The effect of PNF stretching and therapeutic massage combination treatment on markers of exercise induced muscle damage

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Abstract

The purpose of this study was to examine the effects of a Combination Treatment (PNF & therapeutic massage) on biochemical markers (enzymatic levels) and some functional characteristics of exercise-induced muscle damage. Sixteen non-athlete male students with no delayed onset muscle soreness participated in the study. Subjects were randomly assigned into control and experimental subgroups. An eccentric biceps curls exercise program using bilateral arms was performed to induce muscle damage. The following outcome variables were derived at baseline, immediately after exercise, and at 24, 48, 72 and 96 hours after post-exercise: Relaxed arm circumference, flexed arm circumference, elbow resting angle, forearm circumference, range of motion of flexed elbow, range of motion of extended elbow, exercise-induced muscle damage, maximal voluntary isometric and isokinetic strength and Serum Creatine Kinase as well. Results showed that combination treatment had an effect on muscle soreness, maximal voluntary isometric during timing (p<0.05). These findings illustrate that combination treatments were effective on maintenance isometric strength and decreased pain delayed onset of muscle soreness and pain intensity rate.

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Introduction

It is clear that muscle damage is caused by unaccustomed, high intensity exercise; in particular eccentric exercise, where the muscle is actively lengthened (Tidball, 2005). Damage caused may lead to various symptoms such as muscle soreness (Smith et al., 2007; Takahashi et al., 2006), decreased maximal voluntary contraction (Peterson et al., 2007), leakage of myofibrillar proteins such as creatine kinase (CK) and lactate dehydrogenase (LDH) into the blood (Nosaka & Clarkson, 1992), and increased oxidative stress and lipid peroxidation indicated by superoxide dismutase (SOD) and malondialdehyde (MDA), respectively (Neubauer et al., 2008; Suzuki et al., 2006). Symptoms of exercise-induced muscle damage can range from muscle tenderness to severe debilitating pain. These symptoms may last up to 5-7 days, typically peaking between 24 and 72 hours (Cheung et al., 2003).

Several researchers have investigated interventions thought to reduce and/or prevent the severity of muscle damage. From a clinical point of view, preventative intervention is preferred because it reduces the cost of treatment, time lost from training or rehabilitation, and the likelihood of sustaining further injury, and it also maintains the ability to exercise (O'Connor & Hurley, 2003; Weerapong et al., 2004). Several interventions such as warm-up, stretching, massage, acupuncture, anti-inflammatory drugs have been researched in order to find interventions that successfully alleviate the severity of muscle damage (Weerapong, 2005). The results are inconclusive mainly due to the variety of exercise-induced muscle damage protocols, the types of intervention protocols, and the doses of application.

Many coaches and athletes hold the belief, based on observations, experiences, and anecdotal evidence, that warm-up, stretching, and massage can provide several benefits to the body prior to participating in sports activities and exercise. More specifically, warm-up, stretching, and massage are used as pre-exercise activities to enhance performance and reduce the risk of muscle damage through biomechanical, neurological, and psychological mechanisms. Studies however have not shown the effectiveness of static stretching in the prevention of exercise-induced muscle damage (EIMD) (Johansson et al., 1999). Several studies have demonstrated that proprioceptive neuromuscular facilitation (PNF) stretching techniques were more effective than static stretching for increasing the flexibility of the hamstring muscle (Etnyre & Abraham, 1986). The PNF stretching techniques are widely applied in the rehabilitation of patients with musculoskeletal system injury, as it reduces the resistance of the muscle in a pain free manner (Sullivan, 1992). Hence, if the PNF stretching techniques are applied in EIMD for alleviating its symptoms, it may much more effective than static stretching. 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. Massage has shown varying results that may be attributed to the time of massage application and the type of massage technique used. Massage is considered to have a number of physiological and psychological benefits that may contribute to pain modulation, and tissue repair aided in part by increased circulation and lymphatic flow (Wright & Sluka, 2001). Tiidus and Shoemaker (1995) have shown that massage has no effect on blood flow to the muscle. Furthermore, these investigators noted light exercise was better than massage for improving blood flow and temporarily reducing soreness. While one study showed improved lymphatic flow to the skin in humans (Mortimer et al., 1990), muscle lymphatic flow and edema have not been shown to improve with massage (Callagan, 1993). Despite these findings, Holbert, Spratt and Smith (1994) found a reduction in serum creatine kinase (an indirect marker of muscle tissue damage) and soreness when massage was delivered 2 hour (hr.) after eccentric exercise. However, changes in creatine kinase can be highly variable and are not necessarily reflective of muscle damage (Connolly et al., 2003). Smith (1994) also showed that massage prolonged the neutrophil activity associated with inflammation and therefore may alleviate EIMD. If massage is able to diminish muscle damage and the effects of the inflammatory response, then it may be able to alter muscle soreness. To date, the effects of massage on measures
of physical performance have been limited and little is known about whether or not massage can enhance actual physical performance during activities such as vertical jumping ability. According to our knowledge, the effects of pre-exercise PNF stretching and post-exercise massage has not yet been evaluated in patients with EIMD and it may prove that the use of pre-exercise PNF and post-exercise massage could have some potential benefit as a prophylactic and therapeutic effect. Therefore, the purpose of this study was to determine if pre-exercise PNF stretching and post-exercise massage has an effect on EIMD and physical performance. We hypothesized that pre-exercise PNF stretching and post-exercise massage (Appropriate prophylactic and therapeutic strategies) would reduce the severity of soreness and improve muscle function.

Methods

Participants

Sixteen non-athlete male students (age: 22.93 ± 2.23 years; height: 173.75 ± 3.65 cm and body mass: 74.12 ± 11.70 kg) participated in this study. Subjects were randomly divided into subgroups with control and experimental arms. Subjects had no delayed onset muscle soreness for at least 6 months prior to the beginning of the study. Moreover, they had not been involved in any type of upper body resistance training or extensive physical activity in the past six months.

Procedure

The study employed an intra-subject design by using a contralateral limb model (Zainuddin, Newton, Sacco, & Nosaka, 2006). One limb received a specialized treatment in prior and following an eccentric exercise intervention while the contralateral limb acted as a control and received no treatment. Dominant and non-dominant arms were randomly chosen for the control and experimental conditions. Clinical assessment indicates that the subjects are suitable for active exercises. The experimental arms of the subjects underwent PNF stretching before muscle damage induction and, therapeutic massage after muscle damage induction as well. The control arms of the subjects received only muscle damage induction. The dependent variables then were measured at before exercise as baseline, 24, 48 and 72 hrs. after exercise treatment. Changes in the measures over time were compared between experimental and control arms. Dependent variables consisted of maximal voluntary, isometric and isokinetic elbow flexor strength, creatine kinase activity, perceived muscle soreness rating, range of motion and swelling.

Induction of muscle damage

An eccentric biceps curls exercise program using bilateral arms was performed to induce muscle damage. The subjects performed 50 eccentric contractions (60% maximal eccentric contraction) with both arms for a 3 second period. Each contraction was separated by a 10 seconds rest period. This eccentric exercise protocol was previously used in our laboratory to induce muscle damage.

Treatments

Therapeutic protocol

Therapeutic protocol that was selected for this study includes a combination treatment program of PNF stretching (pre-exercise) and therapeutic massage (post-exercise).

Before eccentric exercise

The PNF technique (hold-relax) was performed for stretching. The subjects performed a 10-second isometric contraction followed by a 5-second relaxation and finally a 20-second of stretching (92). They were also undergone daily for a period of 3 days before eccentric exercise. The exercises took 6 sessions, 2 sessions each day (10 am, 5 pm) and each session lasted 10 minutes.

After eccentric exercise

The subjects were treated with 5-minute effleurage massage, 5-minute tapotement massage, 12-minute pettrissage massage and then 2-minute effeurage massage. Immediately after eccentric Exercise (almost 3 hours) a qualified sports masseur applied
the massage directly to the skin by using circular and stroking motions for a period of 24 min to the elbow flexors. The treatments took 5 set with a resting period of 60 minutes between each set (Weber et al., 1994).

**Criterion measurements**

*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 degrees of elbow extension, and too 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 degrees. 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 post-exercise), allowed to clot for 1 hr. 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).

*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 deltid 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 arms 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 degrees). The site on the subject's arms 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**

Statistical analyses were undertaken using Statistical Program for Social Sciences (SPSS Inc., version 15). An dependent-samples t-test was employed to compare the baseline measurements between the groups' arms at the beginning and post of training. ANOVAs with repeated measures were employed followed with Bonferroni post hoc for comparing the dependent variables.

**Results**

Baseline values for the all dependent variables showed no differences between the subgroup (arms) (Table 3).

### Table 1. Muscle soreness for experimental subgroup after the damaging bout of exercise.

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>before-exercise</th>
<th>immediately post-exercise</th>
<th>24-Hours</th>
<th>48-Hours</th>
<th>72-Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Hours</td>
<td>Md=-0.725</td>
<td>Md=-0.456</td>
<td>Md=2.68</td>
<td>Md=3.25</td>
<td>Md=2.36</td>
</tr>
<tr>
<td>72-Hours</td>
<td>*P=.043</td>
<td>*P=1</td>
<td>*P=0.00</td>
<td>*P=.000</td>
<td>*P=0.000</td>
</tr>
<tr>
<td>48-Hours</td>
<td>Md=-3.08</td>
<td>Md=-1.90</td>
<td>Md=0.325</td>
<td>Md=0.894</td>
<td>*P=.011</td>
</tr>
<tr>
<td>24-Hours</td>
<td>Md=3.98</td>
<td>Md=2.80</td>
<td>Md=0.569</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48-Hours</td>
<td>Md=3.41</td>
<td>Md=2.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72-Hours</td>
<td>Md=-1.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>immediately post-exercise</td>
<td>Md=-1.18</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Table n.** Mean Difference * Indicates a significant time effect.

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

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>before-exercise</th>
<th>immediately post-exercise</th>
<th>24-Hours</th>
<th>48-Hours</th>
<th>72-Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Hours</td>
<td>Md=-1.17</td>
<td>Md=-3.70</td>
<td>Md=4.60</td>
<td>Md=-3.44</td>
<td>Md=-1.27</td>
</tr>
<tr>
<td>72-Hours</td>
<td>*P=0.009</td>
<td>*P=0.000</td>
<td>*P=0.00</td>
<td>*P=0.00</td>
<td>*P=0.011</td>
</tr>
<tr>
<td>48-Hours</td>
<td>Md=-3.04</td>
<td>Md=-2.42</td>
<td>Md=3.33</td>
<td>Md=2.16</td>
<td></td>
</tr>
<tr>
<td>24-Hours</td>
<td>Md=2.81</td>
<td>Md=0.837</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48-Hours</td>
<td>Md=-3.43</td>
<td>Md=0.906</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72-Hours</td>
<td>Md=2.52</td>
<td>*P=0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**md=mean Difference  *= Indicates a significant time effect.**

**Relaxed arm circumference, flexed arm circumference, forearm circumference, elbow resting angle**

Baseline arm and forearm circumference, elbow resting angle were not different between the subgroups (P>0.05). Between-subgroups comparison of limb girth and elbow resting angle showed no difference in the control subgroup compared with the experimental subgroup (P>0.05) (Table 3).
Range of motion flexed elbow
A decrease was seen in range of motion flexed elbow for the control subgroup in all sessions (Table 3). In the control subgroup, the range of motion flexed elbow decreased immediately post-exercise (24 hours) by 4.23% below baseline, compared with a 3.49% decrease in the experimental subgroup. This detrimental trend was shown in the control subgroup at 48 hours (by 6.69%) and 72 hours (by 6.27%), whereas decreases of 4.49% and 4.68% were demonstrated at 48 and 72 hours, respectively, in the experimental subgroup. By 96 hours, a mean percentage decrease of 3.59% was still evident in the control subgroup, compared with a 3.1% decrease in the experimental subgroup (P > .05). But between-subgroups comparisons of range of motion flexed elbow didn't show a significant difference evident between the control and experimental arms (P > .05). (Table 3, Figure 1).

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 After Exercise</th>
<th>After Exercise</th>
<th>72-Hours After Exercise</th>
<th>96-Hours After Exercise</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 arms</td>
<td>28.8±4.35</td>
<td>29.4±3.64</td>
<td>29.39±3.40</td>
<td>29.21±3.33</td>
<td>28.98±3.36</td>
</tr>
<tr>
<td>The Control arms</td>
<td>28.8±3.39</td>
<td>29.5±3.68</td>
<td>29.7±3.38</td>
<td>29.3±3.38</td>
<td>29.00±3.38</td>
</tr>
<tr>
<td>P value</td>
<td>.988</td>
<td>.932</td>
<td>.792</td>
<td>.942</td>
<td>.992</td>
</tr>
<tr>
<td>flexed arm circumference, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental arms</td>
<td>30.0±3.52</td>
<td>30.2±3.51</td>
<td>30.55±3.68</td>
<td>30.55±3.51</td>
<td>30.16±3.51</td>
</tr>
<tr>
<td>The Control arms</td>
<td>30.06±3.58</td>
<td>30.26±3.50</td>
<td>30.3±3.82</td>
<td>30.73±3.69</td>
<td>30.28±3.55</td>
</tr>
<tr>
<td>P value</td>
<td>.980</td>
<td>.972</td>
<td>.837</td>
<td>.886</td>
<td>.925</td>
</tr>
<tr>
<td>elbow resting angel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental arms</td>
<td>163.18±4.11</td>
<td>158.00±5.59</td>
<td>153.93±8.09</td>
<td>154.25±5.67</td>
<td>160.75±3.87</td>
</tr>
<tr>
<td>The Control arms</td>
<td>163.09±4.34</td>
<td>156.93±5.67</td>
<td>151.50±8.09</td>
<td>152.8±6.93</td>
<td>160.56±4.11</td>
</tr>
<tr>
<td>P value</td>
<td>.950</td>
<td>.982</td>
<td>.604</td>
<td>.526</td>
<td>.895</td>
</tr>
<tr>
<td>range of motion flexed elbow, degree</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>The experimental arms</td>
<td>146.68±5.71</td>
<td>141.56±5.17</td>
<td>140.09±4.00</td>
<td>139.8±3.72</td>
<td>142.12±3.44</td>
</tr>
<tr>
<td>The Control arms</td>
<td>146.9±4.60</td>
<td>140.68±5.88</td>
<td>137.06±4.17</td>
<td>137.68±3.43</td>
<td>141.6±3.63</td>
</tr>
<tr>
<td>P value</td>
<td>.748</td>
<td>.658</td>
<td>.083</td>
<td>.104</td>
<td>.692</td>
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<tr>
<td>range of motion extended elbow, degree</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental arms</td>
<td>178.68±2.12</td>
<td>176.00±5.26</td>
<td>173.06±6.92</td>
<td>172.8±7.02</td>
<td>176.75±4.34</td>
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<tr>
<td>The Control arms</td>
<td>178.78±2.36</td>
<td>176.25±3.83</td>
<td>168.31±8.17</td>
<td>168.18±7.93</td>
<td>175.37±5.00</td>
</tr>
<tr>
<td>P value</td>
<td>.901</td>
<td>.879</td>
<td>.086</td>
<td>.091</td>
<td>.413</td>
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<tr>
<td>Forearm circumference, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental arms</td>
<td>27.13±4.25</td>
<td>27.4±4.29</td>
<td>27.31±4.20</td>
<td>27.56±3.22</td>
<td>27.38±3.29</td>
</tr>
<tr>
<td>The Control arms</td>
<td>26.93±4.29</td>
<td>27.36±4.28</td>
<td>27.52±4.23</td>
<td>27.45±3.24</td>
<td>27.21±3.26</td>
</tr>
<tr>
<td>P value</td>
<td>.795</td>
<td>.956</td>
<td>.987</td>
<td>.887</td>
<td>.909</td>
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<tr>
<td>maximum isometric torque, Nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The experimental arms</td>
<td>54.3±4.74</td>
<td>53.0±4.85</td>
<td>53.1±4.75</td>
<td>53.06±4.74</td>
<td>53.8±4.74</td>
</tr>
<tr>
<td>P value</td>
<td>.963</td>
<td>.109</td>
<td>.028</td>
<td>.048</td>
<td>.066</td>
</tr>
<tr>
<td>maximum isokinetic torque, Nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>The experimental arms</td>
<td>34.37±5.37</td>
<td>31.89±6.96</td>
<td>32.21±5.20</td>
<td>32.78±4.85</td>
<td>33.63±5.14</td>
</tr>
<tr>
<td>The Control arms</td>
<td>34.27±5.17</td>
<td>30.22±8.10</td>
<td>28.89±6.99</td>
<td>29.77±6.39</td>
<td>31.98±5.73</td>
</tr>
<tr>
<td>P value</td>
<td>.959</td>
<td>.537</td>
<td>.138</td>
<td>.445</td>
<td>.399</td>
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<tr>
<td>PERCEIVED MUSCLE SORENESS</td>
<td></td>
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<td></td>
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<tr>
<td>The experimental arms</td>
<td>0.13±4.293</td>
<td>3.54±9.00</td>
<td>4.11±7.87</td>
<td>3.2±0.965</td>
<td>0.85±0.750</td>
</tr>
<tr>
<td>The Control arms</td>
<td>0.18±4.442</td>
<td>3.63±8.47</td>
<td>4.79±1.05</td>
<td>3.8±0.888</td>
<td>1.36±0.905</td>
</tr>
<tr>
<td>P value</td>
<td>.675</td>
<td>.779</td>
<td>.047</td>
<td>.035</td>
<td>.696</td>
</tr>
</tbody>
</table>

Range of motion extended elbow
A decrease was seen in range of motion extended elbow for the control subgroup in all sessions (Table 3). In the control subgroup, the range of motion
extended elbow decreased immediately (24 hours) post-exercise by 1.41% below baseline, compared with a 1.49% decrease in the experimental subgroup (P > .05). This detrimental trend was shown in the control group at 48 hours (by 5.85%) and 72 hours (by 5.92%), whereas decreases of 3.14% and 3.28% were demonstrated at 48 and 72 hours, respectively, in the experimental subgroup (P < .05). By 96 hours, a mean percentage decrease of 1.90% was still evident in the control group, compared with a 1.08% decrease in the experimental subgroup (P > .05). Between-subgroups comparisons of range of motion extended elbow showed significant difference evident between the control and experimental arms at 48 and 72 hours post-exercise (P < .05). (Table 3, figure2).

Muscular Strength
A decrease was seen in maximal isometric torque for the control subgroup at the 90 degrees elbow angle in all sessions (Table 3). In the control subgroup, the torque decreased immediately post-exercise (24 hours) by 11.17% below baseline, compared with a 2.41% decrease in the experimental subgroup (P > .05). This detrimental trend was shown in the control group at 48 hours (by 15.21%) and 72 hours (by 13.83%), whereas decreases of 2.28% and 2.35% were demonstrated at 48 and 72 hours, respectively, in the experimental subgroup. By 96 hours, a mean percentage decrease of 11.23% was still evident in the control subgroup, compared with a 0.92% decrease in the experimental subgroup (P < .05). But between-subgroups comparisons of maximal isometric torque didn’t show a significant differences evident between the control and experimental arms (P > .05). (Table 3, figure3).

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 post exercise) in the experimental and control subgroups over time (P > .05). Maximal isokinetic torque at 90°/s (correct all degree designations) changed over time within the control subgroup, decreasing by 11.81% low baseline immediately post-exercise (24 hours) and by 15.69% and 13.13% at 48 and 72 hours, whereas decreases of 7.21%, 5.73% and 4.62% were demonstrated at 24, 48 and 72 hours, respectively, in the experimental subgroup. By 96 hours, a mean percentage decrease of 6.68% was still evident in the control subgroup, compared with a 2.15% decrease in the experimental subgroup (P > .05). As illustrated in Table 3 and Figure 4, between-subgroups comparisons of maximal isokinetic torque didn’t show a significant differences evident between the control and experimental arms (P > .05).

Perceived muscle soreness rating (DOMS)
Before eccentric exercise, no participant reported any soreness during assessments. The DOMS developed after eccentric Exercise in both subgroups (Table 1, 2). At 24 hours, the mean pain increment equated to 3.54 and 3.63 in the experimental and control subgroups, respectively (P > .05). The control subgroup reported greater perception of post exercise DOMS (4.79 and 3.88) than the experimental subgroup (4.11 and 3.21) by 48 and 72 hours, respectively. Between-subgroups comparisons of perceived muscle soreness rating did show a significant differences evident between the control and experimental arms (P < .05). (Table 3, Figure5).

Plasma CK Activity
At 48 hours post-exercise, the CK peak value was 39.86% higher than in 24 Hours after Eccentric Exercise. In addition, the CK-level percentage increments at 24, 72 and 96 hours were higher than in baseline (P < .05). At 96 hours, post-exercise CK level was 32.31% above baseline (Figure 6).

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 symptoms of EIMD (Pain intensity rate) and strength loss (Maximal
voluntary isometric), but had no 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, maximal voluntary 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 post-exercise assessment time, in accordance with our findings.

The magnitude of strength loss (Maximal voluntary isometric) was 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 pre-exercise levels in the subsequent 96h and concur with other previous literature (Isabell et al., 1992; Eston & Peters, 1999; Miyama & Nosaka, 2004a; 2004b; Howatson et al., 2007). Muscle strength is one of the best muscle damage indicators, which is normally reduced after eccentric Exercise with slow recovery (Nosaka & Newton, 2002). Strength losses of up to 60% are evident directly after exercise, and these can last up to ten days (Clarkson & Stayers 1999). It was hypothesized 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 & 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 result of this study demonstrated the efficacy of PNF stretching (the effect of prophylactic) with therapeutic massage (the effect of therapeutic) on improve muscle strength. We found no differences between subgroups for range of motion flexed elbow and range of motion extended elbow (P>0/05).

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 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). Stretching 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). But, the result of our study did not demonstrate the efficacy of PNF stretching (the effect of prophylactic) with therapeutic massage (the effect of therapeutic) on improve muscle flexibility.

The results revealed that there were significant differences evident between the control arms and the experimental arms for delayed onset of muscle soreness and pain intensity rate (p<0/05). Thus, that therapeutic protocol was effective in improving disability marker for Visual analog scale (VAS) at immediately post-exercise 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 sensitization of these nerves and thus pain. He supports this theory by rationalizing 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 sensitize the pain nerve endings and thus pain results. The result of our study was dissimilar 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.

As was found in a previous study by Tiidus et al. (1995) massage may induce an analgesic effect on
muscle sensory receptors or induce a psychological relaxation response that reduces the perception of DOMS, thus allowing the athlete to believe they are feeling better and performing better than they may actually be, these changes were observed in this investigation.

The results of this study suggest that PNF stretching prior to exercise, therapeutic massage Post-exercise could potentially increase the psychological recovery from intense performance.

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 & Nosaka, 2004).

Conclusions
A combination treatment program including PNF stretching as pre-exercise and therapeutic massage as post-exercise could reduce the symptoms of muscle damage (pain intensity rate). Results of this study suggests that applying a Combination Treatment help to attenuate symptoms of EIMD (pain intensity rate) and the magnitude of strength loss (maximal voluntary isometric) after a bout of eccentric exercise in the elbow flexors.

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**Fig. 6.** Changes in ck level (iu/l) before (pre-exercise) and 24, 48, 72.
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Smith LL, McKune AJ, Semple SJ, Sibanda E,


