Approval of the Graduate School of Social Sciences

Assoc. Prof. Dr. Sabri Koç

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Assist. Prof. Gazanfer Doğu

We certify that we have read this thesis and in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Physical Education.

THE RELATIONSHIP of BLOOD CREATINE PHOSPHOKINASE and LACTATE DEHYDROGENASE ENZYME LEVEL with MUSCULAR SORENESS in FEMALE GYMNASTS FOLLOWING AN ISOMETRIC CONTRACTION

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MANOLYA AKIN

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January, 1992

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Assist. Prof. Dr. Gül Tiryaki

Examining Committee in Charge

Assoc. Prof. Dr. Kemal Tamer

Assist. Prof. Dr. Fehmi Tuncel

ABSTRACT

THE RELATIONSHIP OF BLOOD CREATINE PHOSPHOKINASE AND LACTATE DEHYDROGENASE ENZYME LEVEL WITH MUSCULAR SORENESS IN FEMALE GYMNASTS FOLLOWING AN ISOMETRIC CONTRACTION

AKIN,Manolya

M.S. in Physical Education and Sport

Supervisor: Assist.Prof.Dr. Gül TIRYAKI

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In this study, to find the relationship of CPK and LDH enzyme level with muscular soreness in female gymnasts following an isometric contraction was investigated.

Eleven volunteer female gymnasts aged from 7 to 13 years were participated as subjects. Two of the subjects were eleminated from this study because one of them dropped out from the study and the other's blood sample did not separate into serum.

The Cybex II equipment was used for an isometric contraction. 100% maximum work load was used for

quadriceps muscle group. Test applied four times as a one minute contraction, five minute rest. 2 cc blood from the cubital fossa were drawn at the pre-exercise period then 6, 12 and 24 hours later. The blood was allowed to clot for ten minute at room tempearature and then centrifuged from ten minutes to seperate into serum. After the centrifugation all serum samples were frozen at -20°C until analysis for CPK and LDH activity. The CPK and LDH activities were assessed in dublicate samples at 37° with the Dacos Analyzing Machine which has a spectrophotometer part. In each time, the blood were taken the questionaire for soreness was applied.

As a result, CPK and LDH levels increased for 6 hours linearly then decreased to 24 hours. Soreness levels showed logarithmic decrease for 24 hours and found direct relationship between CPK and LDH enzyme levels, but not significant relation between two enzymes with soreness.

Keywords: Creatine Phosphokinase, Lactate Dehydrogenase, Isometric Contraction, Muscular Soreness,Female Gymnasts.

Science Code: 224.19.01

ÖZ

BAYAN CİMNASTİKÇİLERDE İZOMETRİK KAS KASILMASI SONRASINDA CPK VE LDH ENZİM SEVİYELERİYLE KAS AĞRILARININ İLİŞKİSİ

AKIN, Manolya

Yüksek Lisans Tezi, Beden Eğitimi ve Spor Anabilim Dalı

Tez Yöneticisi: Y. Doç. Dr. Gül TİRYAKİ

Ocak 1992, 65 sayfa

Bu tezde izometrik kas kasılması sonrasında bayan cimnastikçilerde CPK ve LDH enzim seviyeleri ile kas ağrıları arasındaki ilişki araştırıldı. Yaşları 7 ile 13 arasında 11 gönüllü bayan cimnastikçi denek olarak kullanıldı. Deneklerden ikisi şartlara uymadığından çalışma dışı bırakıldı. Quadriceps kas grubu için %100 maksimum çalışma yükü ile izometrik kas kasılması sağlandı ve ölçümler için Cybex II cihazı kullanıldı. Test 4 sefer birer dakika kasılma ve 5 dakika dinlenme şeklinde uygulandı. Egzersiz öncesi, hemen sonrası ve 6, 12, 24 saat sonra kübital fossadan kan alınarak analiz edildi.

Sonuç olarak CPK ve LDH seviyeleri birbirleri ile doğru orantı gösterdi. Ağrı düzeyi ile enzimler arasında anlamlı bir ilişki bulunamadı.

Anahtar Kelimeler: Creatine Phosphokinase, Lactate Dehydrogenase, Izometrik kas kasılması, Kas ağrısı, Bayan cimnastikçiler.

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LIST OF SYMBOLS

AST	Aspartate transaminase
CPK	Creatine Phosphokinase
FAD	Flavo adenine dinucleatide
G-6-PDase	Glucose 6-phosphate dehydrogenase
GOT	Glutamic oxalacetic transaminase
HAPT	Haptoglobin
Hb	Hemoglobin
Hct	Hematocrit
HSD	High intensity, short duration
HST	Harvard step test
LDH	Lactate Dehydrogenase
LLD	Low intensity, long duration
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
PC	Phosphocreatine
PFK	Phospho-Fructokinase
PGOT	Plasma level of glutamicoxalacetic transaminase
TCK	Total serum creatine kinase

CHAPTER I

INTRODUCTION

Gymnastics is one of the popular and rapidly growing sports in Turkey. Gymnastics perhaps more so than any other sport, encompasses an endless quantity of movements that place stress on the muscles, tendons and joint structures of the human body. While the main objective of the modern day gymnast is to perfect movement skills for the greatest aesthetic appeal, several physical fitness components are obviously highly developed in the process. (Nelson,et al.,1983).

In gymnastics, certain events such as the beam and floor exercise requires the use of a considerable degree of flexibility and strength in each performer's routine (Nelson, et al.,1983). This means that gymnasts should have a strong skeleton, neuromuscular strength, strong connective tissues, and flexibility but should not be overly fat (Morehouse and Miller, 1976). So, gymnastics is a sport which requires a great deal of muscular strength, relative to body weight, muscular endurance, and flexibility (Brzycki,1985).

Strength for gymnastics may be defined as the maximum amount of force that can be exerted by a muscle, and endurance which refers to the ability of a muscle to exert a force repeatedly over a period of time. In gymnastics these two types of strength are very important (Liemohn,1988).

To obtain the optimum resuts in the execution of the complicated movements which are typical in gymnastics, a right balance between muscular strength, resistance, speed and shifting body mass is required (Reggiani,1989).

According to the Weiker (1985) it must be looked at gymnastics and its time factor in order to identify the required energy system that must be trained. Gymnastics contains events of short duration requiring, at times, extremely high intensity work, strength, power, and flexibility. Vaulting events are quite short,and the longest events, the floor exercise and balance beam, last just under 90 seconds. The Adenosine Triphosphate (ATP) required as the constant energy source for contraction-relaxation cycle of muscle can be generated by three ways; phosphogen system, glycolysis, and oxidative phosphorylation. The ATP stores in phosphogen system, skeletal muscle are short-lived during contraction, providing energy probably for less than one second of contraction. In slow skeletal muscle, which has abundant

O_2 stores in myoglobin, oxidative phosphorylation is the major source of ATP regeneration. Fast skeletal muscles regenerate ATP from glycolysis, mainly (Martin and at all, 1983).

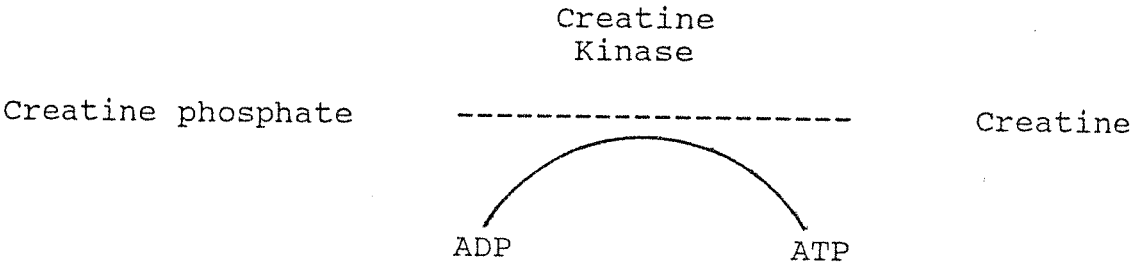
The energy supply for sports is related to the time that it takes to complete the activity. For activities that take less than 10 seconds to complete, the primary sources of fuel are the stored phosphogens; ATP and phosphocreatine (PC).

Gymnastics, which requires from a few seconds to about 90 seconds, then falls into the category of sports that is considered to be anaerobic. That is the energy for these sports predominantly comes from the stored phosphogens and glycolysis (Weiker,1985).

Fox (1976) and Weiker (1985) reported that energy for gymnasts is derived from ATP-PC+LA is 90% and LA+02 is 10%. Therefore, anaerobic training is more important than aerobic in gymnastics.

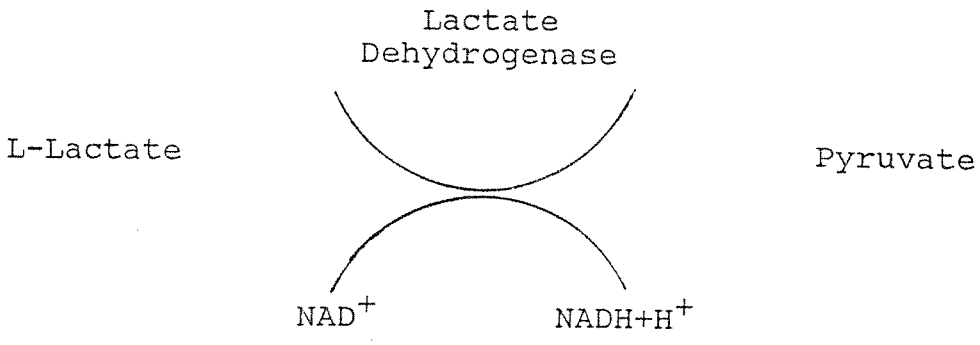
Phosphogens, such as creatine phosphate prevent the rapid depletion of ATP by providing a readily available high-energy phosphate, which is all that is necessary to re-form ATP from Adenosine Diphosphate (ADP). Creatine phosphate is formed from ATP and creatine at times when the muscle is relaxed and ATP demands are not so great. The enzyme catalyzing the phosphorylation

of creatine is Creatine Phosphokinase (CPK), a muscle specific enzymes with clinical utility in the detection of acute or chronic disorders of muscle (Martin and at all 1983).



Transfer of high-energy phosphate between ATP and creatine.

If anaerobic conditions prevail, the reoxidation of Nicotinamide Adenine Dinucleotide (NADH) by transfer of reducing equivalents, through the respiratory chain, to oxygen is prevented. Pyruvate is reduced by the NADH to lactate, the reaction being catalyzed by Lactate Dehydrogenase (LDH).



LDH reaction

Tiidus and Januzzo (1983) reported that high intensity, short duration exercise resulted in greater serum enzymes (CPK and LDH) activities than low intensity, long term exercise, and the largest increase corresponded with the highest perception of muscle soreness.

Hunter and Critz (1971) who suggested that the trained skeletal muscle may have a greater concentration of ATP which may serve to maintain the integrity of the cell membrane during work and thus reduce the enzymes into the plasma.

It is difficult to compare quantitatively the results from the various investigations reported in the literature because of major differences in the methods used. Little work has been done on the effect of short-term intensive exercise on serum enzyme levels in young athletes (Song 1990). Also, no work has been done on the relationship of CPK and LDH enzymes level with the perception of muscular soreness in female gymnasts following an isometric contraction. Thus, this researcher attempted to investigate this relationship.

The perception of delayed muscular soreness has been related to muscle or connective tissue damage brought about by exercise. Muscular damage has been related to the release of certain intramuscular enzymes into the blood (Tiidus and Ianuzzo 1983).

Several types of exercise, especially those involving eccentric actions, and possibly isometric actions with high mechanical forces, have been shown to produce changes generally considered evidence of muscle damage. The changes observed after these exercises include increase in serum enzymes, development of muscle soreness as well as histological and ultrastructural evidence of muscle fiber disruption. Regular Training has been shown to reduce or prevent these changes (Triffletti and at all 1988).

Serum enzymes are used, for example, as a measure of degree of cellular injury in the presence of various organic lesions. Moreover, serum enzyme activity can rise in many nonspecific temporary states, including physical exertion. The release of enzymes from skeletal muscle following exercise may occur as a result of muscle hypoxia, or other conditions that cause a change in cell membrane permeability. Researcher have proposed that changes in membrane permeability of, or damage to, muscle cells after high- intensity exercise can be minimized by regular physical training (Spitler and et al., 1984).

1.1 *Statement of the Problem*

The problem of this study was to investigate the relationship of CPK and LDH enzyme level with muscular

soreness in female gymnasts following an isometric contraction.

1.2 Subproblem

1.Relationship between isometric contraction with the level of CPK.

2.Relationship between isometric contraction with the level of LDH.

1.3 Aim of the Study

This study aims at finding out the relationship CPK and LDH enzymes level with muscular soreness in female gymnasts following an isometric contraction.

1.4 Null Hypothesis

1.There will be no significant relationship between CPK enzyme level with muscular soreness.

2.There will be no significant relationship between LDH enzyme level with muscular soreness.

3.There will be no significant relationship between CPK and LDH enzyme levels at pre and post-isometric contraction.

1.5 *Limitations*

1.All the subjects were selected from ASKI gymnastic club female gymnasts and most of them were from Turkish national team. Therefore, the result of this study was limited to ASKI Gymnastics Club gymnasts.

2.All subjects were volunteers.

3.Subjects number were limited to eleven person and age between 7 to 13 years old trained female gymnast.

4.Training status could not be controlled, which could effect the results.

5.The motivational and psychological states of each gymnast during testing could not be controlled.

6.Blood samples of all subjects were taken with heparinized capillary tubes by steril blood lancet.

1.6 *Assumptions*

1.Gymnasts applied their maximum effort during the test.

2.The measurement of serum enzyme level by "Dacos analyzier" machine was valid.

soreness in female gymnasts following an isometric contraction.

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1.6 *Assumptions*

1.Gymnasts applied their maximum effort during the test.

2.The measurement of serum enzyme level by "Dacos analyzier" machine was valid.

3.Blood samples were taken and analyzed correctly.

4.Gymnasts gave the correct sensation about muscle soreness questionnaire.

5.On the Cybex machine seat belt used around the subjects waist to prevent extraneus movement which fixed the waist correctly.

6.Subjects did not participate in any kind of physical activity prior to measurement on the test days.

7.Percent training load was intense enough to produce muscular soreness.

1.7 *Significance of the Study*

The perception of delayed muscular soreness has been related to muscle or connective tissue damage brougth about by exercise (Tiidus, 1983). It has been suggested that increase in plasma enzyme activities following exercise may be related to subjective muscular soreness or similar clinical syndromes (Nuttall, 1968). Muscular damage has been related to the release of certain intramuscular enzyme into the blood.

Some researchers reported that high intensity,

short duration exercise resulted in greater serum enzyme activities than low intensity, long term exercise, and the largest increase corresponded with the highest perceptions of muscle soreness.

However, Critz (1972),suggested that trained skeletal muscle may have a greater concentration of ATP which may serve to maintain the integrity of the cell membrane during work and thus reduce the enzymes into the plasma and therefore trained subjects may have less muscle soreness.

It is now the curiosity of matter that differences in the muscle enzyme level of the body affect the muscular soreness and also performance level.

In the present study, the purpose was to investigate the relationship of the CPK and LDH enzymes with muscular soreness in trained female gymnast following an isometric training.

Values taken with this investigation may be helpful for selecting the type of training program by the trainer and the amount of work which should be performed by the gymnasts to prevent muscle soreness and to have better performance.

1.8 *Definition of Terms*

Adenosine Diphosphate (ADP) : A Complex chemical substance which, when combined with inorganic phosphate (Pi) forms ATP. It contains one adenosine and two phosphate (Fox, 1988).

Adenosine Triphosphate (ATP) : A complex compound formed with the energy released from food and stored in all cells, particularly in muscles. It contains one adenosine and three phosphate molecules (Fox, 1988).

Aerobic : The process of using energy in the presence of oxygen (Astrand, 1986).

Anaerobic: The process of using energy in the absence of oxygen (Astrand, 1986).

Anaerobic Glycolysis : The incomplete chemical breakdown of carbohydrate (Fox, 1988).

ATP-PC System : An anaerobic energy system in which ATP is manufactured when phosphocreatine (PC) is broken down (Fox, 1988).

Creatine Phosphokinase (CPK) : CPK is the enzyme that catalyzing the ATP regenaration. A muscle specific enzymes with clinical utility in the detection of acute or chronic disorders of muscle (Dirix, 1988).

Energy : The capacity or ability to perform work

(Fox, 1988).

Enzyme : A protein compound that speeds up a chemical reaction (Fox, 1988).

Glycogen : A polymer of glucose; the form in which glucose (sugar) is stored in the body, mainly in muscles and the liver (Fox, 1988).

Hypoxia : Lack of adequate oxygen due to a reduced oxygen partial pressure (Fox, 1988).

Isometric contraction : Contraction in which tension is developed, but there is no change in the length of the muscle (Fox, 1988).

Lactate Dehydragenase(LDH) : LDH is enzyme that catalyzes the reaction of pyruvic acid to lactic acid. The formation and turnover of lactate during heavy exercise is catalyzed and regulated by the enzyme LDH (Hess, 1963).

NAD(Nicotinamide adenine dinucleotide) and FAD(Flavo adenine dinucleotide) : Serve as hydrogen acceptors. These are acetyl inorganic group, function of both NADH and FADH2 is to carry electrons through the electron transfer system (Martin, 1983).

Phosphagen : A group of compounds:collectively refers to ATP and PC (Fox, 1988).

Phosphocreatine(PC) : A chemical compound stored

in muscle, which when broken down aids in manufacturing ATP (Fox, 1988).

Soreness : Severe muscular discomfort 1-3 days after unusual heavy physical exercise. The major symptoms are muscular stiffness, and pain, especially when making active movements (Devries, 1974).

CHAPTER II

REVIEW OF LITERATURE

Effect of Serum Enzyme

The effects of serum enzymes on human body were extensively studied by many of researchers which are listed below and its importance has always been emphasized. Measurements under consideration are especially focused on CPK and LDH enzymes due to fact that these are the most important predictors which display the muscle abnormalities.

Pentti and Konttinen (1962) studied the effect of physical exercise on some enzymes in the serum. Subjects were twenty healthy soldiers. They were young men just undergoing basic military training and so accustomed to physical exercise. The exercise consisted of hard marching lasting two hours. Half-way through the march there was a 5 min pause. During these 2 hrs the men walked 16 km, each carriying a pack weighing 8 kg, and a rifle weighing 4.6 kg. Thus the exercise was strenuous but not exhausting. The experiment was carried out on

four different days under similar conditions. The blood samples were taken at intervals of 2 hrs. The men were not allowed to smoke or eat until the recovery. Sample had been taken 2 hrs after the end of the march. Three dehydrogenases enzymes, namely (LDH), malic dehydrogenase (MDH), and sorbital dehydrogenase (SDH) were studied. As a result, activities of serum LDH and MDH increased after the exercise. On the other hand, the activities of SDH showed no significant changes.

Rosalki (1967) improved procedure for serum CPK determination. The CPK activity of serum is determined by a procedure in which ATP, liberated by the action of enzyme, is linked to the reduction of nicotinamide-adenine dinucleotide phoshpate (NADP) and the formation of reduced NADP followed spectrophometrically. The enzyme substrate for the reaction is prepared in bulk, freeze-dried, and distributed in gelatine-covered capsules. The capsule contents are reconstituted immediately before use by the addition of distilled water, and CPK activity is determined by measuring the increase in optical density of 340 mu following serum addition. The method is convinient, more sensitive, and less time-consuming than other procedures for serum CPK determination in common laboratory use.

Serum creatine kinase (CK) and glutamic oxalacetic transaminase (GOT) activity following a

standardized 6 min period of moderately severe exercise has been studied in 7 normal women and 7 normal men at the begining. Following a 3 to 5 week period of physical conditioning this number decreased to 11 samples (Nuttall and Jones 1968). Before physical conditioning the exercise resulted in a marked increase in serum CK activity with a peak rise at 8 to 16 hours. There was also a slight, but statistically significant increase in serum GOT with a peak at 16 to 24 hours. Following a period of physical conditioning, the same exercise resulted in little or no change in either the serum CK or GOT level. This appears to represent another manifestation of muscle adaptation to increased work.

Hunter (1971) studied the effect of training on plasma enzyme levels in twelve university male students (20-27 years of age). Twelve subjects trained (bicycle ergometer) 10 weeks at a work load producing a heart rate of 150/min, for 30 min, 3 times/week. Maximal Oxygen uptake (VO_2 max), physical work capacity at a heart rate of 150/min (PWC_{150}). and the Harvard step test (HST) were measured before and after training. Plasma were analyzed for CPK, GOT and LDH activity before and after the VO_2 max tests, before and after a training session. Training increased VO_2 max, PWC_{150}, and HST scores. Resting LDH activity was higher after training. Before training, all three plasma enzymes were elevated after submaximal exercise. It was found that training reduced CPK and

eliminated the LDH responses to maximal exercise, and reduced the GOT and eliminated the CPK response to the same submaximal exercise load.

Critz (1972) proposed to measure plasma levels of glutamic- oxolacetic transaminase (PGOT), CPK and LDH in the same subjects after 12 min of running, 12 min of swimming and 12 min of basketball. The eleven subjects had a mean age of 26.4 years (18-36 years) and participated voluntarily. Except two subjects, the others were not elite athletes, they were from physical education department. Heparinized venous blood samples were taken immediately before and between 1st and 2nd min post-exercise for each event. CPK activity was determined according to the method of Rosalki. The method of Babson and Phillips was used to determine LDH activity. Each type of exercise resulted in increases in all three plasma enzymes and blood lactate. The increase in all three plasma enzymes were larger after running than after basketball. There was a larger increase in plasma LDH after running than after swimming. It was concluded that different changes in serum enzyme level resulted from sports specificity.

Goto (1974) studied the effect of CPK isoenzymes in Neuromuscular disorders. Serum was obtained from 101 patients with neuromuscular disorders and 9 patients with myocardial infarction. Normal subjects were used as a

control group. Serum was examined immediately or stored at -20°C for a period not exceeding 48 hrs. Researcher suggest that in neuromuscular disorders and in myocardial infarction, the determination of the serum CPK isoenzyme is useful in identifying not only skeletal muscle damage but also myocardial injury.

Sjodin and co-workers (1975) studied total LDH activity and LDH isoenzyme pattern in muscle biopsies obtained from m. vastus lateralis after 1) "Aerobic" training performed as interval and extreme distance running, respectively (3 subjects): and 2) "Anaerobic" training for two mounths, carried out as repeated maximal bursts of approximately 1 min running (6 subjects). After the "Anaerobic training" no changes in LDH properties could be detected, although running performance improved. The extreme distance running resulted in a decrease in total LDH activity and an increase in relative activity of the heart specific isoenzymes. A relationship was also shown between the relative activity of these isoenzymes and the training distance covered. The relatively more aerobic conditions prevailing during distance running as compared to "anaerobic training" were proposed to decrease muscle specific subunits and/or increase synthesis of heart specific subunits in both muscle fibre types.

Hickson, Heusner, and Huss (1976) studied

specifically designed programs of sprint and endurance running. They tried to determine how different types of training would affect enzyme activities in selected energy metabolism pathways. Three types of rat skeletal muscles were studied. After 8 weeks of training, small but significant decreases in LDH activity (15%) were found in the soleus and vastus lateralis muscles of the sprint animals. Decreased levels of phosphoglucomutase and LDH (approximately 20%) of the white lastus lateralis muscles of the endurance group were observed at the same time. By 16 weeks of training, fumarase activity increased approximately two fold in the white vastus muscles and 45% in the soleus and plantarus muscles of the endurance group. Similarly, increased fumarase activity (42%) was seen in the soleus muscles of the sprint group. In all muscles, phosphoglucomutase and LDH activities generally were lower in the endurance animals than in the control animals. No significant differences were found between the sprint and endurance groups at either eight or sixteen weeks of training. These results suggest that similar enzyme adaptations occur over time with both types of training.

Szasz, Gruber, and Bernt (1976) determined to establish optimum conditions for CK activity measurement with the creatine phosphate and creatine reaction, they re-examined all kinetic factors relevant to an optimal

and standardized enzyme assay at 30 and 25°C. They determined the pH optimum in various buffers, considering the effect of the type and concentration of the buffer, as well as the influence of various buffer anions on the activity. The relation between activity and substrate concentration was shown and the apparent Michaelis constants of CK for CP and ADP were evaluated. They tested the effect on CK measurement of the concentration of substrates (glucose, $NADP^+$) in the auxiliary and indicator reactions, especially the influence of the added auxiliary (hexokinase) and indicator (glucose 6-phosphate dehydrogenase) enzymes on the lag phase, at different temparatures. The $NADP^+$ concentration proved to be the factor limiting the duration of constant reaction rate. They studied the inhibition of CK and adenylate kinase by AMP and established a convenient AMP concentration. For reactivation of CK, N-acetyl cysteine as sulfhydryl compound was introduced. Finally, they examined the relationship between activity and temparature.

The effect of high intensity, short duration chronic exercise (HSD) and low intensity, short duration chronic exercise (LLD) on the selected enzyme activities of three muscles of rats were studied by Gillespie and co-workers (1982). Control enzymes were CPK, Phosphofructo Kinase (PFK), and LDH. Results of this study indicate that the HSD group selectively increased

its glycolytic power, while the LLD group increased only its oxidative ability.

Armstrong, Ogilvie, and Schwane (1983) studied skeletal muscle pathology resulting from eccentric-biased exercise in rats. The effects on the muscles of running on a treadmill on a 0^{o} incline (similar amounts of concentric and eccentric contractions), down a 16^{o} incline (primarly eccentric contractions), and up a 16^{o} incline (primarly concentric contractions) at 16 $m.min^{-1}$ for 90 minute were assessed by following post-exercise changes in 1) plasma CK and LDH activities, 2) glucose 6-phosphate dehydrogenase (G-6-PDase) activity (bio- and histochemically) in the physiological extensor muscles, and 3) histological appearance of the muscles. The data indicate the following; 1) Whereas all exercise protocols resulted in elevations of plasma enzymes immediately after running, only eccentric exercise caused late phase elevations 1.5-2 days postexercise. 2) Significant increases in muscle G-6-PDase activity, which are always associated with accumulations of mononuclear cells, always occured within some muscles of both extensor group 1-3 days following downhill and uphill running and did not occur following level running; the increases in activity were usually of lower magnitude in the muscles of uphill runners that in those of downhill runners; the deeply located predominanty slow-twitch muscles were most

affected by both down- and uphill running. 3) Muscle histology demonstrated localized disruption of normal bending patterns of some fibres immediately after exercise and accumulations of macrophages in the interstitium and in some (<5%) muscle fibers by 24 hour postexercise in the deep slow muscles of the antigravity groups. Although the data generally indicated that eccentric exercise causes greater injury to the muscles, questions still remain.

The proportion of CK isoenzyme activity was increased in skeletal muscle.Biopsies obtained from five long-distance runners by Apple and at all (1984). Both 2 hrs before and 30 min after a marathon race, as compared with that in biopsies from five non runners. Further, mitochondrial CK and CK-BB isoenzymes were present in homogenates of the runners' skeletal muscle samples but not in those of the nonrunners. However, there were no substantial differences in the mean total CK activities per gram of muscle tissue among premarathon samples, postmarathon samples, and nonrunners' samples. It was concluded that the metabolically active gastrocnemius muscle of low distance runners is qualitatively similar to the heart muscle in its CK isoenzyme composition.

Spitler (1984) investigated the potential value of specific cellular responses to acute and chronic exercise as indices of physical fitness. Muscle enzymatic

and hemolytic responses following a progressive cycle-ergometer test to maximal aerobic capacity were studied in 12 women and 12 men, aged 27 to 55 yrs, who had been previosly assigned to "high" and "low" fitness groups. Venous blood samples were obtained at rest prior to the cycle test, immediately following maximal effort, and 1, 2, 4, 6 and 24 hrs post exercise. The samples were analyzed for hematocrit (Hct), hemoglobin (Hb), LDH, GOT, CK, CK isoenzymes, and heptoglobin (Hept). This study demonstrated that serum Hept levels, but not serum levels of the enzymes CK, LDH, and SGOT-differ between high-fitness and low-fitness groups of men and women and, therefore, may serve as index of physical fitness. These results indicated that one progressive test to maximal aerobic capacity is not sufficient to induce significiant muscle enzymatic or hemolytic stress responses.

Apple (1985) investigated the alterations of CK isoenzymes, specifically CK-MB, that occur in the skeletal muscle and serum of highly trained long-distance runners in response to the chronic stress of marathon training and the acute stress of marathon racing. The CK isoenzyme composition was determined in serial gastrocnemius muscle biopsies obtained from 12 male marathon runners. The mean muscle CK-MB composition significantly increased after chronic exercise (training) from 5.3% (pretraining) to 7.7% (premarathon) as well as after acute exercise (postmarathon) to 10.5% of the total

CK activity. However, no significant differences in total CK and CK-MB isoenzymes were present in muscle homogenerates. A significant correlation was observed in the increase in mean serum total CK and CK-MB activities 24 hrs after the race. These results show that gastrocnemius muscle adapts to long- distance training and racing with increased CK-MB activities and imply that skeletal muscle is the major source of elevated serum CK-MB activities in marathon runners.

Rogers (1985) studied forty trained male (mean age: 31.3 ± 4.1yr) and 8 trained female (mean age: 32.0 ± 5.6yr) marathon runners. In an attempt to provide additional data relative to the effect of prolonged, intensive exercise on the release of enzymatic activity from skeletal muscle, this study assessed the total CK and CK-MB activities in the distance runners following a 42.2 km marathon race. Serum samples were obtained pre-marathon and at 24, 48, 72, 96 hrs post-race to quantitate total serum creatine kinase activity (TCK) was measured enzymatically. Both the men's and the women's mean serum TCK activity peaked at 24 hrs post-marathon race. Serum TCK activities for both men and women remained significantly elevated above pre-race values until 72 hrs. The man had significantly greater mean serum TCK activities than did the women at all time points. These data suggest a greater amount of damage to the skeletal musculature in men vs. women.

Newham, Jones, and Clarkson (1987) studied on five women and three men (aged 24-43 year) performed maximal eccentric contractions of the elbow flexors (for 20 minute) on three occations, spaced 2 weeks apart. Muscle pain, strength and contractile properties, and plasma CK before and after each exercise bout were analyzed. Muscle tenderness was greatest after the first bout and thereafter progressively decreased. Very high plasma CK levels (1,500-11,000 IU/I) occured after the first bout, but the second and third bouts did not significantly affect the plasma CK. After each bout the strength was reduced by 50% and after 2 weeks had only recovered to 80% of preexercise values. Each exercise bout produced a marked shift of the force-frequency curve to the right which took approximately 2 weeks to recover. The recovery rate of both strength and force-frequency charecteristics was faster after the second and third bouts. Since the adaptation occured after the performance of maximal contractions, it cannot have been a result of changes in motor unit recruitment. The observed training effect of repeated exercise was not a consequence of the muscle becoming either stronger or more resistant to fatigue.

Song (1990) investigated the effect of anaerobic exercise on serum enzymes LDH, CPK; and Aspartate amino-transferase AST on five elite junior male and five alpine

skier females who ranged in age from 12 to 15 years. An intensive treadmill run to exhaustion (10.5 km/hr, 20% grade) was performed. Blood samples were drawn from the antecubital vein before and 5min after the exercise. None of the enzyme activities had changed after exercise, and the performance time of treadmill run (male: 53.8 sec and female 52.6 sec) was similar between groups but the serum CPK activities of the boys were consistently higher than those of the girls and the differences were significant ($p<0.05$). It was concluded that the intensity and duration of the treadmill running used was not enough to cause changes in serum enzyme activities.

Muscular Soreness

Friden, Sjostrom, and Ekblom (1980) studied morphological way of delayed muscular soreness. Biopsies, taken up to 1 week post-exercise, from the soleus muscle of five healthy males (20-34 years old) suffering from pronounced exercise induced delayed muscular soreness were analyzed morphologically. There was no evidence for ischemic tissue injury or mechanical fibre disruption. However, at the subcellular level frequent myofibrillar disturbances, especially with regard to the Z-bands, were noted. Thus, the contractile machinery of overloaded muscle fibres seemed to be partially distorted several days following exercise.

Schwane and co-workers (1983) tested the hypothesis that running down on incline, during which muscles primarly perform eccentric contractions, causes greater delayed-onset muscular soreness and greater increases in plasma enzyme activities than does running on the level, during which muscles perform similar amounts of concentric and eccentric contractions. Subjective sensations of muscular soreness and plasma activities of CPK and LDH were assessed in seven subjects at 0, 24, 48, and 72 hours after 45 minute of running (one time on the level and a second time down a 10% incline). Following downhill running (57% of VO_{2max}), significant delayed-muscular soreness was experienced in gluteal, quadricep, anterior leg, and posterior leg muscules, and plasma CPK (but not LDH) activity was significantly increased (351% at 24 hour). In contrast, following level running (78% of VO_{2max}), no statisticaly significant soreness occured in any muscle group, and plasma CPK and LDH activity were not elevated. Thus, the results generally support the hypothesis. Secondarily, they investigated whether delayed onset soreness with downhill running is accompanied by increases in peripheral white blood cell counts suggestive of inflammation. No such association was observed. It was suggested that both delayed onset of muscular soreness and plasma enzyme activities are affected by structural changes in muscle tissue resulting from eccentric

contractions.

Byrness and co-workers (1985) were assessed perceived muscle soreness ratings, serum CK activity, and myoglobin levels in three groups of subjects following two 30-min exercise bouts of downhill running (-10^{o} slope). The two bouts were separeted by 3, 6, and 9 week for groups 1, 2, and 3, respectively. Criterion measures were optained pre-and 6, 18, and 42 hour post-exercise. On bout one, the three groups reported maximal soreness at 42 hour post-exercise. Also, relative increases in CK for groups 1, 2, and 3 were 340, 272, and 286%, respectively. Corresponding values for myoglobin were 432, 749, and 407%. When the same exercise was repeated, significantly less soreness was reported and smaller increases in CK and myoglobin were found for groups 1 and 2. For example, the percent CK increases on bout 2 for groups 1 and 2 were 63 and 62(u/l), respectively. Group 3 demonstrated no significant difference in soreness ratings, CK activities, or myoglobin levels between bouts 1 and 2. It was concluded that performance of a single exercise bout had a prophlactic effect on the generation of muscle soreness and serum protein responses that lasts up to 6 weeks.

In Bobbert, Hollander, and Huijing (1986) studies 11 subjects performed exercise resulting in delayed onset muscular soreness in m.gastrocnemius with one leg, as the

experimental leg. The other leg served as control. Pre-exercise and 24, 48, and 72 hours postexercise, soreness perception, resting EMG level of m. gastrocnemius, and volume and skin temperature of both legs were measured, and a leukocyte count was performed. Perception of soreness in m.gastrocnemius reported 24, 48, and 72 hours postexercise was not accompanied by an increase in resting EMG level. This result indicates that soreness perception is not related to a tonic localized spasm in sore muscles. A rise in volume of the experimental leg relative to volume of the control leg was found 24, 48, and 72 hours postexercise ($p<0.05$). It is suggested that the volume rise is due to edema formation in the experimental leg and that this edema formation is responsible for soreness perception. Since granulocytosis was not found, the hypothesis that edema formation reflects muscle inflammation is not substantiated.

Isometric Contraction

Mayer (1984) was to determine whether increased mechanical distruption of the muscle associated with a greater number of muscle contractions was a key factor in CK efflux from skeletal muscle following isometric exercise. Nine collage-age males volunteered to participate. Data collection took place over four test sessions spaced at least three days apart. Session I

consisted of a familiarization day and baseline knee extension maximal voluntary contraction (MVC) measures which was made with padded bench equipped with an ankle cuff with a cable attached to a strain gauge (200 lb). The calibrated strain gauge was connected to a Beckmen pen recorder. The cable length was adjusted to give a femur-tibia angle of 110^{o} at peak tension. The actual pattern of development of MVC was standardized with a build up phase of no more than 2 sec. Session III consisted of a series for 60 sec. isometric knee-extension contractions at 40% MVC with 60 sec inter-trial rests until exhaustion. The number of contractions was determined and used to set the other two exercise regimens. The second sessions consisted of twice the number of isometric contractions at 40% MVC while the duration of each contraction and rest was half that of the first regimen. The third regimens consisted of four times the number of contraction from condition I, while the duration of each contraction and rest was one fourth its length in the first regimen (15 sec contraction, 15 sec rest). The force level of the 40% MVC was calculated from the baseline MVC measures and held constant for each contraction-time trial. The force level was maintained at the calculated level through use of the Beckman pen recorder. The resting, pre-exercise blood sample was drawn from the antecubital vein after the subject had been resting for 15 min. Repeated blood samples were

drawn following each exercise session at 6, 18, and 24 hours blood was allowed to clot at room temperature, centrifuged, and preserved at -30°C until the analysis. Activity of CK was determined using a Boehringer test kit. As a result in this study the isometric exercise condition with the longest duration of each contraction resulted in the highest serum CK levels. It is tempting to suggest that the CK efflux from muscles may be related to blood pressure.

Mccully and Faulker tested the hypothesis that lengthening contractions result in greater injury to skeletal muscle fiber than isometric or shortening contractions in 1985. Mice were anesthetized with pentobarbital sodium and secured to platform maintained at 37°C. The distal tendon of the extensor digitorum longus muscle was attached to a servomotor. A protocol consisting of isometric, shortening, or lengthening contractions was performed. After the contraction protocol the distal tendon was reattached, incisions were closed, and the mice were allowed to recover. The muscle was removed after 1-30 days, and maximum isometric force (P^o) was measured in vitro at 37°C. Three days after isometric and shortening contractions and sham operations, histological appearance was not different from control, and P^o was 80% of the control value. Three days after lengthening contractions, histological

sections showed that 37 ± 4% of muscle fibers degenerated and P^o was 22 ± 3% of the control value. Muscle regeneration, first seen at four days, was nearly complete by 30 days, when P^o was 84 ± 3% of the control value. They concluded that, with the protocol used, lengthening, but not isometric or shortening conractions, caused significant injury to muscle fibers.

Clarkson and at all (1985) suggest that the work to rest ratio of isometric exercise as well as the order of testing are possible factors associated with the magnitute of CK response. The purpose of this study was to compare serum creatine kinase (CK) activity following two forearm flexion isometric exercise regimens differing in work to rest ratio, and examine the CK response to a repeated bout of isometric exercise. Eleven untrained males were tested on two sessions (bouts) spaced 1 week apart. For bout 1, five subjects (group A) performed a forearm flexion; isometric exercise consisting of 40 maximal 10-s contractions with 20-s inter-trial (10:20), while six (group B) performed 40 maximal 10-s contractions with 5-s inter-trial rests (10:5). The increase in serum CK activity following the 10:20 exercise (143%) was significantly greater than that following the 10:5 (52%). The 10:20 exercise was also associated with greater tension generation over trials. One week later, both group performed a bout of 10:20 exercise. A substantial reduction in the serum CK

response was found following this second bout. As a result the data suggest that for bout 1 the isometric exercise associated with the greater overall tension levels resulted in the greater CK response. However, when the 10:20 exercise was repeated 1 week later, a substantial reduction in the CK response was found which was unrelated to the tension generated.

Triffletti and at all (1988) examined the adaptation to isometric exercise with regard to changes in serum CK activity and muscle soreness. Forty-five college age males were placed into six groups, each performing two bouts of strenuous isometric exercise of the knee extensors. In experiment 1 (N=27), after performing the first bout of exercise, groups A,B, and C performed the second bout 3,6, and 9 week later, respectively. In experiment 2 (N=18), groups D,E, and F performed the second exercise bout 1,2, and 3 week after the first bout, respectively.In experiment 3, group D performed two additional exercise bouts, thus, this group performed a total of four bouts spaced 1 week apart. Muscle soreness and CK were assessed prior to and 6, 18, and 24 (or 42) hours following each exercise. In experiment 1, no significiant difference in soreness or serum CK was found between bouts 1 and 2. In experiment 2, a significant decrease in the CK and soreness responses was found on bout 2 compared with bout 1

($P<0.05$). In experiment 3, serum CK and soreness responses were highest following bout 1 while bouts 2, 3, and 4 were not significantly different from one another. Performance of this isometric exercise resulted in an adaptation that lasts approximately 3 weeks, with the greatest adaptation occuring after one bout.

Each test session consisted of blood sampling and the assessment of muscle soreness via questionnaire at pre-exercise, and 6, 18, and 42 hours post-exercise. The muscle soreness questionnaire required the subject to rate the degree of soreness in the exercised leg. The questionaire used a scale ranging from 1 (normal), 5 (sore), to 10 (very sore). As a conclusion the reduction in the serum CK and soreness response observed after a physical conditioning program is due largely to an adaptation induced by the first exercise bout.

CHAPTER III

METHODS and PROCEDURES

The purpose of this study was to investigate the relationship of CPK and LDH enzyme level with muscular soreness in female gymnast following an isometric contraction.

Selection of Subjects

The subjects of this study consisted of 11 volunteer female gymnasts aged 7-13 years from Ankara Su Kanalizasyon Idaresi (ASKI) Gymnastics Club. Two of the subjects were eliminated from this study because one of them did not participate in the last part of the study and the other one's blood sample did not separate into serum. Oral consent and instructions concerning to the experiment were given to each subject prior to their involvement in the study.

The Collection of Personal Data

The collection of personal information was done by interviewing subject and recording the information on

individual form shown in appendix A.

Method of the Study

The Cybex II equipment was used as an isometric contraction apparatus. They performed an exercise four times as follows : holding the weight (100% max. work load) which uses the quadriceps muscle for 1 min with 5 minutes recovery period. This ratio was chosen due to gymnastics rules. In women's competition there are four events, and in each of the event competition takes between 70 to 90 seconds with the ecception of vaulting horse which takes 20 to 30 seconds. Three minutes warming up practice is given before each event and 2 minutes recovery is available.

Two cc blood samples were drawn at the pre-exercise period then in the post-exercise period sampling was separated with 6, 12 and 24 hours intervals. Namely 6, 12, and 24 hours after the exercise blood samples were drawn from the cubital fossa into a serum seperation tube. The blood was allowed to clot for 10 minutes at room temperature and then centrifuged from 10 minutes to separate the serum. After centrifugation, all serum samples were frozen at -20^{o}C until analysis for CPK and LDH activity. The CPK and LDH activities were assessed in duplicate samples at 37^{o}C with the Dacos analyzing

machine which has a spectrophotometer part.

These blood samples were taken from the female gymnasts at the Sporcu Egitimi Saglik ve Arastirma Merkezi (SESAM) and then were sent to Pediatric Biochemistry Laboratory in Hacettepe University Hospital for determination of CPK and LDH levels. In each time the blood was taken, the questioneria for soreness was applied. Questioneria was prepared with the scale ranging from 1 (normal), 5 (sore), to 10 (very sore).

Statistical Analysis

For each valunteer; averages, means and standard deviations of CPK, LDH and soreness values were calculated in all time periods. Then, in each time period, CPK and LDH enzymes were compared individually by means of T-test. Regression analysis was used to find the correlation between the soreness values and time. In order to find a relationship between the values of CPK for pre-exercise period and for the period right after the exercise, T-test method was carried. Same procedure repeated for LDH values also. Finally, using the method MANOVA, CPK, LDH and soreness values were then investigated two by two.

The 0.05 confidence level was used to test for the significance of differences.

CHAPTER IV

RESULTS AND DISCUSSION

There were eleven female gymnasts participated in this study. All the participants were the members of ASKI Gymnastics Club, and five of them were in the Turkish National Gymnastics Team.

Data for two of the volunteers have been discarded, due to the lack of attendance of one of the volunteers and the blood serum of the other one could not be discriminated from the sample blood which were taken for analysis. Thus, researcher was able to collect the data only from nine participants. The data compiled from the nine participants made possible to analyse the systematical behavior of the two enzymes with respect to time, at least to construct a relation between them. On the other hand, soreness is a relative quantity which may differ from one person to the other and it is believed that any measurement in connection to the soreness must contain error. Namely, asked to answer by a number from 1 to 10 indicating the soreness. i.e., 1 is no sore and 10 is extremely sore. Thus, it was possible to investigate the soreness in response to the variations occured in to

enzymes, CPK and LDH respectively.

Cybex II equipment was used to achieve the 100% maximum isometric contraction. Blood samples drawn from each volunteers with regular time intervals, who were subjected to the experiment, i.e.,0 (right after exercise), 6, 12 and 24 hours. Samples were also drawn in the pre-exercise and post-exercise periods.

During the collection of blood sample, it has been found to be useful to use intrakit, considering the ages of the participants, which is an instrument avoiding the repetation of the precedure during the drawing blood. Also heparine was used to get rid of the coagulation of the blood. Results of the measurements of enzymes CPK and LDH obtained from the Dacos analyzing machine together with the data for soreness compiled from the interviews of the participants are displayed in Table 1.

In order to take into account the variations of measurements between the participants data of the same kind grouped together and their means, standard deviations and variances were calculated. Table 2 shows CPK values of each participants for different times. Statistical calculations are also tabulated.

LDH values with respect to times are given in Table 3.

TABLE 1. RAW DATA

		Sample no								
		1	2	3	4	5	6	7	8	9
PREEX	CPK (U/L)	109	87	111	61	82	66	90	65	95
	LDH (U/L)	207	224	233	165	220	146	177	202	263
	Soreness	1	1	1	1	1	1	1	1	1
POSTEX	CPK (U/L)	106	88	83	58	66	63	85	60	87
	LDH (U/L)	210	208	245	162	317	144	197	207	270
	Soreness	2	3	3	5	6	3	3	7	4
6 HRS	CPK (U/L)	109	75	81	71	72	65	119	102	88
	LDH (U/L)	318	270	236	219	328	296	292	237	326
	Soreness	2	1	1	1	3	2	1	1	2
12 HRS	CPK (U/L)	89	72	61	70	61	57	114	98	81
	LDH (U/L)	262	262	202	208	231	171	242	225	261
	Soreness	1	1	1	2	2	1	1	2	1
24 HRS	CPK (U/L)	86	66	62	56	56	60	98	76	81
	LDH (U/L)	200	182	214	170	199	182	210	218	238
	Soreness	1	2	1	3	2	1	2	1	1

The relative soreness values are follows as in Table 4.

Table 2. CPK values of each participant

Sample	Pre	Post	6 hrs	12 hrs	24 hrs
1	109.000	106.000	109.000	89.000	86.000
2	87.000	88.000	75.000	72.000	66.000
3	111.000	83.000	81.000	61.000	62.000
4	61.000	58.000	71.000	70.000	56.000
5	82.000	66.000	72.000	61.000	56.000
6	66.000	63.000	65.000	57.000	60.000
7	90.000	85.000	119.000	114.000	98.000
8	65.000	60.000	102.000	98.000	76.000
9	95.000	87.000	88.000	81.000	81.000
average	85.111	77.333	86.889	78.111	71.222
std	17.407	15.377	17.885	18.089	13.966
variance	924.640	236.444	967.360	327.210	632.090

Table 3. LDH values of each participant

Sample	Pre	Post	6 hrs	12 hrs	24 hrs
1	207.000	210.000	318.000	262.000	200.000
2	224.000	208.000	270.000	262.000	182.000
3	233.000	245.000	236.000	202.000	214.000
4	165.000	162.000	219.000	208.000	170.000
5	220.000	317.000	328.000	231.000	199.000
6	146.000	144.000	296.000	171.000	182.000
7	177.000	197.000	292.000	242.000	210.000
8	202.000	207.000	237.000	225.000	218.000
9	263.000	270.000	326.000	261.000	238.000
average	204.111	217.778	280.222	229.333	201.444
std	34.327	50.079	39.256	29.672	19.984
variance	1178.32	6525.600	1541.062	5525.840	399.358

Table 4. Soreness values of each participant

Sample	Pre	Post	6 hrs	12 hrs	24 hrs
1	1.000	2.000	2.000	1.000	1.000
2	1.000	3.000	1.000	1.000	2.000
3	1.000	3.000	1.000	1.000	1.000
4	1.000	5.000	1.000	2.000	3.000
5	1.000	6.000	3.000	2.000	2.000
6	1.000	3.000	2.000	1.000	1.000
7	1.000	3.000	1.000	1.000	2.000
8	1.000	7.000	1.000	2.000	1.000
9	1.000	4.000	2.000	1.000	1.000
average	1.000	4.000	1.556	1.333	1.556
std	0.000	1.563	0.685	0.471	0.685
variance	0.090	2.444	0.640	0.222	0.640

Furthermore, the mean of nine participants for each parameter, namely CPK, LDH and soreness are tabulated with respect to time in which the blood sample was drawn. Table.5 also includes the statistics of these mean values.

Table 5. Statistics of total mean values

	CPK	LDH	Soreness
Pre	85.111	204.111	1.000
Post	77.333	217.778	4.000
6 hrs	86.889	280.222	1.556
12 hrs	77.714	239.714	1.286
24 hrs	71.222	201.444	1.556
average	79.654	228.654	1.879
std	5.699	29.129	1.080
variance	32.473	848.523	1.167

Thus, mean values formed a basis for the analysis of variance as well as the investigation of the relation of the parameters by means of two sample t-test and regression.

So far, according to mean values variation of enzyme CPK with respect to time is plotted in Figure 1. It is seen that CPK reaches its maximum nearly 6 hours after the exercise and decrease smoothly as time goes on. Note that pre-exercise measurements were not included in the figure.

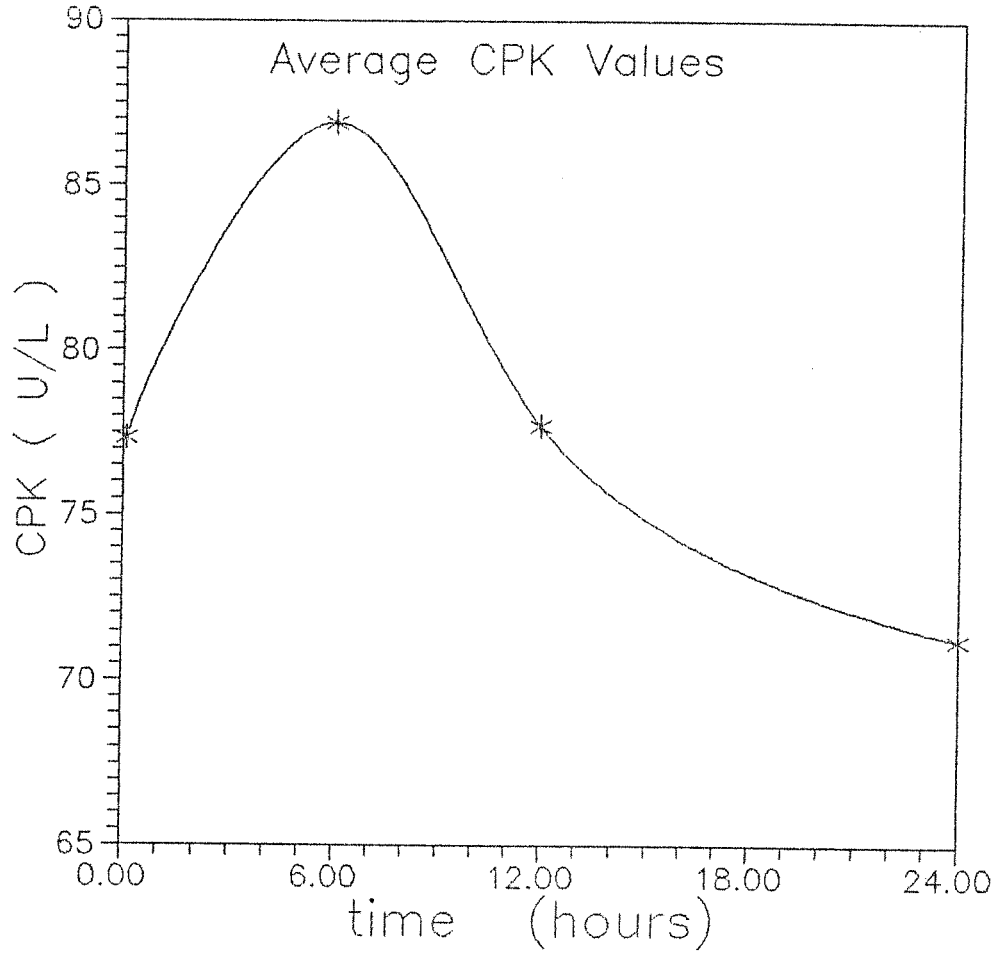


Figure 1. CPK with respect to time for post exercises

In Figure 2 variation in mean LDH values with respect to time is given. If carefully examined, it is clear that time variation in this graph is very similar to the CPK graph.

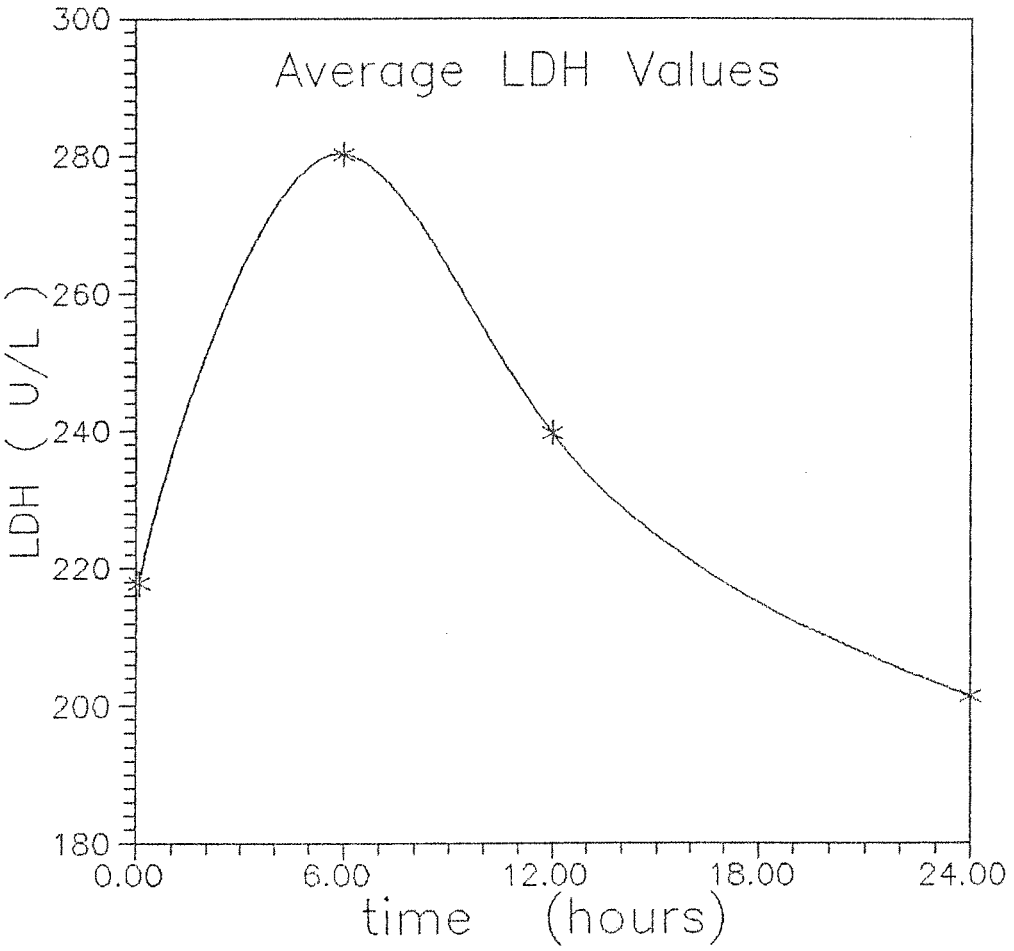


Figure 2. LDH with respect to time for post exercises

Soreness as a function of time is also plotted in Figure 3. It can be seen that there is a decrease of mean soreness values by time.

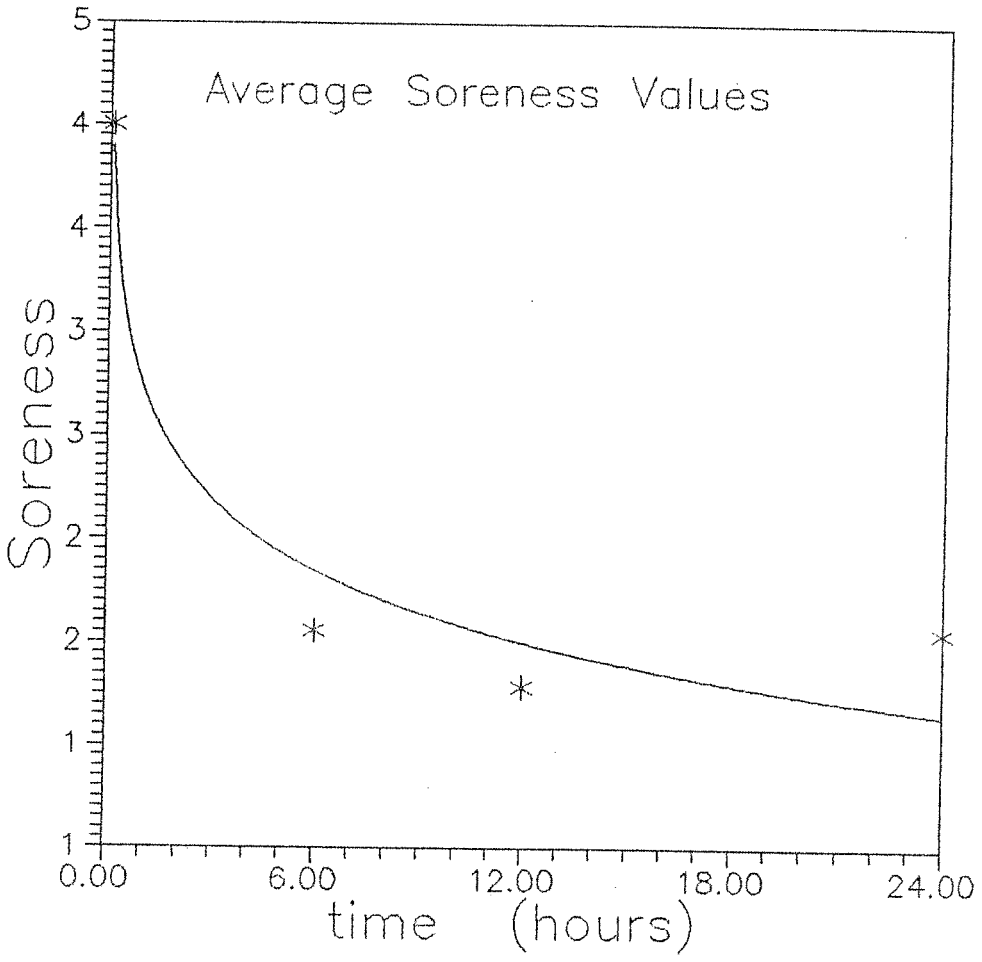


Figure 3. Soreness with respect to time for post exercises

In order to find a preliminary relation between the two enzymes; CPK and LDH, LDH is plotted with respect to CPK in Figure 4. It is clear that a linear proportionality exists between CPK and LDH, as can be formulated;

$$LDH = 5.11 * CPK - 165.41$$

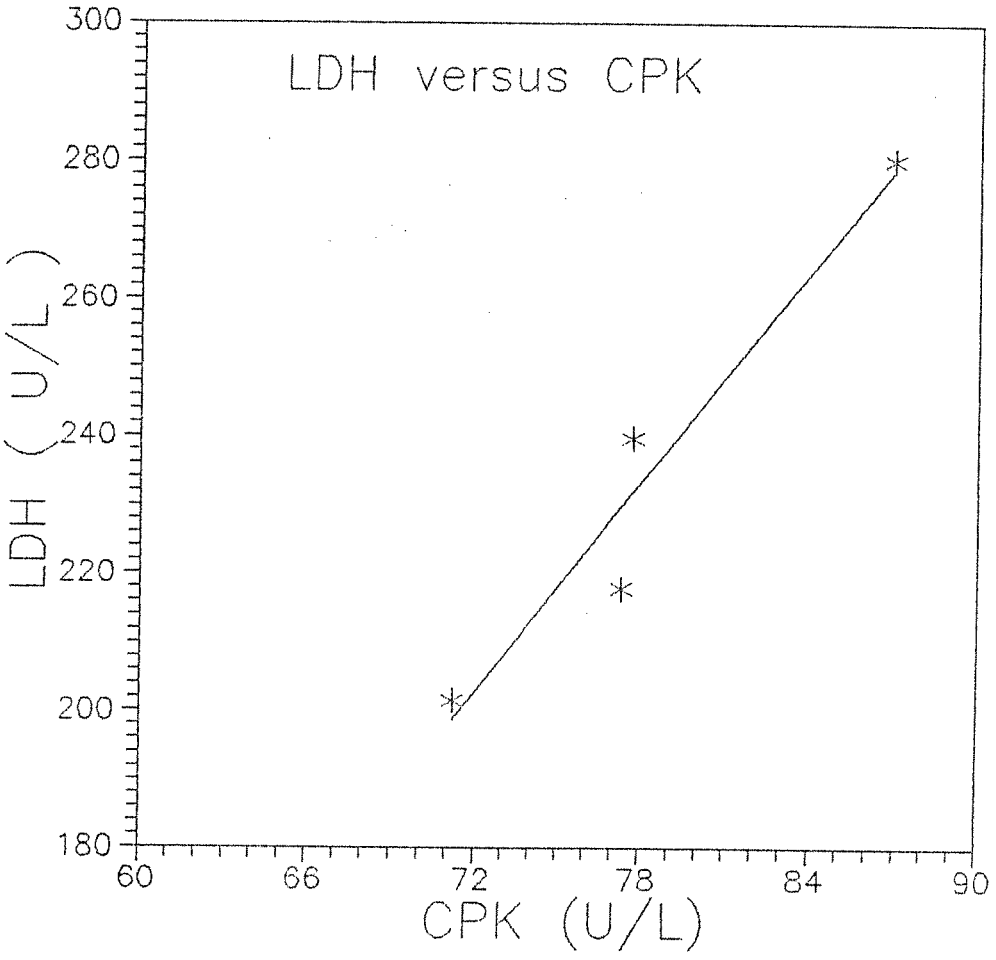


Figure 4. The correlation of CPK and LDH

To understand the correlation of the CPK and LDH values easily, the CPK and LDH values with time is drawn at the same figure (Figure 5).

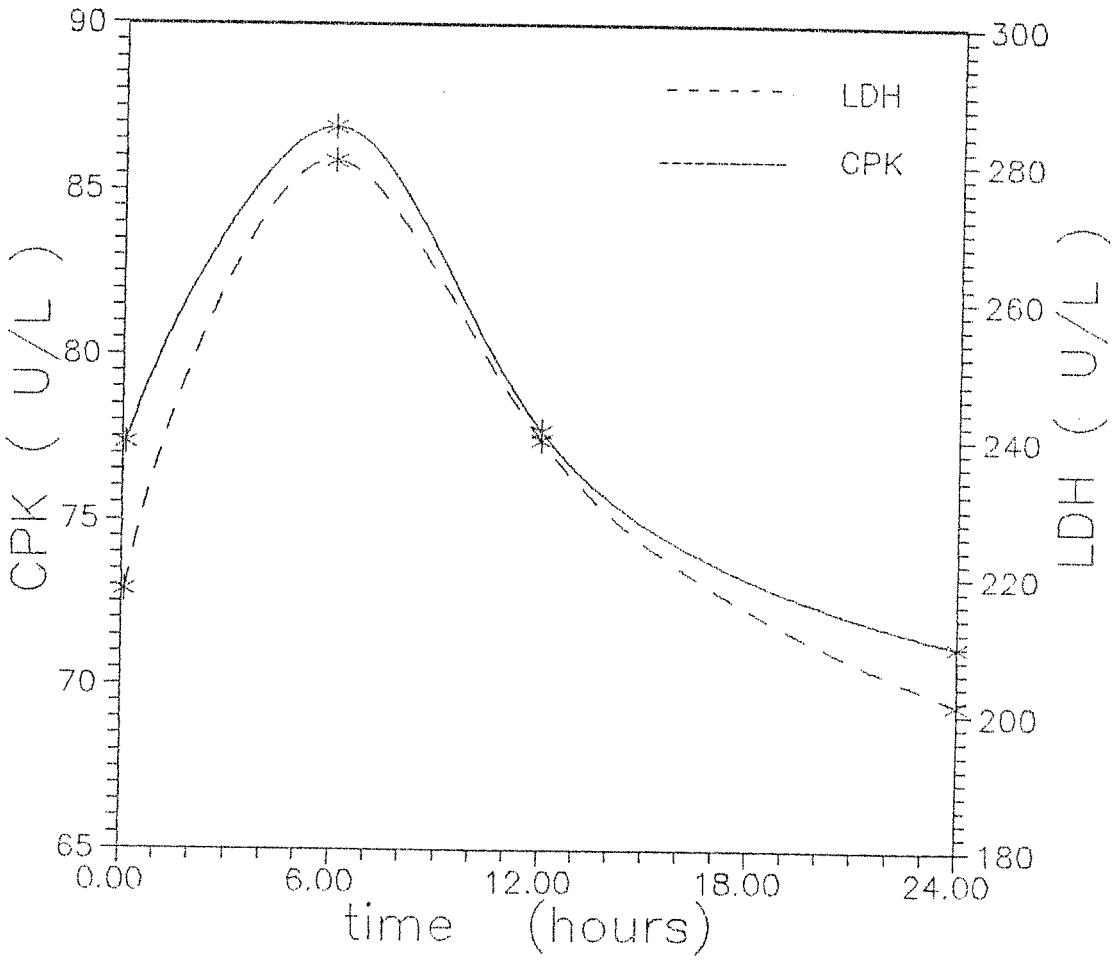


Figure 5. The CPK and LDH values with time

Statistical analysis was essential in order to interpret the results. Thus, by means of t-test, the relation between all the parameters were examined.

T-test for CPK and LDH resulted in the rejection of null hypothesis. Therefore, according to Table 6-10, meaningful relation between CPK and LDH is accepted for all periods.

Table 6. T-test for CPK and LDH

Pre-exercise	N	Mean	std	SE mean	T(cal)
CPK	9.000	85.111	17.407	6.200	-8.750
LDH	9.000	204.111	34.327	12.000	

Table 7. T-test for CPK and LDH

Post-exercise	N	Mean	std	SE mean	T(cal)
CPK	9.000	77.333	15.377	5.400	-7.580
LDH	9.000	217.778	50.079	18.000	

Table 8. T-test for CPK and LDH

6 hrs	N	Mean	std	SE mean	T(cal)
CPK	9.000	86.889	17.885	6.300	-12.680
LDH	9.000	280.222	39.256	14.000	

Table 9. T-test for CPK and LDH

12 hrs	N	Mean	std	SE mean	T(cal)
CPK	9.000	78.111	18.089	6.400	-12.310
LDH	9.000	229.333	29.672	10.000	

Table 10. T-test for CPK and LDH

24 hrs	N	Mean	std	SE mean	T(cal)
CPK	9.000	71.222	13.966	4.900	-15.110
LDH	9.000	201.444	19.984	7.100	

Then, the relation of CPK values are examined for just before and after the isometric contraction. Results are summarized in Table 11.

Table 11. T-test for pre and post exercises

	N	Mean	std	SE mean	T(cal)
CPK (Pre)	9.000	85.111	17.407	6.200	0.950
CPK(Post)	9.000	77.333	15.377	5.400	

It is found that CPK values show no relation due to the fail in rejection of null hypothesis. This means that, CPK enzyme does not vary during pre and post-exercises. However, relation of CPK with time in Figure 1 says that there exist a relation of CPK with time. Therefore, it is concluded together with the t-test that once post-exercise time has passed CPK changes with time.

In the same manner, LDH is also examined and resulted in with the failure in rejection of null hypothesis.

Table 12. T-test for pre and post exercises

	N	Mean	std	SE mean	T(cal)
LDH (Pre)	9.000	204.111	34.327	12.000	-0.640
LDH(Post)	9.000	217.778	50.079	18.000	

CPK and LDH calculations, obviously are related to each other as the correlation analysis indicates. Due to the analysis, it is found that both enzymes are strongly related, as expected from Figure 4. A 0.98 correlation coefficient which is 98% of correlation also shows that CPK and LDH are positively correlated.

Having soreness and time under consideration, R-square test leading the regression put the logarithmic dependence of soreness with respect to time may be formulated as

$$\text{Soreness} = 2.75 - 0.498 * \ln(\text{time})$$

due to the logarithmic curve fitting.

Table 13. Analysis of variance for soreness

Regression of Soreness with time

Predictor	Coeff	std	t-ratio	p
Constant	2.753	0.221	12.480	0.006
ln(time)	-0.498	0.089	-5.620	0.030

s	0.3775
R-sq	94.1 %
R-sq (adj)	91.1 %

R-square found from above analysis indicates model was good with 94.1%.

Finally, two way analysis of variance (two way ANOVA) is applied to enzymes CPK and LDH and time. Results are shown in Table 14.

Table 14. Analysis of variance (two way ANOVA) with F(table) = 2.45 ,4.00,2.45

Two way ANOVA for LDH CPK and Soreness

Source	DF	SS	MS	F (cal)
CPK	4.000	23613	5903	6.660
LDH	1.000	485174	485174	5.470
Interact	4.000	14728	3682	4.150
Error	80.000	70998	887	
Total	89.000	594514		

To investigate the relation between soreness and CPK enzyme was difficult, because soreness values construct a discrete function and fit in a logarithmic function more than a straight line where CPK values are continuous. Then, soreness and CPK values are drawn on the same figure (Figure 6) to make a prediction.

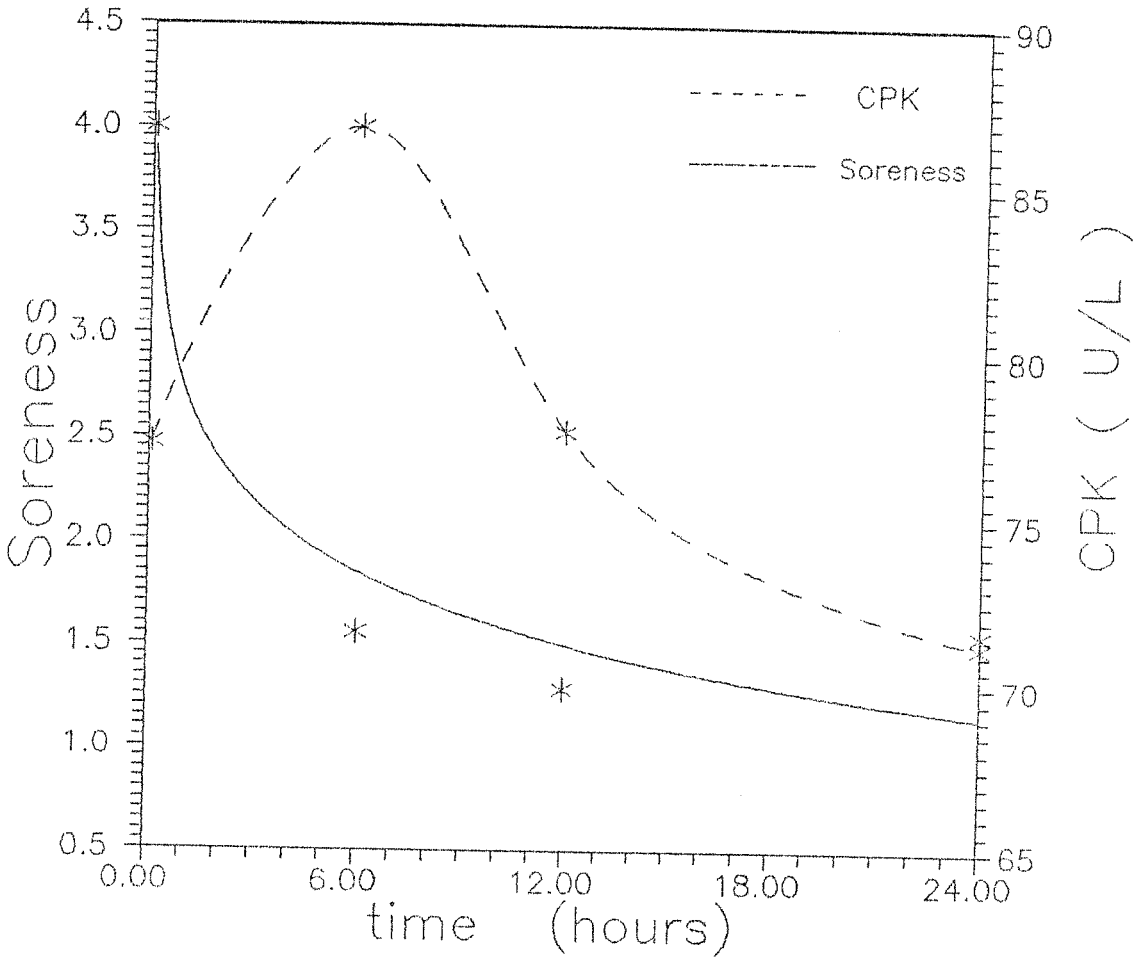
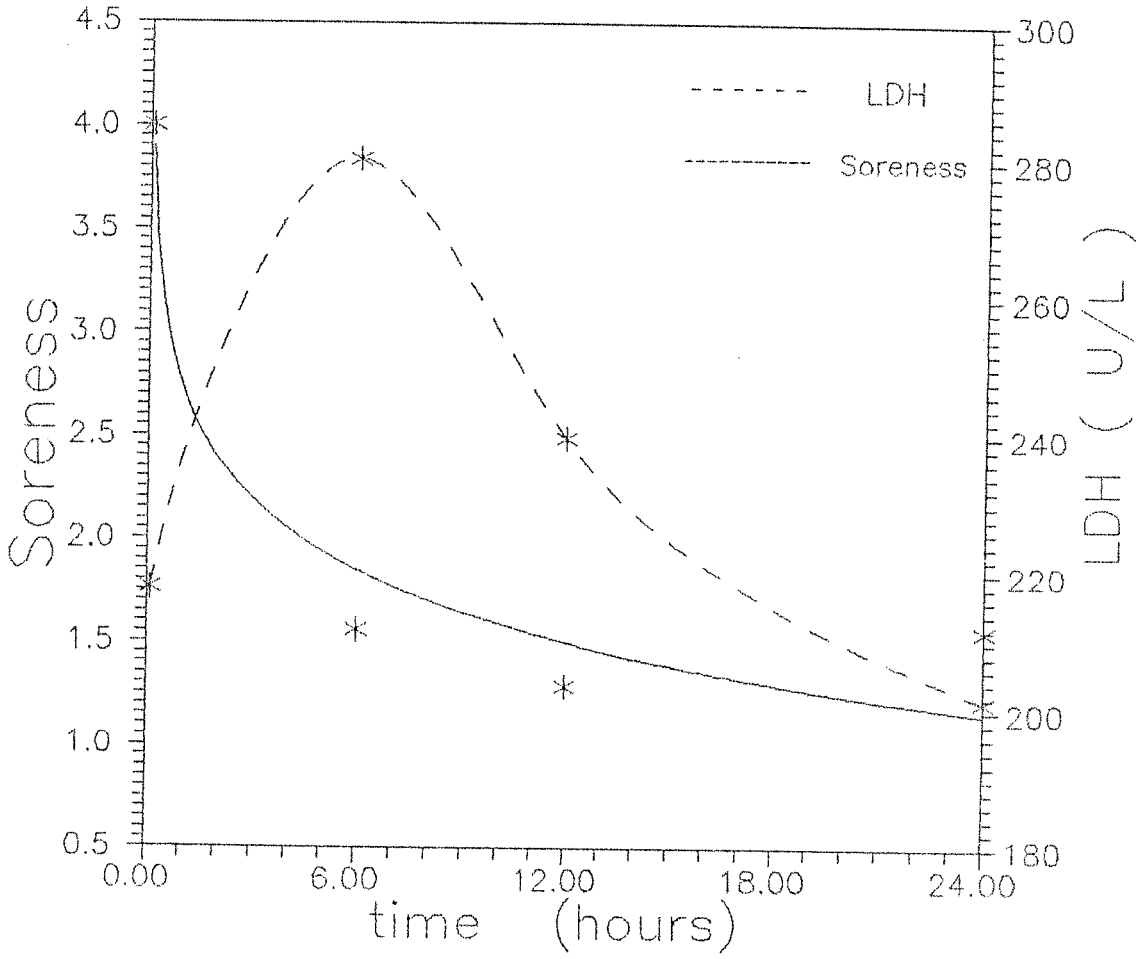


Figure.6 Soreness and CPK values with time

Soreness and LDH correlation impossibility occurs for the same reasons at soreness and CPK correlation. Figure 7 was drawn to see the relation between soreness and LDH.



Results obtained from statistical analysis indicated that a linear proportionality exist between enzyme types and are in coincidence with the previous works carried by different researchers. No relation between the pre-exercise and right after the exercise periods have been found among CPK and LDH, as expected. As indicated by t-test for the enzymes CPK and LDH have time dependences and once post-exercise period has passed enzyme, values changes with time. It is also noted that enzymes reach their maximum levels after 6 hours from the exercise as can be seen the peaks from Figure 1, and Figure 2. Correlation analysis also supported the above findings and a very strong positive correlation was established.

However, for the soreness case the time dependence found is logarithmic rather than linear. This may be due to the number of participants as well as their training level.

There was not any statistical difference between CPK and LDH as it was found by Schwane et al (1983). They also found a relationship between CPK and soreness but no significant correlation between LDH and soreness. However, statistical analysis, which was carried throughout this work resulted in with no correlation between either CPK and soreness or LDH and soreness; while there existed a strong correlation between the two

enzymes. Tiidus and Ianuzzo (1983) in their work concerning with soreness and enzyme activity found that there was a relationship between enzyme activity and soreness. They also stated the time dependence of enzyme activity. In their work, exercises are analyzed in relation to the intensity of the exercise and duration of the exercise. However, Hunter and Critz (1971) putted forward that enzymes are reduced due to the ATP concentration of trained skeletal muscle. Hickson et. al. (1976) also supported them with animal experiments on adaptation of enzymes in trained sample. There is a time dependence of enzymes as reported in Tiidus and Ianuzzo (1983). However, present work considers isometric contraction, with trained saubjects. And main differences with Tiidus and Ianuzzo (1983), such as finding no relation between CPK and soreness, are due to this fact. It is concluded that training effects the enzyme levels and there was no significant relation of soreness with CPK and LDH for trained person.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

1.There is no significant relationship between CPK enzyme level with muscular soreness.

2.There is no significant relationship between LDH enzyme level with muscular soreness.

3.There is linearly depending relationship between CPK and LDH enzyme levels after the isometric exercise.

It is recommended to include in the experiment wide range of participants, having in mind the effects of the other factors such as weight, age, sex and training levels. Also, reduction of the sampling of blood to small time intervals beside the extention of measurements up to 72 hours would increase the accuracy of the experiment, while conclusions drawn from this study holds an adequate accuracy for a behavioral analysis approach.

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APPENDIX A

PERSONAL DATA FORM

Name:

Surname:

Date of Birth:

Weight:

Height:

How many years have you been in gymnastics:

Weekly training program:

Home address and telephone:

Soreness Sensation

1 2 3 4 5 6 7 8 9 10

Pre-exercise:

Post-exercise:

6 hours later:

12 hours later:

24 hours later: