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THE EFFECTS OF A COMBINATION TREATMENT (PRE-EXERCISE VITAMIN C & PNF STRETCHING, POST-EXERCISE ULTRASOUND TREATMENT) 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 effect of prophylactic (prior to exercise) and therapeutic (post-exercise) effects of a Combination Treatment (PNF & vitamin C, Ultrasound) on muscle damage is lacking. Objective: To investigate the effects of a Combination Treatment (PNF& vitamin C, Ultrasound) on biochemical (enzymatic levels) and functional (elbow angle, arm circumference, pain rate, etc) markers 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 hand and experimental hand. Intervention(s): Exercise program was used for induce exercise-induced muscle damage involved Preacher curl test (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, 72 and 96 hours after post-exercise. Serum creatine kinase was measured at baseline, immediately after exercise, and at 24, 48, 72 and 96 hours post-exercise. Results: The experimental subgroup showed a reduction in DOMS symptoms in the form of less range of motion flexed elbow and range of motion extended elbow, less maximal isometric and isokinetic voluntary strength loss ($P < .05$) compared with the control subgroup. However, no effect on relaxed arm circumference, flexed arm circumference, elbow resting angle, forearm circumference was evident ($P > .05$). Conclusion: This Combination Treatment on maximal voluntary isometric strength, delayed onset of muscle soreness and pain intensity rate during timing was effective. Eventually, results suggest that combination treatments are effective treatment on maintenance isometric strength and decrease of delayed onset of muscle soreness and pain intensity rate.

Keywords: Exercise-induced muscle damage (EIMD), PNF (proprioceptive neuromuscular facilitation), vitamin C, Ultrasound.

INTRODUCTION

Exercise-induced muscle damage (EIMD) is a common experience for elite athletes as well as novice ones. Its symptoms may vary from muscle laxity to severe debilitating pain. EIMD increases the intensity of muscle discomfort in the hours post-exercise, reaching its peak after 24-48 hours and is usually solved within one week[1]. The mechanisms, treatment strategies and impact on the athletic performance are still nuclear despite the high incidence of exercise-induced muscle damage (EIMD). EIMD is more prevalent in the beginning of the sports season when the athletes are returning to training after a period of reduced activity. EIMD is also common among athletes when they are first introduced to certain types of activity, regardless of the time of the year. Eccentric activities induce to micro injuries more frequently and severity than other kinds of muscular actions. Eccentric exercises are part of regular rehabilitation as well as sports training. Unusual eccentric exercises cause muscle damage, which appears as muscle pain[2], loss of isometric and dynamic strength[3,4],loss of range of motion[4], swelling[3], increase in muscle-specific proteins in the blood (CK, LDH)[3] and increased passive stiffness[5]. Muscle injury means mechanical rupture of the sarcomeres and pain is a result of the inflammatory response of the synthesis of prostaglandin and leucotriene[6]. The levels of creatine kinase increase with onset of delayed muscular pain, indicating rupture or alterations in permeability in the plasmatic membrane. Such symptoms reduce the capacity to exercise and may be harmful if the individual continues exercising. Exercise intensity and duration are important additional factors in EIMD. At least six theories have been proposed for the EIMD mechanism, namely: lactic acid, muscle spasm, muscle damage in the conjunctive tissue, muscle damage, inflammation and the enzymatic efflux theories. However, the interaction of two or more theories seems to explain the muscle pain. EIMD may affect athletic performance causing reduction in joint range of motion, attenuation of the shock and peak torque. Alterations in the muscular sequencing and recruiting patterns may also occur, causing unusual stress in the muscular ligaments and tendons. These compensation mechanisms may increase the risk of additional damage if early return to sport occurs. A series of treatment strategies was introduced to help to alleviate the EIMD severity and to restore the maximum function of the muscles as soon as possible[7]. Many interventions such as warming-up, stretching, massage, acupuncture, anti-inflammatory medications and estrogen supplements were researched with the aim to find interventions to successfully relieve the muscle damage severity. The findings of investigations which evaluated treatments for delayed onset of muscle soreness (DOMS) were not conclusive and conflicting[8]. The results were mainly inconclusive due to the variety of protocols for DOMS, the kinds of intervention protocols and the application dosage[9]. From the practical point of view, prevention strategies are chosen by many practitioners since they reduce the time away from training, treatment cost, besides decreasing the risk of additional damage[10]. Thus, this article emphasizes on prevention of eccentric DOMS. Suggestions of appropriate prevention strategies to muscle damage caused by eccentric exercise are offered. Non-steroid anti-inflammatory drugs demonstrated effects dependent on the dosing, which may also be influenced by the administration time. Similarly, massage presented many results which can be attributed to the application time as well as kind of massage technique used. Cryotherapy, stretching, homeopathy, ultrasound and electric current modalities have not shown effect of muscle pain alleviation or other DOMS symptom. Exercising is an effective way of relieving pain during DOMS; however, the analgesic effect is also temporary. There are still many unanswered questions concerning DOMS and many potential for future investigation. Few investigations studied about the impact of a combined intervention and many variables on exercise-induced muscle damage. Thus, the present study had the aim to evaluate the effects of combined interventions in markers and determine its prophylactic effects (before the exercise) and therapeutic effects (post-exercise).

METHODS

Participants

Participation was voluntary and the subjects were recruited through verbal invitation at the Azad University and physical education classes. The subjects (mean± sd age 22.50 ± 2.07 years; height 172.50 ± 4.27m and body mass 65.12 ± 4.91kg) had no history of musculoskeletal injury of upper extremities, flexion, extension range of motion of the shoulder and elbow joints totally pain-free and were not engaged in any kind of resistance training or extensive physical activity in the last six months.

Procedures

After careful examination and physiotherapeutic selection by a doctor, besides the first measurements, the subjects (n = 16) were divided in subgroups with control and experimental hands. The clinical evaluation indicates that the subjects are apt for active exercises. The exponential subgroups (experimental sides) received an exercise treatment, while the control subgroup did not receive it. Moreover, they were estimated before the exercise, 24, 48, and 72-96 hours after exercise for measurement of the dependent variables. Measurement alterations during the time were compared between the experimental and control subgroups. The dependent variables consisted of elbow isometric and isokinetic voluntary maximum flexor strength, creatine kinase activity, perceived muscle pain rate, range of motion and swelling.

The Ethics in Research Committee of the University, in agreement with the Declaration of Helsinki, approved all the procedures before the beginning of the investigation; all volunteers filled out a medical selection questionnaire and provided the written consent form before participation.

Muscle damage induction

Muscle damage was induced through the Scott biceps curl test. The subjects performed 50 eccentric contractions (60% of maximum eccentric contraction) with both hands. The eccentric contractions lasted three seconds; each of them was separated by 10-second recovery time.

TREATMENTS

Therapeutic protocol

This program includes pre-exercise drugs (vitamin C) and PNF stretching (prophylactic) and post-exercise ultrasound treatment (therapeutic).

Pre-exercise

Drugs: for that purpose, the subjects considered received prevention doses of vitamin C (250mg three times a day in a total of 750mg a day) for three days before the activity, at the day of the activity (total of four days).

The PNF technique (contract-relax) was performed for stretching. The subjects were treated with 10 seconds of isometric contraction and after five seconds of relaxation, and finally 20 seconds of stretching (92). Moreover, they were daily treated for three days before the test. The exercises were divided in six sessions, two sessions a day (10 o'clock in the morning and five o'clock in the afternoon) and each session lasted 10 minutes.

Post-exercise

For that task, the frontal and forearm muscles were kept under maximal tension while supported any of the subjects. Such position was kept for one minute and the muscles were then induced to deep warm-up for five minutes in an ultrasound device of continuous frequency of 1MHz and intensity of 1.5W/CM2. The ultrasound was performed with 10-seconds intervals; three times (immediately post--exercise, approximately three to four hours after the test).

Measurement criterion

The dependent variables for the indication of damage were maximal voluntary contraction (MVC) of flexing elbow, creatine kinase activity (CK), muscle pain (DOMS), range of motion (ROM) (elbow angle at rest, range of motion of flexed elbow, range of motion of extended elbow) and swelling (circumference of relaxed arm, circumference of flexed arm, circumference of forearm) which were used in previous research [11]. Variables measurements were recorded with basal values, immediately after exercise, and 24, 48 (and 72, 96) hours post-exercise. Serum creatine kinase was measured in basal values, immediately post-exercise and 24, 48 (and 72, 96) hours post-exercise.

Maximum voluntary contraction (MVCd)

Maximal isometric voluntary contraction (MVC): MVC was evaluated with the use of an isokinetic dynamometer (Cybex 6000, Ronkonkoma, NY, USA). The instrument was prepared according to the manufacturer's recommendations for exercise of elbow flexors. The MVC torque was measured in steady joint angles of 90° of elbow extension, MVC isokinetic torque in concentric velocities of 90°·s-1. The subjects were encouraged to produce a continuous maximal contraction of elbow flexors for three seconds against a steady lever of the Cybex 6000 isokinetic dynamometer with elbow joint angles steady at 90°. Each repetition lasted 3s intervaled with 60s rest, and the peak torque generated from three attempts was recorded as the MVC.

Creatine kinase activity (CK): a 5ml sample of venous blood was collected from the basilic vein portion at every measurement moment (basal, immediately post-exercise and 24, 48 (and 72, 96) hours post-exercise), allowing coagulation for 1h at room temperature and was centrifuged for serum separation from the rest of the blood constituents. The serum was removed and immediately frozen at –70°C for subsequent analysis. The serum CK concentrations were determined using the *RANDOM ACCESS* 1000 system.

Perceived muscle pain rate (PMP): muscle pain was assessed with the use of a visual analog scale. That scale had a 10cm-line with the words "pain absence" in one of the ends and "extremely painful" on the other. The subjects should indicate their pain level on the line while their elbow flexors were: 1) palpated (three sites on the upper part of the arm: medial face of the biceps brachii, 3cm above and below the medial face), 2) extended, and 3) flexed by the researcher. During palpation the highest score of the three sites was used for future analysis[7,12].

Range of motion (ROM)

Elbow angle at rest: the elbow angle at rest was determined by the angle formed on the elbow when it was held by the side while the subject did not try to fully extend his arm (the subject tried to extend his arm while it was relaxed) with the elbow laterally held and the hand at half elbow pronation in order to touch his shoulder with the supinated palm.

Range of motion of flexed elbow: it was determined by the angle made on the elbow when it was laterally held while the subject tried to completely flex the elbow joint to touch his shoulder with supinated palm.

Range of motion of extended elbow: it was determined as the angle formed on the elbow joint when the subject tried to completely extend his arm with elbow laterally held and hand at half pronation. In order to have consistent measurements, four marks were made on the skin with a semipermanent ink marker: one laterally close to the deltoid tuberosity level a second one on the humeral lateral epicondyle; a third one on the wrist medial point and the fourth one laterally to the radius styloid process. A plastic goniometer (Sammons Preston Rolyan, Illinois, USA) was used to record the measurements.

Swelling: Circumference of relaxed arm: the diameter of the upper limb was medially measured between the acromion process and the humeral lateral epicondyle using an anthropometric tape while the arm was naturally extended along the body. The site at the subject' hands was three times measured and the means were reported. The skin was marked with a semipermanent ink marker for consistency on the following days. Circumference of flexed arm – the circumference above the arm was medially assessed with flexed arm (arm flexed at 90 degrees). The site on the hands of the subjects was measured three times and the means reported. The skin was marked with a semipermanent ink marker for consistency on the following days.

Circumference of forearm: it was evaluated on the maximal diameter using an anthropometric tape while the elbow was flexed at 90 degrees of supination.

STATISTICAL ANALYSIS

The SPSS (version 15; SPSS Inc, Chicago, IL) was used for the analysis. The statistical significance level for all measures was set at 0.05. Descriptive statistics and suitable parametric tests were used to describe the participants. A *t* test for independent samples was used to compare the basal measurements between the subgroups in the beginning and after training. Data were analyzed with each group (dependent means) repeating measurements with time factors and treatment used (adjustment for multiple comparisons: Bonferroni).

RESULTS

Basal values for all dependent variables did not demonstrate any difference between subgroups (table 1).

Circumference of relaxed arm, circumference of flexed arm, circumference of forearm, elbow angle at rest. Circumference of arm at basal value and forearm and elbow angle at rest were not different between subgroups ($P > 0.05$). Intersubgroup comparison of limb diameter and elbow angle at rest did not present any difference in the control subgroup compared with the experimental subgroup ($P > 0.05$).

Range of motion of flexed elbow

Decrease in range of motion of flexed elbow was observed for the control subgroup in all subgroups and in all sessions (table 1). In the control subgroup, range of motion of flexed elbow immediately decreased (24 hours) post-exercise in 2.06% below the basal value compared with 1.36% of decrease in the experimental subgroup ($P > 0.05$). This trend of decrease was evidenced I the control subgroup in 48 hours (in 3.5%) while decrease of 1.62% was demonstrated in 48 hours in the experimental subgroup ($P < 0.05$), in 72-96 hours, mean percentage decrease of 1.98%, 0.41% was also evident in the control group, compared with decrease of 1.09%, 0.35% in the experimental subgroup ($P > 0.05$). The range of motion comparison of flexed elbow between subgroups presented significant differences between control and experimental hands in the 48 hours post-exercise (P < 0.05), but range of motion of flexed elbow comparisons did not present evident significant differences between the control and experimental hands in 72-96 hours post-exercise (P < 0.05) (table 1, figure 1).

Table 1. Muscle pain for the experimental subgroup after a bout of damage exercise.

	96 hours	72 hours	48 hours	24 hours	Immediately post-exercise
Pre-exercise	Dm = -0.888 *P = .37231	Dm = 2.35 *P = 003	Dm = -2.69 *P = 000	Dm = -1.84 *P = 000	Dm = -2.56 *P = .995
Immediately post-exercise	Dm = -0.631 P = 1	Dm = -2.09 *P = .008	Dm = -2.43 *P = 000	Dm = -1.58 *P = 000	
24 hours	Dm = 0.956 *P = 1	Dm = 0.506 P = 1	Dm = -0.850 P = .470		
48 hours	Dm = 1.80 *P = .005	Dm = 0.344 P = 1			
72 hours	Dm = 1.46 *P = 0.028				

MD = mean difference; * = indicates significant time effect.

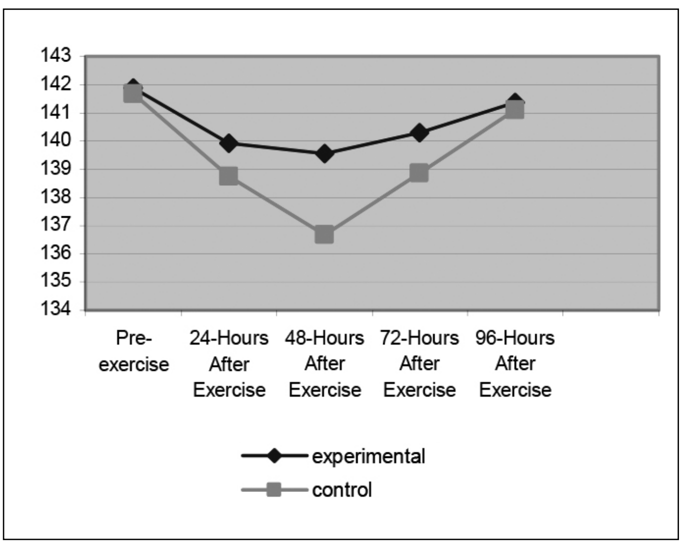

Figure 1. Alterations in range of motion of flexed elbow (degree) before (pre--exercise) and 24, 48, 72 and 96 hours post-exercise for the control and experimental subgroups.

Range of motion of extended elbow

Decrease in range of motion of extended elbow was observed for the control subgroup in all sessions (table 1). In the control subgroup, range of motion of extended elbow decreased immediately (24 hours) post-exercise in 1.71% below the basal values, compared with decrease of 0.07% in the experimental subgroup (P < 0.05). This tendency to decrease was observed in the control group in the 48 hours (in 3.05%) and 72 hours (in 1.93%), while decrease of 1.22% and 0.69% was demonstrated in 48 and 72 hours, respectively, in the experimental subgroup (P < 0.05). In the 96 hours, mean percentage decrease of 0.47% was still evident in the control group, compared with decrease of 0.07% in the experimental subgroup (P > 0.05). Intersubgroup comparisons of range of motion of extended elbow presented evident significant differences between control and experimental hands in 24, 48 and 72 hours post-exercise (P < 0.05) (table 1, figure 2).

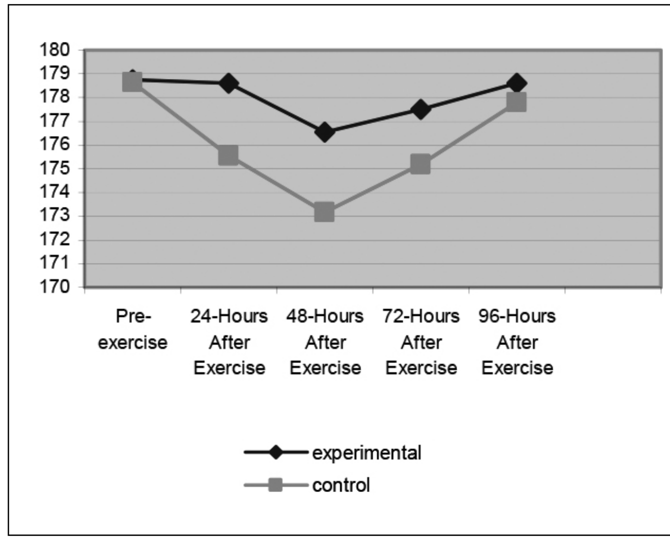

Figure 2. Alterations in range of motion of extended elbow (degree) before (pre--exercise) and 24, 48, 72 and 96 hours post-exercise for the control and experimental subgroups.

Muscle strength

Decrease in maximal isometric torque was observed for the control subgroup in all angles in all sessions (table 1). Concerning the control subgroup, torque decreased immediately post-exercise (24 hours) in 1.70% below the basal value, compared with decrease of 0.84% in the experimental subgroup (P > 0.05). This decrease tendency was presented in the control group in the 48 hours (in 6.56%) and 72 hours (in 5.47%), while decrease of 2.94% and 2.49% was demonstrated in the 48 and 72 hours, respectively, in the experimental subgroup (p < 0.05). In the 96 hours mean percentage decrease of 2.53% was still evident in the control subgroup compared with decrease of 0.67% in the experimental subgroup (P > 0.05). However, intersubgroup comparisons of maximal isometric torque presented evident significant differences between the control and experimental hands in the 48 and 72 hours post-exercise (p > 0.05) (table 1, figure 3).

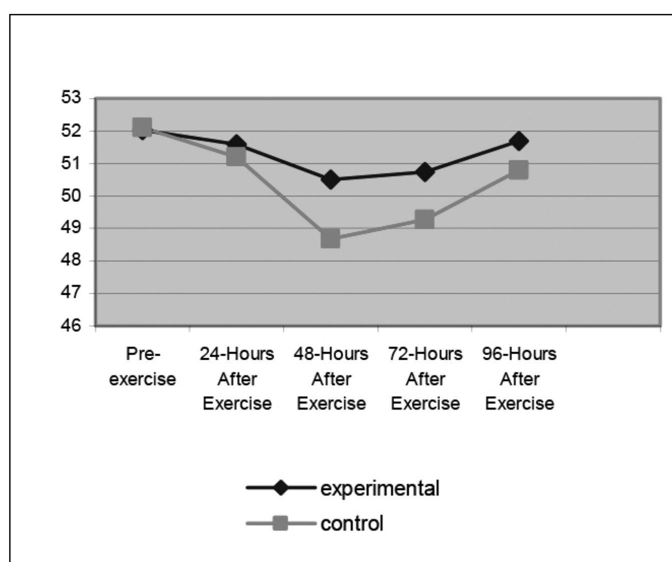

Figure 3. Alterations in maximum isometric torque (Nm) before (pre-exercise) and 24, 48, 72 and 96 hours post-exercise for the experimental and control subgroups.

Tie effect was found in the maximal isokinetic torque in the 90º/s between basal values and any of the other sessions (48, 72 hours pot-exercise) in the experimental and control subgroups along time (p > 0.05). Maximal isokinetic torque in the 90º/s altered with time within the control subgroup, decreasing in 4.12% below the basal value immediately post-exercise (24 hours) and in 7.09% and 6.25% in 48 and 72 hours, while decrease of 2.86% and 2% was demonstrated in the 48 and 72 hours, respectively, in the experimental subgroup (P < 0.05). In the 96 hours, mean percentage decrease of 4.40% was evident in the control subgroup, compared with decrease of 0.60% in the experimental subgroup (P > 0.05). However, intersubgroup comparisons of maximal isokinetic torque evidenced significant differences between the control and experimental hands in 48 and 72 hours post-exercise (p < 0.05) (table 1, figure 4).

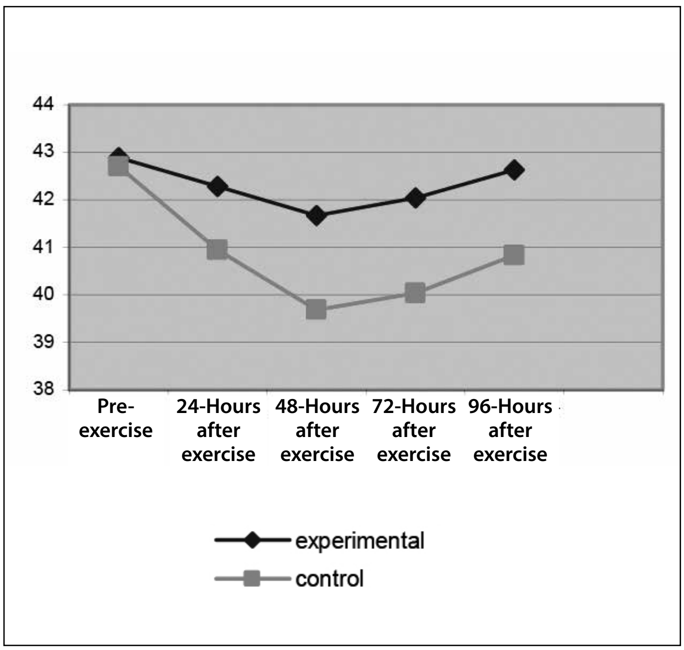

Figure 4. Alterations in maximum isokinetic torque (Nm) before (pre-exercise) and 24, 48, 72 and 96 hours post-exercise for the experimental and control subgroups.

Perceived muscle pain rate (PMP)

Before the exercise, none participant reported pain during the evaluations. The DOMS developed after the exercise in both subgroups (table 1). The control subgroup reported higher DOMS perception post-exercise (3.58 and 4.40) than the experimental subgroup (1.97 and 2.82) in 24 and 48 hours, respectively (p < 0/05). In the 72 hours, mean pain increase was equal to 3.66 and 2.48 in the experimental e control subgroups, respectively (P > 0.05). Intersubgroup comparisons of perceived muscle pain rate presented evident significant differences between the control and experimental hands in the 48 and 72 hours post-exercise (P < 0.05) (table 2, figure 5).

Plasma CK activity

The percentage increase of the CK level in the 24, 48, 72 and 96 hours were higher than in the basal values (P < 0.05). In the 96 hours post-exercise, the CK level was 56.34% above the basal value (table 3, figure 6).

Table 2. Muscle pain for the control subgroup for after the bout of damage exercise.

	96 hours	72 hours	48 hours	24 hours	Immediately post-exercise
Before exercise	Dm=-0.950 P=0.260	Dm=-3.49Dm *P=000	Dm=-4.22D *P=000	Dm=-3.41 *P=0.222	Dm=-0.350 *P=0.296
Immediately post-exercise	Dm=-0.600 P=1	Dm=-3.14 *P=000	Dm=-3.87 *P=000	Dm=-3.06 *P=000	
24 hours	Dm=2.43 *P=0.001	Dm=-0.081 P=1	Dm=-0.813 P=0.040		
48 hours	Dm=-3.27Dm *P=0.000	Dm=0.731 P=0.270			
72 hours	Dm=2.54 *P=0.000				

MD = mean difference; * = indicates significant time effect.

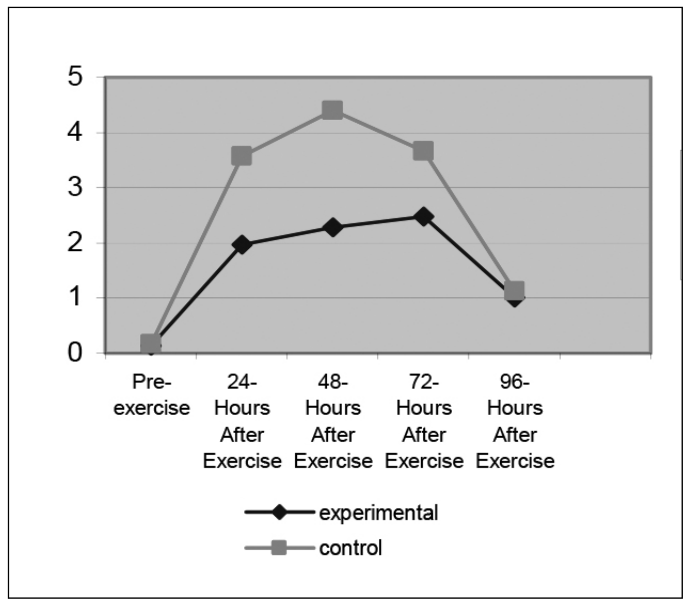

Figure 5. Alterations in perceived muscle pain (cm) before (pre-exercise) and 24, 48, 72 and 96 hours post-exercise for the experimental and control subgroups.

DISCUSSION

The possible prophylactic (before exercise) and therapeutic effect (post-exercise) of a combined treatment in the biochemical (enzymatic levels) and functional markers (elbow angle, circumference of elbow, circumference of arm, pain rate) of exercise-induced muscle damage was investigated. The combined treatment had a relief effect in the exercise-induced DOMS responses concerning the alterations in range of motion (flexibility), muscle pain, maximal voluntary isometric strength and isokinetic strength; however, it did not present relief effect in the exercise-induced DOMS responses concerning the circumference of relaxed arm, circumference of flexed arm, elbow angle at rest, circumference of forearm and plasma CK activity.

No differences were found between the subgroups for circumference of relaxed arm, circumference of flexed arm and circumference of forearm (P > 0.05). Limb diameter did not present evident difference between subgroups and consequently provided

Table 3. Alterations in result measurements before (pre), immediately, 24, 48, 72 and 96h post-eccentric exercise of PNF and control subgroups, mean + MSE.

Variable, group and P value	Pre-exercise	24 hours post-exercise	48 hours post-exercise	72 hours post-exercise	96 hours post-exercise
Circumference of relaxed arm , cm					
Experimental hand	28.06±3.98	28.20±4.10	28.35±4.09	28.24±4.03	28.03±3.98
Control hand	28.10±3.99	28.28±4.05	28.73±4.01	28.58±3.96	28.06±3.98
P value	0.975	0.952	0.792	0.820	0.982
Circumference of flexed arm. cm					
Experimental hand	29.71±4.13	29.84±4.21	30.13±4.26	30.05±4.19	29.68±4.13
Control hand	29.76±4.12	29.84±4.18	30.38±4.17	30.25±4.11	29.73±4.12
P value	0.976	1	0.868	0.889	0.976
Angle of elbow at rest. degree					
Experimental hand	163.93±3.73	161.62±4.14	157.87±7.57	160.75±4.72	163.25±3.78
Control hand	163.56±3.30	159.25±4.41	154.93±7.17	158.09±5.13	162.68±3.43
P value	0.766	0.127	0.268	0.138	0.663
Range of motion of flexed elbow. degree					
Experimental hand	141.87±2.80	139.93±2.95	139.56±3.44	140.31±2.54	141.37±2.64
Control hand	141.68±2.84	138.75±3.02	136.68±3.89	138.87±3.24	141.09±2.60
P value	0.852	0.270	0.035	0.174	0.764
Range of motion of extended elbow					
Experimental hand	178.75±1.65	178.62±1.62	176.56±2.15	177.50±1.67	178.62±1.70
Control hand	178.63±1.64	175.56±2.63	173.18±2.99	175.18±2.22	177.79±1.57
P value	0.848	0.001	0.002	0.003	0.163
Circumference of forearm. cm					
Experimental hand	26.19±2.89	26.25±2.89	26.56±2.75	26.43±2.73	26.16±2.89
Control hand	26.25±2.86	26.34±2.85	26.96±2.65	26.73±2.63	26.24±2.85
P value	0.956	0.932	0.674	0.754	0.937
Maximum isometric torque. Nm					
Experimental hand	52.03±2.17	51.59±2.19	50.50±2.32	50.73±1.89	51.68±2.06
Control hand	52.10±2.20	51.21±3.50	48.68±2.35	49.28± ±1.94	50.78±2.16
P value	0.923	0.719	0.036	0.041	0.235
Maximum isokinetic torque. Nm					
Experimental hand	42.89±3.16	42.28±3.08	41.66±2.66	42.03±2.68	42.63±3.06
Control hand	42.71±3.25	40.95±2.70	39.68±2.12	40.04±2.28	40.83±2.52
P value	0.880	0.206	0.028	0.031	0.080
Perceived muscle pain					
Experimental hand	0.13±.277	1.97±1.10	2.82±1.60	2.48±1.88	1.01±1.40
Control hand	0.17±.286	3.58±.855	4.40±.980	3.66±1.51	1.12±1.34
P value	0.664	0.000	0.002	0.059	0.829

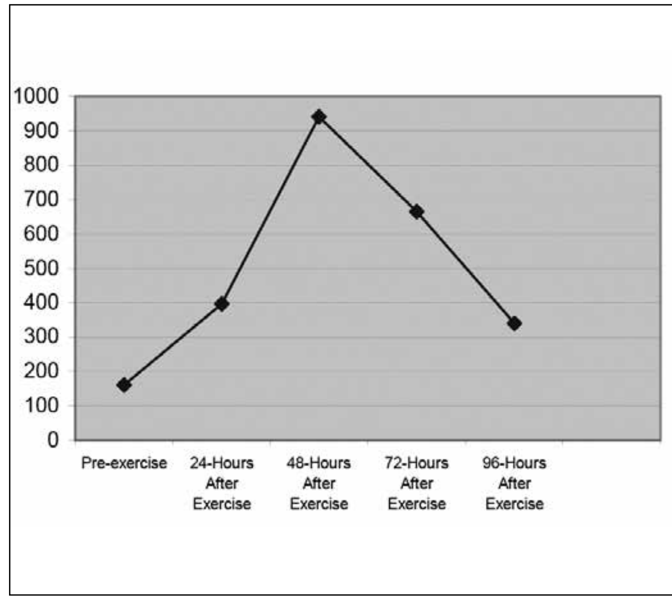

Figure 6. Alterations in the perceived muscle pain rate (IU/L) before (pre-exercise) and 24, 48, 72 and 96 hours post-exercise.

indirect evidence that the intervention was not successful in causing these alterations. Nevertheless, some authors previously[13] attributed the DOMS pain to the edema and swelling of the exercised muscle fibers. Yet, Smith[6] and Armstrong[14] discussed that there is monocytes buildup, which convert to macrophages after injury, and they produce substances which on their turn, sensitize the type III and IV nervous terminations within 24 to 48 hours. Moreover, Buroker and Schwane[15] and Gulick et al.[12] found that diameter measures of eccentrically exercised limbs did not increase in any moment of the post-exercise evaluation, in agreement with our findings.

Swelling was observed as response to DOMS and acute inflammation; even though, DOMS was different from an acute inflammatory response[16]. When pulsed ultrasound was confirmed[17] as successful in the swelling treatment, it was in fact being used to treat an acute inflammatory response. Moreover, it is interesting to note that the use of anti-inflammatory drugs presented a too small effect in the passage of time of DOMS[18]. Thus, we could believe that the process occurred here is not strictly not to reduce an inflammatory process. Another finding is that the combined treatment did not reduce swelling in DOMS.

Magnitude of strength loss was different between subgroups ($P > 0.05$). Maximal voluntary isometric and isokinetic strength response in this investigation presented remarkable decrease immediately post-exercise and general recovery concerning the pre-exercise levels in the 96 subsequent hours and agrees with the literature[19]. Muscle strength is one of the best indicators of muscle damage, which are normally reduced after exercise with slow recovery[20]. Strength loss of up to 60% is directly evident after exercise and it can last up to10 days[21]. It was initially hypothesized that such fact is due to pain inhibition, but strength loss is observed much earlier than pain perception. It is believed that the excess of stretching in the sarcomeres and coincidence of actin and myosin reduction is the main cause of this strength loss[22]. Westerbald et al.[23] suggested that fatigue caused by reduction in the damaged sarcoplasmic reticulum calcium production may lead to incapacity to generate strength.

Prevention of exercise-induced muscle damage with the use of PNF showed positive effect on muscle strength[24]. The result of our study was similar to this study, but it was different from previous studies by High et al.[25], Johansson et al.[26].

Additionally, pulsed ultrasound was used in the past for stable cavitation and microstreaming to heal injured muscles. Hasson et al.[27] observed that the serves in basal percentage for isometric contraction, maximal extension torque and knee extension work were significantly lower in the 48 hours for subjects who received pulsed ultrasound compared with placebo treatment and control subjects. The authors concluded that pulsed ultrasound accelerates restoration of normal muscle performance, and hence, was efficient in decreasing DOMS.

More recently, other authors found conflicting results. Plaskett et al.[28] found that there were no differences in the pain levels or muscular performance between the experimental group receiving pulsed US at 1.0Wcm and a placebo group. However, in this study there was no control group and groups with few subjects, which may limit the conclusions. The result of our study was different from this study. Vitamin C and PNF stretching and ultrasound treatment as used in our study did not significantly decrease the DOMS effects in strength.

Differences between the subgroups were found for range of motion of flexed elbow and range of motion of extended elbow ($P < 0.05$). Range of motion (ROM) presented visible difference between the subgroups and, consequently, provided indirect evidence that the intervention was successful in this study.

In the present study, a different technique (contract-relax PNF and ultrasound treatment) was applied. This technique is a combination of both static and dynamic stretching. As a result, some positive effects of the PNF and ultrasound treatment were observed in the symptoms of exercise-induced muscle damage (EIMD) concerning sensory perception and muscular function.

Application of PNF before exercise and the ultrasound treatment after exercise were the aim in the preparation of the chosen muscle for prevention and treatment of EIMD symptoms.

The contract/relax PNF technique was used for preparation of the elbow flexors with passive and active movements, which may improve muscular flexibility via autogenic inhibition and mutual inhibition. The benefits of active warm-up may be the minimization of muscular stiffness through movement of the muscle groups through their range of motion. As a result, warm-u with PNF stretching may release ligations between actin and myosin and hence, reduce passive muscular stiffness. Such fact may contribute with increased rate of strength development and increase in efficiency of the muscle worked during eccentric exercise[29].

Stretching exercises also affect the mechanical properties of the muscle-tendon unit (MTU), e.g., to reduce tension in the muscle--tendon unit which affects the tissue viscoelastic component leading to increase in muscle conformity and reduction in muscle stiffness; consequently, lower tension will be produced in the muscle during specific stretching. Improvement resulting from muscle flexibility possibly reduces muscle and connective tissue damage after exercise[30]. Consequently, the combined treatment may improve muscular flexibility and significantly decrease the EIMD effects in range of motion.

The results revealed that there were significantly differences between the control and experimental hands for delayed onset of muscle soreness and pain intensity rate ($p < 0.05$). Thus, this therapeutic protocol was able to improve the marker of deficiency for the visual analog scale (VAS) in 24h, 48h and 72h after exercise--induced muscle damage.

Many reasons were proposed for the EIMD pain cause. Smith[6] believes that the intracellular swelling and edema are the cause for the compression in the nervous terminations sensitive to pain. Such fact may lead to sensitization of these nerves, and consequently, pain. This author supports such theory when states that this is the reason for pain being only felt in movement and palpation but not during rest. As the muscle is placed under mechanical stress, inner pressure increases and compression of nervous terminations takes place.

However, Clarkson and Newham[22] believe that the mediators released in the inflammatory process such as bradykinin, serotonin and histamine which sensitize the nervous terminations to pain, and hence, result in pain. As a result, a combined treatment (vitamin C and PNF stretching and ultrasound treatment) as the one used in our study, significantly decreased the effects of EIMD in pain perception.

The CK intracellular release was used as an indirect marker of EIMD for many years[7]. The CK response in this investigation showed peak 48h post-exercise, which is the same response of previous data using a similar protocol for damage induction. Therefore, the majority of CK responses following eccentric exercise which causes damage to the upper limb tends to be slightly delayed and presents peak 24h after[20]. Although the reason for this fact is not clear[19], one can speculate that the upper limb is less used to eccentric load and hence is more prone to damage than the lower limb; consequently, CK is more delayed and of greater magnitude in the upper limb[19].

CONCLUSIONS

The possible prophylactic (before exercise) and therapeutic effects (post-exercise) of a combined treatment in exercise-induced muscle damage were investigated. Based on these results, vitamin C and PNF stretching before exercise and ultrasound treatment post-exercise may reduce the symptoms of muscle damage (AMD, strength loss, muscle pain and pain intensity) more than in the control subgroup. The results of this study suggest that the application of a combined treatment helps to attenuate the EIMD symptoms in the elbow flexors.

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