage induced by lengthening contractions is common in exercise, training, or competitions, and its typical symptoms include muscle weakness and delayed onset muscle soreness or DOMS (7,8,39). Although these symptoms are generally subsided within a week, interventions to attenuate muscle damage and facilitate recovery are important for athletes to perform better in subsequent training sessions and/or matches and for general population to stay physically active (6). It is clear that prevention is better than treatment (9), and many studies have endeavored to find prophylactic modalities to attenuate the symptoms of muscle damage with limited success (6,10,23).

One such modality is stretching, and preexercise static stretching has been advocated to be beneficial for prevention of soft tissue (e.g., muscle, tendon, ligament) injuries (6,10). However, it does not seem that preexercise stretching is beneficial for the attenuation of DOMS or other symptoms of eccentric exercise–induced muscle damage. In fact, several studies (21,25,40,47) failed to find its prophylactic effects on eccentric exercise–induced muscle damage. It is also documented that preexercise static stretching has even negative effects on strength, speed, or power performance (16,20,43). On the other hand, it is possible that a long-term flexibility training is beneficial for attenuating eccentric exercise–induced muscle damage. McHugh et al. (34) reported that the subjects whose hamstrings were compliant had significantly less symptoms of muscle damage after eccentric exercise of the knee flexors than those who had stiff hamstrings. If a long-term flexibility training reduces muscle stiffness or increase muscle flexibility, it is possible that the magnitude of eccentric exercise–induced muscle damage is attenuated.

Address for correspondence: Trevor C. Chen, Ph.D., Department of Physical Education, National Chiayi University, 85 Wenlong Village, Minsyong Township, Chiayi County 62103, Taiwan; E-mail: trevorchen@mail.ncyu.edu.tw.
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To the best of our knowledge, only two previous studies (14,28) have examined the effect of flexibility training on eccentric exercise–induced muscle damage. LaRoche and Connolly (28) showed that 4 wk of static or ballistic stretching of the knee flexors performed three times a week increased the range of motion (ROM) of the hip joint but did not affect muscle soreness and stiffness after eccentric exercise. Eston et al. (14) reported that 5 wk of proprioceptive neuromuscular facilitation (PNF) training of the knee flexors performed twice a week increased flexibility and enhanced the recovery of isometric muscle strength at a long muscle length but did not affect muscle soreness and changes in isometric strength at a short muscle length and ROM after eccentric exercise. These findings do not strongly suggest that flexibility training reduces the symptoms of muscle damage and do not necessarily support the findings of McHugh et al. (34) showing that more compliant (flexible) muscles were less susceptible to eccentric exercise–induced muscle damage. It has been documented that a shift of the optimum angle to a longer muscle length attenuates the magnitude of eccentric exercise–induced muscle damage in animal studies (30,31). However, the aforementioned flexibility training studies (14,28) did not report changes in optimum angle of the knee flexors. It may be that if a flexibility training shifts the optimum angle to a longer muscle length, the magnitude of eccentric exercise–induced muscle damage is attenuated.

Some studies have documented that PNF training is more effective in increasing flexibility than static stretching (42,45) and showed that an increase in ROM was greater for PNF than static stretching (18,49). If this is the case, PNF training would shift the optimum angle to a longer muscle length to a greater extent and would attenuate the magnitude of eccentric exercise–induced muscle damage greater compared with static stretch training. Therefore, the purposes of this study were to test the first hypothesis that an 8-wk static stretch and PNF training of the knee flexors would make the muscles less susceptible to maximal eccentric exercise and the second hypothesis that PNF training would be more effective than static stretching in attenuation of muscle damage.

METHODS

Subjects and study design. Thirty young men, who had not performed regular resistance, aerobic, or flexibility training in the past 1 yr and with no previous muscle, joint, or bone injuries of the lower extremities, gave informed consent to participate in the study. The study was approved by the institutional ethics committee and was conducted in conformity with the policy statement regarding the use of human subjects by *Medicine & Sciences in Sports & Exercise®*. Because previous flexibility training studies (14,28) used only men as subjects, and gender difference in the magnitude of eccentric exercise–induced muscle damage might exist as shown in previous studies (17,24,44), only men were recruited in the present study. Their mean ± SD age, height, and body weight of the subjects were 20.8 ± 2.3 yr, 172.7 ± 6.2 cm, and 66.9 ± 7.0 kg, respectively. The subjects were assigned to one of the three groups (n = 10 per group): control (CON), static stretch (SS), or PNF by matching the baseline maximal isokinetic ($60°\cdot s^{-1}$, $1.05\ rad\cdot s^{-1}$) concentric strength of the knee flexors among the groups. Other factors such as pretraining flexibility and the level of physical activities in daily life were not considered for the subject allocation; however, no significant differences in age, height, body mass, concentric strength, and straight-leg-raise ROM at the hip were evident among the groups. The number of subjects was determined by a sample size estimation using the data from previous studies in which the effect of PNF training (14) or static stretching (28) of the knee flexors on eccentric exercise–induced muscle damage was investigated. On the basis of the effect size of 1, α level of 0.05, and a power ($1-\beta$) of 0.80, the minimum number of subjects was calculated for a minimum estimated difference in the knee flexion muscle strength at 3 d after eccentric exercise between SS and control (10%), between PNF and control (10%), and between PNF and SS groups (15%). The estimation showed that 10 subjects per group were necessary.

The subjects in the SS and PNF groups performed an 8-wk static stretching and PNF training program, respectively, three times per week with a minimum of a 24-h interval between sessions under supervision. Both legs received the flexibility training similarly to minimize any muscle imbalances that could cause injuries (14,41); however, only the left leg performed eccentric exercise. The subjects in the CON group were instructed to maintain their normal lifestyle and not to perform any resistance, aerobic, or flexibility training for the 8-wk period and performed the eccentric exercise 8 wk after the baseline measures. All subjects were asked and reminded to refrain from unaccustomed exercise or vigorous physical activity, to maintain their normal diet, and not to take any anti-inflammatory drugs or nutritional supplements such as amino acid or protein during the experimental period. These instructions were included in the information letter, and the investigators asked the subjects to report if they were not able to follow the instructions. As shown in Figure 1, the subjects performed eccentric exercise of the knee flexors 4 d after the last SS or PNF training session or 8 wk after the baseline measures (control). The indirect muscle damage markers (criterion measures) shown below were measured 1 d (i.e., 3 d after flexibility training for the SS and PNF groups) and immediately before eccentric exercise and immediately after and 1–5 d after the exercise. The changes in the criterion measures over time were compared among the groups.

Static stretching. After jogging for 5 min on a treadmill at $6.4\ km\cdot h^{-1}$ with 1% grade, the subjects in the SS group performed a standard static stretching of the hamstrings. Each subject sat on a padded floor while keeping the knee joint of one of his legs straight and the heel of his other leg touching the groin of the extended leg and bent forward at the hip joint while extending the upper back. The investigator pushed the upper back until the point where the subject felt mild discomfort and held there for 30 s, and

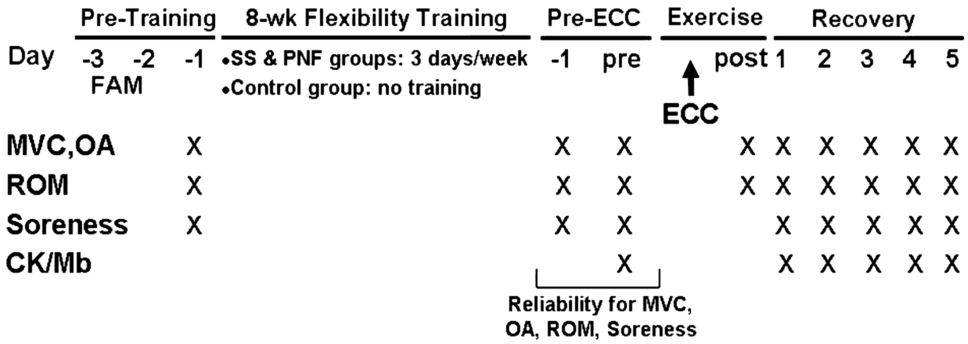

FIGURE 1—Schematic illustration of the study protocol. The study consisted of familiarization session (FAM), baseline measures (1 d before flexibility training), 8-wk flexibility training (SS, PNF), postflexibility training measures, maximal eccentric exercise (ECC), and posteccentric exercise measures. The dependent variables included maximal voluntary concentric strength (MVC), optimun angle (OA), ROM, muscle soreness (soreness), plasma CK activity, and Mb concentration. The time points of these measures are indicated in X (pre, immediately before ECC; post, immediately after ECC).

this was repeated 30 times with a 30-s rest between repetitions (2). Thus, the total stretching time per session was 900 s (15 min).

PNF. The "contract–relax–agonist–contract" PNF stretching protocol, which was similar to that used in a previous study (41), was performed after the 5-min jogging and the five sets of 30-s static stretching of the knee flexors, which were performed in a similar manner to that described in the static stretch section. The static stretch was included before PNF because it is generally included as a warm-up of PNF and was also included in previous studies (14,41). In the "contract–relax–agonist–contract," each subject lay in a supine position, and one investigator moved one leg slowly to a point of mild discomfort while the second investigator kept the other leg on a padded table. The subject was asked to maximally contract the hamstrings isometrically for 10 s at the point against the shoulder of the investigator followed by a 10-s relaxation. After the subject maximally contracted the quadriceps for 5 s, the investigator moved the leg to a further stretched position of the hamstrings and held it there for 10 s. This procedure was carried out two more times. One minute later, the subject sat while fully extending his legs on a table and was asked to flex the trunk maximally forward to reach his toes and to perform a 10-s maximal isometric contraction of the hamstrings against the table, while the investigator supported his lumbar region. After the contraction, the subject flexed his trunk forward by maximally contracting the quadriceps and holding for 10 s. The procedure was carried out two more times with a 30-s rest between repetitions.

Eccentric exercise. All subjects performed six sets of 10 maximal lengthening contractions of the left knee flexors on an isokinetic dynamometer (Biodex System Pro 3; Biodex Medical Systems, Inc., Shirley, NY). The exercise protocol was modified from a previous study (22). Each subject lay prone on the platform of the dynamometer, and the upper and lower back regions and the upper region of the left leg were strapped to the platform. The knee joint of the exercised (left) leg was aligned with the rotation axis of the dynamometer, and the ankle of the leg was strapped to the pad connected to the dynamometer level arm. In this position, a gravity correction for the limb mass was made in accordance with the manufacturer's instructions. The subjects were instructed to contract the knee flexors maximally to resist the knee extending action of the dynamometer that moved the knee joint from a flexed (130°, 2.25 rad) to an extended position (0°) at the angular velocity of 30°·s^{-1}. After each lengthening contraction, the lever arm passively returned the knee joint to the starting position at 10°·s^{-1}, which gave a 13-s rest between contractions. This was repeated 10 times, and a 1-min rest was given between sets to complete six sets. The subjects received strong verbal encouragement during exercise to generate maximal force. Torque and displacement signals of each contraction were saved in a computer (Model P4P800-TAYZ; ASUSTeK Computer, Inc, Taiwan) connected to the isokinetic dynamometer, and peak torque and work (the area under the torque curve) of each contraction were calculated using a software from Biodex Medical Systems (Systems 3).

Criterion measures. Criterion measures consisted of maximal voluntary concentric torque at 60°·s^{-1}, optimum angle, ROM, visual analog scale (VAS) for muscle soreness, plasma creatine kinase (CK) activity, and myoglobin (Mb) concentration. These measures except for CK and Mb were obtained before and 3 d after the last flexibility training session (SS and PNF groups) or 8 wk after the baseline measures (control group), immediately before and after, and at 24-h interval for five consecutive days after eccentric exercise. Plasma CK activity and Mb concentration were measured before and 1–5 d after eccentric exercise (Fig. 1).

Maximal voluntary concentric torque and optimum angle. Maximal voluntary concentric torque and the optimum angle were determined by a modified method of that described in the previous studies (4,13). The measurements were taken at the same position that was described for the eccentric exercise using the same isokinetic dynamometer. Three maximal voluntary concentric knee flexions and extensions were performed continuously at the angular velocity of 60°·s^{-1} (1.05 rad·s^{-1}) for the ROM of 120° (2.08 rad). Each subject was instructed to maximally flex his knee joint from a knee-extended position (0°, 0 rad) to a knee-flexed position (120°, 2.08 rad) and maximally extend his knee joint from the knee-flexed position to the knee-extended position. Strong verbal encouragement was provided during contractions. Torque, position (joint angle), and displacement (ROM) signals of each contraction were saved in a computer connected to the isokinetic dynamometer, and the raw data were filtered and smoothed, then peak torque and the joint angle (optimum angle) at peak torque values during flexion and extension were analyzed using the software from Biodex Medical Systems. Peak torque (highest torque of the smoothed data) and the joint angle (optimum angle) at the peak torque of each trial were obtained, and the average of the three trials was used for subsequent analysis (13).

ROM. A straight-leg-raise ROM test was used to assess the flexibility of the knee flexors based on a previous study (41). It has been reported that the straight-leg-raise ROM is highly correlated with hamstring stiffness (34,35). Each subject lay supine on a padded table, and the subject's knee of the left leg was splinted by a brace (PHC.com, Las Vegas, NV) to prevent bending the knee joint, and the pelvis was strapped to the table to prevent any pelvic rotation or other extraneous movement. A Leighton Flexometer (Leighton, Spokane, WA) was secured to the lateral epicondyle of the femur of the splinted leg. The other leg was held straight by the investigator, whereas the second investigator moved the splinted leg to the position when the subject indicated that the maximum ROM was reached by "stop." The angular displacement from the supine position (0°) to the maximal ROM was measured three times with a 60-s rest between the trials, and the mean value of the three measures was used for further analysis. The same investigator performed all flexibility measurements.

Plasma CK activity and Mb concentration. A 10-mL sample of venous blood was collected by a standard venipuncture technique from the cubital fossa region into a plasma separation tube. The blood was centrifuged for 10 min to obtain plasma, and the plasma samples were stored at −80°C until analysis. Plasma CK activity was determined spectrophotometrically by an automated clinical chemistry analyzer (Model 7080; Hitachi Co., Ltd., Tokyo, Japan) using a test kit (Sigma Diagnostics, St. Louis, MO). Plasma Mb concentration was measured by an automated clinical chemistry analyzer (Model ADVIA-Centaur; Bayer Co., Ltd., Leverkusen, Germany) using a test kit (Denka-Seiken Co., Ltd., Tokyo, Japan). Samples were analyzed in duplicate, and the mean value was used for further analysis. The normal reference ranges for CK and Mb are 38–174 $\text{IU}\cdot\text{L}^{-1}$ and <110 $\mu\text{g}\cdot\text{L}^{-1}$, respectively, based on the manufacturers' information.

Muscle soreness. Muscle soreness was assessed using a VAS consisting of a 100-mm continuous line anchoring "no pain" at one end (0 mm) and "very, very painful" at the other (100 mm) (5). Each subject lay prone on a padded table and asked to indicate the soreness level on the line when an investigator passively extended the knee joint of the left leg from a flexed position (120°) to an extended position (0°).

Test–retest reliability. The test–retest reliability of the criterion measures was examined by an intraclass correlation coefficient (R) using the data taken 1 d and immediately before the maximal eccentric exercise. R values for the concentric strength, optimum angle, ROM, and VAS of muscle soreness were 0.96, 0.88, 0.93, and 1.00, respectively. Coefficient of variation (CV) was also checked for the baseline measures, and the CV for the concentric strength, optimum angle, ROM, and VAS was 7.8%, 11.3%, 6.7%, and 0%, respectively.

Statistical analyses. Data were assessed by the Shapiro–Wilk normality test and the Levene test for the homogeneity of variance assumption before performing an ANOVA. These tests showed that the data of all criterion measures were normally distributed, the cases were independent, and the variance was homogenous. The baseline measures were compared among the groups by a one-way ANOVA. Changes in muscle strength, optimum angle, and ROM before and after the 8-wk flexibility training were compared among the groups by a two-way repeated-measures ANOVA with the Student's *t*-test of Bonferroni adjustment as a *post hoc* test. A Pearson product–moment correlation coefficient (r) was used to determine the relationships between the magnitude of change in knee flexor muscle strength, rightward shift of the optimum angle, and change in ROM from before to after flexibility training. Changes in muscle strength, optimum angle, ROM, muscle soreness, plasma CK activity, and Mb concentration after eccentric exercise were compared among the groups by a two-way repeated-measures ANOVA. When the ANOVA indicated a significant group × time interaction effect, a series of two-way ANOVA was performed to compare between two groups (CON vs SS, CON vs PNF, and SS vs PNF). If a significant interaction effect (groups × time) was observed, a Bonferroni *post hoc* test was conducted to compare between groups for each time point. Using the pooled data of 30 subjects, the relationship between the preeccentric exercise straight-leg-raise ROM or optimum angle of the knee flexors and muscle strength immediately after to 5 d after eccentric exercise, peak plasma CK activity, peak Mb concentration, and peak muscle soreness after the exercise were determined by a Pearson product–moment correlation coefficient. Statistical significance was accepted at $P < 0.05$. For the flexibility training effects, 95% confidence intervals of the criterion measures before and after training were obtained. Data are shown as mean ± SEM, unless otherwise stated.

RESULTS

Effect of flexibility training. Before the flexibility training, no significant differences in ROM, maximal voluntary isokinetic concentric strength of the knee flexors and extensors, and optimal angle were found among the groups (Table 1). After 8 wk of flexibility training, ROM increased significantly for SS (24° ± 3°) and PNF (28° ± 4°) groups. The optimum angle of the knee flexors increased significantly after the flexibility training similarly for the SS (9° ± 2°) and PNF (10° ± 2°) groups. The SS and PNF groups also showed

TABLE 1. Changes (means ± SEM, 95% confidence interval) in passive ROM, maximal voluntary concentric torque of the knee flexors (MVC-Flx) and extensors (MVC-Ext), and optimum angle (OA) before (Pre) and 8 wk after flexibility training (Post) for the control (CON), static stretching (SS), and PNF groups.

	Pre			Post		
	CON	SS	PNF	CON	SS	PNF
ROM (°)	97.6 ± 3.0 (90.4–104.9)	96.1 ± 3.6 (87.3–105.0)	95.5 ± 3.4 (86.3–104.7)	99.0 ± 3.1 (91.5–106.6)	120.1 ± 3.7*† (110.9–129.3)	123.1 ± 3.5*† (114.7–131.4)
MVC-Flx (N·m)	70.4 ± 3.9 (60.4–80.4)	72.7 ± 3.2 (66.7–78.7)	70.8 ± 3.7 (60.8–80.8)	71.0 ± 4.0 (62.1–79.9)	79.0 ± 3.0*† (71.8–86.1)	81.8 ± 3.9*† (72.6–89.0)
MVC-Ext (N·m)	117.1 ± 6.7 (96.9–137.1)	124.9 ± 7.8 (101.8–148.8)	121.9 ± 7.0 (103.3–144.5)	119.3 ± 6.8 (98.6–139.6)	128.7 ± 8.3 (103.9–154.2)	129.3 ± 6.6* (111.7–150.7)
OA (°)	35.6 ± 2.4 (29.5–41.7)	36.5 ± 2.2 (30.8–42.1)	34.1 ± 2.3 (28.3–39.9)	34.5 ± 2.8 (27.2–41.8)	27.9 ± 2.6*† (21.6–34.2)	23.9 ± 2.7*† (17.1–30.7)

* Significant ($P < 0.05$) difference from the Pre value.
† Significant ($P < 0.05$) difference from the CON group.

significant increases in isokinetic concentric strength of the knee flexors (SS = 11% ± 3%, PNF = 16% ± 4%). Only the PNF group showed a significant increase (6% ± 2%) in concentric strength of the knee extensors after flexibility training compared with baseline. No significant difference in the changes in ROM, concentric strength of the knee flexors, or optimal angle was evident between the SS and PNF groups. The control group did not show any significant changes in these variables. When looking at the relationship between the changes in the knee flexors strength, optimum angle, and ROM for the SS and PNF groups separately, a significant correlation was found between the strength and optimum angle changes ($r = 0.65$ for SS, $r = 0.56$ for PNF) and between the optimum angle and ROM ($r = 0.83$ for SS, $r = 0.74$ for PNF), but no significant correlation was evident between the strength and ROM ($r = 0.48$ for SS, $r = 0.35$ for PNF).

Peak torque and work during eccentric exercise. Figure 2A shows the average peak torque of 10 lengthening contractions of each set during six sets. The decrease in peak torque from the first to the last set was significantly greater for the CON group (18% ± 4%) compared with the SS (12% ± 2%) and PNF (10% ± 2%) groups, without a significant difference between the SS and PNF groups. The work during the eccentric exercise in the SS and PNF groups was also significantly greater than that of CON, but no significant difference between the SS and PNF groups was found (Fig. 2B).

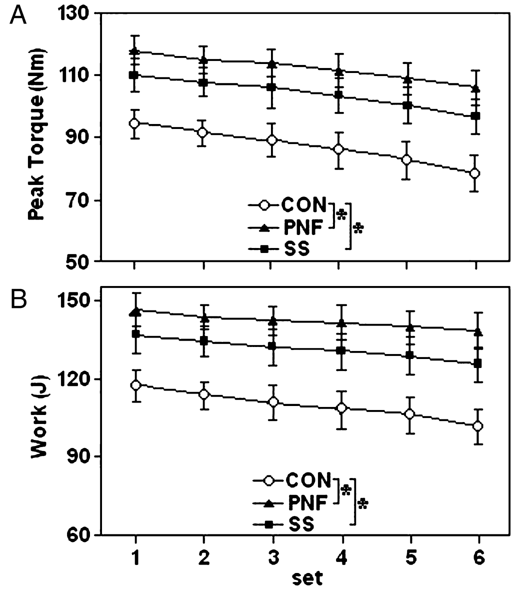

FIGURE 2—Changes (means ± SEM) in peak torque (A) and work (B) during six sets of 10 maximal eccentric contractions of the knee flexors for the PNF, static stretching (SS), and control (CON) groups. *Significantly ($P < 0.05$) different from the CON group.

Effect of flexibility training on muscle damage. After maximal eccentric exercise, significant changes in all criterion measures were seen for all groups; however,

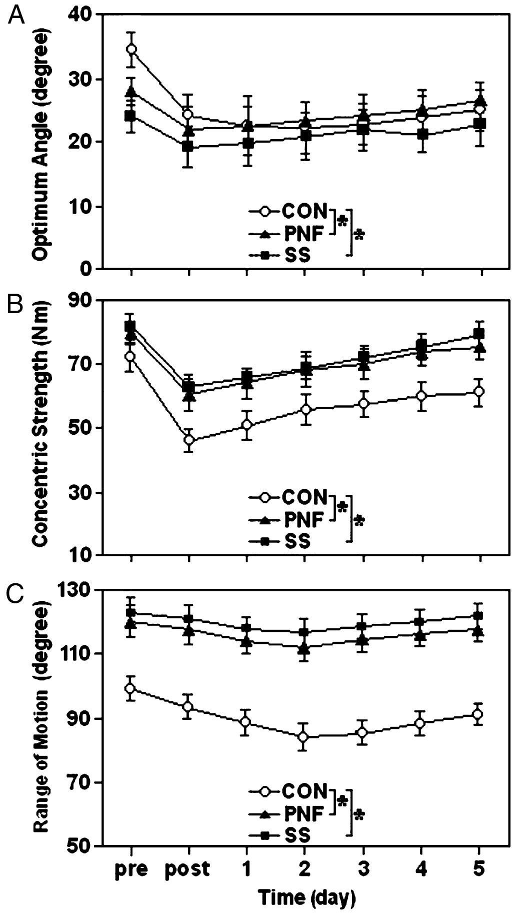

FIGURE 3—Changes (means ± SEM) in optimum angle (A), maximal isokinetic concentric torque of knee flexion at the optimum angle (B), and range of motion at the hip (C) before (pre), immediately after (post), and 1–5 d after maximal eccentric exercise for the control (CON), static stretching (SS), and PNF groups. *Significantly ($P < 0.05$) different from the CON group.

significant differences in the changes were evident between the control and flexibility training (SS and PNF) groups, without significant differences in any of the measures between the SS and PNF groups (Figs. 3 and 4).

Figure 3 presents changes in optimum angle, peak torque at the optimum angle, and ROM. All groups showed a significant decrease in optimum angle after eccentric exercise, indicating a shift toward a longer muscle length. The magnitude of the shift was significantly greater for the CON group (12° ± 4°) compared with the SS (6° ± 2°) and PNF (5° ± 2°) groups (Fig. 3A). All groups showed significant decreases in concentric strength immediately after exercise; however, the changes were significantly greater for the CON group compared with the SS and PNF groups (Fig. 3B). The recovery of concentric strength after eccentric exercise was significantly greater for the PNF and SS groups than that for the CON group such that the concentric strength returned to the baseline by 5 d after exercise for the SS and PNF groups but was still 16% ± 3% lower than the baseline for the CON group.

As shown in Figure 3C, the decrease in ROM after eccentric exercise was significantly greater for the CON group compared with that for the SS and PNF groups. The maximal change in ROM for the CON group (15° ± 4°) was significantly greater than that of the SS (8° ± 3°) and PNF (7° ± 2°) groups. ROM returned to the baseline by 5 d after exercise for the SS and PNF groups; however, ROM was 8° ± 3° smaller from the baseline for the CON group.

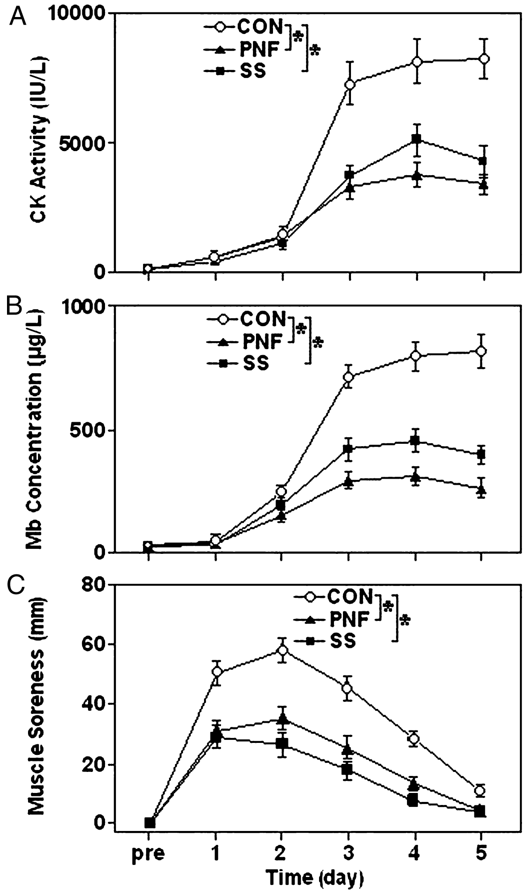

FIGURE 4—Changes (means ± SEM) in plasma CK activity (A), Mb concentration (B), and muscle soreness (C) before (pre) and 1–5 d after maximal eccentric exercise for the control (CON), static stretching (SS), and PNF groups. *Significantly (*P* < 0.05) different from the CON group.

Figure 4 shows changes in plasma CK activity, Mb concentration, and muscle soreness. All groups showed significant increases in plasma CK activity and Mb concentration; however, the increases were significantly smaller for the SS and PNF groups compared with the CON group (Figs. 4A and B). Muscle soreness developed for all groups after eccentric exercise; however, the SS and PNF groups showed significantly smaller values compared with the CON group (Fig. 4C).

Correlation between flexibility and muscle damage indices. The preeccentric exercise flexibility was significantly correlated with the magnitude of change in the markers of muscle damage. Both ROM and optimum angle (OA) were correlated ($P < 0.01$) with the magnitude of decrease in maximal voluntary concentric strength (MVC) immediately after exercise (Figs. 5A and B), 1 d (ROM: $r = 0.65$, OA: $r = -0.69$), 2 d ($r = 0.66$, $r = -0.62$), 3 d ($r = 0.59$, $r = -0.63$), 4 d ($r = 0.65$, $r = -0.62$), and 5 d (Figs. 5C and D) after exercise. A high correlation ($P < 0.01$) was also found between ROM or optimum angle and peak CK activity (Figs. 5E, F) or peak Mb concentration (ROM: $r = -0.79$, OA: $r = 0.55$). Although significant, the correlation between ROM or optimum angle and peak muscle soreness was not high (Figs. 5G and H). The results indicate that the greater the preeccentric exercise ROM and the smaller the preeccentric exercise optimum angle (i.e., optimum angle at a longer muscle length), the less the magnitude of muscle damage induced by the eccentric exercise.

DISCUSSION

The present study was the first to show that the SS and PNF training shifted the optimum angle of the knee flexors to a longer muscle length, together with increases in ROM and concentric strength of the knee flexors (Table 1). Both training attenuated the changes in ROM, concentric strength, optimal angle, muscle soreness, plasma CK activity, and Mb concentration after maximal eccentric exercise, without significant differences between SS and PNF groups (Figs. 3 and 4). These results strongly support the first hypothesis that both flexibility training protocols were effective in attenuating eccentric exercise–induced muscle damage but do not support the second hypothesis that the protective effect would be greater for PNF than SS training. The correlation analyses (Fig. 5) clearly revealed that flexible muscles are less susceptible to eccentric exercise–induced muscle damage, supporting the previous report by McHugh et al. (34).

It is important to note that the present study investigated the effect of an 8-wk static stretching or PNF training on one bout of maximal eccentric exercise, and an acute effect of

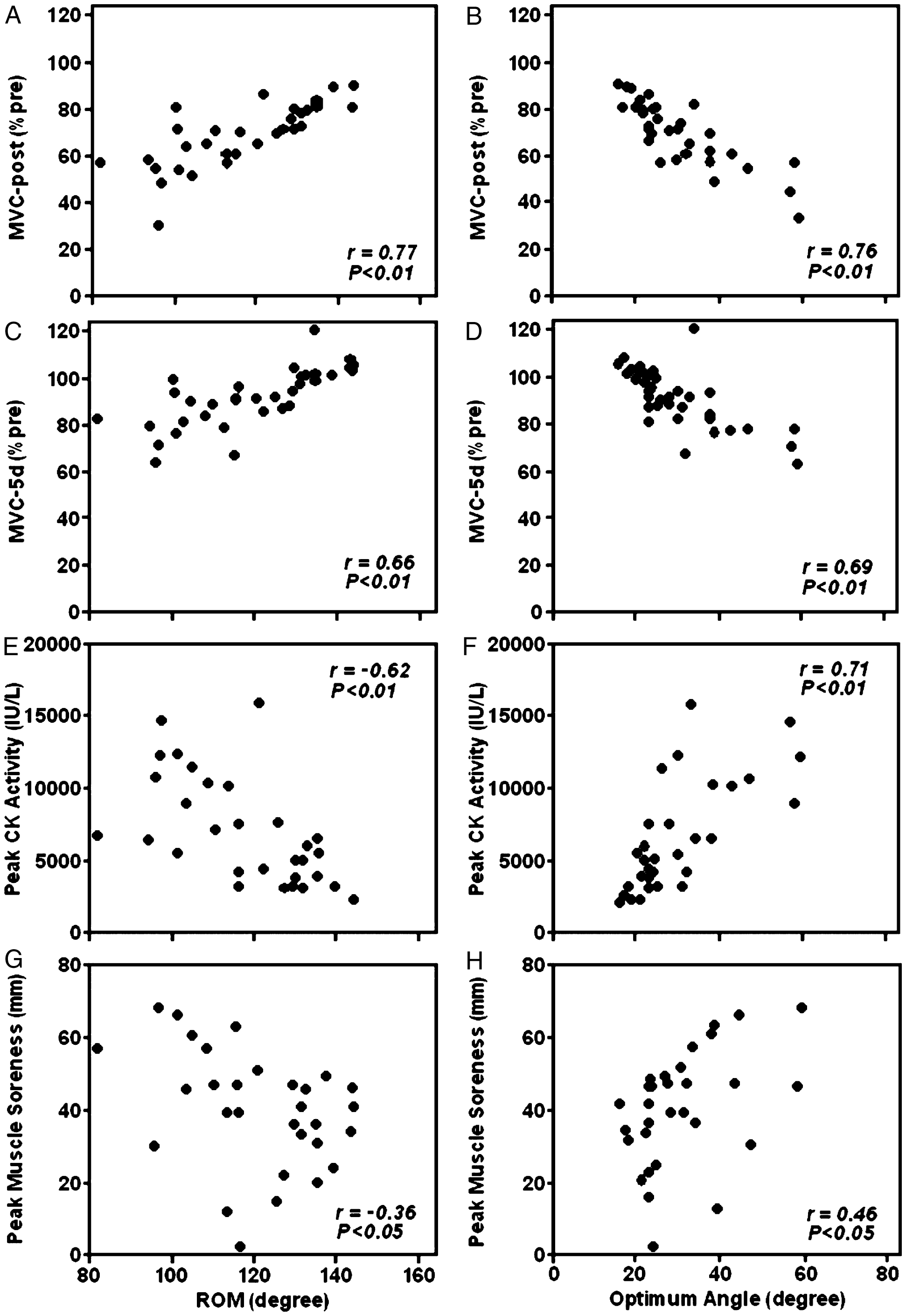

FIGURE 5—Correlations between preeccentric exercise ROM of the hip joint or optimum angle of the knee flexors and percent changes in maximal concentric strength (MVC) immediately after (A, B) and 5 d after exercise (C, D), peak CK activity (E, F), and peak muscle soreness (G, H) based on pooled data from the three groups (n = 30).

stretching on muscle function and on muscle damage should be considered separately from the findings of the present study. In fact, it has been shown that preexercise static stretching has negative effect on muscle function (16,20,43) and no effect on eccentric exercise–induced muscle damage (21,25,40,47). As shown in Table 1, both SS and PNF training in the present study were effective in improving flexibility such that the ROM increased more than 20° and the optimal angle shifted to a long muscle length. In addition, the flexibility training increased the knee flexor muscle strength more than 10% (Table 1). Previous studies (19,27,48) also reported significant increases in ROM and muscle strength after flexibility training of the knee flexors. For example, Worrell et al. (48) reported that static stretching and PNF training of the knee flexors for 3 wk (5 d·wk^{-1}) increased ROM by 8°–10° and knee flexor strength by 10%.

APPLIED SCIENCES

It may be that the longer (3 vs 8 wk) training period and greater number of sessions in the training (15 vs 24 sessions) in the present study resulted in greater increases in ROM compared with the previous study (48). It seems that the isometric contractions of the knee flexors performed during the flexibility training were responsible for the increases in the knee flexor strength of both SS and PNF groups. A significant increase in knee extensor strength was evident only for the PNF group (Table 1), and this appeared to be due to the concentric contractions of the knee extensors (six maximal contractions per session) performed in the PNF. The increases in knee flexor strength are reflected in the greater force production and work during the eccentric exercise in the PNF and SS groups compared with the control group (Fig. 2).

Alonso et al. (1) reported that no significant difference in peak isometric torque of knee flexors was found between less flexible and "normally" flexible subjects, but the knee joint angle to generate the peak torque was larger (i.e., optimum muscle length was shorter) in the less flexible subjects compared with the normal subjects. The present study found a significant correlation between the increase in knee flexors strength and the shift of the optimum angle to a longer muscle length after the static stretching and PNF training. It may be that increases in muscle strength are associated with the shift of the optimum angle (i.e., an increase in sarcomere numbers in series). The changes in the optimum angle and ROM after flexibility training were significantly correlated, but the changes in strength and ROM were not significantly correlated in the present study. It seems that the increases in ROM were associated with the shift of the optimum angle to a longer muscle length. No significant correlation between the strength and ROM may be related to the different movement of the knee flexors during measurements; the knee joint was flexed in the strength measure but was extended in the ROM measure.

The present study showed a significant shift (~10°) of the optimum angle toward a longer muscle length after both SS and PNF training (Table 1). It was hypothesized that the shift of the optimum angle would be greater for the PNF training than SS training, because isometric contractions at a longer muscle length, which have been shown to shift the optimum angle to a longer muscle length (38), were only performed in the PNF training. However, the SS group also showed a similar change in optimum angle to that of the PNF group (Table 1). Ferreira et al. (15) reported a shift of the optimum angle of the knee flexors to a long muscle length (4°) after a 6-wk static stretching training (five sessions per week). Animal studies (11,12) showed that static stretching (3 $\mathrm{d \cdot wk^{-1}}$, 40 $\mathrm{min \cdot d^{-1}}$) during a 3-wk period of the latissimus dorsi or soleus muscles resulted in a 4%–25% increase in the number of sarcomeres in series. These suggest that static stretching shifts the optimum angle to a longer muscle length by increasing the sarcomere number in series (4,39); however, further human studies are necessary to investigate the relationship between optimal angle shift and sarcomerogenesis in flexibility training. It should be noted that static stretching was included in the PNF in the present study, although the volume of the static stretching in the PNF (5 sets of 30 s per session) was less than 20% of that in the static stretching (30 sets of 30 s per session). It might be that static stretching in the PNF played a major role in the shift of the optimal angle to a longer muscle length. However, Handel et al. (19) reported that a contract–relax PNF training (10 $\mathrm{min \cdot d^{-1}}$, 3 $\mathrm{d \cdot wk^{-1}}$, 8 wk) shifted the optimum angle of the knee flexors to a longer muscle length, although the magnitude of the shift (4°) was smaller than that of the present study. Thus, it seems likely that the shift of the optimum angle after PNF training shown in the present study was the combination effects of the static stretching and the PNF-specific protocols.

The most striking finding of the present study was that the changes in the criterion measures after eccentric exercise were significantly smaller for the PNF and SS groups compared with the control group as shown in Figures 3 and 4. The changes in muscle strength and muscle soreness after eccentric exercise of the control group were similar to those reported in a previous study (14) in which a similar maximal eccentric exercise of the knee flexors to that of the present study was performed. Thus, it seems reasonable to state that the PNF and SS groups had attenuated responses to the eccentric exercise. It should be noted that the force and work during the eccentric exercise were greater for the PNF and SS groups compared with the control group (Fig. 2). Because muscle damage is generally greater with greater force production during eccentric contractions (32,46), the magnitude of muscle damage should have been greater for the PNF and SS groups than that for the control group if the flexibility training induced no protective effect; however, the opposite was found. This strongly suggests that the flexibility training provided prophylactic effects on eccentric exercise–induced muscle damage. In contrast, previous studies (14,28) did not find strong protective effects of flexibility training (4–5 wk) on muscle damage induced by eccentric exercise of the knee flexors, despite significant increases in the flexibility after the training. LaRoche and Connolly (28) found a 10% increase in ROM, and Eston et al. (14) reported a 14% increase in sit-and-reach test. It is possible that the duration of the flexibility training and the total time that the muscles under stretching were not long enough to produce a protective effect against eccentric exercise–induced muscle damage in the previous studies (14,28). The similar protective effects conferred by SS and PNF training are likely to be explained by the similar changes in ROM, optimum angle, and muscle strength of the knee flexors between the two flexibility training protocols. The magnitude of the shift of the optimum angle after the flexibility training of the present study (~10°) was comparable to the difference in the optimum angle (~10°) between the normal group and the group with less hamstring flexibility shown in a previous study (1).

It is important to note that significant correlations between preeccentric exercise ROM/optimum angle and changes in muscle damage indices were evident, especially for MVC, peak CK activity, and Mb concentration (Fig. 5). This suggests that the greater the ROM or the longer the optimum muscle length, the less the muscle damage developed after eccentric exercise. This supports the previous study by McHugh et al. (34), reporting that subjects who had greater hamstring flexibility were less susceptible to eccentric exercise–induced muscle damage of the hamstrings. Thus, it seems reasonable to assume that flexibility of the muscle is a factor determining the magnitude of eccentric exercise–induced muscle damage. It may be that a large variability in the magnitude of muscle damage among subjects reported in previous studies (e.g., Clarkson et al. [8] and Nosaka et al. [36]) can be, at least partially, explained by a difference in muscle flexibility or optimum angle of the muscle.

The mechanisms by which a flexibility training confers the protective effect against the subsequent bout of maximal eccentric exercise are not clear, but some speculations can be made. As mentioned previously, McHugh et al. (34) showed that compliant hamstrings were less susceptible to muscle damage after eccentric exercise of the knee flexors and postulated that the tendon–aponeurosis complex of compliant muscles can absorb lengthening and limit muscle strain. Because no measures of the tendon or muscle–tendon unit were made in the present study, further studies are necessary to investigate the changes in muscle–tendon behavior during eccentric contractions after static stretching and PNF training in relation to muscle damage. An increase in sarcomere number in series might also be related to the protective effect conferred by the flexibility training. When the sarcomere number in series increases, it is likely that the tension across the muscle during eccentric contractions decreases, which could attenuate the severity of muscle damage (33). Proske and Morgan (39) stated that a shift of the optimum angle to a longer muscle length could represent an increase in the number of sarcomeres in series. In the present study, the optimum angle of the knee flexors after flexibility training shifted to a long muscle length by 10° (Table 1), suggesting that the flexibility training increased the number of sarcomeres. Further studies are necessary to investigate changes in sarcomere number in series after flexibility training in humans and how this affects the sarcomere behavior during eccentric contractions. Moreover, Lavender and Nosaka (29) reported that eccentric exercise with a light dumbbell (10% of MVC), which did not induce significant changes in any of the indirect markers of muscle damage, attenuated decreases in isometric strength and ROM and increases in muscle soreness after a subsequent bout of eccentric exercise with a heavier (40% of MVC) dumbbell performed 2 d later. This type of muscle adaptation is often called the repeated bout effect (7,8,33,37). An animal study (26) reported that a nondamaging passive stretch and isometric exercise produced a protective effect against eccentric exercise. Although muscle damage markers were not measured during the flexibility training in the present study, it seems unlikely that the flexibility training induced muscle damage. It is possible that the flexibility training provided the effects similar to the repeated bout effect.

In summary, the present study showed that both the 8-wk PNF training and static stretching shifted the optimum angle to a longer muscle length, and increased the flexibility and strength of the hamstrings, and similarly attenuated muscle damage induced by maximal eccentric exercise of the knee flexors. The protective effect conferred by the flexibility training is likely to be associated with the addition of sarcomeres and/or changes in tendon compliance, but these speculations should be investigated further. Because it has been reported that men and women respond differently to eccentric exercise–induced muscle damage (17,24,44), and a difference in flexibility exists between sexes (3), it is necessary to investigate women in a similar experimental setting to that of the present study. It is also important to note that the present study used subjects who did not participate in any specific training or sports to minimize confounding factors, therefore caution is required when applying the findings of the present study to a practical situation. It is also of concern how the beneficial effect of the flexibility training on muscle damage affects strength, speed, or power performance. It has been reported that preexercise static stretching has negative effects on power, speed, and strength performance, but dynamic stretching did not affect such performance (16,20,43). It is interesting to examine how a combination of flexibility training and preexercise stretching affects eccentric exercise–induced muscle damage, or whether preexercise dynamic stretching is effective for prevention of muscle damage. In conclusion, flexibility training is effective in not only improving flexibility and strength but also attenuating the extent of eccentric exercise–induced muscle damage, and flexible muscles are less susceptible to the damage.

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REFERENCES

1. Alonso J, McHugh MP, Mullaney MJ, Tyler TF. Effect of hamstring flexibility on isometric knee flexion angle–torque relationship. *Scand J Med Sci Sports*. 2009;19(2):252–6.
2. Alter MJ. *Sport Stretch*. 2nd ed. Champaign (IL): Human Kinetics; 1998. p. 106.
3. Blackburn JT, Riemann BL, Padua DA, Guskiewicz KM. Sex comparison of extensibility, passive, and active stiffness of the knee flexors. *Clin Biomech*. 2004;19(1):36–43.
4. Brockett CL, Morgan DL, Proske U. Human hamstring muscles adapt to eccentric exercise by changing optimum length. *Med Sci Sports Exerc*. 2001;33(5):783–90.
5. Chen TC, Chen HL, Lin MJ, Wu CJ, Nosaka K. Muscle damage responses of the elbow flexors to four maximal eccentric exercise bouts performed every 4 weeks. *Eur J Appl Physiol*. 2009;106(2):267–75.
6. Cheung K, Hume PA, Maxwell L. Delayed onset muscle soreness:

treatment strategies and performance factors. *Sports Med*. 2003; 33(2):145–64.

7. Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil*. 2002;81(11):S52–69.
8. Clarkson PM, Nosaka K, Braun B. Muscle function after exercise-induced muscle damage and rapid adaptation. *Med Sci Sports Exerc*. 1992;24(5):512–20.
9. Cleak MJ, Eston RG. Delayed onset muscle soreness: mechanisms and management. *J Sports Sci*. 1992;10(4):325–41.
10. Connolly DAJ, Sayers SP, McHugh MP. Treatment and prevention of delayed onset muscle soreness. *J Strength Cond Res*. 2003; 17(1):197–208.
11. Coutinho EL, Gomes AR, Franca CN, Oishi J, Salvini TF. Effect of passive stretching on the immobilized soleus muscle fiber morphology. *Braz J Med Biol Res*. 2004;37(12):1853–61.
12. Cox VM, Williams PE, Wright H, et al. Growth induced by incremental static stretch in adult rabbit latissimus dorsi muscle. *Exp Physiol*. 2000;85(2):193–202.
13. Cramer JT, Beck TW, Housh TJ, et al. Acute effects of static stretching on characteristics of the isokinetic angle–torque relationship, surface electromyography, and mechanomyography. *J Sports Sci*. 2007;25(6):687–98.
14. Eston RG, Rowlands AV, Coulton D, McKinney J, Gleeson NP. Effect of flexibility training on symptoms of exercise-induced muscle damage: a preliminary study. *J Exerc Sci Fit*. 2007;5(1):33–9.
15. Ferreira GNT, Teixeira-Salmela LF, Guimarães CQ. Gains in flexibility related to measures of muscular performance: impact of flexibility on muscular performance. *Clin J Sport Med*. 2007;17(4): 276–81.
16. Fletcher IM, Anness R. The acute effects of combined static and dynamic stretch protocols on fifty-meter sprint performance in track-and-field athletes. *J Strength Cond Res*. 2007;21(3):784–7.
17. Fredsted A, Clausen T, Overgaard K. Effects of step exercise on muscle damage and muscle Ca^{2+} content in men and women. *J Strength Cond Res*. 2008;22(4):1136–46.
18. Funk DC, Swank AM, Mikla BM, Fagan TA, Farr BK. Impact of prior exercise on hamstring flexibility: a comparison of proprioceptive neuromuscular facilitation and static stretching. *J Strength Cond Res*. 2003;17(3):489–92.
19. Handel M, Horstmann T, Dickhuth HH, Gülch RW. Effects of contract–relax stretching training on muscle performance in athletes. *Eur J Appl Physiol*. 1997;76(5):400–8.
20. Herda TJ, Cramer JT, Ryan ED, McHugh MP, Stout JR. Acute effects of static versus dynamic stretching on isometric peak torque, electromyography, and mechanomyography of the biceps femoris muscle. *J Strength Cond Res*. 2008;22(3):809–17.
21. High DM, Howley ET. The effects of static stretching and warm up on prevention of delayed onset muscle soreness. *Res Q Exerc Sport*. 1989;60(4):357–61.
22. Hilbert JE, Sforzo GA, Swensen T. The effects of massage on delayed onset muscle soreness. *Br J Sports Med*. 2003;37(1):72–5.
23. Howatson G, van Someren KA. The prevention and treatment of exercise-induced muscle damage. *Sports Med*. 2008;38(6):483–503.
24. Hubal MJ, Rubinstein SR, Clarkson PM. Muscle function in men and women during maximal eccentric exercise. *J Strength Cond Res*. 2008;22(4):1332–8.
25. Johansson PH, Lindstrom L, Sundelin G, Lindström B. The effects of preexercise stretching on muscle soreness, tenderness and force loss following heavy eccentric exercise. *Scand J Med Sci Sports*. 1999;9(4):219–25.
26. Koh TJ, Brooks SV. Lengthening contractions are not required to induce protection from contraction-induced muscle injury. *Am J Physiol Regul Integr Comp Physiol*. 2001;281(1):R155–61.
27. Kokkonen J, Nelson AG, Eldredge C, Winchester JB. Chronic static stretching improves exercise performance. *Med Sci Sports Exerc*. 2007;39(10):1825–31.
28. LaRoche DP, Connolly DAJ. Effects of stretching on passive muscle tension and response to eccentric exercise. *Am J Sports Med*. 2006;34(6):1000–7.
29. Lavender AP, Nosaka K. A light load eccentric exercise confers protection against a subsequent bout of more demanding eccentric exercise. *J Sci Med Sport*. 2008;11(3):291–8.
30. Lynn R, Morgan DL. Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *J Appl Physiol*. 1994;77(3):1439–44.
31. Lynn R, Talbot JA, Morgan DL. Differences in rat skeletal muscles after incline and decline running. *J Appl Physiol*. 1998;85(1):98–104.
32. McCully KK, Faulkner JA. Characteristics of lengthening contractions associated with injury to skeletal muscle fibers. *J Appl Physiol*. 1986;61(1):293–9.
33. McHugh MP, Connolly DAJ, Eston RG, Gleim GW. Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Med*. 1999;27(3):157–70.
34. McHugh MP, Connolly DAJ, Eston RG, Kremenic IJ, Nicholas SJ, Gleim GW. The role of passive muscle stiffness in symptoms of exercise-induced muscle damage. *Am J Sports Med*. 1999;27(5): 594–9.
35. McHugh MP, Kremenic IJ, Fox MB, Gleim GW. The role of mechanical and neural restraints to joint range of motion during passive stretch. *Med Sci Sports Exerc*. 1998;30(6):928–32.
36. Nosaka K, Newton M, Sacco P. Delayed onset muscle soreness does not reflect the magnitude of eccentric exercise–induced muscle damage. *Scand J Med Sci Sports*. 2002;12(6):337–46.
37. Nosaka K, Sakamoto K, Newton M, Sacco P. How long does the protective effect on eccentric exercise–induced muscle damage last? *Med Sci Sports Exerc*. 2001;33(9):1490–5.
38. Philippou A, Bogdanis GC, Nevill AM, Maridaki M. Changes in the angle–force curve of human elbow flexors following eccentric and isometric exercise. *Eur J Appl Physiol*. 2004;93(1–2):237–44.
39. Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol*. 2001;537(Pt 2):333–45.
40. Rodenburg JB, Steenbeek D, Schiereck P, Bär PR. Warm-up, stretching and massage diminish harmful effects of eccentric exercise. *Int J Sports Med*. 1994;15(7):414–9.
41. Rowlands AV, Marginson VF, Lee J. Chronic flexibility gains: effect of isometric contraction duration during proprioceptive neuromuscular facilitation stretching techniques. *Res Q Exerc Sport*. 2003;74(1):47–51.
42. Rubini EC, Costa ALL, Gomes PSC. The effects of stretching on strength performance. *Sports Med*. 2007;37(3):213–24.
43. Samuel MN, Holcomb WR, Guadagnoli MA, Rubley MD, Wallmann H. Acute effects of static and ballistic stretching on measures of strength and power. *J Strength Cond Res*. 2008; 22(5):1422–8.
44. Sewright KA, Hubal MJ, Kearns A, Holbrook MT, Clarkson PM. Sex differences in response to maximal eccentric exercise. *Med Sci Sports Exerc*. 2008;40(2):242–51.
45. Sharman MJ, Cresswell AG, Riek S. Proprioceptive neuromuscular facilitation stretching: mechanisms and clinical implications. *Sports Med*. 2006;36(11):929–39.
46. Warren GL, Hayes DA, Lowe DA, Prior BM, Armstrong RB. Materials fatigue initiates eccentric contraction-induced injury in rat soleus muscle. *J Physiol*. 1993;464:477–89.
47. Wessel J, Wan A. Effect of stretching on the intensity of delayed-onset muscle soreness. *Clin J Sport Med*. 1994;4(2):83–7.
48. Worrell TW, Smith TL, Winegardner J. Effect of hamstring stretching on hamstring muscle performance. *J Orthop Sports Phys Ther*. 1994;20(3):154–9.
49. Yuktasir B, Kaya F. Investigation into the long-term effects of static and PNF stretching exercises on range of motion and jump performance. *J Bodyw Mov Ther*. 2009;13(1):11–21.