The Effects of Electrical Stimulation and Isokinetic Exercise on Serum Enzymes and Electrolytes

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Master of Science

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by

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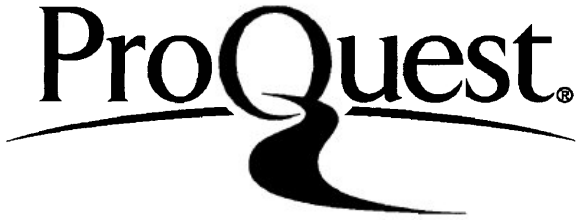

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ABSTRACT

Title of Thesis: The Effects of Electrical Stimulation and Isokinetic Exercise on Serum Enzymes and Electrolytes

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The purpose of this study was to determine the effects of short-term electrical stimulation of the quadriceps muscle, isokinetic leg extension and flexion exercise, and combined electrical stimulation and isokinetic exercise on serum enzyme and electrolyte concentrations. The subjects used in the study were eleven healthy female university students, 20 to 23 years of age. The subjects reported to the laboratory once each week for three consecutive weekends. On the first weekend electrical stimulation exercises were conducted. Isokinetic exercise and combined electrical stimulation and isokinetic exercise were performed on the second and third weekends respectively. All testing was carried out in the morning between 10:00 AM and 12:00 PM. Blood samples were obtained from an atecubtial vein just prior to, immediately after, and five minutes after each exercise. Serum enzymes (lactate dehydrogenase, creatine kinase, aspartate aminotransferase and alkaline phosphatase)

and electrolytes (sodium, potassium, chloride and total carbon dioxide) were measured using the Abbott Laboratories VP Bichromatic Analyser and the Nova Biochemical NOVA-4 Specific Ion Electrode Analyser respectively.

Minor, but statistically significant ($p < 0.05$) changes in lactate dehydrogenase and aspartate aminotransferase occurred after exercise. No significant changes were found in creatine kinase. Serum alkaline phosphatase levels were increased significantly after both electrical stimulation and isokinetic exercise. This suggested that the electrical stimulation and isokinetic exercise may stimulate bone metabolism. Small but statistically significant ($p < 0.05$) changes in serum sodium and total carbon dioxide occurred after the electrical stimulation and isokinetic exerise. No significant changes were noted in serum potassium and serum chloride.

There was no evidence from this study, to suggest that either electrical stimulation or isokinetic exerise had detrimental effects on muscles. No other research dealing with the effects of electrical stimulation and isokinetic exercise on serum enzymes and electrolytes were available for comparison with this study.

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CHAPTER 1

INTRODUCTION

Statement of the Problem

An extensive literature review has failed to reveal any studies dealing with the effects of electrical stimulation on serum enzyme activities and/or electrolyte balance in humans. Most recent studies on the effects of electrical stimulation have dealt with muscle development (Knight, 1980; Romero, Sanford, Schroeder and Fahey, 1982; Standish, Valiant, Bonen and Belcastro, 1982; Lainey, Walmsley and Andrew, 1983). Some work has been performed on enzyme and electrolyte content of muscle after electrical stimulation (Jaweed, Herbison, Ditunno and DeGroof, 1982a; Jaweed, Alam, Herbison and Ditunno, 1982b and Eriksson, Häggmark, Kiessling and Karlsson, 1981). The purpose of this study was to determine the effects of short term electrical stimulation of the quadriceps muscle, isokinetic leg extension and flexion exercise, and combined electrical stimulation and isokinetic exercise on serum enzyme and electrolyte concentrations.

Significance of the Study

Electrical stimulation has been used as a treatment mode especially for muscular disorders since the eighteenth century. In 1744 Christian Gottlieb Kratzewstein reported

that he used an electrostatic generator to treat paralysed patients and claimed beneficial results. By 1791 Luigi Galvani had developed the galvanic cell and was conducting experiments on electrical stimulation of muscles and nerves. Interest in this mode of treatment grew rapidly so that, by the beginning of the twentieth century, it was used by many medical practitioners to treat all manner of ailments (Almekinders, 1984). However, many of the early electrical stimulators were ineffective or potentially dangerous, and their use declined until the advent of solid state electronics allowed the production of easily controlled, variable wave form instruments (Stamford, 1983). There is again an upsurge in the use of electrical stimulation. It has proven to be effective in preventing muscle atrophy, and improving muscle strength. Stamford (1983) warns against improper use of electrical stimulation as an alternative to exercise in weight reduction.

Interest in electrical stimulation as a training mode for athletes has developed since the 1972 Olympic Games when it was rumored that Russian athletes were using it in their training programs. A report on the methods used in the Soviet Union was given by Kots (1977) at a symposium held at Concordia University in 1977. His protocol was a ten second maximum tetanic contraction followed by a fifty second rest period. This was repeated ten times per session and the treatments were given five days a week. He reported a 30 to

40% increase in muscle strength after 20 to 25 sessions. Since that time, many attempts have been made to duplicate Kots' results with varying degrees of success (Lainey, Walmsley and Andrew, 1983).

Most work with serum enzymes and electrolytes has been concerned with athletes performing either short term, intensive exercise, such as on a bicycle ergometer, or long term, less intensive exercise such as distance running. This study has added to the knowledge of the effects electrical stimulation and isokinetic exercise on the levels of enzymes and electrolytes in serum.

Limitations of the Study

1. The inability to control the subjects in following instructions, and providing full and honest co-operation in all aspects of the study.
2. The pre-exercise blood sample during each test was used to determine baseline variable levels.
3. The use of female volunteers from a selected group limits the study in terms of generalizing the results to other populations.
4. An alpha level of 0.05 was established as the level of significance for statistical tests.

Delimitations of the Study

1. Eleven female university students were used as subjects.
2. Three studies were conducted.
    1. Electrical stimulation of the quadriceps muscle alone.

2. Isokinetic leg extension and flexion exercise.
3. Combined electrical stimulation and isokinetic leg exercise.

3. The enzyme assays performed were creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and alkaline phosphatase. Electrolyte assays were sodium, potassium, chloride, and total carbon dioxide.

Definitions

Alkaline phosphatases (ALP) A group of enzymes which catalyse the hydrolysis of phosphate esters. They are present in bone, intestine, liver, placental and renal tissue. Plasma ALP is derived mainly from bone and liver. There are eight isoenzymes of ALP.

Aspartate aminotransferase (AST) A group of enzymes which transfer amino groups from amino acid to alpha keto acids. They are found in both mitochondria and cytoplasm. Serum AST is derived mainly from cytoplasm. Isoenzymes of AST are not well defined.

Chloride (Cl) Chloride is the major extracellular anion.

Creatine kinase (CK) A group of enzymes which catalyse the phosphorylation of creatine by adenosine triphosphate. They are most abundant in muscle. There are three isoenzymes of CK.

Electrical stimulation The transmission of a high frequency electrical current through electrodes placed on the skin surface in order to stimulate muscle contraction.

International unit (IU) That amount of enzyme which will catalyse one micromole of substrate per minute. Enzyme activities are usually given in international units per litre (IU/L).

Isokinetic contraction Muscle contraction in which the tension developed by the muscle while shortening at constant speed, is maximal over the full range of movement.

Lactate dehydrogenase (LD) A group of enzymes which catalyse the oxidation of L-lactate to pyruvate. They are found in most tissues. There are five isoenzymes of LD.

Potassium (K) Potassium is the major cation of the intracellular fluids. Its concentration in serum is about 1/20th of it concentration in intracellular fluids.

Reference (Expected) range The range of assay values that 95% of the general population would normally have.

Serum alkaline phosphatase 36 - 92 IU/L.

Serum aspartate aminotransferase 10 - 30 IU/L.

Serum chloride 95 - 105 mmol/L

Serum creatine kinase 45 - 235 IU/L.

Serum lactate dehydrogenase 109 - 193 IU/L.

Serum potassium 3.5 - 5.0 mmol/L.

Serum sodium 134 - 145 mmol/L.

Serum total carbon dioxide 25 - 30 mmol/L.

The reference values as given above, were obtained from unpublished data supplied by McKellar Clinical Laboratories, Thunder Bay, Ontario.

Sodium (Na) Sodium is the major extracellular cation.

Total carbon dioxide Total carbon dioxide is the sum of the bicarbonate, carbonic acid and dissolved carbon dioxide present in serum.

CHAPTER 2

REVIEW OF THE LITERATURE

Electrical Stimulation of Muscle

It has been demonstrated that electrical stimulation is a useful mode of treatment for preserving muscle strength during periods of forced immobility, and has a place in the training of athletes for certain sports activities. It also appears to be useful as an adjunct to various other forms of exercise in an athlete's training regimen. Houston (1983) in his review cautions that, while electrical stimulation is useful, it may be abused if employed by persons unfamiliar with the physiological principles of its use. As well, he cautioned that care must be taken to ensure that injury to the skin and underlying tissues does not occur through the prolonged use of surface electrodes.

Effects of Electrical Stimulation on Muscle Strength

Romero et al. (1982) examined eighteen young women aged from 20 to 27 years. Nine were subjected to electrical stimulation, and nine were used as controls. The subjects were not trained athletes, and were assigned randomly to test and control groups. Thigh girth and leg strength were measured before, and at the conclusion of the experiment.

Members of the experimental group were given faradic stimulation twice a week for five weeks, using a model SP-5 Teca low-voltage therapeutic generator. Each stimulation period was fifteen minutes in duration. The stimulus was pulsed four seconds on, and four seconds off. The intervals between treatments were one day, and then four days over the period of the study. The authors found that isometric knee strength increased by 21% in the dominant leg, and by 31% in the non-dominant leg of the test group. No changes were observed in the control group. There was no increase in thigh girth in any of the subjects.

Effects of Electrical Stimulation on Immobilized Muscle

Standish et al. (1982) examined twelve patients who had knee surgery which entailed immobilization of the leg in a cast. Six subjects, who were used as controls, were not given electrical stimulation. The other six subjects were given electrical stimulation starting ten days after surgery, and continuing until four weeks after removal of the cast. Needle biopsies were taken from the vastus lateralis muscle before surgery, ten to fifteen days later after removal of the cast, and six weeks later. Their results showed a reduction in muscle ATPase in the control group, but not in the experimental group. No difference in muscle glycogen content between the two groups was observed. The authors concluded that electrical stimulation was

effective in preventing the decrease in muscle ATPase which results from prolonged immobilization of a limb.

In another study on patients with knee injury, Lainey, Walmsley and Andrew (1983), used a regimen which combined electrical stimulation with isokinetic exercise. They used a crossover design for their experiments. The subjects were assigned to two groups, each group consisted of one female and two males. The first group started with isokinetic exercise alone, followed by isokinetic exercise plus electrical stimulation. They then reverted to isokinetic exercise alone and finished with exercise plus stimulation. The second group was given the reverse exercise/electrical stimulation sequence. They found that during the first two weeks of training, subjects who received isokinetic exercise alone showed a greater increase in strength than those on exercise plus electrical stimulation. However, during the next four weeks, those receiving both isokinetic exercise and electrical stimulation demonstrated a greater increase in strength than those on exercise alone. The authors suggested that there were two possible explanations for this: 1. The subjects needed a period of adaptation before they could tolerate sufficient stimulation to be effective, or 2. That electrical stimulation is more effective when the subject can generate a higher degree of tension than is possible immediately after surgery.

The effects of electrical stimulation on immobilized muscles was also studied by Knight (1980). He found that muscle atrophy was prevented in a basketball player with a sprained ankle, who was required to wear a cast for seven weeks. Electrical stimulation was applied to the muscles through holes cut in the cast. Each muscle was stimulated for one minute on the first day, and the time was increased by one minute a day to a maximum of five minutes. This was then continued for the rest of the treatment period. A set of ten repetitions of isometric exercise were also used before and after each electrical stimulation.

Isokinetic Exercise Versus Resistance Training

In a study involving 47 male marines ranging in age from 19 to 30 years, Massey, Nelson, Sharkey, Comdon and Otott (1965) found that electrical stimulation was not as effective as weight training and static exercise in developing grip strength and arm flexion. They determined that the increase in muscle strength was roughly proportional to the length of the training period and speculated that the actual training time for electrical stimulation was less than that for the other exercises. Massey et al. (1965) also noted that electrical stimulation had no effect on arm extension and concluded that this was probably due to the position of the elbow during stimulation and the placement of the elctrodes. They used an Isotron low voltage, high frequency (100 - 3000 cps) stimulator.

Currier and Mann (1983) compared electrical stimulation with various resistance training modes. The subjects were 15 males and 19 females ranging in age from 18 to 32 years. They were divided into four groups: 1. controls (n = 9), 2. exercise only (n = 8), 3. electrical stimulation (n = 8), and 4. electrical stimulation and exercise (n = 9). The exercise used was isokinetic contraction at the rates of 100°/sec, 200°/sec and 300°/sec. Electrical stimulation was applied to the quadriceps femoris muscle at a stimulus intensity which produced 60% of the maximum voluntary isokinetic contraction for each subject. Thigh girth and leg strength were measured before and after a five week experimental period. From the results of their study, the authors concluded that high intensity electrical stimulation augments torque when subjects train with isometric contractions. No increase in thigh girth was noted in any of the subjects over the five week training period. A similar study by Laughman, Youdas, Garrett, and Chao (1983) also showed that electrical stimulation of the quadriceps femoris muscle produced an increase in muscle strength like that developed by isokinetic exercise.

A study by McMiken, Todd-Smith and Thompson (1983) compared electrical stimulation with isometric exercise, using fifteen female and three male student volunteers, aged 19 to 27 years. Initially, all subjects were tested for maximum voluntary isometric contraction of the quadriceps

muscle. They were then divided randomly into two groups. One group trained daily with isometric exercise and the other with electrical stimulation. The training was continued for a period of two weeks and they were then retested. The authors found that the average increase in muscle strength was 22% with electrical stimulation and 25% with isometric exercise. They did not find any significant difference between the two modes of training.

Kaada (1984) tested the effects of transcutaneous nerve stimulation (TCN) on the performance of 21 well trained athletes in various sports. The athletes were given either a placebo stimulation (i.e., with the voltage set at or near zero), or low frequency stimulation (2 Hz) for 45 minutes. There was a significant improvement in performance after transcutaneous nerve stimulation when compared with the performance after placebo stimulation in long distance runners and swimmers, and in performance on the bicycle ergometer. There was no significant change, however, in performance in isometric endurance or hand grip strengths.

Biochemical Changes Caused by Electrical Stimulation

Some data have been obtained on biochemical changes elicited by electrical stimulation of muscle using rats. Jaweed, Herbison, Ditunno and DeGroof (1982a), using Wistar rats, demonstrated that electrical stimulation of both normal and denervated muscle caused an increase in calcium

content of muscle. They concluded that electrical stimulation causes an increase in calcium metabolism in muscles.

In a second study Jaweed, Alam, Herbison and Ditunno (1982b) showed that prostaglandin (PGE_2) increased by 110.6% in electrically stimulated normal muscle, and by 45.9% in electrically stimulated denervated muscle. From this they concluded that low frequency (10 Hz) electrical stimulation of denervated muscle may retard the synthesis of PGE_2.

Eriksson, Häggmark, Kiessling and Karlsson (1981) investigated the acute and adaptive effects of electrical stimulation on the quadriceps muscle of healthy male physical education students, aged 20 to 41 years. Four series of tests were performed. In series I (n = 6), they evaluated the acute effects of electrical stimulation by analysing muscle biopsies taken before, and after treatment. Biopsies from the untreated legs were used as controls. The treatment protocol was 15 seconds of stimulation interrupted by 15 second rest periods for a total of six minutes of effective stimulation. In series II (n = 6), the adaptation of muscle to repeated electrical stimulation was tested. The same treatment protocol as in series I was used and it was repeated four to five times a week for five weeks to give an average of 150 minutes of effective electrical stimulation. Series IIIA (n = 6) was similar to series II, except that the periods of galvanic stimulation and rest were shortened to six seconds. The test was repeated 15

times to give a total effective stimulation time of 90 minutes. The subjects in series IIIB (n = 4) were used as controls for series IIIA. They performed dynamic training on the quadriceps muscle using the same number of contractions as in series IIIA. The authors demonstrated that there was a significant decrease in muscle concentration of adenosine triphosphate, creatine phosphate and glycogen, and an increase in muscle lactic acid as a result of acute electrical stimulation. No changes in muscle enzyme activities were noted in either the acute or long term series. Serum enzyme activities were not measured.

Serum Enzymes and Exercise

This literature review has revealed two major areas of research which deal with serum enzymes and exercise. The first, and most easily standardized, was the effect of short term intensive bursts of exercise on serum enzyme levels. The second dealt with less intensive, long term exercise such as marathon running and long distance swimming. The enzymes most frequently determined were lactate dehydrogenase (LD, LDH), aspartate aminotransferase (AST, GOT) and creatine kinase (CK, CPK). Other subjects dealt with were the isoenzymes of CK, including isoelectric focusing of CK-MM, and isoenzymes of LD, in muscles and sera from marathon runners. The results obtained with athletes were compared with those found in patients with myocardial infarction. The effects of vitamin E on serum enzyme levels

during exercise (Helgheim, Hetland, Nilsson, Inger and Stromme, 1979), and theories with regard to the effects of adenosine triphosphosate depletion on enzyme efflux have also been investigated (Griffiths, 1966; Sweetin and Thomson, 1973; Thomson, Sweetin and Hamilton, 1975).

The Effects of Short Term Exercise on Serum Enzyme Levels

Fowler, Gardner, Kazerunian and Lauvstad (1968) studied sixty normal student volunteers with ages ranging from 17 to 31 years. Using standardized exercise regimens they showed that serum AST and LD activities increased during intensive short term exercise. Blood samples were obtained before, immediately after, and five minutes after exercise. In all cases the subjects exercised to exhaustion. The exercises used were 1. treadmill running at 5 mph (8 km/h) with grades varying from three to eleven degrees, and 2. the bicycle ergometer at work loads of 750, 1000, and 1300 kpm/min. The bicycle ergometer exercise periods were three minutes at each work load, with five minutes rest between the first and second periods.

There was considerable variation between subjects but it was concluded that serum AST levels rose in proportion to the type and intensity of work. The increases observed in well conditioned athletes were proportionately less than in untrained subjects. Significant increases in serum AST levels were not seen until the eighth treadmill grade was reached. Serum LD levels rose in proportion to the work

load but were not statistically significant until the eleventh degree treadmill grade. Factors influencing serum LD levels were: duration of exercise, physical conditioning, and type of exercise. Similar findings were observed for other serum enzymes, but in the case of serum CK levels fluctuated considerably during exercise on the bicycle ergometer. In addition, there was no relationship between work duration, intensity of work, or total work performed.

Ledwich (1973) examined seventy subjects in an effort to correlate electrocardiographic changes with serum CK levels before and after submaximal exercise on the bicycle ergometer. He did not find evidence to support the hypothesis that a greater efflux of muscle CK occurred in those who were physically less fit that the other subjects. He concluded that the increase in serum CK levels seen in some of his subjects was due to enzyme efflux across the cell membrane of an ischemic myocardium and not from skeletal muscle.

In another study Chanine, Kasantzis, Luchi, Raizner and Gyorkey (1976), using a routine treadmill test, examined 100 subjects for serum enzyme changes after exercise. They obtained blood samples before, immediately after, four hours after, and twenty-four hours after exercise. The results at four and twenty-four hours after exercise did not show a significant increase in enzyme activities. Only two of the 100 subjects showed a rise in AST levels above the upper

limit of the reference range. The serum LD levels did not show any significant variation from the baseline levels at any time after exercise, except in five subjects. In seven subjects the serum CK levels rose slightly above the upper limit of the reference range. Minor variations occurred in the other subjects but they were within the reference range, and the mean values for the group showed no significant variation at any time after exercise.

Forssell, Norlander, Nyquist, Orinius and Styrelius (1975) studied seventeen untrained, middle-aged subjects using the bicycle ergometer. Their mean age was 50 years. Blood samples were taken 30 minutes before, one hour after, and then every two hours for 18 to 49 hours after exercise. The load was increased every six minutes, and the subjects exercised to exhaustion. They found that the maximum increase in serum CK levels was 32 IU/L and except for one subject, the serum CK levels remained below the upper limit of the reference range (i.e., less than 130 IU/L).

Metivier, Poortmans, Vanroux and Gauthier (1980) examined 11 male subjects at two levels of work intensity: 1. 50% $\dot{V}O_{2\ max}$ and 2. 66% $\dot{V}O_{2\ max}$. They found that serum AST activity increased significantly after the 66% $\dot{V}O_{2\ max}$ exercise but found no significant change after the 50% $\dot{V}O_{2}$ max exercise. They concluded that an increase in muscle enzyme efflux was related to work intensity and not to duration for short term (30 minutes) exercise.

Using exhaustive "square-wave" endurance exercise, Gimenez and Florentz (1984) showed that serum LD activity increased by 20.5% immediately after exercise but returned to resting levels at five minutes after exercise. Creatine kinase on the other hand increased by 17.3% and remained elevated at five minutes after exercise.

Parikh and Ramanathan (1977) used a less conventional form of exercise; staircase running. The subjects ran up and down a flight of stairs for thirty minutes. The stairs had 26 steps and a riser height of 14.2 cm. Blood samples were obtained before and immediately after exercise. The serum enzymes measured were AST and alanine aminotransferase (ALT). They found that serum enzyme levels increased with exercise. They also noted that there was great variation between individuals.

The Effects of Long Term Exercise on Serum Enzyme Levels

Many workers have found that long term exercise causes an increase in serum levels of AST, LD, and CK. A variety of forms of exercise have been used, such as handball (King, Statland and Savory, 1976), endurance running (Misner and Williams, 1973), long distance swimming (Haralambie and Senser, 1980) and jogging (Kaman, Goheen, Patton and Raven, 1977).

Schnohr (1974) examined three well trained male physicians before and after a 100 km jogging race. He found that in all subjects the serum AST, LD and CK levels in the

blood samples taken between five and twenty-five minutes after the race were much higher than the basal levels. The serum CK showed the greatest increase. The mean pre-race serum CK was 67 IU/L and the post-race mean was 1900 IU/L. Serum AST and LD levels showed a less dramatic increase.

Kaman et al. (1977) perfomed a similar experiment on five well trained, healthy, middle-aged males who were chronic joggers. To establish a baseline they refrained from running for five days prior to the race. On the morning of the race venous blood samples were obtained, and after a 10 to 17 kilometre run, further samples were taken at one hour post-exercise and at eight hour intervals for four more days. During this time no further exercise was permitted. They found significant increases in all enzymes during the post-exercise period. Serum CK levels were elevated at eight hours and remained elevated for up to 72 hours. Elevated serum LD levels occurred at one hour and remained elevated for up to 24 hours after the race. An increase in LD_1 similar to that found in myocardial infarction was demonstrated by electrophoresis (i.e., LD_1-LD_2 flip). The exercise regimen for the Kaman experiment was not as rigorous as in the Schnohr (1974) experiment and the increase in CK was not as dramatic.

Kaman et al. (1977) were not able to detect creatine kinase MB (CK-MB) isoenzyme and therefore concluded that

CK-MB could be used to distinguish between exercise induced and post-myocardial infarction enzyme patterns.

A similar experiment was carried out by Noakes and Carter (1976), in which they examined seven men and five women before and after a 65 to 160 km race. They found that serum LD, AST, and CK levels were increased after the race and noted that the serum CK showed the greatest increase. In another experiment, Haralambie, Senser and Sierra-Chavez (1981) examined nine female athletes before and after a 25 km race. They demonstrated that the activities of both serum CK and LD rose significantly after the race.

Long distance swimming also caused an increase in serum enzyme levels. Haralambie and Senser (1980) investigated sixteen young adults immedialtely before and immediately after they had completed a 5299 $\pm$ 618 metre swim in 90 minutes. The mean baseline serum CK was 81.8 $\pm$ 52.1 IU/L. Five minutes after the swim the mean serum CK was 1216.1 $\pm$ 52.8 IU/L. In another study, Millar (1978) examined 13 boys before and after a regular summer training session. He found that the serum enzyme levels increased in direct proportion to the degree of exercise.

King et al. (1976) examined four healthy male volunteers before and after a one hour game of handball. Blood samples were obtained prior to, one hour after the exercise, and eight additional times up to 93 hours post-exercise. They found an average increase in serum CK, AST, and LD of

+116%, +41%, and +32%, respectively. All the enzyme levels remained above the baseline for 53 hours or longer.

From the above experimental data, it is apparent that severe, prolonged exercise causes an increase in the efflux of intramuscluar enzymes which is reflected in a marked increase in serum enzyme levels. However, in none of these experiments was the effect of changes in intravascular volume considered.

Effects of Duration and Type of Exercise on Serum Enzymes

In order to assess the importance of the duration of exercise on serum enzyme levels, Chanine, Kasantzis, Luchi, Raizner and Gyorky (1976) divided their subjects into two groups. One group exercised for less than six minutes and the other group for more than six minutes. They did not detect any differences in enzyme activities between the two groups.

Sanders and Bloor (1975) examined two groups of athletes. Group 1 ran for 45 minutes three times a week for five weeks. Group 2 played an average of one and one half 45 minute periods of handball per week for five weeks. Both groups were examined before and after each exercise session. They found that pre-exercise serum CK activities increased progressively, while the pre-exercise AST did not change. The post-exercise serum CK increased by 4 to 12% in group 1, and by 8 to 20% in group 2. Post-exercise serum AST levels

were 46 to 64% above the baseline levels in group one and 38 to 56% in group two.

Berg and Haralambie (1978) examined eighteen groups of male athletes with ages ranging from 17 to 42 years. There were 166 subjects in total. Exercise protocols were: 1. bicycle ergometer, 2. cross-country ski racing, and 3. impact-type exercises (running, skating and walking). They determined that serum CK activities rose in proportion to the degree of exercise up to 300 minutes. After 300 minutes the increase was accelerated and was no longer proportional to the degree of work.

Untrained male and female volunteers, with ages ranging from 20 to 45 years, were used by Tiidus and Ianuzzo (1983) to study the effects of concentric muscle contractions on serum enzyme levels. They used a dynamic leg extension apparatus (Global Gym) which was calibrated with a cable tensiometer. The exercise was performed by extending the knee to approximately 90% of full extension, which raised the weights vertically to 0.5 m. The weights were lowered slowly to allow eccentric contraction. They concluded that high intensity, short duration exercise resulted in greater enzyme efflux than long term low intensity exercise. Creatine kinase showed the greatest increase (greater than 300%) post-exercise. Lactate dehydrogenase and AST levels also increased, but not to the same extent as the CK. Serum enzyme levels were highest at 8 to 24 hours after exercise.

The subjects noted that muscle soreness began to occur 8 to 24 hours after exercise, and was at a maximum at 48 hours. Thus the highest muscle soreness occurred approximately 24 hours after the peak enzyme activities.

CK and LD Isoenzymes and Exercise

During the past few years there has been a resurgence of interest in exercise induced increases in serum CK and LD levels. This was because of the increasing popularity of jogging among the general public, and the symptoms which can occur with over exertion that mimic those of acute myocardial infarction. The main interest has centered around the CK-MB and LD_1 isoenzymes which are more abundant in myocardial muscle than other muscles. Lott and Strong (1980) and Werner, Brooks, Mohrbacher and Wasserman (1982) suggested that the two most useful serum enzyme measurements for the diagnosis of myocardial infarction, are CK-MB and LD_1 if measured on two consecutive days. However, many other workers have shown that CK-MB and LD_1 are increased in the sera of athletes after prolonged severe exercise. This could be misleading in an athlete admitted to hospital with chest pain from overexertion.

In one experiment Schnohr, Grande and Christiansen (1980) showed that while there was a rise in the serum CK-MB of two volunteers after a 26 kilometre jogging race, the results were not high enough to interfere with the diagnosis of myocardial infarction. Bark, Bergström, Hendriksson and

Lindberg (1979-80) also reported an increase in the serum level of the B sub-unit of creatine kinase after severe exercise. Since they used an immunological method specific for the B sub-unit, they were unable to distinguish between CK-MB and creatine kinase BB isoenzyme (CK-BB). They concluded that it was probably CK-MB because CK-BB levels were not likely to increase unless brain damage occurred, and that myocardial damage was more likely to result from severe exercise than brain damage. Their findings were similar to those of Noakes and Carter (1976) who examined the sera from seven men and five women before, and after completing a 160 km race.

In a more recent study, Kaste and Sherman (1982) found increased levels of CK-MB in blood samples taken from marathon runners after they had completed a race. Their conclusion was that the isoenzymes were probably from the myocardium and suggested that marathon running and other forms of severe exercise may cause damage to the myocardium. Stansbie, Aston, Powell and Willis (1982) conducted a similar study in which they examined 70 runners who had completed the London, U.K. marathon race. They found that eighteen had CK-MB levels greater than 6% of the total CK after the race. Robinson, Williams, Worthington and Carten (1982) also demonstrated CK-MB in the sera of 52 of 335 members of the 1980 British Olympic team. In contrast to these studies, Munjal, McFadden, Matix, Coffman and Cattaneo

(1983), who examined 61 male and 23 female runners, concluded that their serum CK-MB did not exceed the expected range for non-myocaridial infarct patients. However, they stored the sera at -20° C for up to two weeks before measuring CK-MB activity which could account for the low values they obtained. Creatine kinase isoenzymes are unstable and no data were included to demonstrate their stability under the conditions of storage.

Origin of CK-MB in the sera of marathon runners

Silverman, Siegel, Madar, Evans and Straight (1982), in an effort to determine the origin of CK-MB in the sera of marathon runners, examined muscle biopsies (lateral gastrocnemius) from 25 runners and six non-runners for total CK and CK-MB. They found that the difference in total muscle CK for the two groups was not statistically significant. CK-MB levels on the other hand were markedly different. On the average, muscle CK-MB for runners was 8.9% of the total CK activity, while the mean CK-MB for non-runners constituted only 2.3% of the total.

A similar study was conducted by Sylven, Jansson, Brandt and Kallner (1983) who examined muscle biopsies from highly trained long distance runners, and compared their findings with those obtained in muscle biopsies taken from untrained individuals. They found that the CK-MB activity was three times higher, and the LD_1 activity about two times higher in muscles from the trained athletes than in the

muscles of untrained persons. In spite of the increased levels of CK-MB and LD_1, they found that the CK-MB/total CK and LD_1/total LD ratios were lower than those found in patients with myocardial infarction. Similar conclusions were reached by Kettunen, Kala and Rehunen (1982) who examined muscle biopsies from junior ice hockey players. They found that although the CK-MB levels were increased the CK-MB/CK-MM ratios were within the expected range.

In another study, Silverman, Lubahn, Siegel and Evans (1984) examined sera from runners and untrained persons after severe eccentric exercise. They found that the sera of untrained persons contained an increased level of total CK which was shown to be all CK-MM, while the sera from marathon runners contained 8.0% CK-MB. To further elucidate the origin of CK-MB in the sera from athletes, Apple and McGue (1983) measured both CK-MB and aldolase in sera from two male runners during marathon training. Since both CK-MB and aldolase were elevated they concluded that the CK-MB was from skeletal muscle because heart muscle does not contain more than a trace of aldolase. Further studies by Silverman et al. (1984) demonstrated changes in muscle LD patterns in marathon runners with a selective increase in the LD_1 and LD_2 isoenzyme fractions. They also found that serum LD patterns post-marathon resembled the LD_1, LD_2 flip as seen in myocardial infarction. They concluded that the CK and LD isoenzyme patterns found in marathon runners showed a

complex adaptation of skeletal muscle to strenuous exercise. Another study was conducted by Apple, Rogers, Sherman, Costill, Hagerman and Ivy (1984a) who examined muscle biopsies from five marathon runners. The biopsies were obtained two hours before and thirty minutes after a marathon race. They compared their results with those taken from the muscles of non-runners. From their results, they concluded that the active gastrocnemius muscle of long distance runners is metabolically similar to heart muscle in its CK composition.

In another study by Apple, Rogers, Yashmineh, Sherman and Ivy (1984b) muscle biopsies were obtained before, and 30 minutes after a race, from five marathon runners. They compared the CK isoenzyme composition of these samples with those from five non-runners. They found that the muscles from the marathon runners had CK-MB activities of 7.7 $\pm$ 2.4% before, and 7.2 $\pm$ 1.2% of the total CK after the race. In the non-runners the CK-MB was less than 1% of the total CK. It was also noted that mitochondrial CK and CK-BB were also present in the muscle samples from the marathon runners and were not detected in the muscles from the non-runners. From their findings, the authors concluded that the gastrocnemius muscle of marathon runners is quantitatively similar to heart muscle in its CK isoenzyme composition.

Isoenzymes of CK-MM in muscle

Apple et al. (1984b), using isoelectric focusing on polyacrylamide gel, determined which isoenzymes of CK-MM were present in the gastrocnemius muscle and sera of both male and female marathon runners after a marathon race. They compared their results with those obtained in patients with myocardial infarction, and found that the isoenzyme patterns in the sera were similar. Muscle biopsies taken from eight men 48 hours before and 24 hours after a race, showed two major forms of CK-MM. They were MM-1 (pI = 6.9, 85%) and MM-2 (pI = 6.62, 15%). There was no difference in the CK-MM isoenzyme patterns before or after the race. The sera from both males and females showed similar isoenzyme patterns. The post-marathon serum samples yielded three major CK-MM isoenzymes. They were MM-1, MM-2 and MM-3 (pI = 6.36). Three additional minor forms of CK-MM were found in the sera from the men; MM-4 (pI = 8.2) and two previously unreported forms designated MM-1B (pI = 6.76) and MM-2B (pI = 6.49). No percentage of the total serum CK-MM content were given for these isoenzymes. From their experiments they concluded that the post-marathon results obtained in runners concurred with the patterns found in post-myocardial infarction.

CK-BB in the sera of athletes

Kaste and Sherman (1982) found increased levels of both CK-MB and CK-BB in sera from marathon runners. Since there

was no apparent brain damage in any of the runners, they concluded that the CK-BB was from some other source. The reseachers noted that other tissues contain CK-BB, and gave the approximate content of each tissue as a percentage of the content of the brain: prostate and intestine 35%, kidney and lung 10%, skeletal muscle, liver and spleen less than 0.5%. However, in spite of its low content of CK-BB, they concluded that skeletal muscle was the most likely source because of its large bulk when compared to the brain and other tissues.

Phillips and Horner (1982) also detected CK-BB in the sera from ten male marathon runners. They observed that five men had levels which were equal to those found in patients with severe concussion. They speculated on the possibilty of brain damage in mararthon runners but were inclined to think that the CK-BB was from sources other than the brain. Other workers, (Apple, Rogers, Sherman and Ivy, 1983; Apple and McGue, 1983) also demonstrated CK-BB in the muscles and sera from marathon runners.

Liver Enzyme Induction and Exercise

Frenkl, Gyore, Mezarose and Szeberenyi (1980) studied the liver microsomal activity in athletes by observing the rates of elimination of aldactone and antipyrine. They found that the rates of elimination of these substances were significantly higher in athletes than in non-athletes, and concluded that exercise caused induction of liver enzymes

similar to that seen in drug induced liver enzyme activity (e.g., barbiturates). They also noted that the rate of antipyrine elimination was directly related to the degree of physical fitness, and could probably be used as a measure of physical fitness.

Vitamin E and Serum Enzymes During Exercise

Helgheim et al. (1979), using a double blind study, demonstrated that vitamin E loading did not influence the rate of increase in serum enzymes during exercise. They were unable to demonstrate CK-MB isoenzymes using an electrophoretic technique, and concluded that the increase in total serum CK was due to efflux of muscle CK-MM.

Adenosine Triphosphate Depletion and Muscle Enzyme Efflux

There is evidence to support the hypothesis that adenosine triphosphate (ATP) is necessary for intracellular retention of enzymes. It was demonstrated by Sweetin and Thomson (1973) that, during moderate exercise, ATP declined rapidly to a level lower than the resting level and then remained constant. It was further reduced only by prolonged heavy exercise which produced exhaustion. Their findings were in agreement with the work of Griffiths (1966) who demonstrated that serum CK levels did not increase unless the exercise was severe and prolonged. Thomson et al. (1975) using cat leg muscle, demonstrated that CK efflux did not occur until the capacity for work had markedly declined.

They used the capacity for work as an indirect measure of the ATP content of muscle.

Serum Electrolytes and Exercise

Research concerning the effects of exercise on serum electrolytes has dealt mainly with short term, intensive exercise, and long term, endurance exercise, as evidenced by this literature review. Sodium, potassium, chloride and bicarbonate (total carbon dioxide) were the electrolytes most studied. Changes in blood volume and the composition of plasma, as well as electrolyte loss due to exercise, were studied in conjunction with serum electrolytes.

Effects of Short Term Intensive Exercise on Blood Volume and Electrolytes

Coester, Elliot and Luft (1973) examined ten male volunteers for changes in blood gases, plasma electrolytes and electrocardiographs (ECG) before, during, and after exhaustive exercise on a bicycle ergometer. Two sets of experiments were conducted; one with the subjects breathing room air, and the other with them breathing room air with added carbon dioxide at a partial pressure of 15 $\pm$ 2 mm Hg (about 2% at sea level). Baseline measurements were made with the subjects breathing room air. Arterial blood samples were obtained through an indwelling plastic catheter inserted into either the radial or brachial artery and were heparinized to prevent clotting. Samples were taken at

rest, during the last minute of exercise, and at 1, 4, 10, 20, and 30 minutes after exercise. For the first three minutes of exercise the work load was 300 kpm/min, and was increased by 75 kpm/min each minute until the subject could no longer maintain a pedalling rhythm of 50 rpm, at which time the exercise was terminated. Blood pH, pCO_2 and, pO_2 were measured and the bicarbonate (HCO_3^-) calculated using the Henderson Hasselbach equation. Serum sodium, potassium, chloride, total calcium, and inorganic phosphorus were also measured.

The blood gas results demonstrated that there was a significantly greater degree of acidosis in the subjects when they breathed room air with added carbon dioxide, than when they breathed room air alone. All plasma electrolytes were increased above the resting levels immediately after exercise. Plasma potassium levels decreased faster than those of the other electrolytes during the recovery period and were below the resting levels at four minutes, but returned to resting levels by the 10th minute post-exercise Plasma potassium levels did not correlate with the amplitude of the T waves of the electrocardiographs which suggested that acute changes in plasma potassium do not reflect intra-muscular potassium levels. There was a better correlation between the degree of acidosis and T wave amplitude, but it was not statistically significant. The other electrolytes declined more slowly than the potassium during the recovery

period, and apart from calcium and inorganic phosphorus, which remained slightly elevated, were at resting levels by thirty minutes post-exercise. No allowances were made for changes in plasma volume in this experiment.

In another experiment, van Beaumont, Strand, Petrofsky, Hipskind and Greenleaf (1974) examined six male volunteers for changes in plasma electrolytes and total proteins at $\dot{V}O_2$ max during and after exercise on a bicycle ergometer. All experiments were carried out at 25° C and the subjects started at 0 Watts (W) work rate. The work rate was increased by 25 W/min until exhaustion. All the subjects reached their $\dot{V}O_{2\ max}$ at either 9.5 or 10 minutes. Duplicate venous samples were taken without stasis five minutes before, and 0.5, 1.0, 1.5, and 2.0 minutes after exercise. Additional samples were taken at 25 and 60 minutes post-exercise. No fluids were allowed during the recovery period. Percentage changes in plasma volume were calculated from hematocrit values. The raw hematocrit data were corrected for trapped plasma by multiplying by the factor 0.96, and to whole body hematocrit by multiplying by 0.91. The combined factor was 0.874. The authors noted a 15.5% decrease in plasma volume immediately after exercise. Van Beaumont et al. (1974) were able to demonstrate that there was a 4% loss of protein from the vascular system after exercise despite an apparent 13% increase in plasma proteins. This suggested that there was increased permea-

bility of the capillary walls during exercise. Using the same methods to calculate potassium, sodium, and chloride levels, they determined that there was a significant drop in plasma sodium and chloride immediately after exercise. The sodium levels were at pre-exercise levels 25 minutes after exercise, but the chloride levels were still significantly lower than the pre-exercise levels. After 60 minutes recovery, the potassium levels were significantly elevated. The authors postulated that conclusions based on changes in the concentrations of plasma components without evaluation of concommitant changes in plasma volume may lead to erroneous interpretations.

Using Evans' blue and ^{131}I tagged albumin dilution methods, Greenleaf, van Beaumont, Brock, Morse and Mangseth (1979) assessed the validity of the formula proposed by van Beaumont et al. (1974) to assess changes in plasma volume which used hematocrit alone, and the formula of Dill and Costill (1974) which employed both the hemoglobin and the hematocrit values. They concluded that for studies of short duration (i.e., less than two hours), calculated percent changes in plasma volume based on hematocrit values alone were valid.

Studies were conducted by van Beaumont, Underkofler and van Beaumont (1981) on changes occurring in erythrocyte volume during exercise and the factors influencing them. Their study was divided into three parts: 1. Submaximal

exercise in which five male volunteers performed thirteen exercise bouts, each of thirty minutes duration and at exercise levels between 40 and 75% of their $\dot{V}O_{2\ max}$ using a bicycle ergometer. Heparinized blood samples were taken five minutes before, and 1, 2, 3, 30, and 60 minutes after exercise. The samples were analysed for hematocrit, hemoglobin, pH, pCO2, pO_2, plasma sodium, potassium, chloride, calcium, and total proteins. 2. Maximal exercise, in which twelve athletes exercised to exhaustion on a bicycle ergometer (10 to 12 minutes). Except for the one minute post-exercise sample, blood sampling was the same as for the submaxiamal exercise. The same blood parameters were also measured. The only additional measurement was lactic acid. 3. Thermal dehydration effects were measured on four male subjects. They rested in a semi-reclining position for 2.5 to 3.0 hours at 58° C and 27 Torr vapour pressure, or until 2.5% dehydration was achieved. Blood samples were taken five minutes before, at 30, 60, and 135 minutes during heat stress, and 20 minutes after returing to a cool environment (25° C).

They found there was little change in mean corpuscular volume (MCV) with submaximal exercise. With maximal exercise, the MCV showed a significant increase during the first three minutes of recovery when the blood pH was less than 7.1, and the plasma lactate was above 90 mg/100 mL. However, the calculated change in plasma volume, using

hematocrit values alone, was within one percent of the value obtained with both the hemoglobin and hematocrit values. With heat exposure, the hematocrit method for fluid shifts gave values 2.5 to 3.0% lower than those calculated from both hemoglobin and hematocrit values. In this study, the hematocrits were measured in quadruplicate by the micro-hematocrit method and were corrected for trapped plasma by multiplying by 0.96.

In all the preceeding experiments microhematocrit values were corrected for 4% trapped plasma. This factor, according to Miale (1982), is valid only for the macro (Wintrobe) method. He stated that the amount of trapped plasma in the microhematocrit is negligible, and values obtained by this method should not be corrected for trapped plasma. An average error of 3% will be introduced though inappropriate use of the correction factor.

Greenleaf, van Beaumont, Brock, Morse and Mangseth (1979) measured plasma volume and electrolyte shifts in four men with ages ranging from 26 to 45 years, before and for 60 minutes after a continuous peak oxygen uptake ($\dot{V}O_{2\ peak}$) on a bicycle ergometer. Two experiments were conducted; one in the sitting, and the other in the supine position. They found that there was no significant difference in the $\dot{V}O_2$ peak between subjects in the sitting and supine positions ($\dot{V}O_{2\ peak}$ sitting = 3.16 $\pm$ 0.32 L/m and supine $\dot{V}O_{2\ peak}$ = 3.13 $\pm$ 0.33 L/m). It was noted that the mean plasma volume

decreased by 477 mL (-16.1%) in the sitting position and by 548 mL (-17.6%) in the supine position, and that the greatest net loss of plasma occurred during the first ten minutes of submaximal exercise. The percentage losses of protein, total calcium and ionized calcium were about half that of plasma volume, indicating that there was selective retention of these substances. They also noted that the plasma osmolality returned to the control values within 1.5 minutes after the supine exercise. From their findings they concluded that the changes in plasma proteins which occurred were not of sufficient magnitude to account for the shifts in plasma volume, and that changes in hydrostatic and/or systemic blood pressure were most likely the driving force for the restoration of plasma volume after exercise.

Differences in fluid responses were noted by Senay, Rogers and Jooste (1980) when they compared subjects who exercised on a treadmill with another group who exercised on a bicycle ergometer. For the treadmill, exercise $\dot{V}O_{2\ max}$ was determined on three separate occasions at ten day intervals. This exercise was at 6 km/h and the grade was increased by 3% every three minutes. Blood samples were taken before and during each successive increase in treadmill grade and the exercise was terminated when the subject could no longer continue. Nine black and two white subjects participated in the study. No significant differences were noted between the two races. The second experiment was made

on untrained men who exercised on a bicycle ergometer. The exercise was at 50 rpm and the workload was increased by 0.5 kp/2 min. Blood samples were obtained before and during the final minute at each exercise level. In both experiments blood samples were obtained through indwelling needles which were kept open with 0.9% weight to volume sodium chloride solution. No relationship was found between $\dot{V}O_{2\ max}$ and vascular volume shifts in the treadmill exercise. A significant relationship was found, however, between the exercise level and hemoconcentration during the bicycle ergometer exercise. They concluded that the fluid dynamics in the two forms of leg exercises were significantly different.

A study by Hagan, Francisco, McMurray and Horvath (1980) demonstrated that careful attention must be paid to changes in posture during studies of changes in plasma constituents induced by exercise. Their subjects were four healthy males with a mean age of 31.4 years. The exercise used was the bicycle ergometer, and each subject exercised in both the upright and low sit positions. Each subject rested on a bed for 30 minutes before the exercise. A catheter was then inserted into either the median, cubital or cephalic vein, and an initial blood sample taken. The subject then sat on the bicycle ergometer in either the low sit or upright position for 60 minutes. Blood samples were obtained at 0, 15, 30, 45, and 60 minutes. The exercise was then performed for 45 minutes and blood samples were taken

between 15 and 20, 30 and 35, and, 40 and 45 minutes of exercise. The samples were analysed for hematocrit, hemoglobin and total serum proteins,and the mean corpuscular hemoglobin concentration was calculated. They found that change from the supine to the low sit produced a 6% decrease in plasma volume after 15 minutes with no further change occurring during the next 45 minutes. In the upright position, the volume decrease was 14.1% after 30 minutes with no further change occurring during the next 30 minutes. During exercise in the low sit position, a rapid decrease in plasma volume occurred, followed by a slight increase. The volume then remained constant for the rest of the exercise period. The percent changes in plasma volume were 10.9%, 11.5%, and 15.7% at 31%, 51%, and 69% $\dot{V}O_{2\ max}$, respectively. Exercise in the upright position produced a 10.1% reduction in plasma volume at 31% $\dot{V}O_{2\ max}$. After 45 minutes recovery the plasma volumes were at or slightly above the pre-exercise levels.

The Effects of Long Term Exercise on Blood Volume and Electrolytes

Milledge, Bryson, Catley, Hasp, Luff, Minty, Older, Payne, Ward and Withey (1982) conducted an extensive study on five male subjects before, during and after five consecutive days of hill walking. During a four day pre-exercise period baseline values for blood volume, total body water,

electrolytes, plasma renin activity and leg volume were established. By the end of the exercise period, they found that the subjects retained 264 ± 85 mmol of sodium, while the plasma sodium remained constant. This indicated that an expansion of the intracellular space of 1.84 litres had occurred. They also noted that there was a positive water balance of 0.9 litres, and a net shift of 0.94 litres of fluid from the intracellular to the extracellular space. There was a decrease in packed cell volume from 43.9% to 37.9% which represented about a 0.9 litre increase in plasma volume. It was suggested that the remaining 0.04 litres could account for the the increase in leg volume which occurred. There was an increase in both plasma aldosterone and plasma renin activity during the exercise period which correlated significantly with sodium retention. These findings, coupled with increase in leg volume, suggested that edema may result from prolonged exercise.

In another study involving hill walking Withey, Milledge, Williams, Minty, Bryson, Luff, Older and Beeley (1983) examined six male subjects over a period of thirteen consecutive days. The regimen consisted of an initial four day control period and a final four day recovery period at an altitude of 900 metres, during which time the subjects were sedentary. During the middle five day exercise period, the men participated in hill walking for about seven hours each day between altitudes 2,678 and 3,629 metres. Each

subject was on a strictly regulated diet containing about 138 mmol of sodium and 84 mmol of potassium, with an energy content of 2,850 kcal. Blood samples were taken daily at 14:30 hours during the control and recovery periods, and within two minutes post-exercise during the exercise period. Their findings were similar to those of Milledge et al. (1982) whose experiments were conducted at sea level. From this it was apparent that exercise at high altitude does not place an extra burden on the homeostatic mechanisms for water and electrolyte balance.

Rose, Carroll, Lowe, Peterson and Cooper (1970) studied 80 highly trained male volunteers who participated in the 1969 Boston marathon run. Blood samples were taken one hour before, and one minute after the race. The samples were examined for changes occurring in serum electrolyte levels, The results showed a significant increase in serum total proteins, sodium and potassium, a significant decrease in serum magnesium and no change in serum levels of calcium or chloride. The authors recommended that electrolyte replacement solutions be used in long distance running since electrolytes are lost in sweat. They also suggested that the electrolyte solutions should have a high content of magnesium because of the marked decrease in serum magnesium after a marathon run as shown in their studies.

In another study, Noakes and Carter (1976) examined two groups of long distance runners. The first group consisted

of seven men who ran 160 km. The second group contained five men and one woman who each ran between 65 and 249 km. They found that the serum sodium and chloride levels dropped in the majority of runners, while the serum potassium and calcium levels increased in some and decreased in others. From this study, they concluded that serum electrolytes were maintained within acceptable limits during prolonged exercise.

A study involving long-distance swimmers was conducted by Haralambie and Senser (1980). Sixteen male volunteers from 18 to 26 years of age swam an average of 5,200 metres in 90 minutes. Blood samples were taken before, after, and 24 hours after the exercise. Serum sodium and potassium were found to be significantly elevated post-exercise, and increased again 24 hours after the swim. Serum magnesium showed a significant decrease after exercise, but recovered slightly 24 hours later. Inorganic phosphate increased significantly post-exercise, but returned to pre-exercise levels after 24 hours. Serum chloride levels showed no statistically significant increase until 24 hours after exercise. Serum calcium did not change significantly at any time during the study period. The authors concluded that, apart from magnesium, serum electrolyte changes were less pronounced in swimming than in other forms of exercise of similar stress and duration.

A more recent study by Schidler and Stumpfhauser (1984) was not entirely in agreement with the preceeding studies. They examined fifteen physically fit men 23 to 50 years of age, before and after an eight week training session for marathon runners. They found that the serum magnesium and ionized calcium levels were increased, although the total calcium levels were decreased at the end of the study.

Ross and Attwood (1984) measured the leucocyte count, red cell count, hemoglobin, and hematocrit on blood samples taken from healthy male volunteers during a three week, heavy training session. All measurements were made on a Coulter-S cell counter. The subjects ranged in age from 20 to 35 years. Twenty-one of the original 91, completed the program. The authors found that there was a decrease in hemoglobin and hematocrit values during the first two weeks of the training program, but not during the third week. They concluded that their results could be explained on the basis of plasma volume expansion and that hemolysis was not a significant factor.

CHAPTER 3

METHODOLOGY

Subjects

Eleven healthy female university students volunteered to participate in this study. The physical characteristics of the subjects are shown in Table 1. Prior to the experiment, the basic premise of the investigation was explained to the students. Each subject was then required to fill out a concent form (Appendix B) before taking part.

Testing Procedures

All testing for this study was carried out at Lakehead University. The subjects reported to the laboratory once a week for three consecutive weekends. The electrical stimulation experiment was conducted on the first week, with the isokinetic exercise, and combined isokinetic exercise and electrical stimulation experiment on the second and third weeks, respectively. All testing was carried out in the morning between 10:00 AM and 12:00 PM. Once the testing schedule had been established, each subject was tested at the same time on each occasion. The subjects were advised to keep their daily routines as regular as posible, and to avoid any vigorous physical activity for 48 hours before each testing.

Table 1

Physical Characteristics of Subjects

Subjects	Age (yr)	Weight (Kg)	Height (cm)
1	21	51.8	159.5
2	22	67.0	165.0
3	22	59.1	163.5
4	20	65.9	167.5
5	20	56.8	160.0
6	23	63.6	161.5
7	20	68.2	174.0
8	20	77.3	157.5
9	21	75.0	163.5
10	21	62.0	166.0
11	22	76.0	175.5
Mean	21.1	65.7	164.9
SD	1.0	8.2	5.7

Isokinetic Exercise

A Cybex II Dynamometer was used in experiments 2 and 3. The device was calibrated according to the Cybex user manual before the exercises. Knee extension and flexion speed was set at 60 degrees per second. All subjects performed six contractions on the Cybex as a warm up exercise. The regime for experiments 2 and 3, consisted of six sets of ten contractions separated by a one minute rest period.

Electrical Stimulation

Electrical stimuluation of the quadriceps muscle, in experiments 1 and 3, was performed using an Ultra Pulsator Model 4 (Medelco Ltd., Downsview, Ontario). The variable adjustment was set on the surge program, which allowed the stimulation and rest times to be set independently. The instrument delivered 50 pulses per second, each pulse being 200 microseconds in width. Six sets of ten contractions were performed on all subjects, following a warm up and familiarization period consisting of six repetitions at a lower setting, which induced sufficient muscle contraction. There was a rest period of one minute between each set.

The subjects were put in the supine position on a table, with their right leg bent at 120 degrees by means of a roll placed under the knee. Sandbags were draped over the ankle to prevent the leg from moving. Two 3 by 5 inch damp rubber/metal electrodes were positioned diagonally across

the muscle, and were fastened to the leg with rubberized velco straps. The superior pad, located proximally over the belly of the muscle, was connected to the positive lead. The negative lead was connected to the inferior pad placed over the distal portion of the muscle, just above the knee. Subjects were asked to endure any pain associated with the contraction in order to receive the maximum amount of current. Each electrical stimulus induced an isometric contraction which was 10 seconds in duration. Contractions were separated by a 30 second rest period.

Combined Electrical Stimulation and Isokinetic Exercise

A similar protocol was used for this experiment as in that for experiments 1 and 2 above. Changes were made in the surge and rest times, and in the fact that the subjects were sitting when they received electrical stimulation. The stimulator was adjusted to give a two second contraction phase with a one second relaxation period. The warm up exercise consisted of 10 contractions. Each subject was instructed to extend her leg when electrical stimulus was applied, and to return her leg to a 90 degree flexion after contraction to be ready for the next repetition. Six sets of ten contractions were performed with a one minute rest between each set.

Blood Samples

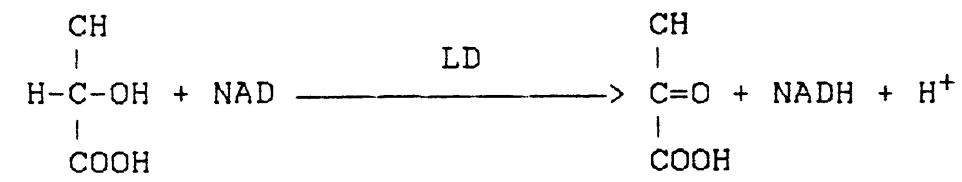
All blood samples were drawn from an antecubital vein into 7 mL serum separator Vacutainer tubes. These were used to collect blood for the enzyme and electrolyte determinations. Samples were taken just before, immediately after, and five minutes after exercise.

Enzyme Determinations

The Abbott Laboratories VP Bichromatic Analyser [Abbott Laboratories (Canada) Ltd., Toronto, Ontario.] was used for the enzyme determinations. The reagents used for the enzyme assays were supplied by Abbott Laboratories Diagnostics Division, Pasadena, California 91030.

Measurement of serum LD

Serum LD (EC 1.1.1.27) was measured by a modification of the method of Armadore (Henry, Cannon and Winkelman, 1974). In this method, lactic acid is converted to pyruvic acid by the action of the LD in the sample. Nicotinamide adenine dinucleotide (NAD) is required as a coenzyme and is reduced to NADH during the reaction. The activity of LD is measured by the increase in absorbance of NADH with time, as measured at 340 nm. The equation for the reaction is given below:



L-lactic acid pyruvic acid

Measurement of serum CK

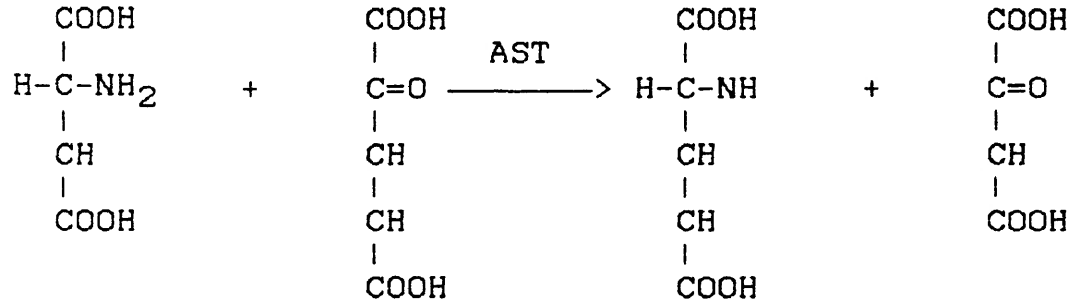
Serum CK (EC 2.7.3.2) was measured by a modification of the method of Rosalki (Henry et al., 1974; Szasz, Gruber and Bernt, 1976; Szasz, Gerhardt and Gruber, 1977; Szasz, Waldenstrom and Gruber, 1979). In this procedure CK removes a phosphate group from creatine phosphate and transfers it to adenosine diphosphate (ADP) to form ATP. The reaction is coupled with a second enzyme, hexokinase (HK), which converts glucose to glucose-6-phosphate. The amount of glucose-6-phosphate formed is limited by the ATP produced in the first reaction because ATP is required as a coenzyme for the second reaction. In order that the reaction rate may be measured at 340 nm, a third enzyme system is introduced which uses NAD as a coenzyme. The enzyme is glucose-6-phosphate dehydrogenase (G-6-PD) which converts glucose-6-phosphate to 6-phosphogluconate (6-PG). The reactions are summarised below:

1. creatine phosphate + ADP $\xrightarrow{CK}$ creatine + ATP
2. ATP + glucose $\xrightarrow{HK}$ glucose-6-phosphate + ADP
3. glucose-6-phosphate + NADP $\xrightarrow{G\text{-}6\text{-}PD}$ 6-PG + NADPH + H^+

Measurement of serum AST

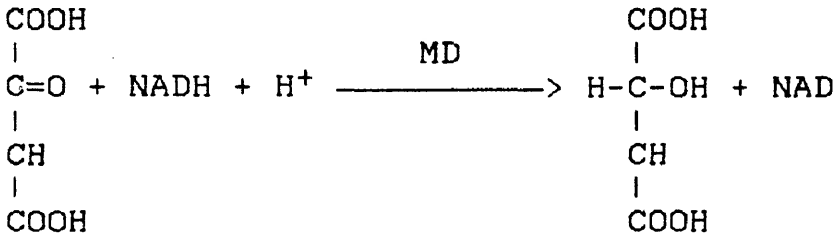
Serum AST (EC 2.6.1.1) activity was measured by a modification of Karmen's method (Henry et al., 1974). In this method, AST in the sample catalyzes the transfer of the

aspartate amino group to alpha-ketoglutarate forming oxal-acetate and glutamate, as shown below:



L-aspartate alpha-ketoglutarate L-glutamate oxalacetate

In order that the reaction may be measured at 340 nm, malic dehydrogenase (MD), which requires NADH as a coenzyme is used, as seen in the following equation:



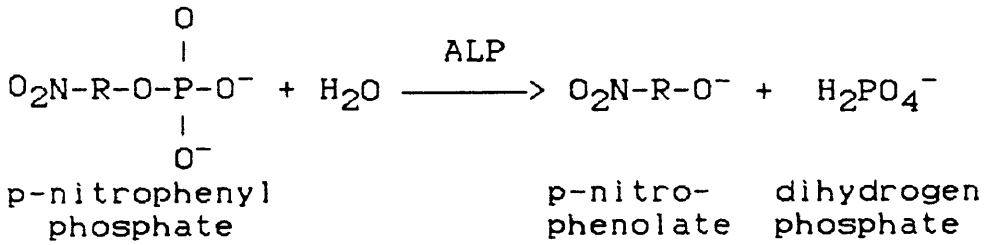
oxalic acid malic acid

This allows the conversion of oxalic acid to malic acid with concomitant conversion of NADH to NAD. As the second reaction is limited by the amount of oxalic acid formed in the first reaction, the change in absorbance at 340 nm is a measure of serum AST activity.

Measurement of serum ALP

Serum ALP (EC 3.1.3.1) was measured by a modification of a method described by Bessey, Lowry and Brock (Kaplan and Pesce, 1984). This enzyme is used to catalyze a hydrolytic reaction in alkaline solution (pH 10), which is activated by

magnesium ions. The product of the reaction, p-nitrophenolate, absorbs much more strongly at 415 nm than the substrate, p-nitrophenyl phosphate. The reaction may be followed by measuring the increase in absorbance at this wavelength. The rate of hydrolysis is directly related to the activity of the alkaline phosphatase. The reaction is given below:



where R is a phenol group

Electrolyte Determinations

Measurments of serum sodium, potassium, chloride and total carbon dioxide were made on a NOVA-4 specific ion electrode Electrolyte Analyser (NOVA biochemical, Waltham, Massachusetts 02154).

Quality Control

Within run and between run quality control sera were used with all assays. The quality control materials were supplied by Wellcome Diagnostics, Dartford, England DA1 5AH. If the assay control results were not within acceptable limits, the test was repeated.

Analysis of the Data

A two way analysis of variance (ANOVA) was conducted to determine any treatment effect. When a statistically significant F-ratio was calculated, differences between the means were tested for significance by employing the Tukey test. The significance level was set at 0.05.

CHAPTER 4

RESULTS

Means and standard deviations for serum enzymes and electrolytes before, immediately after, and five minutes after exercise are given in Tables 2 and 3, respectively.

Lactate dehydogenase

Results of statistical analyses for serum lactate dehydrogenase are given in Table 4. There was a small but significant increase ($p < 0.05$) of 3.8% in serum LD immediately after the isokinetic exercise. No significant changes were noted with either electrical stimulation alone or with combined electrical stimulation and isokinetic exerise. Four of the serum LD levels were above the upper limit of the reference range of 109 - 193 IU/L. Of these, three occurred in a single subject whose before exercise level was above the upper limit of the range. In another subject only the before exercise level was increased and was not in keeping with her other results, which suggested that this spurious result may have been due to undetected hemolysis.

Table 2

Means and Standard Deviations of Serum Enzymes Before, Immediately After and Five Minutes After Exercise and Electrical Stimulation on Three Treatments

Enzyme	Treatment	Before Exerise	Immediately After	5 Minutes After
LD	Elec. stim.	159 ± 25	161 ± 23	161 ± 32
	Isok. exer.	159 ± 35	165 ± 17	162 ± 18
	Comb. exer.	129 ± 22	133 ± 23	133 ± 24
CK	Elec. stim.	111 ± 59	110 ± 59	113 ± 60
	Isok. exer.	134 ± 55	133 ± 58	131 ± 60
	Comb. exer.	129 ± 67	140 ± 72	131 ± 68
AST	Elec. stim.	16.2 ± 8.5	16.2 ± 8.5	16.6 ± 9.4
	Isok. exer.	17.0 ± 3.6	18.6 ± 4.0	19.2 ± 5.3
	Comb. exer.	14.3 ± 3.8	15.2 ± 3.7	15.4 ± 4.2
ALP	Elec. stim.	58.6 ± 17.4	59.0 ± 16.8	59.7 ± 18.5
	Isok. exer.	59.4 ± 20.0	62.7 ± 20.8	60.0 ± 21.3
	Comb. exer.	56.5 ± 22.5	58.9 ± 23.6	56.0 ± 23.0

Note. Elec. stim.= Electrical stimulation, Isok. exer.= Isokinetic exercise, Comb. exer.= Combined isokinetic exercise and electrical stimulation. Units : IU/L.

Table 3

Means and Standard Deviations of Serum Electrolytes Before, Immediately After and Five Minutes After Exercise on Three Treatments

Electrolyte	Treatment	Before Exercise	Immediately After	5 Minutes After
Sodium	Elec. Stim.	146 ± 6	147 ± 7	147 ± 3
	Isok. Exer.	142 ± 2	145 ± 1	143 ± 2
	Comb. Exer.	143 ± 1	144 ± 2	143 ± 2
Potassium	Elec. Stim.	5.2 ± 0.5	5.4 ± 0.4	5.5 ± 0.4
	Isok. Exer.	5.2 ± 0.5	5.3 ± 0.3	5.5 ± 0.4
	Comb. Exer.	5.4 ± 0.4	5.6 ± 0.7	5.5 ± 0.6
Chloride	Elec. Stim.	110 ± 4	109 ± 4	109 ± 3
	Isok. Exer.	104 ± 1	105 ± 2	105 ± 2
	Comb. Exer.	105 ± 1	105 ± 2	105 ± 1
Total CO_2	Elec. Stim.	23.8 ± 2.8	24.8 ± 2.8	24.8 ± 2.5
	Isok. Exer.	26.8 ± 2.2	25.9 ± 3.1	24.2 ± 2.8
	Comb. Exer.	25.2 ± 1.0	24.8 ± 2.0	23.4 ± 2.2

Note. Elec. stim.= Electrical stimulation, Isok. exer.= Isokinetic exercise, Comb. exer.= Combined isokinetic exercise and electrical stimulation. Units : mmol/L.

Table 4

Analysis of Variance for Serum Lactate Dehydrogenase

Source	DF	SS	MS	F
A (treatment)	2	19181	9591	6.57*
Between subject error	30	43806	1460	
B (time)	2	254	127	1.18
AB interaction	4	711	178	1.65
Within subject error	60	6470	108	
Total	98	70742		

*$p < 0.05$.

Creatine Kinase

The results of statistical analyses for serum creatine kinase are given in Table 5. The F ratios showed that there were no significant changes in serum creatine kinase levels with any of the exercise regimens. Four of the serum CK levels were above the expected range of 45 - 235 IU/L. Three of these occurred in a single subject who had a level above the expected range before the combined exercise. The other occurred in a subject at five minutes after isokinetic exercise. She had a before exercise level which was close to the upper limit of the range. All the results were above the mean for the expected range.

Aspartate Aminotransferase

The results of statistical analyses on serum aspartate aminotransferase are given in Table 6. Electrical stimulation alone did not produce any significant changes in serum AST. However, isokinetic exercise and combined isokinetic exercise and electrical stimulation did cause significant changes. The average increase in serum AST immediately after isokinetic exercise was 9.4% and at five minutes after was 12.9%. Immediately after the combined isokinetic exercise and electrical stimulation the average increase in serum AST was 6.3% and at five minutes after exercise it was 7.7%. Three values were above the upper limit of the reference range of 10 - 30 IU/L. They all occurred in one

Table 5

Analysis of Variance for Serum Creatine Kinase

Source	DF	SS	MS	F
A (treatment)	2	11702	5851	0.65
Between subject error	30	271464	9049	
B (time)	2	126	63	0.05
AB interaction	4	669	167	0.13
Within subject error	60	78417	1307	
Total	98	362378		

Table 6

Analysis of Variance for Aspartate Aminotransferase

Source	DF	SS	MS	F
A (treatment)	2	186	93	0.85
Between subject error	30	3273	109	
B (time)	2	27	13.5	5.87*
AB interaction	4	8	2	0.87
Within subject error	60	135	2.3	
Total	98	3629		

*$p < 0.05$.

subject whose serum AST level prior to the exercise was 39.8 IU/L.

Alkaline Phosphatase

The statistical data for serum alkaline phosphatase are given in Table 7. The results indicate that there was a significant increase ($p < 0.05$) in serum levels both immediately after and five minutes after isokinetic and combined isokinetic exercise and electrical stimulation. The average increase in serum ALP immediately after isokinetic exercise was 5.6% and five minutes after was 1.0%. The increase immediately after exercise with the combined isokinetic exercise and electrical stimulation was 4.2% and a five minutes after 1.0%. A smaller (1.9%) but statistically significant increase ($p < 0.05$) in serum ALP occurred at five minutes after exercise with electrical stimulation. One subject had serum ALP levels which were above the upper limit of the reference range of 36 - 92 IU/L throughout the study period. In the other subjects the serum ALP levels remained below the upper limit even after exercise.

Table 7

Analysis of Variance for Alkaline Phosphatase

Source	DF	SS	MS	F
A (treatment)	2	18.2	9.1	0.01
Between Subject Error	30	37486.8	1249.6	
B (time)	2	78.1	39.1	43.44*
AB interaction	4	141.0	60.3	67.00*
Within subject error	60	50.9	0.9	
Total	98	37875.0		

*$p < 0.05$.

Sodium

Results of the statistical analyses for serum sodium are give in Table 8. The F ratios revealed that there were significant changes ($p < 0.05$) in the serum sodium levels after exercise. The Tukey tests showed that there were significant increases ($p < 0.05$) in serum sodium levels immediately after exercise, with electrical stimulation (0.7%), isokinetic execise (2.1%) and combined isokinetic exercise and electrical stimulation (1.0%). The increase was sustained at five minutes after exercise only with electrical stimulation. Twenty-seven percent of the sodium values were above the upper limit of the reference range of 134 - 145 mmol/L. Only one value was below the lower limit of the reference range.

Potassium

Results of statistical analyses for serum potassium are given in Table 9. No significant changes were found in serum potassium levels with any of the exercise regimens. Eighty-two percent of the potassium values were above the upper limit of the reference range of 3.5 - 5.0 mmol/L. None were below the lower limit of the range.

Table 8

Analysis of Variance for Serum Sodium

Source	DF	SS	MS	F
A (treatment)	2	246	123	6.09*
Between subject error	30	605	20.2	
B (time)	2	51	25.5	
AB interaction	4	12	3.0	10.2*
Within subject error	60	148	2.5	1.2
Total	98	962		

*$p < 0.05$.

Table 9

Analysis of Variance for Serum Potassium

Source	DF	SS	MS	F
A (treatment)	2	1.20	0.60	1.09
Between subject error	30	16.37	0.55	
B (time)	2	0.35	0.18	2.00
AB interaction	4	0.27	0.08	0.89
Within subject error	60	5.21	0.09	
Total	98	23.40		

Chloride

Statistical data for serum chloride are given in Table 10. No significant changes were found in serum chloride levels with any of the exercise regimens. Forty-nine percent of the chloride values were above the upper limit of the reference range of 95 - 105 mmol/L. None were below the lower limit of the range.

Total Carbon Dioxide

Results of statistical analyses for serum total carbon dioxide are given in Table 11. With electrical stimulation, there was a slight but statistically significant increase ($p < 0.05$) in serum total carbon dioxide immediately after and five minutes after exercise. The mean increase immediately after exercise was 4.2%, and remained at that level at five minutes after exercise. Both the isokinetic exercise and combined isokinetic exercise and electrical stimulation produced a statistically significant decrease ($p < 0.05$) in total serum carbon dioxide. Immediately after isokinetic exercise the mean decrease was 3.5% and at five minutes after 9.7%. The mean decrease after combined isokinetic exercise and electrical stimulation was 1.5% immediately after and 7.1% five minutes after exercise. Only one total carbon dioxide value was above the upper limit of the reference range of 25 - 30 mmol/L. Thirty-three percent were below the lower limit of the range.

Table 10

Analysis of Variance for Serum Chloride

Source	DF	SS	MS	F
A (treatment)	2	462.2	231.1	15.39*
Between subject error	30	450.5	15.0	
B (time)	2	0.3	0.15	0.07
AB interaction	4	4.4	1.1	0.53
Within total error	60	124.3	2.1	
Total	98	1042.7		

*$p < 0.05$.

Table 11

Analysis of Variance for Serum Total Carbon Dioxide

Source	DF	SS	MS	F
A (treatment)	2	24	12	0.98
Between subject error	30	368	123	
B (time)	2	37	18.5	6.49*
AB interaction	4	45	11.3	3.96*
Within subject error	60	171	2.85	
Total	98	645		

*$p < 0.05$.

CHAPTER 5

DISCUSSION

Serum Enzymes

The results of this study, using electrical stimulation and isokinetic exercise, were similar to those found by other researchers cited in the literature review, who used short term, intensive exercise regimens. (Chanine et al, 1976; Forssell et al, 1975; Fowler et al, 1968; Gimenez and Florentz, 1984; Ledwich, 1973; Metevier et al, 1980). The only enzyme examined which, to the author's knowledge, had not previously been studied in conjunction with short term exercise was alkaline phosphatase.

Lactate dehydrogenase

The only statistically significant changes in serum LD in this study occurred immediately after the isokinetic exercise, and all tests had returned to pre-exercise levels five minutes after exercise. No statistically significant changes occurred with electrical stimulation alone or with the combined electrical stimulation and isokinetic exercise.

The lack of significant increases in serum LD after electrical stimulation and combined electrical stimulation and isokinetic exerecise, and the minor changes which occurred immediately after isokinetic exercise alone, were probably due to the relatively short duration and low

intensity of the exercises used. Fowler et al. (1968) demonstrated that there was no change in serum LD until the eleventh treadmill grade was reached. Chanine et al. (1976) also used a treadmill exercise. They found no change in serum LD immediately after, at 4 or at 24 hours after exercise. None of their values were above the upper limit of the reference range. With exhaustive exercise Gimenez and Florentz (1984) found significant increases in serum LD immediately after exercise, but the levels had returned to resting values at five minutes after exercise. Thus it follows from the results of this study and the works cited above, that significant changes in serum LD do not occur after short term exercise unless it is extremely strenuous. In the present study, subject 7 had serum LD levels slightly above the upper limit of the reference range during the first study period. The increased results could possibly have been from previous long term exercise because her enzyme levels were within the reference range during subsequent studies. The before exercise serum LD levels for all three exercise experiments were evenly distributed about the reference mean.

Creatine kinase

No statisticaly significant changes occurred in serum CK levels with any of the exercise regimens. Ledwich (1973) using submaximal exercise on a bicycle ergometer, found that in the majority of cases, no statistically significant

changes in serum CK levels occurred with exercise. In the few subjects that did show an increase in serum CK, he concluded that it was due to enzyme efflux from an eschemic myocardium. Chanine et al. (1976) who used a treadmill exercise, also reported no statistically significant change in serum CK after exercise. Similar findings were made by Forssell et al. (1975), who examined 17 untrained middle-aged men before and after a bicycle ergometer exercise. They noted that the maximum increase in serum CK for any subject was 32 IU/L. In this study, the greatest increase was 16 IU/L after isokinetic exercise. With electrical stimulation, the highest increase was 12 IU/L, and with combined electrical stimulation and isokinetic exercise was 10 IU/L. The majority of the values for serum CK were evenly distributed about the reference mean and only four values were above the upper limit of the expected range. From the results of this study it is apparent that electrical stimulation and isokinetic exercise have no significant effect on serum CK levels.

Aspartate aminotransferase

Electrical stimulation did not cause any statistically significant changes in serum AST levels. Both isokinetic exercise and combined electrical stimulation and isokinetic exercise produced statistically significant ($p < 0.05$) changes in serum AST immediately after and at five minutes

after exercise. Only subject 7 had serum AST levels above the upper limit of the reference range. In all other cases the pre-exercise levels were well within the expected range and were evenly distributed around the reference mean. The results for serum AST in this study were similar to those of Fowler et al. (1968) who found no statistically significant changes in AST until the eleventh treadmill grade had been reached, and Metivier et al. (1980), who did not observe statistically significant results until the 66% $VO_{2\ max}$ exercise level.

Alkaline phosphatase

Alkaline phosphatase showed the greatest response to electrical stimulation and isokinetic exercise of all the enzymes studied. This was suprising because it is not a muscle enzyme and is mainly confined to bone, liver and intestine, and is not found in muscle in signifcant amounts. It was included in this experiment to determine the possible effects of electrical stimulation on bone metabolism. The increase in this enzyme could indicate that bone metabolism is affected by electrical stimulation and isokinetic exercise. These findings appear to concur with those of Jaweed et al. (1982a) who demonstrated that electrical stimulation caused an increase in the calcium content of both normal and denervated rat muscle. As ALP is increased during periods of bone formation and dissolution, the

increased muscle calcium may be associated with an increase in serum ALP.

Only two of the authors cited in this text studied serum ALP levels. Both studies were long term in nature, and neither had significant results. Noakes and Carter (1976) observed that serum ALP levels were slightly elevated in athletes after a 160 km marathon. Millar (1978) found that, out of 22 swimmers studied over the duration of a six month training camp, serum ALP rises were seen only in the pre-pubertal children and could not be related to training load.

The effects of isokinetic exercise and combined electrical stimulation and isokinetic exercise were significantly greater than that of electrical stimulation alone. Apart from subject 10 who had serum ALP levels above the upper limit of the reference range thoughout the study, all values were within the reference range.

The small changes in serum enzymes which occurred in this study indicate that the effects of electrical stimulation and isokinetic exercise on muscle metabolism were minimal. Isoenzymes were not measured because changes in the enzyme activties were not great enough to allow for their acurate determination. Available methods require dilution of the samples, and will not detect small changes in isoenzymes.

Electrolytes

The changes in electrolytes in this study were smaller than those found by Coester et al. (1973) and van Beaumont et al. (1974). This was probably because the exercises were less intensive and of shorter duration than those used by these researchers. In addition, it is difficult to compare the results of this study with those of Coester et al. (1973) because they took samples through indwelling catheters and were able to obtain blood for analysis within seconds of cessaton of the exercise, while in the present study samples were taken by venipuncture. Although no time was lost in obtaining samples, the time between completion of the exercise and drawing of blood, was from ten to twenty seconds. Coester et al. (1973) found that serum potassium levels were elevated immediately after exercise but returned to below resting levels four minutes after exercise. Since there was a time lag between cessation of exercise and the drawing of blood samples in this study, a possible increase in potassium was probably missed.

The exercise periods used in this study were considered to be too short to allow significant changes in blood volume to occur (unpublished data, Song, 1986). Plasma volume changes due to posture changes were also not considered. Dixon and Paterson (1978) have shown that the time required for plasma volume adjustments due to posture change to take place were much longer than the exercise periods used in

this study. The authors who found significant changes in plasma and blood volumes (Coester et al., 1973; Dill and Costill, 1974; Hagen et al., 1980; van Beaumont et al., 1974) used exercise regimens which were of longer duration and of greater intensity than those used in the electrical stimulation and isokinetic exercise studies.

Potassium

Although no statistically significant changes in serum potassium occurred with any of the experiments, it was interesting to note that 82% of the values obtained were above the upper limit of the reference range of 5.0 mmol/L. This suggested that young female athletes may form a sub-population who have serum potassium levels above the range generally accepted for the total population. With more exhaustive forms of exercise such as bicycle ergometer, (Coester et al., 1974; van Beaumont et al., 1973) serum potassium levels rise sharply immediately after exercise and fall rapidly to below resting levels within two minutes post-exercise.

Sodium

There was a small but statistically significant ($p < 0.05$) increase in serum sodium immediately after isokinetic exercise and combined electrical stimulation and isokinetic exercise. The increase with electrical stimulation alone was smaller but still statistically significant ($p < 0.05$). The levels had returned to pre-exercise values at five

minutes after exercise. Although the changes were statistically significant, they were very small and probably of no physiological significance. There are no previous studies available showing the effects of electrical stimulation or isokinetic exercise on serum sodium. However, other workers (Coester et al., 1974; van Beaumont et al., 1973) who used exhaustive exercise on a bicycle ergometer obtained results which were similar to those found in this study. Van Beaumont et al. (1973) found that there was no significant increase in serum sodium immediately after exercise, but that there was a significant increase 60 minutes later. Coester et al. (1974) found a small but statistically significant increase in sodium immediately after exercise, but after four minutes it had decreased to pre-exercise levels.

Chloride

Serum chloride levels did not change significantly with any of the exercise regimens. Fifty percent of the values were above the upper limit of the reference range of 105 mmol/L for the general population. This could mean that young physically active females form a sub-population with serum chloride levels slighly higher than the reference range for the general population. No previous studies were available for comparison with the results obtained in this study. Other workers (Coester et al., 1984; van Beaumont et al., 1973) who used more strenuous exercises obtained

results which were similar to those found in this study. Van Beaumnt et al. (1973) had their subjects exercise to exhaustion on a bicycle ergometer. They found that there was no significant increase in serum chloride levels immediately after exercise. Coester et al. (1974) who used a similar exercise found a small but statistically significant increase immediately after exercise. At four minutes, the levels had returned to resting levels.

Total carbon dioxide

Statistically significant decreases ($p < 0.05$) in total carbon dioxide occurred immediately after and five minutes after both isokinetic exercise and combined isokinetic exercise and electrical stimulation Paradoxically, there was a small but statistically significant rise ($p < 0.05$) in the serum total carbon dioxide immediately after and five minutes after electrical stimulation. The changes in total carbon dioxide were very small. No previous studies on the effects of electrical stimulation and isokinetic exercise on serum total carbon dioxide were available for comparison with the results of this study. More strenuous exercise such as bicycle ergometer (Coester et al., 1974; van Beaumont et al., 1973) produced a significant decrease in serum total carbon dioxide.

CHAPTER 6

Summary, Conclusions and Recommendations

Summary

The purpose of this study was to determine the effects of electrical stimulation, isokinetic leg extension and flexion exercise, and combined electrical stimulation and isokinetic exercise on the serum enzymes and electrolytes. Eleven female university student volunteers, with ages ranging from 20 to 23 years, were used as subjects. The subjects participated in the exercise regimens once a week for three consecutive weeks. The first weekend they were given electrical stimulation, the second weekend isokinetic exercises, and the final weekend combined electrical stimulation and isokinetic exercise. Each subject was tested at the same time of day for each exercise to avoid diurnal physiological changes which could possibly occur. Blood samples were obtained from an antecubital vein just prior to, immediately after, and five minutes after exercise.

Statistically significant ($p < 0.05$) changes in serum LD and AST occurred after exercise but they were minor. No statistically significant changes were observed with creatine kinase. The most significant ($p < 0.05$) changes in serum enzymes were in ALP after both electrical stimulation and isokinetic exercise. Changes in serum sodium and total carbon dioxide were statistically significant ($p < 0.05$) but

minor. Changes in serum potassium and chloride were not statistically significant.

From the results of this study, it was apparent that electrical stimulation and isokinetic exercise had only minor effects on serum enzymes and electrolytes. Results obtained were similar to those found by other workers who used short term intensive exercise regimens.

Conclusions

1. From the data obtained in this study it was seen that electrical stimulaton and isokinetic exercise had a minimal effect on muscle metabolism.

2. Results of the serum alkaline phosphatase determinations indicated that electrical stimulation and isokinetic exercise may affect bone metabolism.

3. Serum potassium and chloride results indicated that young physically acitive females may form a sub-population with potassium and chloride levels above the accepted reference range for the general population.

4. The results of the experiments have indicated that the level of electrical stimulation used in this study was a safe procedure and had little effect on muscle metabolism.

5. This study has added to the body of knowledge regarding biochemical and physiological changes occuring with exercise and electrical stimulation.

Recommendations

To further elucidate the effects of electrical stimulation and isokinetic exercise on the levels of serum enzymes and electrolytes, the following experiments could provide useful information:

1. Subjects of a variety of age groups and levels of fitness could be compared.

2. The effects of varying the duration and intensity of electrical stimulation and isokinetic exercise could be studied.

3. A comparsison of the effects of electrical stimulation and isokinetic exercise on serum enzymes and electrolytes in untrained and trained individuals would be useful.

4. A study of the effects of electrical stimulation on bone metabolism could provide an answer to the question posed by the increased levels of alkaline phosphatase found in this study.

5. Examination of serum and muscle biopsies obtained simultaneously for enzymes and electrolytes could provide useful information on the rate of efflux of these substances from muscle after exercise.

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APPENDICES

APPENDIX A

Raw Data for Serum Enzymes and Electrolytes

APPENDIX B

Consent form

APPENDIX A

Table A-1

Raw Data For Lactate Dehydrogenase

	Group								
	Electrical Stim.			Isokinetic Exer.			Combined Exer.		
Subject	B	A	5A	B	A	5A	B	A	5A
1	144	136	136	165	163	153	127	123	114
2	131	139	132	129	142	135	112	111	111
3	165	169	200	187	187	182	139	148	152
4	186	177	174	209	166	158	126	121	129
5	130	140	131	123	130	130	106	108	102
6	137	148	146	146	169	175	106	121	114
7	207	211	213	152	176	164	118	137	129
8	171	164	124	176	179	190	174	191	182
9	175	181	200	161	166	171	133	133	124
10	145	143	142	160	159	161	162	139	176
11	162	165	168	145	179	165	113	128	126
Mean	159	161	161	159	165	162	129	133	133
SD	25	23	32	35	17	18	22	23	26

Note. Units : IU/L. B = before, A = immediately after & 5A = 5 min. after exercise. Stim.= Stimulation, Exer.= Exercise.

Table A-2

Raw Data for Creatine Kinase

	Group								
	Electrical Stim.			Isokinetic Exer.			Combined Exer.		
Subject	B	A	5A	B	A	5A	B	A	5A
1	43	42	42	53	55	55	45	43	42
2	79	80	81	78	80	78	71	77	77
3	82	87	89	148	157	146	72	71	70
4	101	99	98	237	241	243	174	178	180
5	45	48	49	164	169	166	93	95	92
6	227	232	239	129	127	124	105	116	109
7	188	185	186	131	137	129	144	161	154
8	124	107	108	103	101	95	280	304	288
9	72	69	82	138	97	104	100	151	95
10	103	104	105	209	216	225	186	179	183
11	158	155	161	83	85	81	145	160	154
Mean	111	110	113	134	133	131	129	133	133
SD	59	59	60	55	58	60	22	23	24

Note. Units : IU/L. B = before, A = immediately after & 5A = 5 min. after exercise. Stim.= Stimulation, Exer.= Exercise.

Table A-3

Raw Data for Aspartate Aminotransferase

	Group								
	Electrical Stim.			Isokinetic Exer.			Combined Exer.		
Subject	B	A	5A	B	A	5A	B	A	5A
1	11.1	10.4	8.2	12.7	13.0	13.8	10.4	9.6	9.6
2	10.9	10.4	9.8	13.0	16.5	14.3	12.5	13.3	14.3
3	17.3	17.8	20.9	14.1	25.7	26.3	17.0	20.0	18.6
4	13.3	10.1	11.1	18.3	15.1	14.9	10.6	12.2	12.7
5	10.1	11.7	12.2	14.9	15.4	15.9	13.3	13.8	13.8
6	12.2	13.5	15.1	16.2	23.6	23.6	10.4	11.4	11.9
7	39.8	40.6	46.7	16.7	17.8	17.3	13.5	17.3	15.1
8	11.7	12.7	10.9	13.5	16.2	15.4	14.1	14.9	14.9
9	14.6	14.3	14.1	17.5	18.6	17.3	13.8	14.6	13.3
10	16.2	15.1	16.7	19.6	19.4	27.9	21.8	20.4	23.1
11	20.7	22.0	22.0	21.0	22.8	24.9	19.6	20.2	33.0
Mean	16.2	16.2	16.6	17.0	18.6	19.2	14.3	15.2	15.4
SD	8.5	8.8	9.4	3.6	4.0	5.3	3.8	3.7	4.2

Note. Units : IU/L. B = before, A = immediately after & 5A = 5 min. after exercise. Stim.= Stimulation, Exer.= Exercise.

Table A-4

Raw Data for Alkaline Phosphatase

	Group								
	Electrical Stim.			Isokinetic Exer.			Combined Exer.		
Subject	B	A	5A	B	A	5A	B	A	5A
1	40.2	44.3	43.9	48.4	47.6	45.1	44.7	43.5	39.0
2	55.4	55.4	55.8	56.2	59.5	51.7	63.2	64.0	60.0
3	61.9	63.2	62.8	52.5	55.8	53.3	53.1	52.9	52.1
4	34.5	34.9	34.1	32.0	34.1	33.2	30.4	30.8	29.5
5	55.0	57.4	60.3	52.9	55.0	53.7	48.4	47.2	45.9
6	40.2	39.8	39.4	37.7	43.9	41.8	25.0	30.4	27.5
7	59.1	59.5	58.7	60.7	67.3	61.9	50.9	55.4	52.5
8	57.8	55.0	56.2	52.9	55.8	51.3	57.0	59.9	56.6
9	71.0	71.8	73.4	80.4	74.3	73.8	61.9	65.2	62.8
10	95.2	93.5	95.6	99.7	104.2	104.6	105.4	108.7	106.2
11	74.3	74.7	75.9	80.4	92.7	89.4	82.0	90.2	84.1
Mean	58.6	59.0	59.7	59.4	62.7	60.0	56.5	58.9	56.0
SD	17.4	16.8	18.5	20.0	20.8	21.3	22.5	23.6	23.0

Note. Units : IU/L. B = before, A = immediately after & 5A = 5 min. after exercise. Stim.= Stimulation, Exer.= Exercise.

Table A-5

Raw Data for Sodium

	Group								
	Electrical Stim.			Isokinetic Exer.			Combined Exer.		
Subject	B	A	5A	B	A	5A	B	A	5A
1	130	142	145	143	145	143	144	144	144
2	144	145	148	142	144	142	142	143	141
3	150	148	148	143	145	143	143	143	143
4	149	149	149	144	145	145	144	145	144
5	148	149	148	143	145	144	143	144	143
6	149	149	149	139	144	141	143	147	145
7	152	151	151	145	148	146	144	146	145
8	150	152	150	142	144	142	140	143	141
9	147	148	148	139	142	141	141	142	141
10	142	142	142	143	144	144	143	143	141
11	142	142	143	141	145	143	144	147	145
Mean	146	147	147	142	145	143	143	144	143
SD	6	7	3	2	1	1	1	2	2

Note. B = before, A = immediately after amd 5A = 5 minutes after exercise. Stim.= Stimulation, Exer.= Exercise. Units : mmol/L.

Table A-6

Raw Data for Potassium

	Group								
	Electrical Stim.			Isokinetic Exer.			Combined Exer.		
Subject	B	A	5A	B	A	5A	B	A	5A
1	4.0	5.3	5.1	5.1	5.7	5.1	5.3	5.4	4.9
2	5.2	6.0	5.6	5.6	5.4	5.4	5.5	5.3	5.5
3	5.1	5.2	5.4	4.8	5.5	5.3	5.2	4.8	5.2
4	5.3	5.4	5.7	5.3	5.5	5.0	5.6	6.4	5.9
5	5.6	5.1	6.3	4.6	4.6	5.2	5.2	5.0	4.9
6	5.1	5.3	5.2	4.9	5.1	4.6	4.7	4.9	4.8
7	5.6	5.1	5.2	5.0	5.4	5.1	5.2	5.9	5.5
8	5.5	5.9	5.9	6.5	5.7	5.5	6.1	6.8	6.8
9	5.9	5.9	5.6	5.4	5.4	5.6	5.9	5.8	5.8
10	5.4	5.3	5.5	5.4	5.2	5.7	6.1	6.1	6.0
11	4.6	5.0	4.9	4.7	4.8	4.7	5.1	4.8	4.8
Mean	5.2	5.4	5.5	5.2	5.3	5.2	5.4	5.6	5.5
SD	0.5	0.4	0.4	0.5	0.3	0.3	0.4	0.7	0.6

Note. B = before, A = immediately after and 5A = 5 minutes after exercise. Stim.= Stimulation, Exer.= Exercise. Units : mmol/L.

Table A-7

Raw Data for Chloride

	Group								
	Electrical Stim.			Isokinetic Exer.			Combined Exer.		
Subject	B	A	5A	B	A	5A	B	A	5A
1	116	105	106	106	106	107	106	106	106
2	106	108	111	105	104	106	105	104	106
3	106	107	107	105	105	106	106	104	105
4	107	110	108	103	103	103	106	107	106
5	113	113	111	105	106	106	104	104	104
6	113	113	112	104	106	103	104	104	104
7	115	114	114	106	108	108	106	106	105
8	111	113	110	105	104	103	106	104	104
9	110	111	111	103	103	103	102	103	103
10	104	103	104	102	102	104	104	103	104
11	105	105	105	103	104	104	107	107	107
Mean	110	109	109	104	105	105	105	105	105
SD	4	4	3	1	2	2	1	2	1

Note. B = before, A = immediately after and 5A = 5 min. after exercise. Stim.= Stimulation, Exer.= Exercise. Units : mmol/L.

Table A-8

Raw Data for Total Carbon Dioxide

	Group								
	Electrical Stim.			Isokinetic Exer.			Combined Exer.		
Subject	B	A	5A	B	A	5A	B	A	5A
1	20	29	28	27	30	28	26	25	25
2	27	28	25	29	27	27	26	27	25
3	24	25	24	28	30	25	25	26	24
4	22	25	25	26	28	26	25	26	19
5	22	22	22	25	22	22	24	23	23
6	24	23	24	29	29	21	25	21	20
7	21	23	22	25	24	23	24	23	25
8	23	26	26	41	25	28	27	28	26
9	23	24	21	24	25	24	26	24	24
10	28	30	28	25	24	21	25	26	24
11	28	29	28	26	21	21	24	24	22
Mean	24	25	25	27	26	24	25	25	23
SD	3	3	3	2	3	3	1	2	2

Note. B = before, A = immediately after and 5A = 5 minutes after exercise. Stim.= Stimulation, Exer.= Exercise. Units : mmol/L.

APPENDIX B

LAKEHEAD UNIVERSITY

HUMAN PERFORMANCE LABORATORY

I, , authorize Lakehead University to perform a series of procedures which constitute the following studies:

1. Isokinetic exercise

2. Electrical muscle stimulation

3. Isokinetic exercise with electrical muscle stimulation

4. Collection of a small blood sample from finger tip and/or vein in my arm. Complications of such blood sampling rarely arise but may include hematoma (swelling), bruising or thrombosis.

I understand that I have the option to stop the test(s) at any time and/or omit any part of any test. In agreeing to these tests, I accept all responsibility and waive my legal resource against Lakehead University and members of their staff from any and all claims resulting from personal injuries sustained from these tests. I understand that any data resulting which may be of a personal nature will be confidential. I further consent to the use of information obtained from these tests by Lakehead University.

I have read and understand the above.

DATE:_____ SIGNATURE: _____

WITNESS:_____