The Effects of a Combination Treatment (PNF Stretching "Pre-exercise", Ice Massage Plus Static Stretching-30s "Post-exercise) on Markers of Exercise-Induced Muscle Damage

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Abstract: Context: Numerous recovery strategies have been used in an attempt to minimize the symptoms of delayed-onset muscle soreness (DOMS). However, scientific evidence to support the protective effects of a Combination Treatment on muscle damage is lacking. Objective: To investigate the effects of a Combination Treatment on biochemical markers (enzymatic levels) and functional (elbow angel, arm circumference, pain rate …) of Exercise-induced muscle damage. Design: Randomized controlled trial. Setting: University laboratory. Participants: non-Athletic college-age men participated voluntary in this study, which reported no delayed onset muscle soreness for at least 6 months before then subjects were randomly assigned to subgroups with control and experimental hands. Intervention(s): Exercise program was used for induce Exercise-induced muscle damage involved Predcher curl -testing (eccentric contraction in two hands). Main Outcome Measure(s): Relaxed arm circumference, flexed arm circumference, elbow resting angel, forearm circumference, range of motion flexed elbow, range of motion extended elbow, Exercise-induced muscle damage, Maximal voluntary isometric and isokinetic strength were recorded at baseline, immediately after exercise, and at 24, 48( and 72,96) hours after postexercise. Serum creatine kinase was measured at baseline, immediately after exercise, and at 24, 48(and 72, 96) hours after postexercise. Results: No significant difference occurred in relaxed arm circumference, flexed arm circumference, elbow resting angel, forearm circumference, Maximal voluntary isometric and isokinetic strength, delayed onset of muscle soreness and Pain intensity rate between groups(control hand and experimental hand) in intervention (P <.05). The result of Combination Treatment during timing on muscle soreness (at 48 hours after induce DOMs) not effective. Significant difference was observed between hands in range of motion flexed elbow in this Combination Treatmen ts (P <.05). Significant difference was observed between hands in range of motion extended elbow in this Combination Treatments (P <.05). Conclusion: This Combination Treatment on elbow flexion, range of motion extended elbow during timing was effective. Eventually, results suggest that combination treatments are effective treatment on decreased flexibility and Range of Motion due to Exercise-induced muscle damage.

Key words: Exercise-induced muscle damage (EIMD); PNF (proprioceptive neuromuscular facilitation), therapeutic massage; static stretching; cryotherapy.

INTRODUCTION

Exercise-induced muscle damage (EIMD) is the common muscle discomfort experienced when a new physical activity is initiated. EIMD is characterized by (a) a loss of muscle strength immediately after exercise, (b) muscle soreness that peaks within 24 to 48 hours post exercise and lasts 5 to 7 days, (c) swelling (increased limb girth) and pain of the damaged muscle, and (d) increased concentrations of intramuscular protein in serum (creatine kinase) that peak 5 to 7 days post exercise (Clarkson et al. 2002). During the symptomatic period (usually 5-7 days), motor control, flexibility, and task performance (reduced torque production and range of motion) may be impaired (McHugh et al. 2000; Proske and Morgan, 2001; Nosaka and Newton, 2002). For previously sedentary trainees and those who are physically unconditioned, these symptoms might be somewhat more severe and last longer. EIMD often results from unfamiliar, predominantly eccentric (i.e., muscle-lengthening) activities and exercise, such as walking or running downhill or performing squatting exercises (Connolly et al.,2003). Muscle injury entails a mechanical disruption of the sarcomeres (Friden et al., 1983; Friden et al., 1992) and soreness results from the inflammatory response of prostaglandin and leukotriene synthesis (Smith et al., 1991). Blood creatine kinase levels are elevated with delayed onset muscle soreness, indicating disruption or permeability changes in the plasma membrane. Compared with muscle strains in which the muscle injury and resulting tenderness are isolated in a specific part of the muscle EIMD is patchy...
throughout the muscle, and the degree of tenderness varies within the muscle. Swelling results from the movement of cells and fluid from the bloodstream into the interstitial spaces, and it contributes to the pain (Connolly et al., 2003; Smith et al., 1991; Warren et al., 1993). Many researchers have examined various ideas about intervention to reduce or prevent the severity of this kind of muscle damage. From a clinical perspective, preventative intervention is preferable and more important because it reduces the cost of treatment, time lost from training or rehabilitation, and the probability of persisting further injury. It also allows the continuation of exercise and competition (O’Connor and Hurley, 2003; Weerapong et al., 2004). A number of investigations have examined interventions in an attempt to reduce the detrimental effects of EIMD (Eston and Peters, 1999; Howatson et al., 2005; Knitter et al., 2000; Lanier, 2003; Zaimuddin et al., 2005), which have provided equivocal results with regard to their efficacy. Some useful methods to prevent musculoskeletal injuries include traditional interventions such as therapeutic exercise. In clinical practice, for example, we usually do stretching before the exercise as a preventative technique of muscle damage. It has been thought that the compliant muscle can be stretched further before it is damaged during eccentric exercise (Noonan et al., 1993; Safran et al., 1988) or that stretching could reduce muscle spasm after unaccustomed exercise (Herbert and deNoronha, 2007). Studies however have not shown the effectiveness of static stretching in the prevention of EIMD (High et al., 1989; Johansson et al., 1999). It is well-known that eccentric work causes Exercise-induced muscle damage. It is for this reason that experiments relating to muscle pain are designed with this type of work. Some authors have tested the effect of stretching previous to an effort; others after the effort and finally other authors during exercise.

Pre-Exercise Stretching:

Johansson et al., (1999) studied the effect on the occurrence of EIMD of 4 stretching exercises of 20 seconds on hamstrings of one leg only before an eccentric training. No difference was noted between the stretched leg during warm-up and the control leg. In another experiment, Wessel and Wan (1994) also noted the inefficiency of stretching before exercise.

Post-Exercise Stretching:

After a 30 minutes training session containing eccentric exercises on quadriceps and triceps muscles, Buroker et al., (1989) studied a group of athletes that performed static stretching. No pain attenuation was noted in the 3 days following the session when compared to the control group. They also observed an increase in CK (Creatin Kinase) and a decrease of force in painful thighs. They concluded that stretching had no effect on DOMS. In a second experiment, Wessel and Wan (1994) also tested the effect of stretching after exercises. No significant cant effect was found.

Stretching During Exercise:

In a study by Wieman et al., (2000), athletes were performing, during training sessions of force, passive stretching on one leg only. This has resulted in a more painful stretched limb than the unstretched one. Passive stretching adds microtraumatisms to the eccentric effort and worsens the myolysis. However, Howatson and van Someren (2008) highlighted that other as yet uninvestigated stretching protocols, for example proprioceptive neuromuscular facilitation (PNF) may be of benefit, and therefore present a direction for further research. PNF stretching techniques are commonly used in the athletic and clinical settings to enhance both active and passive range of motions with a view to optimizing motor performance and rehabilitation. PNF stretching is one of the most effective stretching techniques which has been claimed to increase muscle flexibility (Sharman et al., 2006; Spernoga et al., 2001). There was also a report using a single set of PNF stretching which demonstrated a significant increase in flexibility (Spernoga et al., 2001).Improving tissue flexibility has also been mentioned as a means to reduce the risk of soft tissue injury and prevent muscle damage (O’Connor and Hurley, 2003; Weerapong et al., 2004). However, PNF technique has not yet been evaluated in patients with EIMD and it may prove that the use of PNF could have some potential benefit as a prophylactic effect. Rodenburg et al., (1994) reported that an intervention involving warm up, stretching and massage produced a significant reduction in muscle soreness.

Ice Massage:

Ice Massage is easy to apply, provides cooling of superficial and deep tissues from a relatively short application period when compared with some other methods (Meeusen and Lievens, 1986). Cryotherapy is documented to constrict capillaries, reduce capillary permeability and blood flow (Meeusen and Lievens, 1986) thereby attenuating swelling and the inflammatory response (Smith, 1991) which may reduce the negative effects associated with damaging exercise. Gulick et al., (1996) found that a single treatment with ice massage had an immediate but short-term beneficial effect on muscle soreness following eccentric exercise. In contrast, Yackzan et al., (1984) reported a single treatment of ice massage administered either immediately after, 24 or 48 h post-exercise to be ineffective in reducing muscle soreness. Although repeated administration of cryotherapy
is advocated following muscle tissue trauma (Knight, 1995) the majority of studies using ice massage have not followed this advised practice. Both Eston and Peters (1999) and Howatson and van Someren (2003) reported that repeated cold-water immersion and ice massage, respectively, following eccentric exercise were effective in reducing plasma CK concentrations, although no effect on perceived soreness or strength were found. However, other authors (Isabell et al., 1992) suggested that ice massage administered repeatedly over a 96 h period post-exercise may be contraindicated in the treatment of exercise-induced muscle damage. It is clear therefore, that current literature addressing the treatment of exercise-induced muscle damage with ice massage is limited and equivocal. This study investigated the effects of a combination of interventions, so it could not provide an estimate of the specific effect of the stretching, in isolation from the other methods employed. Limited research has evaluated the impact of Combination Treatments with and various variables on markers of Exercise-induced muscle damage. Therefore the present research attempted to evaluation the effects of a Combination Treatment on markers and determines its prophylactic (prior to exercise) and therapeutic (post-exercise) effects.

MATERIALS AND METHODS

Participants:
Participation was voluntary and the subjects were recruited by verbal announcements in the University of Azad and physical education classes. The subjects (mean ± s age 22.62 ± 1.99 years; height 173.50 ± 3.09 m and body mass 71.250 ± 10.28 kg) had no prior history of musculoskeletal injury to the upper extremity, full pain-free flexion/extension range of motion of the shoulder and elbow joints and had not been involved in any type of upper body resistance training or extensive physical activity in the past six months.

Procedure:
After careful standard medical examinations and physiotherapeutic screening by the physician, and after the first measurements had been performed, the subjects (n=16) were divided into subgroups with control and experimental hands. Clinical assessment indicates that the subjects are suitable for active exercises. The exponential subgroups (experimental hands) received an exercise treatment and the control subgroups did not. They were also required to estimate at before-exercise, 24, 48, and 72-96 hour post exercise for measurement of the dependent variables. Changes in the measures over time were compared between experimental and control subgroups.

Dependent variables consisted of maximal voluntary, Isometric and isokinetic elbow flexor strength, Creatine kinase activity, perceived muscle soreness rating, Range of motion, Swelling.

The University’s Research Ethics Committee in accordance with the Helsinki Declaration approved all procedures prior to the start of the investigation; all volunteers completed a medical screening questionnaire and provided written informed consent prior to participation.

Induction of Muscle Damage:
Muscle damage was induced through Predcher curl -testing. The subjects performed 50 eccentric contractions (60% maximal eccentric contraction) with both hand. The eccentric contractions were 3 second; each contraction was separated by 10 second rest.

Treatments:
The present study is a clinical and pseudo-experimental one with exercise model of an intra-subject design. Including subgroups with the control and experimental hands. The experimental group underwent a specialized treatment. One limb receives a specialized treatment prior and following an eccentric exercise intervention while the contralateral limb act as a control.

Therapeutic Protocol:
This program includes Pre-exercise PNF stretching, Post-exercise ice massage plus static stretching-30s.

Pre-Exercise: The PNF technique (hold-relax) was performed for stretching. The subjects were treated 10-second isometric contraction, and then 5-second relaxation, finally 20-second stretching (92). They were also treated daily for a period of 3 days before test. The exercises took 6 sessions, 2 sessions each day (10 am, 5 pm) and each session for 10 minutes.

Post-Exercise: The subjects were treated 7- minute ice massage, and then 30-second static stretching. Immediately after exercise (almost 3 hours) a qualified sports masseur applied the ice ball directly to the skin by using circular and stroking motions for a period of 7 min to the elbow flexors, Following the static stretching-30s was given. The Treatments took 5 set, and each set was separated by 60 min rest.
Criterion Measurements: Dependent variables to indicate damage were maximal voluntary contraction (MVC) of the elbow flexor, creatine kinase activity (CK), muscle soreness (DOMS), range of motion (ROM) (elbow resting angle, range of motion flexed elbow, range of motion extended elbow) and swelling (relaxed arm circumference, flexed arm circumference, forearm circumference) which have been used in previous research (Byrne et al., 2004; Howatson and van Someren, 2003; Warren et al., 1999). Dependent variables measurements were recorded at baseline, immediately after exercise, and at 24, 48 (and 72, 96) hours after postexercise. Serum creatine kinase was measured at baseline, immediately after exercise, and at 24, 48 (and 72, 96) hours after postexercise.

Maximal Voluntary Contraction (MVC): Isometric Maximal Voluntary Contraction (MVC): MVC was assessed using an isokinetic dynamometer (Cybex 6000, Ronkonkoma, NY, USA.). The device was set up according to the manufacturer’s recommendations to exercise the elbow flexors. MVC torque was measured at fixed joint angles of 90° of elbow extension, and 90° MVC isokinetic torque at concentric velocities of 90°·s⁻¹. Subjects were exhorted to produce a continuous maximal voluntary contraction of the elbow flexors for three seconds against an immovable lever arm of the Cybex 6000 isokinetic dynamometer at fixed elbow joint angles of 90°. Each repetition lasted 3 s interspersed with 60 s rest, the peak torque generated from three trials was recorded as the MVC.

Creatine Kinase Activity (CK): A 5 ml venous blood sample was collected from a branch of the basilic vein at each measurement time point (baseline, immediately after exercise, and at 24, 48 (and 72, 96) hours after postexercise), allowed to clot for 1 h at room temperature and was spun in a centrifuge to separate serum from the remaining blood constituents. Serum was drawn off and frozen immediately at -70°C for later analysis. Serum CK concentrations were determined using RANDOM ACCESS 1000 system.

Perceived Muscle Soreness Rating (DOMS): Muscle soreness was evaluated using a visual analogue scale. This scale possessed a 10-cm line that had the words “no pain” on one end and “extremely sore” on the other. Subjects were asked to indicate their pain level on that line while their elbow flexors were: (1) being palpated (three sites on the upper arm: mid-belly of the biceps brachii, 3-cm above and below the midbelly), (2) being extended, and (3) being flexed by the investigator. For the palpation measure, the highest score of the three sites was used for further analysis (Howatson et al., 2005; Gulick et al., 1996).

Range of Motion (ROM):

Elbow Resting Angle: Elbow resting angle was determined by the angle formed at the elbow when it is held by the side while the subject didn’t attempt to extend their arm as much as possible (the subject attempted to extend their arm as relax) with the elbow held by their side and the hand in mid pronation the elbow joint to touch their shoulder with the palm of the supinated hand.

Range of Motion Flexed Elbow: Range of motion flexed elbow was determined by the angle formed at the elbow when it is held by the side while the subject attempted to fully flex the elbow joint to touch their shoulder with the palm of the supinated hand.

Range of Motion Extended Elbow: Range of motion extended elbow was determined as the angle formed at the elbow joint when the subject attempted to extend their arm as much as possible with the elbow held by their side and the hand in mid pronation. To obtain consistent measurements four marks were drawn on the skin with a semi-permanent ink pen, one laterally approximating the level of the deltoid tuberosity, the second at the level of the lateral epicondyle of the humerus, a third at the mid-point of the wrist, and the fourth laterally at the styloid process of the radius. A plastic goniometer (Sammons Preston Rolyan, Illinois, USA) was used to record measures.

Swelling:

Relaxed Arm Circumference: Limb girth of the upper arm was taken midway between the acromion process and the lateral epicondyle of the humerus using an anthropometric tape while the arm was hung naturally at the side of the body. The site on the subject’s hands was measured three times and the averages were reported. The skin was marked with a semi permanent marker for consistency on subsequent days.

Flexed Arm Circumference: Upper arm circumference was assessed at midway with the arm flexed (the arm flexed at 90 degree). The site on the subject’s hands was measured three times and the averages were reported. The skin was marked with a semi permanent marker for consistency on subsequent days.

Forearm Circumference: Forearm circumference was assessed at the forearm maximum girth using an anthropometric tape while the elbow was flexed at 90 degrees, supination.

Statistical Analysis: We used SPSS (version 15; SPSS Inc, Chicago, IL) to conduct the analysis. The statistical significance level for all data analyses was set at 0.05. Appropriate descriptive statistics and parametric tests were used to describe the study participants. An independent-samples T test were used to compare the baseline measurements between the subgroups at the beginning and post of training. For data analysis each group (dependent means) repeated measures with factors of time and treatment was used (Adjustment for multiple comparisons: Bonferroni).
**Results:**

Baseline values for the all dependent variables showed no differences between the subgroups (Tables 3).

**Relaxed Arm Circumference, Flexed Arm Circumference, Forearm Circumference:**

Baseline arm and Forearm circumference were not different between the subgroups (P>0.05). Between-subgroups comparison of limb girth showed no difference in the control subgroup compared with the experimental subgroup (P>0.05) (Table 3).

**Elbow Resting Angel:**

A decrease was seen in elbow resting angel for the control subgroup in all sessions (Table 1). In the control subgroup, the elbow resting angel decreased immediately postexercise (24 hours) by 5.98% below baseline, compared with a 4.24% decrease in the experimental subgroup. This decremental trend was shown in the control subgroup at 48 hours (by 7.87%) and 72 hours (by 7.91%), whereas decreases of 5.59% and 5.59% were demonstrated at 48 and 72 hours, respectively, in the experimental subgroup. By 96 hours, a mean percentage decrease of 2.85% was still evident in the control subgroup, compared with a 1.85% decrease in the experimental subgroup (P >.05). But Between-subgroups comparisons of range of elbow resting angel didn't show a significant differences evident between the control and experimental hands (P>0.05). (Table 3, Figure1).

![Fig. 1](image1.png)

**Fig. 1:** Changes in elbow resting angle (degree) before (preexercise) and 24, 48, 72 and 96 hours postexercise for the experimental and control subgroups.

**Range of Motion Flexed Elbow:**

A decrease was seen in range of motion flexed elbow for the control subgroup in all sessions (Table 1). In the control subgroup, the range of motion flexed elbow decreased immediately postexercise by 0.77% below baseline, compared with a 0.47% decrease in the experimental subgroup (P >.05). This decremental trend was shown in the control group at 48 hours (by 5.03%) and 72 hours (by 4.34%), whereas decreases of 1.99% and 1.90% were demonstrated at 48 and 72 hours, respectively, in the experimental subgroup. By 96 hours, a mean percentage decrease of 0.91% was still evident in the control group, compared with a 0.52% decrease in the experimental subgroup (P >.05). Between-subgroups comparisons of range of motion flexed elbow showed a significant differences evident between the control and experimental hands at 48 and 72 hours postexercise (P<0.05). (Table 3, Figure2).

![Fig. 2](image2.png)

**Fig. 2:** Changes in range of motion flexed elbow (degree) before (preexercise) and 24, 48, 72 and 96 hours postexercise for the experimental and control subgroups.
Range of Motion Extended Elbow:

A decrease was seen in range of motion extended elbow for the control subgroup in all sessions (Table 1). In the control subgroup, the range of motion extended elbow decreased immediately (24 hours) postexercise by 1.67% below baseline, compared with a 1.49% decrease in the experimental subgroup (P > 0.05). This decremental trend was shown in the control group at 48 hours (by 8.35%) and 72 hours (by 7.17%), whereas decreases of 3.69% and 2.96% were demonstrated at 48 and 72 hours, respectively, in the experimental subgroup (P < 0.05). By 96 hours, a mean percentage decrease of 0.83% was still evident in the control group, compared with a 0.45% decrease in the experimental subgroup (P > 0.05). Between-subgroups comparisons of range of motion extended elbow showed a significant differences evident between the control and experimental hands at 48 and 72 hours postexercise (P < 0.05). (Table 3, figure 3).

Muscular Strength:

A decrease was seen in maximal isometric torque for the control subgroup at all angles in all sessions (Table 1). In the control subgroup, the torque decreased immediately postexercise (24 hours) by 2.38% below baseline, compared with a 1.02% decrease in the experimental subgroup (P > 0.05). This decremental trend was shown in the control group at 48 hours (by 4.84%) and 72 hours (by 3.78%), whereas decreases of 4.99% and 4.14% were demonstrated at 48 and 72 hours, respectively, in the experimental subgroup. By 96 hours, a mean percentage decrease of 2.23% was still evident in the control subgroup, compared with a 2.38% decrease in the experimental subgroup (P > 0.05). But Between-subgroups comparisons of maximal isometric torque didn't show a significant differences evident between the control and experimental hands (p > 0.05). (Table 3, figure 4).

Fig. 3: Changes in range of motion extended elbow (degree) before (preexercise) and 24, 48, 72 and 96 hours postexercise for the experimental and control subgroups.

Fig. 4: Changes in maximum isometric torque (Nm) before (preexercise) and 24, 48, 72 and 96 hours postexercise for the experimental and control subgroups.
No time effect was found in maximal isokinetic torque at 90°/s between baseline and any other sessions (immediately after or 24, 48, 72, or 96 hours postexercise) in the experimental and control subgroup over time (p>0.05). Maximal isokinetic torque at 90°/s changed over time within the control subgroup, decreasing by 0.99% of baseline immediately postexercise (24 hours) and by 4.28% and 4.88% at 48 and 72 hours, respectively. By 96 hours, a mean percentage decrease of 2.03% was still evident in the control subgroup, compared with a 2.59% decrease in the experimental subgroup (P >.05). But between-subgroups comparisons of maximal isokinetic torque didn't show a significant differences evident between the control and experimental hands (p>0.05). (Table 3, Figure5).

**Fig. 5:** Changes in maximum isokinetic torque (Nm) before (preexercise) and 24, 48, 72 and 96 hours postexercise for the experimental and control subgroups.

**Perceived Muscle Soreness Rating (DOMS):**
Before exercise, no participant reported any soreness during assessments. The DOMS developed after exercise in both subgroups (Table 1). At 24 hours, the mean pain increment equated to 36.02% and 82.25% in the experimental and control subgroups, respectively. The control subgroup reported greater perception of 36.44% and 46.03% postexercise DOMS than the experimental subgroup by 24 and 48 hours, respectively. But between-subgroups comparisons of perceived muscle soreness rating didn't show a significant differences evident between the control and experimental hands (P>0.05). (Table 3, Figure6).

**Fig. 6:** Changes in perceived muscle soreness (cm) before (preexercise) and 24, 48, 72 and 96 hours postexercise for the experimental and control subgroups.

**Plasma CK Activity:**
At 48 hours postexercise, the CK peak value was 40.48% higher (Figure7). In addition, the CK-level percentage increments at 24, 72 and 96 hours were higher than in baseline (P < .05). At 96 hours, postexercise CK level was 47.15% above baseline (Figure7).
Fig. 7: Changes in Perceived muscle soreness rating (IU/L) before (preexercise) and 24, 48, 72 and 96 hours postexercise.

| Table 1: Muscle soreness for experimental subgroup after the damaging bout of exercise. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| before-exercise                 | immediately post-exercise       | 24-Hours                        | 48-Hours                        | 72-Hours                        | 96-Hours                        |
| Md=2.05                         | Md=1.32                         | Md=5.55                         | Md=5.63                         | Md=4.66                         | Md=1.32                         |
| *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=0.725                        | Md=4.31                         | Md=4.38                         | Md=3.33                         | Md=4.38                         | Md=3.38                         |
| P=.360                          | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=2.61                         | Md=0.975                        | Md=0.888                        | Md=1                            | Md=0.888                        | Md=1                            |
| *P=0.000                        | P=1                             | P=0.004                         | P=0.004                         | P=0.004                         | P=0.004                         |
| Md=3.58                         | Md=0.089                        | Md=0.089                        | Md=0.089                        | Md=0.089                        | Md=0.089                        |
| *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=3.50                         |Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |
| Md=1.90                         | Md=5.55                         | Md=5.71                         | Md=4.71                         | Md=1.32                         | Md=1.32                         |
| *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=5.75                         | Md=4.22                         | Md=4.38                         | Md=3.38                         | Md=4.38                         | Md=3.38                         |
| P=.692                          | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=2.81                         | Md=0.837                        | Md=1                            | Md=1                            | Md=0.837                        | Md=1                            |
| *P=0.004                        | P=1                             | P=0.170                         | P=0.170                         | P=1                             | P=0.170                         |
| Md=3.81                         | Md=0.162                        | Md=0.162                        | Md=0.162                        | Md=0.162                        | Md=0.162                        |
| *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=3.65                         | Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |
| Md=.974                         | Md=3.58                         | Md=3.63                         | Md=3.77                         | Md=4.45                         | Md=4.45                         |
| .986                            | .923                            | .958                            | .958                            | .989                            | .974                            |

* = Indicates a Significant Time Effect.

Table 2: Muscle soreness for control subgroup after the damaging bout of exercise.

| Table 2: Muscle soreness for control subgroup after the damaging bout of exercise. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| before-exercise                 | immediately post-exercise       | 24-Hours                        | 48-Hours                        | 72-Hours                        | 96-Hours                        |
| Md=1.90                         | Md=1.32                         | Md=5.55                         | Md=5.71                         | Md=4.71                         | Md=1.32                         |
| *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=5.75                         | Md=4.31                         | Md=4.38                         | Md=3.38                         | Md=4.38                         | Md=3.38                         |
| P=.692                          | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=2.81                         | Md=0.837                        | Md=1                            | Md=1                            | Md=0.837                        | Md=1                            |
| *P=0.004                        | P=1                             | P=0.170                         | P=0.170                         | P=1                             | P=0.170                         |
| Md=3.81                         | Md=0.162                        | Md=0.162                        | Md=0.162                        | Md=0.162                        | Md=0.162                        |
| *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        | *P=0.000                        |
| Md=3.65                         | Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |Md=Mean Difference               |
| Md=.974                         | Md=3.58                         | Md=3.63                         | Md=3.77                         | Md=4.45                         | Md=4.45                         |
| .986                            | .923                            | .958                            | .958                            | .989                            | .974                            |

* = Indicates a significant time effect.

Table 3: Changes in outcome measures before (pre), immediately, 24h, 48h, 72 and 96h following eccentric exercise of the PNF and control subgroups, Mean± SEM.

<table>
<thead>
<tr>
<th>Variable, Group, and P Value</th>
<th>Pre-exercise</th>
<th>24-Hours</th>
<th>48-Hours</th>
<th>72-Hours</th>
<th>96-Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxed arm circumference, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental hand</td>
<td>28.33±3.75</td>
<td>28.85±4.00</td>
<td>28.67±3.83</td>
<td>28.45±3.80</td>
<td>28.36±3.76</td>
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<tr>
<td>The Control hand</td>
<td>28.37±3.66</td>
<td>28.96±4.10</td>
<td>28.73±4.10</td>
<td>28.46±3.78</td>
<td>28.31±3.73</td>
</tr>
<tr>
<td>P value</td>
<td>.977</td>
<td>.939</td>
<td>.965</td>
<td>.989</td>
<td>.974</td>
</tr>
<tr>
<td>Flexed arm circumference, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental hand</td>
<td>29.62±3.84</td>
<td>30.00±4.00</td>
<td>30.16±4.20</td>
<td>30.06±4.04</td>
<td>29.78±3.88</td>
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<tr>
<td>The Control hand</td>
<td>29.65±3.88</td>
<td>29.86±4.00</td>
<td>30.07±4.07</td>
<td>29.98±3.98</td>
<td>29.71±3.83</td>
</tr>
<tr>
<td>P value</td>
<td>.986</td>
<td>.923</td>
<td>.967</td>
<td>.958</td>
<td>.964</td>
</tr>
<tr>
<td>Elbow resting angel, degree</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental hand</td>
<td>162.0±4.09</td>
<td>155.12±6.52</td>
<td>152.93±5.79</td>
<td>153.06±6.39</td>
<td>159.00±5.88</td>
</tr>
<tr>
<td>The Control hand</td>
<td>161.87±4.63</td>
<td>152.18±7.56</td>
<td>149.12±6.11</td>
<td>149.06±6.27</td>
<td>157.25±6.20</td>
</tr>
<tr>
<td>P value</td>
<td>.936</td>
<td>.249</td>
<td>.080</td>
<td>.084</td>
<td>.420</td>
</tr>
<tr>
<td>Range of motion flexed elbow, degree</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental hand</td>
<td>144.12±3.55</td>
<td>143.43±3.42</td>
<td>141.25±3.29</td>
<td>141.37±2.94</td>
<td>143.37±3.13</td>
</tr>
<tr>
<td>The Control hand</td>
<td>143.87±3.84</td>
<td>142.75±3.75</td>
<td>136.62±4.73</td>
<td>137.62±4.58</td>
<td>142.56±4.24</td>
</tr>
<tr>
<td>P value</td>
<td>.850</td>
<td>.592</td>
<td>.006*</td>
<td>.010*</td>
<td>.543</td>
</tr>
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</table>
### Discussion:

We investigated the possible effect of prophylactic (prior to exercise) and therapeutic (post-exercise) a Combination Treatment on biochemical markers (enzymatic levels) and functional (elbow angle, arm circumference, pain rate…) of Exercise-induced muscle damage. The Combination Treatment had an alleviative effect on the responses to DOMS-inducing exercise in terms of changes in range of motion (flexibility). But hadn't an alleviative effect on the responses to DOMS-inducing exercise in terms of changes in Relaxed arm circumference, flexed arm circumference, elbow resting angle, Forearm circumference, muscle soreness, Maximal voluntary isometric and isokinetic strength, plasma CK activity.

We found no differences between subgroups for Relaxed arm circumference, flexed arm circumference, Forearm circumference (P>0.05). Limb girth showed no discernable difference between subgroups and consequently provided indirect evidence that the intervention was unsuccessful in bringing about these changes. However, previous authors (Fride, 1986-1981; Hasson et al., 1989) attributed the pain of DOMS to edema and swelling within the exercised muscle fibers. But, Smith (1991) and Armstrong (1984) argued that monocytes, which convert to macrophages, accumulate after injury and produce substances which, in turn, sensitize the type III and IV nerve endings within 24 to 48 hours. In addition, Buroker and Schwane (1989) and Gulick et al., (1996) found that girth measurements of eccentrically exercised limbs did not increase at any postexercise assessment time, in accordance with our findings.

The magnitude of strength loss wasn't different between the subgroups (P>0.05). The Maximal voluntary isometric and isokinetic strength response in this investigation showed a large decline immediately post-exercise and a general recovery towards preexercise levels in the subsequent 96h and concur with other previous literature (Isabell et al., 1992; Eston and Peters, 1999; Miyama and Nosaka, 2004a; 2004b; Howatson et al., 2007). Muscle strength is one of the best muscle damage indicators, which is normally reduced after exercise with slow recovery (Nosaka and Newton, 2002). Strength losses of up to 60% are evident directly after exercise, and these can last up to ten days (Clarkson and Staer, 1999). It was hypothesised initially that this is due to pain inhibition, but the strength losses are seen well before pain perception. It is believed that over-stretching of the sarcomeres and a reduction in actin and myosin overlap is the main cause for this strength loss (Clarkson and Newham, 1995). Westerbald et al., (1993) suggested that it is fatigue due to a reduction in calcium production from the damaged sarcoplasmic reticulum, that may lead to an inability to generate force.

The prevention of Exercise-induced muscle damage by using PNF has shown the beneficial effect on muscle strength (Peanchai Khamwong, 2010), But The result of our study was dissimilar to this study, But was similar to previous studies High et al., (1989), Johansson et al., (1999).

We found differences between subgroups for range of motion flexed elbow and range of motion extended elbow (P<0.05). Range of motion (ROM) showed discernable difference between subgroups and consequently provided indirect evidence that the intervention was successful in this study.

In this present study, a different stretching technique (PNF-hold-relax and 30-second static stretching) was performed. This technique is a combination of both static and dynamic stretching maneuvers. As a result, some advantageous effects of the PNF and 30-second static stretching were evidenced on Exercise-induced muscle damage (EIMD) symptoms in terms of sensory perception and muscle function.

The application of PNF before exercise and 30-second static stretching after exercise was aimed at preparing the localized muscle to prevent and treatment EIMD symptoms.
PNF technique of hold-relax was used to prepare the elbow flexors with passive and active movement that can improve muscle flexibility via autogenic inhibition and reciprocal inhibition. The benefits of an active warm up may be to minimize muscle stiffness by moving the required muscle groups through their range of motion. As a result, the warm up with PNF stretching may release actin-myosin bonds and thereby reduce the passive stiffness of muscle. This may contribute to an increased rate of force development and an increase in the efficacy of muscle working during eccentric exercise (Bishop, 2003).

Streching exercises also affect the mechanical properties of the muscle-tendon unit (MTU), i.e., reduce the tension on the muscle-tendon unit that affects the visco-elastic component of tissue leading to an increase in the compliance of muscle and a reduction in muscle stiffness; consequently, less tension will be produced in the muscle during a specified stretch. The resulting improvement of muscular flexibility possibly reduces muscle and connective tissue damage after exercise (Weldon and Hill, 2003; Magnusson and Renstrom, 2006). As a result, the PNF stretching (the effect of prophylactic) with 30-second static stretching (the effect of therapeutic) can improve muscle flexibility.

The results revealed that there weren't significant differences evident between the control hand and the experimental hand for delayed onset of muscle soreness and Pain intensity rate (P<0.05). Thus, That therapeutic protocol wasn't effective in improving disability Marker for Visual analog scale (VAS) at immediately post-exercise, 24h, 48h, 72 and 96th following Exercise-induced muscle damage.

Several reasons have been proposed for the cause of pain in DOMS. Smith (1991) believes it is the swelling and the intracellular edema that causes compression on the pain sensitive nerve endings. This may lead to sensitisation of these nerves and thus pain. He supports this theory by rationalising that this is why pain is only felt only on movement and palpation but not at rest. As the muscle is placed under mechanical stress pressure increases within, and compression of nerve endings occurs.

Clarkson and Newham (1995) however believe it is the mediators released in the inflammation process, such as bradykinin, serotonin and histamine that sensitisate the pain nerve endings and thus pain results.

Cryotherapy has been speculated to alter nerve conduction velocity and hence pain tolerance (Algaflly and George, 2007), Denegar and Perrin (1992) observed beneficial effects of cryotherapy (ice packs) on DOMS. These authors documented a further reduction in perceived soreness when the treatment was supplemented with a period of stretching. They proposed that stretching results in stimulation of the Golgi tendon organ, motor inhibition, and reduced muscular tension resulting inacurrent reduction in the pain - spasm cycle (Denegar and Perrin, 1992), however, these changes were not observed in this investigation.

The result of our study was similar to previous studies. High et al., (1989), Johansson et al., (1999) did not demonstrate the efficacy of stretching on muscle soreness in quadriceps and hamstrings, respectively. They applied static stretching before the induction exercises in healthy student volunteers, and their results showed no effect of static stretching on EIMD.

Intracellular release of CK has been used as an indirect marker of EIMD for many years (Manfredi et al., 1991; Howatson et al., 2005).

The CK response in this investigation peaked at 48h post-exercise, which is the same response as previous data using a similar protocol to induce damage. Hence, most CK responses following damaging eccentric exercise in the upper limb tend to be slightly more delayed and peak 24 h later (Howatson et al., 2007; Nosaka et al., 2002); although the reason for this is unclear (Miyama and Nosaka, 2004a), it may be speculated that the upper limb is more unaccustomed to eccentric loading and hence has a greater susceptibility to damage than the lower limb; consequently CK is more delayed and of a greater magnitude in the upper limb (Jamurtas et al., 2005). (Miyama and Nosaka, 2004). Cryotherapy has been speculated to reduce membrane permeability, thereby reducing CK efflux (Eston and Peters, 1999), however, these changes were not observed in this investigation.

Conclusions:

We investigated the possible the possible effects of prophylactic (prior to exercise) and therapeutic (post-exercise) a Combination Treatment on Exercise-induced muscle damage. Based on these results, PNF stretching prior to exercise, massage plus static stretching-30s Postexercise can reduce the symptoms of muscle damage (ROM) than the control subgroup. Results of this study suggests that applying a Combination Treatment help to attenuate symptoms of EIMD (ROM) in the elbow flexors.

ACKNOWLEDGMENT

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REFERENCES


