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The static stretching, eccentric training, nano particles and biochemical enzymes (CK and LDH)

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ABSTRACT: Introduction: Delayed onset muscle soreness is known, muscle pain, soreness and discomfort feeling approximately 24-48 hours after exercise. The increased serum biochemical enzymes after acute exercise or unaccustomed training were reported in some studies. The main purpose of this study was to search the effect of warm-up before eccentric contractions on DOMS, biochemical enzymes changes and Nano particles.

Methods: Twenty player female volunteers were selected. All subjects were right handed and healthy. Subjects were assigned in one of two experimental group (n=10) (age: 22.6±1.91 years, height: 168.46 ± 2.91 cm, weight: 58.24±6.91 kg, and $V_{O2,\ max}$: 33.19±3 $ml.kg^{-1}.min^{-1}$) and, control group (n=10) (age: 23.36 ±5.350 years, height: 160.81±5.16 cm, weight: 55.16±3.42 kg, and maximal oxygen consumption $V_{O2,max}$: 35.1±4.71 $ml.kg^{-1}.min^{-1}$). They performed experimental protocol (80 resistance eccentric contraction) that approved by the university of ethics committee. All subjects were informed of the risks and purposes of the inquiry. Before attempt, their written consent was obtained. Blood samples were drawn from the anticubital vein before, immediately after, 24 and 48 hours after an exercise (n = 20). The study of protocol was Bruce. Ambient temperature during running was 19°C. The experimental groups, the first performed static stretching on shoulder elbow for 20 minutes and then performed incremental resistance eccentric contraction. Control group performed, only incremental resistance eccentric contraction.

Results: We used mean ±SD, tables, graph, chart, ANOVA and Bonferroni post hock tests for analyses by SPSS, version 21 (P≤0.05). After analyzing, data showed an increasing CK and LDH biochemical enzymes levels in two subject's blood. But this increase was higher in experimental group.

Discussion: The results showed warm up by static stretching did not prevent not only the muscular damage cellular damage and soreness, but also induced increasing CK and LDH biochemical enzymes. Muscle sourness and weakness are acute (immediately after exercise) delayed after, 1-2 days after exercise. We approach that it is better, the first general warm up and then static stretching, because muscle fibers and membrane do not damage, destroy and don't deplete metabolism enzymes .So we suggest using Nano particles reduce pain and soreness.

Keywords: *Contraction; CK; LDH; Muscle soreness; Nano particles*

INTRODUCTION

The physical training is one of the factors that can influence immune function, health and exercise performance. Another factors (pathogens, health status, lifestyle behaviors, sleep, recovery, nutrition and psychosocial issues) need to be considered. So exercise

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can induce muscle damage, inflammation and injury that indicated by muscle soreness and swelling, prolonged loss of muscle function, and leakage of muscle proteins, for example: CK and Mb and LDH (Lavender, *et al.*, 2006). It has been reported that, the changes in plasma proteins, enzymes, cytokine and another immune factors concentrations, depends on the intensity and duration of exercise, and the type of the muscle contraction (Malm, *et al.*, 2000). Delayed onset muscle soreness is muscle pain and discomfort experienced approximately 1-3 days after exercise. DOMS is thought to be a result of microscopic muscle fiber tears and is more common after eccentric exercise rather than concentric exercise.

While DOMS is not a disease or disorder, it can be painful and is a reason of the presence of pain and other possible debilitating performance factors, preventing or minimizing the effects of DOMS should be a concern for coaches, athletic trainers, physical therapists, and other sports medicine personnel concern for athletes because it can limit further exercise in the days following an initial training (Sethi, 2012). Following concentric, eccentric and isometric exercise, demonstrated that eccentric contractions affect greater muscular soreness than either concentric or isometric exercise, with the peak occurring after 48 hours, muscular strength also decreased following eccentric contractions and remains depressed throughout the duration of the soreness period (Tiidus, 2010). Clarkson show that concentric arm flexion exercises (bicep curls) minimized the DOMS in comparison to arm flexion eccentric exercise (Clarkson, *et al.*, 1999).

Exercise involving both concentric and eccentric exercises produced a greater perception if soreness (Uchida, *et al.*, 2009). The use of hydraulic resistive devices (hydra fitness) that involve concentric only produced less DOMS 25 hours post-exercise than concentric eccentric combined contractions (Yu, *et al.*, 2003). Increased serum enzyme after acute exercise or unaccustomed training was reported in some studies. Many factors alternative the degree to which the variety of biomarkers enzymes activities in serum, they will increase during and after exercise. Activities of biochemical enzymes that searched in muscle include CK and LDH (Sethi, 2012). These enzymes increase in the intensity and duration of exercise. Peaking them are 24 hours after exercise. Serum enzyme activities the highest after very prolonged exercise (ultra- marathon running, triathlon), eccentric contractions (bench stepping, downhill running), swimming and cycling (Lieber, *et al.*, 1996). Serum enzyme activities increase lower in females, and the train than males and un-train, in altitude and in the heat. Increased serum enzyme activities cause DOMS (dilate onset muscle soreness). Myofibrils damage, Z-disk of sarcomeres disrupt, muscle glycogen deplete, lipid of membrane peroxidase and free radical product (malm, *et al.*, 2004).

These changes are the same occur with acute myocardial infarction. Serum biomarkers CK and LDH activity increase and return to normal after few days. Nutrition (vitamins, electrolytes, water and proteins) and exercise can help to recovery period (Chambers, *et al.*, 1996, Gleeson, 2006). Adaptation to acute exercise training induced muscular damage and another tissue then increased inflammation. Organism systems can repair pathways (Clarkson, *et al.*, 1996). Physical exercise and types of methods training involves

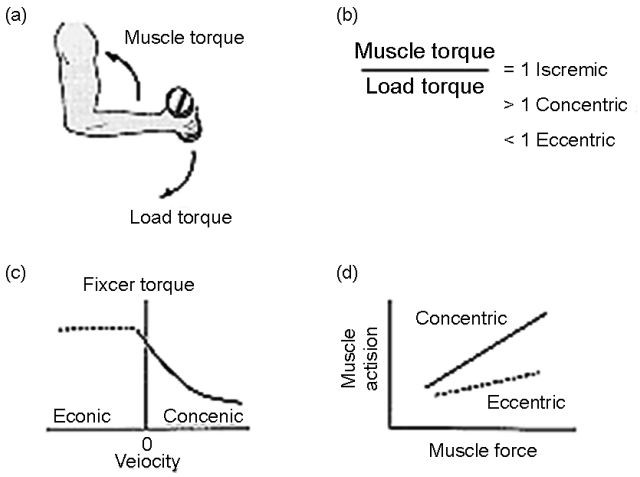

Fig. 1. Comparison of torque in eccentric and concentric contraction.

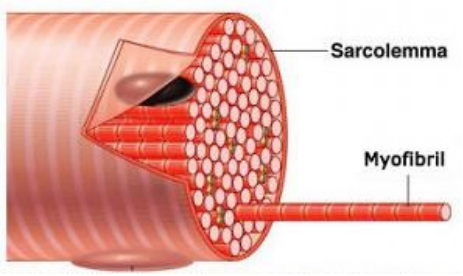

Fig. 2. Damage in myofibril

muscles, blood, tissues, releasing immune cells (leukocytes, neutrophils, granulocytes, macrophages) and adhesion cells (ICAM), chemo-attractive factors and activation of satellite cells (Tidball, 1995). Adaptation in skeletal muscle by exercise training related with muscle development, cell regeneration and satellite cell activation (MacIntyre, *et al.*, 1995). In the recent research in several pieces of evidence indicated that muscle adaptation to exercise training occurs via classical pathway and they showed similar inflammatory changes in skeletal muscle occurred in the exercise and control groups (Malm, *et al.*, 2004). Some of studies demonstrated ultra-structural damage in eccentric versus concentric exercised muscle (Gibala, *et al.*, 2000), but another could not reach any alteration in structure of myofibrils after different modes of eccentric contractures (Yu, *et al.*, 2003). Stupka reported number of leukocyte and inflammatory cells, increased in skeletal muscle 48 h after eccentric contraction (Stupka, *et al.*, 2000). Acute exercise training disrupts of the cytoskeleton and remodel (Nosaka, 2008). This study investigated the effect of warm up by static stretching on DOMS, CK and LDH biochemical enzymes. The main purpose was to determine the effect of static stretching before eccentric contractions (Lund, 1998). Eccentric contractions used in many training, can induced releasing enzymes. Some researchers found that DOMS (disrupt of epimysium), CK and LDH increased in recovery period (Chen, *et al.*, 2009). There is hypothesized, that muscle damage of eccentric contraction is greater than another or prolonged exercise. Acute-phase response and muscle injury will rise 24 h after exercise (Lieber, *et al.*, 1996). In one of studies, eight healthy men in 4 experimental conditions (1: cycle ergometer exercise at 90% VO_{2max}, 5 min, 2: circuit-training standard, 3: cycle ergometer exercise at 60% VO_{2max}, 2 h, 4: cycle ergometer exercise at 60% VO_{2max} 5h set. In circuit-training condition only, levels of CK were elevated significantly 72 h after training. Correlation between biomarkers CK and LDH concentrations, muscle soreness and immune biomarkers of the inflammation response reported (MacIntyre, *et al.*, 1995). Some of searches did study on Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. Finally they concluded that muscular adaptation to physical exercise occurs without preceding muscle inflammation, leukocytes important for repair regeneration and adaptation of human skeletal muscle (Zainuddin, *et al.*, 2006). The patterns of change in plasma keratin kinase (CK) activities between trained and untrained men were strikingly different. When compared with the untrained men, pre-exercise CK levels were significantly higher in the trained subjects. In the untrained men, CK levels increased progressively for 5 days afterwards, reaching a peak value that was 33 times larger than the baseline level. In contrast, the increase in plasma CK level in trained subjects was significant only at 24 h after the exercise bout, reaching a maximum value of 2.3 times larger than the pre-exercise level (Pedersen, 1999).

MATERIAL AND METHODS

In this present quasi-experimental study, 31 volunteers female university student (P.E. students) were randomly selected and divided into 2 groups: experimental group (n=15, age: 21.7±1.81 years, height: 161.54±2.81cm, weight: 56.25 ±6.96 kg and Vo_{2max}: 34.14±2 $ml.kg^{-1}.min^{-1}$) and control group (n=16, age: 24.24±4.30 years, height: 158.81±4.85 cm, weigh: 54.66 ±3.83 kg and Vo_{2max}: 36.2± 3.78 $ml.kg^{-1}.min^{-1}$). They were normal healthy, with no positive clinical signs. All subjects were right handed. Blood sampling of all subjects were taken venues pretest, then experimental group performed static stretching on hands (shoulder, elbow, arm for) 20 minutes, after stretching they performed eccentric contraction with lifting by none dominant hand (80% RM, Seventy unilateral, voluntary, with the elbow flexors 1bout).Control group performed, only eccentric contraction with lifting. Blood biomarkers sampling were obtained immediately, 24 and 48 hours after exercise training protocol, were drawn from elbow vein in the seated position. Muscle soreness at rest, after, 24 h, and 48 h after exercise, was saved by the subjects on a 0–10, Borg scale (soreness, pain, swelling). Serum biochemicals CK and LDH activity was measured using the standard laboratory kit. Data were lastly analyzed using SPSS version 21. The mean and SD of data were calculated using Descriptive Statistics. Data distribu-

Table 1. Changes CK, LDH in 2 groups

Time	M ± SD			
	CK		LDH	
	Con.g	Exp.g	Con.g	Exp.g
Pre test	72.72± 33.11	78.50±17.03	373.18 ±125.63	372.50± 125.63
Post test	107.72 ± 56.14	94.50±20.46	434.09±74.72	470 ±117.61
24 h after	157.27± 41.64	177.50± 31.91	531.81±79.35	570±112.96
48h after	148.18 ± 35.46	261.66 ± 23.42	273.18± 26.57	272.77± 55.46

tion normality and homogeneity of variance were respectively examined with Shapiro-Wilk and Levene's test. The paired-sample t-test and Bonferoni's post hoc analysis were applied to compare the differences within groups and between ones, respectively ($p<0.05$).

RESULTS AND DISCUSSION

Our results showed eccentric contraction induced DOMS (pain, soreness, inflammatory) in their hands and levels of CK and LDH enzymes increased in blood's subjects, after 24 hours. Experimental group had changes higher. Levels of enzymes increased immediately and 24 hours posttest, then decreased until 48 hours after exercise, but did not reach to baseline in 2 groups. Mean ±Standard Deviation and ANOVA results of enzymes have showed in Tables 1, 2 and Figs. 3 and 4. ANOVA results showed that CK and LDH activity in experimental group was increased significantly and Bonferroni test was significant.

DISCUSSION

There are theories that describe the current of events in the process of exercise and muscle damage (High, *et al.*, 1989) and drugs, such as Nano particles. When myofibrils are stretched during contraction, the weaker sarcomeres absorb more of the stretch, and the length-tension ratio is important, these sarcomeres become

Table 2. Result ANOVA ($p<0.05$)

Time / Factor	Pre	Pos	24 h	48 h
	Sig			
Ck	0.07	0.27	0.08	*0.05
LDH	0.27	0.71	0.82	*0.001

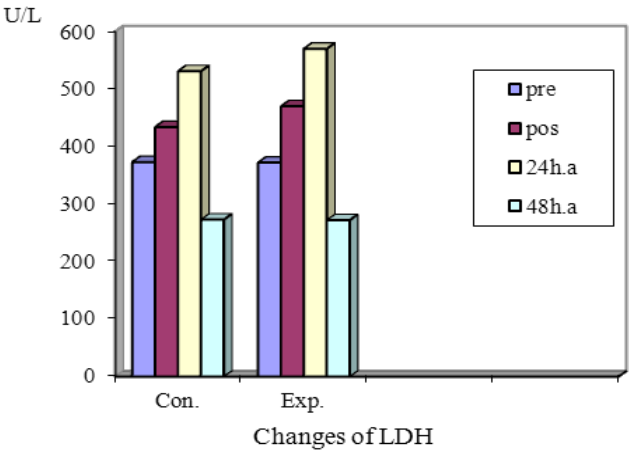

Fig. 3. Changes of LDH

weaker and they don't have any overlap between filaments (Peake, *et al.*, 2005). When muscles relax, the filaments may fail and disrupt sarcomeres. Structural disruption can develop areas of the muscle and then damage to the membranes of the sarcoplasmic reticulum and transverse tubules. This process lead to produce some of the symptoms: loss of muscle function, DOMS, and increases proteins in the circulation (Herbert, *et al.*, 2007). Skeletal muscle can adapt to eccentric exercise and the immune system has key role in the degeneration and regeneration. It shows the static

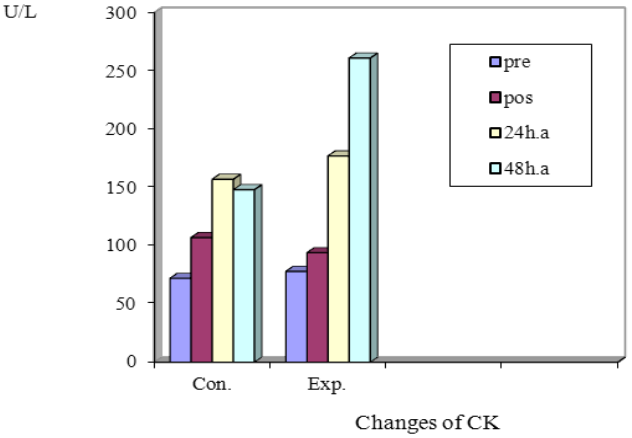

Fig. 4. Changes of CK

stretching has an effect on CK and LDH. The results showed warm up not only did not prevent the muscular damage and did not decrease the pain of cellular damage, but also the warm up by eccentric stretching increased CK and LDH (Denegar and Perrin, 1992). MS (muscle sourness) are acute (Immediately after exercise) or delayed after, 24-48 hours after exercise. That is better static stretching use after general warm up, because muscle fibers and member cells do not damage and deplete enzymes. There were significant correlation between CK in both groups, and LDH in experimental group and CK and LDH biochemical enzymes in recovery period on experimental group. It shows the warm up has a significant effect on CK and LDH correlation in recovery period (Kokkinos, 2009). The results showed warm up prevented the muscular cramp and decrease the pain of cellular damage, also the warm up changes the depletion of CK and LDH after strenuous activities (Denegar and Perrin, 1992). It is suggested that exercise can induce DOMS by activating inflammatory factors present in the epimysium before exercise. Repeated physical training may alter the content of inflammatory factors in the epimysium and thus reduce DOMS and types and intensities of exercise is important (Lieber, *et al.*, 1996). The exercise protocol used in this study resulted in decreased muscle function and inflicted severe muscle discomfort that hindered normal every day activities. DOMS and muscle pain were related to markers of inflammation in the epimysium and significant increases in muscle soreness, serum biochemical enzymes CK and LDH activity. Of course the correlations between immunological variables in human blood and skeletal muscle found in the previous study (Walsh, *et al.*, 2011). Studies interpret that muscular adaptation to physical exercise is fundamentally different from the muscle cell repair mechanisms activated by experimentally inflicted damage, the sensation of DOMS and pain after unaccustomed physical exercise is confined to immunological reactions in muscle epimysium, and individual differences between subjects result in larger deviations when investigating muscle adaptation to physical exercise than when tissue repair after trauma is studied (MacIntyre, *et al.*, 1995). When muscle soreness is caused, tiny tears happen in the muscle in response to weight training.

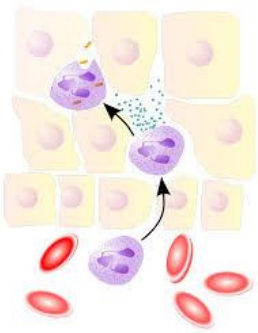

Fig. 5. Releasing macrophages in blood

Stretching of the muscle under tension (eccentric or lowering phase of a movement) causes damage to the muscle fibers and connective tissues that surround the muscle fibers. This is cellular micro trauma, and it is similar to tiny tears in the cell's membrane. Lower a heavy weight induced change that motor units are involved in the lift. There are differences in the muscle proteins connect and detach during contraction. Calcium ions are important, because responsible muscle for contraction. It's possible that all of these, and potentially other things, contribute to cellular micro trauma.

When muscles are involved to any sufficient amount of eccentric exercise, the tissue demonstrate several biomarkers of being damaged. Eccentric muscle action can result from any contracture that involves; weight training, another one, running or something can be a culprit. Any activity can cause soreness. Unaccustomed exercise, particularly eccentric muscle contractions, initiates mechanical muscle damage of varying degrees (Baird, *et al.*, 2012). When you do unaccustomed exercise or activity, you will feel sore the next or two day. But if you are active, you'll stop getting soreness and weakness. Muscle and connective tissues will adapt to the stress (Paulsen, *et al.*, 2010). This is an understanding process that could be the result of several events. The changes in motor unit activation include fibers that handle the weight, massive of the connective tissues around the muscle, big and strong muscles (Nosaka, *et al.*, 2005). These cause, induced muscles adapt to future exercise to the same conditions. Some recent studies have focused on the role of the cytoskeleton and its contribution to the sarcomere injury. Although little has been confirmed regarding the mechanisms involved in the production of delayed muscle soreness, it has been suggested that

the soreness may occur as a result of mechanical factors or it may be biochemical in nature (MacIntyre, *et al.*, 1999). So it is suggested that exercise can induce biomarker DOMS by activating inflammatory factors present in the epimysium before exercise. Repeated physical training may alter the content of inflammatory factors in the epimysium and thus reduce DOMS (Malm, *et al.*, 2004). The exact cause of DOMS is unknown, although it is generally described as the consequence of mechanical and/or metabolic stress (Ingraham, 2017), of course some studies showed the main cause of the DOMS is microscopic tears in the muscle fibers. As the muscle fibers slide against each other to produce tension, tearing can occur, especially during lengthening (eccentric) contractions (Howeon, 2013). DOMS also triggers an inflammatory reaction. The white blood cell count tends to increase after activities that induce muscle soreness; accordingly, some investigators believe that soreness results from inflammatory reactions in the muscle. In fact, substances released from injured muscle can act as attractants, initiating typical inflammatory processes. Monocytes in muscle are activated by the injury and provide chemical signals to circulating inflammatory cells. Neutrophils invade the injury site and release cytokines. Cytokines are immune regulatory substances, which attract and activate additional inflammatory cells. Neutrophils possibly also release oxygen free radicals that can damage cell membranes. Macrophages invade the damaged muscle fibers and phagocytize debris. After the dead tissues are removed, muscle regeneration starts to replace the injured cells (Ma, 2011).

Both DOMS and muscle damage can be influenced by the type of activity, with emphasis on eccentric muscle movements, type of exercise, velocity of the movement, interval period between series, the level of individual fitness, this last primarily affecting beginners. When myotrauma occurs, muscle damage repair is initiated by leukocytes migrating to the injured area, although, the histamines, prostaglandins, kinas and K^+ produced by neutrophils and macrophages stimulate free nerve endings in the muscle, causing the DOMS (Foschini, *et al.*, 2007).

Nutrients can decrease issues of DOMS. Some of results suggest glutamine supplementation attenuates effects, uncomfortable, muscle damage and downfall of performance, a little (Tajari, *et al.*, 2010). Many of results suggest massage, ice and topical cooling can attenuate DOMS (Tseng, *et al.*, 2013). So, Ibuprofen topical gel, Diclofenac acid decrease pain and soreness (Hyldahl, *et al.*, 2010), NSAIDs on recovery (Morelli, *et al.*, 2018) and Curcumin supplementation are useful (Nicol, *et al.*, 2015). Previous studies have evaluated the effectiveness of branched-chain amino acid (BCAA) supplementation for preventing delayed onset muscle soreness (DOMS) and muscle damage induced by eccentric exercise, their findings have been inconclusive. Since taurine has anti-inflammatory and anti-oxidative effects, the present study investigated the combined effect of BCAA and taurine on DOMS and muscle damage (Gyu Ra, *et al.*, 2013).

NANO Particles and DOMS

Nanoparticle such as Omega 3, 6, 9 and vitamin E have all parameters positively. Physical exercise or exercise training results in muscle damages. When exercise is hard, intensity or prolonged sports injuries are long muscle groups such as hamstrings muscle, biceps or shoulder muscle. External factors such as contusion, laceration, or crush muscles can be damaged (Huard, *et al.*, 2002), from trauma, acceleration output or collisions on the exercise training field or by internal injuries such as strains on hamstrings muscle tear (running or kicking). Foam rolling is a form of Self-Myofascial Release Therapy (SMRT). Foam rolling exercises can effortlessly target every part of the body that needs stretching. The important muscles are hamstrings, gluteal muscles, shoulder and the quadriceps (Brockett, *et al.*, 2004). Foam rolling exercises with Nano particles help to muscle rehabilitation and healing after injury. Those benefits are improved core strength and temperature, increase flexibility, release tension, pain and soreness; rehabilitate damage, strain, sprain, and injury prevention. Researchers showed statistically significant differences for use of foam roller exercise with nanoparticle (Omega3, 6, 9, vitamin E) because of inhibit pain and percipience.

CONCLUSIONS

In conclusion, our results showed the static stretch-

ing induced DOMS and then CK and LDH enzymes increased. Static stretches have to use after general warm up. In this study stretching could not effect on the weight training with eccentric contracture. We can use massage, ice, cold, supplementations (curcomin) ibuprofen for to decrease pain, soreness and weakness.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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