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THE EFFECTS OF INCREASED TRAINING VOLUME IN MIDDLE- AND LONG-DISTANCE RUNNERS ON SELECTED SERUM ENZYME ACTIVITIES

by

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the Graduate School of Studies in
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ABSTRACT

Five blood components, including three serum enzyme activity levels, were measured daily throughout one week of normal training and up to one week of increased training in 9 competitive middle- and long-distance runners. The athletes performed their regular training regimen during the control week, after which they were told to increase their daily training volume by either 10% or 20% for the experimental week. Actual increases in training volume ranged from 9% to 26%. The experimental period ranged from 3 to 7 days for the 9 subjects. Blood samples were taken prior to the day's training on 6 consecutive days during the control week starting on the morning of the third day. Blood samples were again taken on the corresponding days of the experimental week. All blood samples were analyzed for serum CPK, ASAT, and LDH activity levels as well as hemoglobin and hematocrit. Percent increases in each of these parameters were determined for each subject for each day during the experimental period by matching it with the corresponding day of the control week. A descriptive approach was taken for the analysis of the results. CPK was seen to be the most responsive of the three enzymes to changes in training volume while LDH was seen to be the least responsive. A trend toward an increase in both CPK and ASAT levels was seen as a result of increased training volume. LDH did not show any consistent response. Neither hemoglobin nor hematocrit showed any consistent response to the increased training volume. The study indicates the further research into the responses of serum enzymes to increases in training volume is warranted.

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CHAPTER I

THE PROBLEM

Training for an athletic event involves the repeated application of an exercise load to systems within the body. A cycle of exercise and recovery is established during which breakdown and then rebuilding occurs (Astrand and Rodahl 1977, Ch. 12). It is the breakdown/rebuilding cycle that forms the basis for the theory of progressive training. Breakdown in exercise-stimulated skeletal muscle fibres is often characterized by protein loss due to degradation and/or efflux from the cell (Dohm et al. 1978). Rebuilding encompasses the restoration of the partially depleted intracellular proteins (Zimmer and Gerlach, 1973).

Symptoms of overtraining can be the result of an imbalance in the breakdown/rebuilding ratio. Exercise of high intensity, duration or frequency can increase protein loss and recovery time (Dohm et al. 1978). Application of an exercise load prior to complete recovery from previous exercise can cause a net depletion of intracellular protein stores. Repetitious loss of protein in this manner can result in a reduction in the contractile and enzymatic capabilities of the muscle fibre and hence a decrease in the performance capabilities of the athlete (Costill 1979).

During the base work phase of training the distance runner gradually and progressively increases his or her training volume (Costill 1979, Ch. 4). It is not uncommon for symptoms of overtraining, including chronic fatigue, muscular soreness and poor performance, to occur during this period. It is possible that depletion of intracellular proteins is a cause of these symptoms.

Serum levels of a number of enzymes have been shown to increase as a result of many pathological and physiological conditions. Early findings showed increases due to myocardial infarction (LaDue, Wroblewski and Karmen 1954), hepatitis (Wroblewski and LaDue 1955a, 1955b), jaundice (Wroblewski and LaDue 1955b) and muscular dystrophy (Ebashi et al. 1959). It has also been established that many of these enzymes have an increased activity in the blood after strenuous physical exercise (Atland and Highman 1961). Studies have revealed that the greater the stress involved in the exercise, the greater is the increase in serum enzyme activity. The time course and degree of the response is different for each enzyme (Sanders and Bloor 1975; King et al. 1976).

Three frequently studied serum enzymes are creatine phosphokinase (EC 2.7.3.2), lactate dehydrogenase (EC 1.1.1.27) and aspartate aminotransferase (EC 2.6.1.1, also commonly refered to as GOT, glutamate oxaloacetate transaminase). Of these, creatine phosphokinase has shown the greatest response to a given exercise and lactate dehydrogenase the least, while the response of aspartate aminotransferase has been seen to be intermediate between that of the former two enzymes (Yakovleva 1980).

The mechanisms responsible for the elevation of serum enzyme activity after exercise are not well understood. That the enzymes originate from skeletal muscle rather than liver, heart or other tissue has been confirmed in isozyme studies (Schnohr 1974; Schnohr et al. 1980; Rose et al. 1970). A number of theories have been suggested to explain their release from muscle cells. It has been suggested that calcium plays a major role in enzyme efflux (Anand and Emery 1980; Soybel et al. 1978), while ATP energy levels (Sweetin and Thomson 1973; Wilkinson and Robinson 1974) and cellular swelling (Deiderichs et al. 1979) have also been considered as possible factors.

Numerous studies have investigated the effects of exercise on serum enzyme levels. However few have looked at serum enzymes over the course of repeated daily exercise and no literature has been found regarding the effects of increased training volume on enzyme activity in the blood.

STATEMENT OF THE PROBLEM

It was the purpose of this study to observe and describe the effect of selected increases in the training volume (see definition following) of competitive middle- and long-distance runners on the subsequent daily serum activity levels of the enzymes creatine phosphokinase (CPK), aspartate aminotransferase (ASAT) and lactate dehydrogenase (LDH), and on hemoglobin concentrations and hematocrit values.

RATIONALE FOR THE STUDY

The choice of the three enzymes was based on a number of different factors. CPK, LDH, and ASAT represent three different segments in the energy production system. Each shows a time course and sensitivity of response to exercise different from the others (Yakovleva 1980; Anand and Emery 1980). In addition, these three enzymes are among the most thoroughly studied of the human serum enzymes.

In terms of the sensitivity of their reponses to activity, CPK is more responsive than either ASAT or LDH. It is sensitive to variations in normal daily activity and the possibility exists that increases due to changes in training may be hidden within normal daily variations. LDH shows the least daily fluctuation and response to exercise, and its relatively small variance may therefore mean that

only a small increase is necessary to register significance. ASAT is intermediate between CPK and LDH in its response to exercise.

Of the many serum enzymes, CPK, LDH and ASAT are among the most thoroughly studied. Their response to exercise is more completely documented than most other enzymes and information is available on each for verification of their response to different types of exercise in trained and untrained people. This information is important in developing a study protocol and for comparison of results from this study to those available from previous research.

The hemoglobin level in the blood is also monitored throughout the study. Hemoglobin is presently a frequently used monitor of an athlete's response to training. It is desired to observe the response of hemoglobin (Hb) to an increase in the training volume of middle- and long-distance runners.

In order to be able to account for the effects of any blood volume changes blood hematocrit is monitored along with the other blood parameters. This is necessary to eliminate hemoconcentration and hemodilution as factors affecting enzyme concentrations or hemoglobin levels.

SCOPE AND LIMITATIONS OF THE STUDY

The scope of this research is limited by constraints on a number of parameters. The athletes studied are competitive middle- and long-distance runners. The period of training during which they are studied is their pre-season base work phase. The experimental training increase includes selected volume changes only; no observations are made of the effects of intensity increases. It may not be valid to generalize the results of this study in order to apply them to (i) competitive athletes other than distance

runners, (ii) recreational or fitness athletes, (iii) athletes in the midst of their competitive season, (iv) changes in training volume outside the 10 to 20 percent increase used in the training protocol for this study, or (v) changes in training parameters other than volume as defined for this study (for example, intensity).

The descriptive approach taken in the analysis of the results of this study serves certain desired purposes. As the study is the first of its kind it should serve as a base from which future study in the area can proceed. The descriptive analysis provides the necessary information for a judgement on the possible value of further more specific study. It is of value to first describe any changes in serum enzyme levels and in hemoglobin and hematocrit as a result of increased training volume before attempting to establish statistical significance of such changes.

DEFINITION OF TERMS AND LIST OF ABBREVIATIONS

In the text a number of terms are used to refer to specific occurrences or events. Definitions, grouped into terms regarding (i) training, and (ii) the exercise/recovery cycle are listed below.

The following terms are used in reference to training.

Progressive Training

Training for an athletic event involves the adaptation of many systems within the body to enable them to accommodate physical stresses. As training proceeds the nature of the training is changed to stress those systems in which one wishes to induce adaptation. Concurrently the volume and intensity are adjusted as the system adapts in

order to further stress the system. It is this progression in the training programme that makes the term "progressive training" suitable.

Overtraining

Overtraining occurs when the progression in training is too rapid to permit the body to adapt to accommodate the increasing stress. The repeated application of stress with a lack, or possible loss of adaptation to this stress is termed overtraining and is often characterized by symptoms including chronic fatigue, muscular soreness, poor performance and sickness.

Training Volume

Training volume is traditionally defined as the amount of work performed in training. Work is the mathematical product of workload and time, or intensity and duration. However, the distance runner commonly refers to training volume meaning not total work performed but total distance run. This latter definition is the one which will be used for the purposes of this study.

The following definitions refer to aspects of the exercise/recovery cycle.

Overload

When exercise is of an intensity or duration sufficient to create a stress on one or more systems within the body and necessitate adaptation of the system the exercise is termed an overload. This definition may not be in complete agreement with more customary definitions of overload. Exercise of an intensity or duration not stimulating adaptation is often considered an overload. For the

purposes of this study, however, adaptation after repeated application of the exercise is necessary to consider it an overload.

Breakdown

Breakdown is the depletion, through efflux or destruction, of those cellular components necessary for a system to maintain its functional performance. Breakdown results in a decrease in the functional capabilities of the affected system.

Rebuilding

Rebuilding, which occurs during the recovery period, is characterized by the regaining of the functional capabilities of the system lost during breakdown. Rebuilding is the repletion of the depleted cellular components to their initial pre-breakdown levels, presumably through resynthesis within the cells and through influx of the necessary components into the cell.

Overcompensation

If the recovery period continues after rebuilding is complete an overcompensation may be seen whereby the replenished cellular components reach concentrations higher than prior to breakdown. This may cause an increase in the functional capability of the system to a level above that of the pre-overload state.

Abbreviations

A	change in absorbance
ADP	adenosine-5'-diphosphate
AK	adenylate kinase (myokinase; EC 2.7.4.3)

a-KG	alpha-ketoglutarate
ALAT	alanine aminotransferase (glutamic pryruvic transaminase; EC 2.6.1.2)
ALD	aldolase (EC 4.1.2.7)
AMP	adenosine-5'-monophosphate
APP	adenosine-5-pentaphosphate
ASAT	aspartate aminotransferase (glutamic oxaloacetic transaminase; EC 2.6.1.1)
ATP	adenosine-5'-triphosphatase
CP	creatine phosphate
CPK	creatine phosphokinase (EC 2.7.3.2)
EDTA	ethyldiaminetetraacetic acid
G-6-P	glucose-6-phosphate
G-6-P DH	glucose-6-phosphate dehydrogenase (EC 1.1.1.49)
H+	hydrogen ion
HK	hexokinase (EC 2.7.1.1)
LDH	lactate dehydrogenase (EC 1.1.1.27)
MDH	malate dehydrogenase (EC 1.1.1.37)
min	minute(s)
NAC	N-acetyl-L-cysteine
NAD	nicotinamide-adenine-dinucleotide
NADH	nicotinamide-adenine-dinucleotide, reduced
NADP	nicotinamide-adenine-dinucleotide phosphate
NADPH	nicotinamide-adenine-dinucleotide phosphate, reduced
OAA	oxaloacetic acid
6-PG	6-phosphogluconate
umole	micromole(s)

CHAPTER II

REVIEW OF LITERATURE

This chapter reviews the current literature pertaining to the study of serum enzymes in exercise, training and overtraining, dealing with both general and specific related topics. An initial overview of the subject matter is followed by in depth discussion on pertinent topics.

The review begins with an introduction to the theory of progressive training. The concept of an exercise/recovery cycle and the phenomenon of overtraining are discussed. The exercise related breakdown/rebuilding cycle is subsequently expanded upon with reference to both glycogen and protein content within the muscle.

In attempting to devise a practical method for estimating the state of protein stores within the muscle, serum enzymes may prove useful. With this in mind, the review briefly traces the history of serum enzyme studies as related to myocardial infarction, liver disease, muscular dystrophy and exercise. The present theories on mechanisms of enzyme release from muscle are discussed.

The three enzymes studied in this investigation are aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK). The function of each of these enzymes within the active skeletal muscle fibre is outlined and is followed by a detailed review of their individual responses to exercise and training. A comparison between these responses is made, with reference to possible mechanisms of enzyme release from muscle cells.

THEORY OF PROGRESSIVE TRAINING AND OVERTRAINING

Physical training can be defined as the process of adaptation of various cardiovascular, muscular, skeletal and neurological functions of the body to enable it to withstand the effects of the application of physical stressors. This adaptation is accomplished through the repeated application of such stressors to the body's systems. The results obtained from a given training programme are influenced by many external factors including sex, age, genetic predisposition, initial fitness level and previous training experience (Fox 1979, Ch. 8). Variables within the programme, for example intensity, duration, frequency or recovery time influence the type and degree of the system's adaptation (Fox 1979, Ch. 8).

In any training programme the following basic theory prevails. During exercise, work is performed and an overload is placed on systems within the body. The overload creates a demand for these systems to function at a greater rate than is customary which in turn requires increased energy production. When the energy or functional demands on the system cannot be met "breakdown" of some form occurs (Astrand and Rodahl 1977, Ch. 12). This breakdown stimulates "rebuilding" when the overload is removed thereby returning the system to, or even beyond, its pre-overload resting state (Astrand and Rodahl 1977, Ch. 12). The term "overcompensation" is applied to the process of rebuilding beyond the pre-overload state. This adjustment means that the same stressor applied to the system again will create less of an overload for it. If the same training exercise and the full overload/recovery cycle are repeated regularly, the system gradually adapts and eventually is able to accommodate the demands of the exercise, at which point an overload no longer exists (Astrand and Rodahl 1977, Ch. 12). This is the nature of the training response.

When the body has adapted to the initial exercise stressor, an increase in the volume or intensity of work is required to produce a new overload and stimulate further training response. With each change in the training exercise stressors new adaptations are stimulated and a gradual progression is seen in the system's ability to accommodate the physical stressors of exercise (Astrand and Rodahl 1977, Ch. 12). This is the theory of progressive training.

Many athletes, in their anxiousness to attain their goals, encounter the phenomenon of overtraining. Overtraining is a result of excessive increases in the intensity, duration or frequency of training sessions, or a combination thereof. With excessive stressors being applied to the body, the recovery portion of the overload/recovery cycle is inadequate to permit complete rebuilding or overcompensation. With no overcompensation occurring no adaptation or training response can occur. If rebuilding is inadequate to return the system to its pre-overload state, ie. if the system becomes unable to accommodate a stressor it previously could, a negative adaptation occurs. Negative adaptation is observed in an athlete as a drop in his or her performance level in competition or as chronic fatigue, muscular soreness, loss of appetite or insomnia (Costill 1979; Daniels et al. 1978).

The pre-season base work training for competitive middle- and long-distance runners consists of relatively high volume, low intensity work. Total training mileage reaches its peak during this training phase (Costill 1979, Ch. 4). If an athlete's training volume (ie. mileage) is increased frequently, or suddenly by a larger amount, his or her body may not be able to adapt to the increased functional and energy demands on its internal systems. This can induce the onset of symptoms of overtraining.

The correct balance in the exercise/recovery cycle is necessary to avoid overtraining. To this end an understanding of the breakdown/rebuilding cycle is desirable.

MUSCULAR EXERCISE AND RECOVERY; THE BREAKDOWN/REBUILDING CYCLE

During exercise the musculoskeletal system is one of the body's internal systems upon which an overload is placed through the application of physical stressors. The stressor is the physical work that is performed. The overload is the demand on the muscle fibres for a high contractile force and/or velocity. The demands of the stressor are met through the recruitment of motor units and through an increased frequency of contraction of the individual units (Guyton 1976, Ch. 11; Astrand and Rodahl 1977, Ch. 4). The contractile proteins within the muscle fibres of each motor unit require energy to perform their function. This need is met by increased carbohydrate and/or fat metabolism utilizing intramuscular stores of glycogen and triglycerides, and glucose, free fatty acids and other substrates supplied by the blood.

Muscle glycogen depletion and repletion is perhaps one of the more well known breakdown/rebuilding cycles initiated by exercise. Numerous studies have shown that the increased carbohydrate metabolism during exercise results in a decrease in the glycogen content of skeletal muscle, heart and liver (Poland et al. 1980; Gaesser and Brooks 1980). During recovery after exercise, repletion of the glycogen content of each of these tissues occurs at different rates. According to Gaesser and Brooks (1980) and Poland et al. (1980) the glycogen content of rat cardiac muscle is fully restored within less than four hours after exhaustive exercise; at four hours an overcompensation is seen, ie. the

myocardial glycogen content is greater than the pre-exercise level. The same studies reveal that both the liver and skeletal muscle require more time to fully replenish their glycogen stores than does the myocardium. Gaessar and Brooks (1980) propose that this may be due to the fact that both these tissues deplete their glycogen stores to a considerably greater extent than does cardiac muscle during exhaustive exercise. In man, full repletion of skeletal muscle glycogen after exhaustive exercise takes 24 to 48 hours with an adequate supply of exogenous carbohydrates (Kochan et al. 1979; Piehl 1974). Post-exercise fasting inhibits restoration of glycogen content (Maehlum and Hermansen 1978) while a carbohydrate-rich post-exercise diet promotes an overcompensation effect and an abnormally high muscle glycogen content (Kochan et al. 1979).

Protein content of skeletal muscle follows a depletion/repletion, breakdown/rebuilding pattern similar to that of intramuscular glycogen. Dohm et al. (1978) have found that the protein content of skeletal muscle of the rat decreases as a consequence of exhaustive exercise. More recently Dohm et al. (1980) have shown that protein degradation is increased and protein synthesis decreased in rat skeletal muscle during exercise. This same result has been determined by others (Zimmer and Gerlach 1973). Numerous studies confirm that strenuous exercise causes an increase in serum activity levels of a number of enzymes involved in oxidation and energy production; isozyme studies reveal that these come from skeletal muscle (Rose et al. 1970; Schnohr 1974) indicating at least a partial depletion of muscle enzymes. Thus there are three responses of the muscle to explain its decreased protein content after exercise - decreased synthesis, increased degradation and increased loss to the extracellular fluids.

During recovery from exercise, protein synthesis increases (Zimmer and Gerlach 1973). Results of studies by

Wenger et al. (1981) on rat skeletal muscle support the idea that this synthesis serves to replace proteins catabolized or lost from the muscle during exercise. Using radioactive leucine uptake techniques, they determined that endurance exercise causes enhanced uptake into both the mitochondrial and the soluble protein fractions of red oxidative muscle while only the mitochondrial fraction of white glycolytic fibres shows the increased uptake. Sprint exercise, on the other hand, enhances uptake into both fractions of white muscle. That amino acid uptake varies according to the exercise, the fibre type and the protein fraction indicates that only those systems overloaded during exercise are affected (ie. sprint exercise does not create an overload on the oxidative portion of red muscle and endurance exercise does not create an overload on the cytoplasmic glycolytic enzymes of the white fibre).

The study of protein cycles relating to exercise may be important to the advancement of the knowledge and understanding of athletic training and performance. However, direct measurements of protein loss and degradation rates require complex techniques using radioactive tracers and muscle biopsies. Serum enzyme activities are relatively simple to measure as only small blood samples are required of the subjects. It is for this reason that the present study uses serum enzyme measurements to investigate the responses to increased training volume in competitive endurance athletes.

A BRIEF REVIEW OF STUDIES OF SERUM ENZYMES

The study of tissue enzymes in the serum as indicators of various pathological or physiological states began in the early 1950's. LaDue, Wroblewski and Karmen (1954) established norms for aspartate aminotransferase (ASAT; commonly referred to as glutamic oxaloacetic transaminase,

GOT) activity in human serum and subsequently traced its activity levels in patients after acute myocardial infarction (M.I.). They found serum ASAT (s-ASAT) to peak at up to 20 times its normal level, 12 to 24 hours after M.I. Their results were confirmed by Stone et al. (1955) who studied the enzyme response to induced M.I. in dogs. Kattus et al. (1956) added that s-ASAT returned to normal in humans by the fifth day post-M.I.; they found the peak to occur 24 to 36 hours after the infarction.

These and other initial studies opened the gates for further research into serum responses of numerous tissue enzymes to M.I. and other disease and pathologic conditions. Wroblewski and LaDue (1955a) observed that serum lactate dehydrogenase (s-LDH) activity increased after both experimental and clinical M.I., while Siegel and Bing (1956) added that aldolase (ALD) and malate dehydrogenase (MDH) were also elevated in the serum after M.I. S-ASAT, s-LDH and serum alanine aminotransferase (s-ALAT) (Wroblewski and LaDue 1955a, 1955b, 1956) were all found to increase in hepatitis, and s-ASAT in cirrhosis, jaundice and acute liver injury by poisoning (Wroblewski and LaDue 1955a, 1955b, 1956; Fleisher and Wakin 1961).

Realizing that ASAT and other enzymes were not only present in heart and liver tissue but in skeletal muscle as well, serum enzyme levels in patients with muscular disease, in particular Duschenne muscular dystrophy (D.M.D.) were studied. ASAT, ALD, LDH and creatine phosphokinase (CPK) serum activities were found to be elevated in muscular dystrophy (M.D.) (Pearson 1957; Ebashi et al. 1959). It was suggested by Pearson (1957) and confirmed by Okinaka et al. (1961) that serum enzyme levels are positively correlated to the rapidity of the progress of the disease.

It was at this stage in the study of serum enzymes that investigations involving exercise were first reported.

Schlang and Kirkpatrick (1961) referred to Wroblewski as reporting the first observations of elevated s-ASAT after exercise in the course of another study. Henley, Schmidt and Schmidt (1960) quoted increases in LDH, ALD, MDH and ASAT in the serum after one hour of "muscular exercise" though the exercise is not further specified. Schlang and Kirkpatrick (1961) found elevated s-ASAT immediately after "strenuous physical exercise", its activity returning to normal within 12 to 24 hours. S-ALAT was not affected by the same exercise. Atland and Highman (1961) exercised rats almost continuously for 16 hours and found increases in serum levels of ALAT, ASAT, LDH and ALD and a decrease in alkaline phosphatase (ALP). The time for recovery of the different enzymes to normal levels ranged from 24 hours to more than 6 days.

Since these first studies, much work has been done on the response of serum enzymes to exercise. The mechanism or mechanisms by which enzymes are permitted to escape from the muscle fibre into the blood has not yet been clearly identified. A number of theories exist to explain the phenomenon and the field is still being researched.

MECHANISMS OF ENZYME RELEASE FROM SKELETAL MUSCLE

That serum enzyme concentrations increase in various pathological and physiological conditions is now accepted as fact. The possible mechanisms for such increases are currently being studied. There appear to be different mechanisms involved in different conditions.

Cellular damage may be the cause of high serum enzyme levels in the presence of diseased or injured tissue. It is generally agreed that after myocardial infarction enzymes are released from the necrotic cardiac muscle and serum concentrations are positively correlated to the amount of infarcted tissue (Stone et al. 1955; Agress et al. 1955).

Wroblewski and LaDue (1955b) found s-ASAT levels to be an indicator of liver cell damage in their study of liver disease, indicating the possibility of enzymes being released from necrotic tissue. S-CPK and s-ALD levels are seen to show a positive correlation to the rate of progress of muscular dystrophy (Okinaka et al. 1961). This suggests that their release may be from recently affected tissue. Skeletal muscle damage due to injury or surgery is also seen to cause increases in s-ASAT (LaDue and Wroblewski 1955). In each of these conditions it appears that damaged cells are releasing enzymes into the blood.

Recently Warhol et al. (1985) looked at skeletal muscle from trained marathon runners after competition. Ultrastructural damage was observed, and it was suggested that this may contribute to enzyme leakage from the muscle. Furthermore it was found that the ultrastructural damage observed was seen only in those myofibres depleted of glycogen.

Many internal physiological factors have been reported to influence serum enzyme concentrations in in-vitro experiments. Included among these are annoxia (Zierler 1956) and hypoxia (Highman and Atland 1960; Loegering and Critz 1971), levels of epinephrine (Highman et al. 1959), norepinephrine (Highman et al. 1959), glucose (Zierler 1956), potassium (Zierler 1956), calcium (Horak et al. 1980), glycolytic inhibitors (Sweetin and Thomson 1973) and ATP (Diederichs et al. 1979; Anand and Emery 1980). A number of theories have been offered to explain the mechanisms for the response of serum enzyme concentrations to these factors.

The mechanisms of skeletal muscle enzyme efflux during exercise have not been well documented. The acute effects of exercise include changes in many of the above-mentioned physiological factors known to influence serum enzymes.

Presumably one or more of these physiological responses to exercise may play a role in facilitating enzyme efflux. Some of the more feasible mechanisms suggested involve ATP levels, calcium stores or myofibre glycogen levels.

Glycogen Levels and Enzyme Release

Warhol et al. (1985) tested the hypothesis that CPK acts as a marker for tissue regeneration in damaged muscle. They studied muscle specimens from competitive runners after a marathon over a timespan of 12 weeks following the race.

It was found that various forms of ultrastructural damage occurred, and that this damage was confined to those muscle fibres depleted of glycogen. They speculated that enzyme leakage is permitted from these damaged fibres.

ATP and Enzyme Release

A number of investigators have suggested a decreased intracellular ATP content as an initiator of enzyme efflux from muscle fibres. Sweetin and Thomson (1973) studied human erythrocytes and demonstrated enzyme efflux under conditions of low intracellular ATP levels due to impaired glycolysis. Wilkinson and Robinson (1974) showed a strong relationship between ATP levels and LDH release using rat lymphocyte suspensions. Enzyme efflux in the absence of potassium efflux, as seen by Sweetin and Thomson (1973) contra-indicates a generalized increase in membrane permeability. They suggest that ATP may help maintain an intracellular protein aggregate incapable of diffusion through the membrane. Disturbance of the aggregate due to low ATP concentrations would lead to efflux of soluble enzymes to re-establish equilibrium. This mechanism in skeletal muscle would permit re-uptake of serum enzymes by the fibres once ATP levels and the aggregate were restored.

If ATP is responsible for enzyme efflux from muscle fibres, explanation of the effects of many other physiological conditions is simplified. Lack of glucose results in depressed glycolysis and hence reduced ATP production. Increased potassium interferes with the sodium-potassium pump whose energy is supplied by ATP. Annoxia leads to increased NADH, failure of the citric acid cycle and inhibition of ATP synthesis. These conditions all result in depletion of ATP stores which could explain why enzyme release is enhanced in each case.

Calcium and Enzyme Release

Calcium is another factor suggested to cause enzyme efflux from skeletal muscle. Incubation of mouse skeletal muscle in a medium containing calcium results in efflux of CPK from the muscle (Soybel et al. 1978). A direct relationship was found between extracellular calcium concentration and CPK efflux. Studies on human skeletal muscle (Anand and Emery 1980) demonstrated CPK, LDH, ASAT and ALAT to respond similarly to high extracellular calcium levels with enhanced release from the cells, CPK and LDH being most responsive. Reduction of calcium concentration showed the response to be reversible -- within an hour s-CPK levels had begun to return to normal while s-LDH continued to rise for three hours prior to returning towards normal levels.

The mechanisms by which calcium affects enzyme efflux cannot be determined from these studies. The non-parallel responses of the different enzymes to calcium again suggest, in accordance with Sweetin and Thomson (1973), that enzyme efflux is not due simply to a generalized non-specific increase in cell membrane permeability, but to a complex mechanism involving a series of steps.

Cell Volume and Enzyme Release

A series of in-vitro experiments using rat skeletal muscle was performed by Diederichs et al. (1979) to study enzyme release as a result of cellular swelling and osmotic pressure; ATP concentration was controlled by poisoning with dinitrophenol (DNP); swelling was controlled through the tonicity of the medium and using sodium pyrophosphate (NaPP). Although enzyme release increased when ATP was depleted, as demonstrated earlier by Sweetin and Thomson (1973), this phenomenon was not seen under conditions in which swelling was reduced using NaPP administration while all other conditions remained identical. A dramatic increase in LDH permeability was seen within a range of relative osmolality between 0.45 and 0.55, regardless of the degree of swelling or the energy conditions of the cell. The size of the increase was influenced by the combination of cellular swelling and ATP levels. A sudden reduction in swelling was associated with the increase in LDH permeability, except under conditions of reduced ATP or calcium and/or magnesium. Under conditions when the reduction in swelling was not observed, i.e. under conditions of low ATP or in the absence of calcium and magnesium, the size of the LDH permeability increase was markedly less than when it was accompanied by the reduction in swelling.

These results indicate that cellular swelling may play a role in the release of intracellular enzymes. The mechanism is again demonstrated to be rather complex. Evidently it may incorporate each of osmotic pressure, ATP energy level and calcium concentration as factors. Further study is required from healthy skeletal muscle fibres. The four theories presented are indicative of the present knowledge in this field.

THE ENZYMES: ASAT, CPK, AND LDH

Numerous enzymes are present in human serum. The three used is this study, CPK, LDH and ASAT are each involved in a different segment of energy production. LDH is necessary in anaerobic metabolism of carbohydrates to convert pyruvate to lactate. CPK is required in anaerobic alactic metabolism to transfer a phosphoryl group from creatine phosphate to adenosine diphosphate. ASAT is involved in amino acid metabolism in the reversible transfer of an amino group from aspartate to alpha-ketoglutarate, forming glutamate and oxaloacetate.

Aspartate Aminotransferase

Aspartate aminotransferase (ASAT), EC 2.6.1.1, is one of a number of aminotransferases or transaminases that catalyze the transfer of an alpha-amino group from an alpha-amino acid to the alpha-carbon of an alpha-keto acid. ASAT is most active in the transfer of an amino group from aspartate to alpha-ketoglutarate forming oxaloacetate and glutamate. It also participates to some extent in other transamination reactions despite the existence, in low concentrations, of aminotransferases specific to each transaminase reaction (Lehninger 1978, Ch. 21).

ASAT is a dimer and has two isozymes which are different in structure, isoelectric pH, metabolic function and cellular location and have different regulatory mechanisms. The anionic ASAT is localized in the cytosol (ASAT-cyt) and is termed "supernatant" ASAT. The cationic isozyme is localized in the mitochondrial matrix and is termed "mitochondrial" ASAT (ASAT-mit). In normal purification procedures only ASAT-cyt is retained, the mitochondrial isozyme being lost in the first preparation stage. Destruction of the mitochondrial membrane, however, releases the ASAT-mit in a soluble form. (Braunstein 1973)

The primary function of ASAT-cyt is the deamination of aspartate and the formation of the amino acid glutamate. The formation of glutamate, common to all transaminase-catalyzed reactions, is a method of accumulating all amino groups in a common form in which they can enter the mitochondrial matrix via a specific transport system. In the mitochondria, glutamate acts as the amino group donor in reactions which convert the amino group into nitrogenous waste products for excretion. Deamination occurs either directly or via ASAT-mit, the former procedure producing ammonia and the latter transferring the amino group to oxaloacetate and producing aspartate to be incorporated into the urea cycle where the nitrogen from the amino group is eventually excreted as urea. (Lehninger 1978, Ch. 21)

Creatine Phosphokinase

Creatine phosphokinase (CPK), EC 2.7.3.2, catalyzes the reversible reaction which transfers a phosphoryl group from creatine phosphate (CP) to adenosine diphosphate (ADP) forming adenosine triphosphate (ATP) and creatine. The equilibrium state strongly favours the formation of ATP and only through a special mechanism is the reverse reaction, the phosphorylation of creatine, possible. For either of the two reactions the presence of magnesium is required to form an active complex by combining with ATP or ADP. (Saks et al. 1978)

The CPK molecule is a dimer and at present four isozymes are known to exist. Three of these are the MM, MB, and BB forms (M for muscle, B for brain) located in the cytoplasm; the fourth is specific to the mitochondrion. (Watts 1973)

The formation of ADP and CP from ATP and creatine takes place in the mitochondrion, catalyzed by the mitochondrial CPK isozyme. In the mitochondrial matrix oxidative

phosphorylation produces ATP and a translocase enzyme on the inner membrane exchanges matrix ATP for extramitochondrial ADP. It is suggested that CPK, which is bound to the outer surface of the inner membrane of the mitochondrion is located at the same site as the tranlocase (Saks et al. 1978). The presence of the translocase ensures a very high local ATP concentration in the vicinity of the CPK which permits the CPK-catalyzed reaction to proceed in favour of CP formation. The ADP produced in this reaction is rapidly transported by the translocase into the matrix, thus maintaining the conditions of high ATP and low ADP concentrations necessary for CP production. The creatine phosphate formed in the mitochondrion is then transported into the cytoplasm and to the active site of the cell where it rephosphorylates ADP. The ATP so formed in the cytoplasm is used locally as a source of energy for the cell to perform its function. Both the dephosphorylated creatine and the regenerated ADP are returned to the mitochondrion where the cycle commences again. (Saks et al. 1978)

Lactate Dehydrogenase

Lactate dehydrogenase (LDH), EC 1.1.1.27, catalyzes the oxidation of lactate to pyruvate and the reverse reaction, the reduction of pyruvate to lactate. In the forward process a molecule of NAD+ is reduced to NADH + H+.

The LDH molecule is tetrameric, composed of a combination of two distinct subunits, the H (heart) and the M (muscle) forms. Five isozymes exist; they are H4, H3M, H2M2, HM3, and M4. LDH is localized in the cytoplasm of the cell. The predominant isozyme in the myocardium is LDH-H4, while in skeletal muscle and liver LDH-M4 predominates. (Lehninger 1978, Ch. 9)

Each of the five LDH isozymes performs differently as a consequence of differences in their sensitivity to substrate

and product inhibition and in their Km and Vmax values. (Km and Vmax values indicates the enzyme's influence on reaction rates.) LDH-M4 reduces pyruvate at a very high rate, while LDH-H4 reduces pyruvate only slowly. LDH-H4 is strongly inhibited by pyruvate; its muscle counterpart is much less sensitive to pyruvate concentrations. These characteristics indicate that under normal circumstances LDH-H4 catalyzes the reverse reduction reaction. Both are inhibited by very high lactate concentrations but, again, the M4 form is less sensitive than the H4 form. In all of these properties the other three intermediate isozymes have characteristics correspondingly distributed between the two extremes. (Holbrook et al. 1973; Lehninger 1978, Ch. 9)

LDH-M4 is active in skeletal muscle in anaerobic metabolism. Pyruvate produced during metabolism is reduced to lactate by the enzyme, which is not significantly inhibited by normal muscle concentrations of pyruvate. The waste product lactate is resynthesized into glycogen during rest or is carried to aerobic tissues to be oxidized.

LDH-H4 is the isozyme responsible for the oxidation of lactate in cardiac muscle. Lactate delivered to the heart is converted to pyruvate under the catalytic influence of LDH-H4 and then to carbon dioxide and water via the citric acid cycle (CAC) (Lehninger, 1978, Ch. 30). As lactate inhibits glycolysis, pyruvate concentrations remain low and LDH-H4 remains uninhibited. When lactate is not present as the metabolic substrate, glycolysis takes place, oxidizing glycogen to pyruvate. LDH-H4 is inhibited by the pyruvate, preventing reduction of the pyruvate to lactate and permitting its complete oxidation to carbon dioxide and water via the CAC.

TABLE I: TECHNICAL CHARACTERISTICS OF THE ENZYMES CPK, ASAT AND LDH

CHARACTERISTIC	CPK		ASAT		LDH	
	DESCRIPTION	REFERENCE	DESCRIPTION	REFERENCE	DESCRIPTION	REFERENCE
IDENTIFICATION	EC 2.7.3.2		EC 2.6.1.1		EC 1.1.1.27	
MOLECULAR WEIGHT	81,000	Barman (1969)	90,000	Barman (1969)	140,000	Barman (1969)
EQUILIBRIUM CONSTANT	7.2×10^{-9} (pH 7.4, 30 deg)	Barman (1969)	0.16-0.17 (pH 7.4, 37 deg)	Barman (1969)	2.76×10^{-6} (pH 7.0, 27 deg)	Barman (1969)
ISOZYMES	1. CPK-MM 2. CPK-MB 3. CPK-BB	Watts (1973)	1. ASAT-cyt 2. ASAT-mit	Braunstein (1973)	1. LDH-H4 2. LDH-H3M 3. LDH-H2M2 4. LDH-HM3 5. LDH-M4	Lehninger (1978)

SERUM ENZYMES IN EXERCISE

The three enzymes just discussed are the ones to be considered in the review of the effects of physical exercise on serum enzyme levels. CPK is very responsive to activity and is elevated to extremely high values after intense exercise of some duration. ASAT is less responsive to exercise than CPK. S-LDH levels appear to be more stable than both s-ASAT and s-CPK levels after exercise (Yakovleva, 1980). Following is a review of the response of the serum activity levels of CPK, ASAT and LDH to physical exercise.

Creatine Phosphokinase in Exercise

CPK activity in the serum has been seen to be elevated to 20 or more times its resting level after prolonged exercise. Numerous studies have confirmed these findings, among them studies of participants in a 100 km run (Schnohr 1974), a 53 mile walk (Griffiths 1966) and a two day 110 km march (Shapiro et al. 1973). Post-exercise CPK levels are correlated to the duration of the exercise, that is, the longer the duration, the greater the increase that is seen (King et al. 1976; Ahlborg and Brohult 1967; Vejjajiva and Teasdale 1965). Furthermore, it would appear that CPK levels may be positively correlated to the relative intensity of the exercise. In Shapiro's study (1973) of untrained men marching 110 km in two days, the group marching at a set pace demonstrated elevations in CPK which were found to show a negative correlation with their maximal oxygen consumption (mVO2) whereas all groups whose paces were adjusted according to mVO2 showed similar increases. This would indicate that elevations in CPK after exercise may be related to fitness level or to the relative intensity of the workload.

Research has produced conflicting evidence on the effects of short duration exercise on CPK levels. For

exercise of less than 30 minutes duration, some studies have revealed no significant change in s-CPK (Pearce et al. 1964; Galteau et al. 1976; Bolter and Critz 1974) while others show significant increases of less than 100 percent (Yakovleva 1980; Forssell et al. 1975; Hunter and Critz 1971).

Perhaps the reason for the discrepancies seen in studying CPK levels after short duration exercise lies in its sensitivity to normal daily activity. Griffiths (1966) demonstrated this in a two-part study. They found that 30 minutes of bed rest produced an 80 percent decrease in s-CPK. They also compared in- and out-patients at a hospital and found the male out-patients to have a 110 percent and the females a 70 percent higher s-CPK level than the corresponding in-patients. These differences were attributed to the higher activity levels of the out-patients. Such findings indicate that pre-study activity levels and conditions may be an important consideration in the assessment of CPK values after short term exercise.

Increased levels of s-CPK after long duration exercise are seen to continue for many hours or days after cessation of the exercise. In fact, peak values are not seen immediately but some 10 to 30 hours after the exercise has been completed (King et al. 1976; Riley et al. 1975). This would indicate a continued release of the enzyme from the muscle for some time after exercise. What little evidence there is does not show similar results for s-CPK levels after short term exercise of less than 30 minutes duration (Forssell et al. 1975; Pearce et al. 1964).

In summary, CPK would appear to be sensitive even to normal daily activity levels. Elevations in s-CPK are higher after longer duration exercise, and are also correlated to the relative intensity of the exercise. After

exercise lasting one hour or more peak values of s-CPK are not seen immediately, but some 10 to 30 hours later.

Aspartate Aminotransferase in Exercise

The response of ASAT to exercise increases as both the intensity and duration of the activity increase. At high intensities, exercise lasting less than 1.5 minutes can elicit an increase in s-ASAT (Nerdrum and Berg 1964). After exercise lasting up to an hour or more, increases in s-ASAT levels of up to 100 percent are seen immediately post-exercise (Ohno et al. 1978; Yakovleva 1980; Metivier et al. 1980). In trained athletes the response at a given absolute workload is less than that in an untrained individual (Hunter and Critz 1971; Fowler et al. 1962). The effect of mVO2 on the response of ASAT is similar to that described for CPK (Shapiro et al. 1973) where there is an inverse relationship between ASAT elevation and mVO2 at a given workload.

After short duration exercise, s-ASAT levels return to normal relatively quickly. In most cases cited s-ASAT returned to or near pre-exercise values within an hour post-exercise (Ohno et al. 1978; Metivier et al. 1980; Fowler et al. 1962). The exact time course of recovery may depend on the increase observed as well as the activity level of the subject during recovery, however this has not yet been determined.

In exercise lasting between approximately 1 hour and 4 hours, increases in s-ASAT are seen immediately after completion of the activity. These vary from 10 percent in a two hour march by soldiers (Halonen and Konttinen 1962) to 80 to 200 percent after an 8 mile cross country run by competitive runners (Fowler et al. 1962). In instances of exercise lasting 14 hours or more, s-ASAT levels have been shown to increase by a factor of 2 to 13 times the pre-

exercise values (Atland and Highman 1961; Schnohr 1974; Shapiro et al. 1973). Again, perhaps fitness level and exercise intensity play a large role in accounting for the differences seen.

After long term exercise, contrary to short term exercise, s-ASAT continues to rise after exercise ceases, reaching its peak at some time as yet not clearly defined. Riley et al. (1975) found ASAT values in the serum to be higher 20 to 30 hours after a marathon run (42.2 km) than immediately after finishing it. With rats exercised for 16 hours, Atland and Highman (1961) found the peak to occur within 24 hours after cessation of exercise; and King et al. (1976) found a peak in their human subjects' s-ASAT levels between 5 and 11 hours after 1 hour of handball. The time required for s-ASAT to return to pre-exercise values after prolonged exercise has not been studied in any detail though King et al. (1976) report a return to normal by 43 hours, while the rats studied by Atland and Highman (1961) still had somewhat elevated levels 6 days after exercise though the values remained constant throughout days 3 to 6.

Lactate Dehydrogenase in Exercise

After exercise of less than 30 minutes duration and of high intensity, increased concentrations of serum lactate dehydrogenase (s-LDH) may be seen. Increases ranging from 10 to 50 percent have been reported by Fowler et al. (1962), Hunter and Critz (1971) and Yakovleva (1980). At low workloads, no change is seen (Hunter and Critz 1971; Statland et al. 1973; Fowler et al. 1962). Very short maximal bursts of activity, less than 1 1/2 minutes in length, elicit no s-LDH response (Fowler et al. 1962; Nerdrum and Berg 1964).

The response of s-LDH diminishes with training, the same absolute workload causing a lesser rise in the enzyme

level in the blood of the trained individual (Fowler et al. 1962). According to Hunter and Critz (1971) the same relative workload may also create a lesser response in the trained than the untrained person.

The time course of recovery of LDH after exercise has not been well documented. Ahlborg and Brohult (1967) report values to remain elevated at 1 day (24 hours) but to have returned to normal by 4 days (96 hours) after 90 minutes of work to exhaustion on a bicycle ergometer. King et al. (1976) found individuals' peak values to occur anywhere between 1 and 19 hours after an hour of handball, and the time to regain pre-exercise values to be 43 hours.

TABLE II: SUMMARY OF RECENT STUDIES OF SERUM ENZYMES IN EXERCISE

REFERENCE	KING ET AL. (1976)	SCHNOHR (1974)	HUNTER & CRITZ (1971)	AHLBORG & BROHULT (1967)
RESTING CPK MEAN VARIABILITY NORMAL	7.8 U range 2.5-13.0 U	67 U/1 range 43-110 U/1 < 50 U/1	43.2 mU/ml SEM 4.0	0.37 nM SD 0.33 < 1 nM
POST-EXERCISE CPK (PEAK) MEAN VARIABILITY	17.4 U range 5.4-28.8 U	2900 U/1 range 1200-6100 U/1	58.7 mU/ml not specified	1.58 nM SD 2.13
CPK ANALYSIS TECHNIQUE	Statzyme CPK kit 7251	Rosalki (1967)	Rosalki (1967)	Bernt & Bergmeyer (1962)
RESTING ASAT MEAN VARIABILITY NORMAL	31 U range 24-37 U	18 U/1 range 15-24 U/1 < 0.4 U/1	13.7 mU/ml SEM 0.9	22 Karmen Units SD 4.5 10-35 Karmen U.
POST-EXERCISE ASAT (PEAK) MEAN VARIABILITY	44 U range 30-59 U	124 U/1 range 67-226 U/1	26.5 mU/ml not specified	27 Karmen Units SD 8.9
ASAT ANALYSIS TECHNIQUE	Boehringer Mannheim kit 15791	Schriewer (1971)	Karmen (1955)	Karmen (1955)
RESTING LDH MEAN VARIABILITY NORMAL	213 U range 158-260 U	199 U/1 range 180-230U/1 160-310 U/1	47.3 IU/1 SEM 1.0	210 Wroblewski U SD 27 100-300 W. U
POST-EXERCISE LDH (PEAK) MEAN VARIABILITY	287 U range 191-385 U	549 U/1 426-736 U/1	51.6 IU/1 not specified	249 Wroblewski U SD 71
LDH ANALYSIS TECHNIQUE	Boehringer Mannheim kit 16314	van der Helm (1962)	Babson & Phillips (1965)	Wroblewski & LaDue (1955a)
EXERCISE STIMULUS	Handball (1 hour)	Running (100 km)	Bicycle Ergometer (mVO2 test)	Bicycle Ergometer (90 minutes)

ISOENZYMES IN SERUM

A number of studies of serum and muscle enzymes during or after exercise have focussed attention on isoenzymes, rather than total enzyme levels, investigating the relative contribution of different isoenzymes to serum and muscle activity levels.

CPK has two isozymes acting as the major contributors to total CPK activity. Kettunen et al. (1982) found no changes in the relative contribution of CPK-MB and CPK-MM to the total muscle CPK activity when looking at athletes before and after a hard training session. In addition, no difference was found in relative contribution of the two isozymes when comparing athletes with non-athletes. This latter finding is not, however, supported by that of Siegel et al. (1983) who studied marathon runners and found their muscle CPK-MB levels to be higher than those of sedentary control subjects, while total CPK was not significantly different. Expressed as a percentage, CPK-MB was higher in the runners than the non-runners.

Apple et al. (1984) also studied marathon runners in comparison with non-runners controls, looking at CPK isoenzymes in the muscles of the leg. Their findings were in agreement with Siegel et al. (1983) in that the runners had higher CPK-MB levels than non-runners. In addition, Apple et al. found that the relative contribution of CPK isozymes did not change after a marathon run, an observation consistent with that of Kettunen et al. (1982).

For CPK, the findings indicate that, at the muscular level, CPK isoenzymes do not change their relative contribution to total CPK after exercise. The relative contribution of CPK-MB does, however, increase after long-term training, while total CPK may or may not be affected by long-term training.

SUMMARY

During exercise an overload is placed on systems within the body. This causes breakdown of some form to occur. Recovery from exercise leads to rebuilding and a replacement of cellular components lost or destroyed as a result of breakdown during exercise. Glycogen depletion during exercise and its repletion during recovery is a familiar example of the breakdown/rebuilding cycle.

Skeletal muscle protein content follows a pattern similar to that of glycogen stores during and after exercise. The depletion of intramuscular protein is a result of a combination of the decreased protein synthesis, increased protein degradation and increased protein loss to the extracellular fluids during exercise.

Intramuscular enzymes are one source of protein lost to the extracellular fluid during muscular activity. The mechanism or mechanisms by which they escape from the muscle is not yet completely understood, however calcium concentrations and intracellular energy (ATP) levels are known to affect enzyme efflux.

The fact that serum enzyme concentrations increase after exercise as well as in many disease states including myocardial infarction, hepatitis, jaundice, cirrhosis and muscular dystrophy, has been documented. Three of the most thoroughly studied serum enzymes are aspartate aminotransferase (ASAT), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). These react to exercise in a manner non-parallel to each other, with CPK generally showing the greatest response, and LDH being the most stable. All show greater elevation after longer duration physical activity.

Overtraining is the result of an unbalanced exercise/recovery cycle. The rebuilding period is

insufficient to restore cellular components to their pre-breakdown state. Protein content of the muscle may become depleted. Serum enzymes may accumulate due to a large efflux of the muscle enzymes from the fibres.

Middle- and long-distance runners, when building their base during pre-season training perform high volume, low intensity work. Their running mileage reaches a peak during this phase of training. Volume-induced overtraining is not an uncommon phenomenon in these athletes when training volume is being progressively increased. It was the purpose of this study to investigate the effects of increased training volume in competitive middle- and long-distance runners on serum activity levels of the enzymes creatine phosphokinase (CPK), aspartate aminotransferase (ASAT) and lactate dehydrogenase (LDH), and on hemoglobin concentrations and hematocrit values.

CHAPTER III

METHODOLOGY

SUBJECTS

This chapter outlines the protocol, techniques and methods used in this study of serum enzymes in endurance athletes during a period of increased training volume. The subjects studied and a table of data on the subjects are discussed first. The four week study protocol is outlined, followed by brief detailed descriptions of the various aspects of the protocol.

Blood analysis techniques are described for the three enzymes CPK, ASAT and LDH, and for hemoglobin and hematocrit. Finally, the statistical analysis used in reporting the results is discussed.

The subjects in this study were all competitive middle- and long-distance runners. Male and female athletes between the ages of 19 and 41 were studied. Subjects were volunteers from a local track club. A prerequisite for participation in the study was the agreement of both the athlete and their coach. It was realized by both the subject and the researcher that adherence to the study protocol was on a strictly voluntary basis on the part of the subject, and that the subject could withdraw from the study of his or her own accord at any time. Informed consent was obtained from each athlete wherein the voluntary nature of their participation was indicated. It was stated, as well, that the researcher may choose to end a subject's participation should any symptoms of overtraining (as

described in the definition of overtraining in Chapter I) become apparent. A sample consent form is included in Appendix A.

The subjects were studied during the base work phase of their training. This phase generally involves relatively low intensity, high volume training.

STUDY PROTOCOL

The study consisted of a maximum of four weeks of monitored training, during which each subject was required to keep a detailed log of his or her training and daily activities. Sample log forms are included in Appendix B.

During the first two weeks, the pre-study weeks, the athletes followed their normal training programme and completed the log forms on a daily basis. These two weeks served two purposes: (i) to permit identification of any abnormal activities prior to the study weeks which may affect the results, and (ii) to familiarize the subjects with the requirements and restrictions of the protocol. The third week was the control week during which training was maintained as close to the athlete's current regular programme as he or she could achieve. Daily blood samples were taken Monday through Saturday of this week and analyzed for serum CPK, LDH and ASAT activities, and hemoglobin and hematocrit levels. The fourth week or part thereof was the experimental period during which half the subjects were instructed to increase their training volume by as close to 10% as possible and the other half by as close to 20% as possible. Daily blood samples were analyzed for CPK, LDH, ASAT, hemoglobin and hematocrit levels. Subjects able to maintain this intended protocol throughout the control week and for an experimental period of three days or more, up to the one week maximum, were included in the analysis to follow.

Throughout the entire period of monitored training including the pre-study weeks, the control week and the experimental period, no attempt was made impose a common daily training schedule on the subjects. Subjects were requested to maintain their regular training programme and to follow the same daily sequence during the control and experimental weeks. This protocol was designed in order to obtain normal blood values for each subject following their normal training programme.

The range for the training volume increases during the experimental week was established as a result of personal communications with the coach of the majority of the subjects and with the knowledge of pilot study results. An increase in training volume of 20 to 25% was regarded as the maximum one could realistically expect an athlete to successfully maintain for a period of one week (personal communications with Ken Parker, C.T.F.A. level III distance coach). Pilot work showed elevations in serum enzyme activities to be no greater after a one week increase in training volume of 19% as compared with 23%. A 25% change was therefore deemed unnecessary and 20% accepted for the largest increase. It was further concluded from previous experience and pilot study data that normal variations in an athlete's training and deviations from the prescribed training preclude the use of any increments smaller than 10%.

The control period and the ideal experimental period were chosen as one week, beginning on Saturday and ending on Friday. One week is the period of one full cycle of training for the athletes used as subjects in this study. A weekly training cycle is common among athletes. Based on the literature review, the full effects (peak and recovery to normal of serum enzyme levels) of the initial days' increased training volume should be observed within one week. One week (seven days) thus corresponds to one

complete cycle of training and allows sufficient time to observe the full effects of the initial increase in training.

Three days was chosen as the minimum acceptable experimental period for further analysis. Findings in pilot work indicated that two days of increased training was required to observe any change in the serum enzymes being studied. In order to be able to obtain a progressive comparison at least two days of such possible changes were necessary. With a minimal experimental period of three days, the second and third day provide this opportunity.

It was not required that subjects complete a full one week experimental period to provide usable data for analysis. It was expected that the application of an intentional overtraining may cause subjects to be unable to continue for a full week at that training volume. Individual responses including fatigue levels and the ability to withstand fatigue were expected to vary. The possibility of subject attrition was therefore minimized by adopting a variable length experimental period, one week being the maximum intended period, with three days being the minimum acceptable period for inclusion in the analysis of results.

Blood samples were obtained from the subjects prior to training, on a daily basis, excluding Sunday. Pilot study data indicated that six days was sufficient time to establish the desired response in the blood. The use of a Sunday sample each week was eliminated since there could be no cumulative effect from one day's training. The first sample of each week was obtained on Monday, after the athletes had completed both Saturday's and Sunday's training, allowing two days for a cumulative response to show.

Daily Log

The daily information required from the subjects was recorded using three different log forms. These were entitled "General Information", "Training - Distance Run" and "Training - Track Workout" (see samples in Appendix B).

The General Information form included information on sleep, diet, physical activities, resting pulse, weight and subjective feelings. This form was filled out each day to obtain pertinent information on the subject's daily activities.

The two training forms were used for the recording of training sessions, one form being filled out after each training session. The Distance Run form was used in cases where the training session was a continuous steady paced run. On it, the time of day for the workout, the distance of the run, a description of the running route, the total time of the run, intermediate times on the run, weather and running conditions, and subjective feelings were recorded. The Track Workout form was used for all other training sessions with an intermittent, interval type nature to them. This included interval training on the track, circuit training, fartlek training and hill training. Information recorded on this form included details of the workout, time of day for the start and finish of the workout, location, weather and running conditions, and subjective feelings. Details of the workout were recorded on a chart which included training activity and time, and recovery activity and time for each interval.

Pre-Study Weeks

Weeks 1 and 2 of the four weeks of monitored training were termed the Pre-Study Weeks. During this time the subjects followed their normal training programme and

completed the daily log. This enabled the subjects to become familiar with the restrictions and requirements placed on them for the period of the study. It also served to establish some control level for their training.

Control Week

Week 3 of the study period was the control week. Each subject's training schedule for this week followed as closely as possible the "norm" established during the Pre-Study Weeks. Training, other activities and other information were recorded using the daily log forms.

Blood samples were taken on a daily basis, Monday through Saturday, from the subjects. Samples were taken prior to their daily training. A fasting state was not required and no restrictions were placed on diet prior to blood sampling. With minor exceptions blood samples were taken at the same time each day to avoid any effects from possible diurnal variations in enzyme levels.

Experimental Week

Week 4 of the study period was the experimental week. The experimental period ranged from a minimum of three days for some subjects to a maximum of seven days for others. The actual length of each subject's experimental period was determined by their ability to continue to follow the protocol, recognizing that the voluntary nature of their participation permitted their withdrawal at any time, and the individual responses to increased training may force abandonment of their programme.

At the end of week 3 the subjects were told to increase their training, by either 10% or 20%. The subjects were randomly chosen for one of these two groups. It was understood that actual increases may deviate from these

values to some extent, and that for each subject the actual increase would be calculated for each day and for the entire study period.

For each subject each day in the experimental period was matched to the corresponding day in the control week. It was required that matching days were the same in terms of (i) the time of day for the blood sampling; (ii) the training (except for the prescribed increase); and (iii) other physical activities. Aspects of training that were matched were (i) time of day for the training session; (ii) running route, for distance runs; and (iii) pace, for both distance runs and track workouts. The subjects referred to their week 3 log to ensure correct matching in each of these aspects.

To adjust training volume to the increased level, distance runs and track workouts were individually changed. Volume was measured in terms of running time for distance runs. Running mileage would serve as the best measurement of volume, however the impracticality of obtaining accurate mileage measurements required the substitution of a more practical measurement, hence running time was used and running pace was monitored. After completing the matched run from the previous week the subject was required to add on extra running time at the end of the run to reach the required increase in training volume. The pace throughout the run was to be kept the same as in the control week and was monitored at checkpoints enroute. It was also required that the pace of the extra distance at the end did not change from that of the rest of the run.

Interval workouts were run at the same pace as during the matched workout of the control week. Additional intervals of the same distance, pace and rest interval were added on at the end of the workout to increase the volume by

the correct percentage. An integral number of intervals was added, and some discrepancy in the exact percentage increase was necessarily accepted.

The time of day for blood sampling was required to be the same as in the control week, as were any physical activities unrelated to training, in order to minimize external influences on the enzyme levels.

Blood Sampling

Both the control week and the experimental period began on Saturday. Blood samples were taken daily, Monday through to one day after the final day of the experimental period during the experimental week. The first blood sample of the week, obtained on Monday morning, was taken after the first two days of the week's training. With the maximal experimental period of one week, the final blood sample was obtained on Saturday after the full week's training. For a shorter experimental period the final blood sample was correspondingly taken the day after completion of the entire experimental period. It was essential that samples be taken at the same time of day on corresponding days from weeks 3 and 4.

Most subjects' blood samples were taken at the University of Ottawa Kinanthropology Laboratory by qualified lab technicians. For the two subjects who were unable to be at the University, an agreement was reached with the nursing staff at their school or office to do the blood sampling.

Blood was drawn alternately from the veins of both arms to avoid excessive scarring, bruising and soreness. Approximately 2 ml was drawn into a heparinized tube for hemoglobin and hematocrit analysis, and 8 ml into a non-heparinized tube for clotting to extract serum for the enzyme determinations.

Blood Analysis Techniques

Two tubes of blood, one containing 2 ml of heparinized blood, the other containing 8 ml of non-heparinized blood were obtained daily from each subject.

The non-heparinized blood was allowed to clot for at least 20 minutes. After clotting the blood was centrifuged at 4750 r.p.m. for 15 minutes and the serum was drawn off the top of the clotted blood. This serum was used for the enzyme analyses, each of which was performed in duplicate.

The heparinized blood was used for the determination of hematocrit and hemoglobin and, as for the enzyme analyses, each test was performed in duplicate. Microhematocrit capillary tubes filled with blood were centrifuged at 11,700 r.p.m. for 10 minutes to separate the cellular mass from the plasma. Hematocrit was measured as the percentage of total blood volume occupied by cells.

Hemoglobin was determined using a laboratory cyanmethemoglobin reagent. 0.02 ml blood was mixed by gentle inversion in 5 ml cyanmethemoglobin solution. After allowing a minimum of 10 minutes for hemolysis to occur, the absorbance of the solution was measured at a wavelength of 540 nm using the Bausch and Lomb Spectrometer. A standard curve for hemoglobin was determined using DMA (Data Medical Associates Inc.) cyanmethemoglobin standard solution. Fixed hemoglobin concentrations of 0, 5, 10, 15 and 20 mg/100ml were obtained by mixing the appropriate volumes of the standard and control solutions and the absorbance of each obtained solution was measured in the spectrometer. A least squares fit to a straight line was determined for a plot of the absorbance versus the hemoglobin concentration. The equation for this line was used to calculate hemoglobin

concentrations of the serum samples from their absorbancy readings. The hemoglobin concentration was expressed in mg/100ml blood.

CPK Analysis

The Boehringer Mannheim kit #126357 was used for the analysis of CPK activity. The principal reactions of the method are as follows:

$$CP + ADP \xleftarrow{CPK} C + ATP$$

$$ATP + GLUCOSE \xleftarrow{HK} ADP + G\text{-}6\text{-}P$$

$$G\text{-}6\text{-}P + NADP \xleftarrow{G\text{-}6\text{-}P\ DH} 6\text{-}PG + NADPH + H$$

Using this kit, the serum to be analyzed is added to a solution in which the final concentrations after the serum is added are: imidazole buffer 100mM, pH 6.7 (30 deg C); glucose 20mM; magnesium acetate 10mM; ADP 2.0mM; AMP 5.0mM; CP 30mM; NADP 2.0mM; NAC 2mM; APP 10mM; EDTA 2.0 mM; HK >2.5U/ml (25 deg C); G-6-P DH >1.5U/ml (25 deg C).

The formation of NADPH is measured spectrophotometrically at 340nm, at which wavelength NADPH demonstrates a relative maximum in absorbancy. For the reaction to proceed to the formation of NADPH the working solution for the test must contain CP, ADP, glucose, HK and G-6-P DH. Szasz et al. (1976) showed optimal conditions for maximal CPK activity and minimal lag time to exist in a solution containing CP 30mM, NADP 2.0mM, glucose between 5 and 100mM, ADP between 2 and 4mM, HK greater than 2000U/l and G-6-P DH greater than 1500U/l. The optimal pH for CPK was found to be 6.7 to 6.8 and the buffer for stabilizing the solution's pH after the serum is added, was found to be 100mM. Both

magnesium salts and sulfhydryl compounds are required for the activation of CPK in the serum. Szasz et al. (1976) found N-acetyl cysteine (NAC) to be the most effective thiol compound in reactivating serum CPK with a concentration of 20mM being suitable. Magnesium acetate in concentrations of 10 to 20mM produced the highest CPK activity of the salts tested.

Serum adenylate kinase (AK, also called myokinase) catalyzes the reaction:

$$2\ ADP \xleftrightarrow{AK} AMP + ATP$$

The production of ATP via this reaction interferes with the reaction sequence producing NADPH from CP, ADP, glucose and NADP. The s-AK must be inhibited in order to obtain valid s-CPK measurements. High AMP concentrations inhibit AK, but if too high, also inhibit CPK. APP also inhibits AK. Concentrations of 5mM and 10mM for AMP and APP respectively were found to be optimal for the inhibition of AK without any inhibition of CPK (Szasz et al. 1976).

A mixture ratio of 2:100 for serum:test solution was used by Szasz et al. and is used in this procedure. 0.05 ml serum is added to 2.5 ml test solution at 37 degrees C. The solution was maintained at 37 degrees in a water bath. The absorbancy of the solution at 340 nm was measured after one minute lag time and again three minutes later. From these measurements the change in absorbancy per minute was calculated.

Enzyme activity is described in International Units per litre (U/l). 1 U/l is defined as the amount of enzyme required to convert one micromole (umole) of substrate to product in one minute at standard temperature and pressure (STP). At 340 nm, the absorbancy coefficient for NADP is 6.22 cm2/umole (Rosalki 1967). This means that with a light

path of 1 cm and with 1 ml of solution the reduction of 1 umole of NADP produces an increase of 6.22 in absorbancy. As there is a one-to-one correspondence between CP conversion and NADP reduction, CPK activity is calculated as:

$$\text{CPK (U/l)} = \frac{\text{A/min} \times \text{assay volume (ml)} \times 1000\ \text{ml/l}}{\text{abs. coeff. (cm2/umole)} \times \text{light path (cm)} \times \text{specimen volume (ml)}}$$

so

$$\text{CPK activity (U/l)} = \frac{\text{A/min} \times 2.55\ \text{ml} \times 1000\ \text{ml/l}}{1.1\ \text{cm2/umole} \times 1\ \text{cm} \times 0.05\ \text{ml}}$$

$$= \text{A/min} \times 82000\ \text{umole/l}$$

LDH Analysis

The Boehringer Mannheim kit #124915 was used for the analysis of LDH activity. The reaction catalyzed by LDH, and on which this analysis is based, is:

$$\text{pyruvate} + \text{NADH} + \text{H+} \xleftrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}$$

This procedure uses pyruvate as the substrate for the LDH catalyzed reaction, following the natural equilibrium of the reaction and permitting a faster reaction rate. Using lactate as substrate gives equal accuracy in measurement though values determined in this manner are approximately 30% of the activity obtained using pyruvate as substrate (Gay et al. 1968).

With the kit, 0.1 ml serum is added to 2.5 ml of a working solution which, when prepared with the serum, contains 50 mM phosphate buffer, pH 7.5; 0.6 mM pyruvate;

and 0.18 mM NADH. The German Society for Clinical Chemistry (1972) has determined that these concentrations are optimal for conversion of pyruvate to lactate in the presence of LDH.

Reduction of pyruvate to lactate and oxidation of NADH occur in equimolar quantities. NADH oxidation rate can be measured spectophotometrically at a wavelength of 340 nm at which wavelength it exhibits a relative maximum in absorbancy.

NADH oxidation was measured over a period of three minutes. As with CPK analysis the solution was kept at 37.0 degrees C. in a water bath. The initial reading was taken after a one minute lag time, the final reading three minutes later.

LDH activity is calculated using the same formula as for the calculation of CPK activity. At 340 nm the absorbancy coefficient of NADH is 6.3 cm2/umole. A light path of 1 cm is used, assay volume is 2.6 ml and specimen volume is 0.1 ml giving a final equation of:

$$LDH\ (U/l) = A/min \times 4127$$

There appears to be conflicting information on the stability of LDH in serum. To avoid possible loss of activity with storage, LDH should be analyzed as soon as possible after obtaining the specimen (Demetriou et al. 1974), as was done in this study.

LDH activity is much higher in erythrocytes than in serum. Hemolysis will interfere significantly with the test procedure and no visible hemolysis should therefore be tolerated. Barely visible hemolysis has been shown to increase LDH activity by 15 to 20 percent (Demetriou et al. 1974).

ASAT Analysis

ASAT was analyzed using the Boehringer Mannheim kit #124435. The test is based on the following two reactions:

$$\text{a-KG} + \text{L-aspartate} \xrightleftharpoons{\text{ASAT}} \text{L-glutamate} + \text{OAA}$$

$$\text{OAA} + \text{NADH} + \text{H+} \xrightleftharpoons{\text{MDH}} \text{L-malate} + \text{NAD}$$

Alpha-ketoglutarate (a-KG) and aspartate react in the presence of ASAT to form glutamate and oxaloacetate (OAA). To prevent the reaction from reaching equilibrium, removal of one of the products is necessary. In the presence of NADH and MDH, the product OAA is reduced to malate; at the same time NADH is oxidized to NAD. It is the oxidation of NADH that is measured spectrophotometrically at a wavelength of 340 nm, at which point NADH exhibits a maximum in absorbancy.

The assay mixture, using the Boehringer Mannheim kit, is obtained by the addition of 0.5 ml of serum to 2.5 ml of a working solution containing a-KG, aspartate and NADH. The final assay mixture contains a phosphate buffer, 80 mM, pH 7.4; L-aspartate 200 mM; MDH > 0.6 U/l; LDH > 1.2 U/l; NADH 0.18 mM; and a-KG 12 mM.

The buffer is necessary to stabilize the pH at 7.4, the value determined by the German Society for Clinical Chemistry (1972) to be optimal for ASAT activity. The buffer was found to permit maximum activity at low concentrations, hence 80 mM was recommended. The same study showed ASAT activity to level off at maximum for aspartate concentrations greater than 200 mM and a-KG concentrations greater than 12mM. Activity of ASAT quickly reached maximal

values at very low NADH and MDH concentrations. 0.18 mM NADH and 0.6 U/l MDH were well within the range producing maximal activity.

The addition of LDH to the assay mixture is required to prevent interference with the test by serum pyruvate. LDH levels greater than 1.2 U/l were found to be adequate for this purpose.

The activity of ASAT in this test procedure is linearly related to the rate of NADH oxidation. As both test reactions proceed to completion the deamination of 1 mole of aspartate is accompanied by the formation of 1 mole of OAA and the subsequent reduction of the OAA to 1 mole of malate. During this latter process, 1 mole of NADH is oxidized. Measurement of NADH oxidation spectrophotometrically is therefore truly representative of ASAT activity.

The oxidation of NADH was measured over a period of three minutes after allowing one minute lag time prior to the initial reading. The temperature of the reaction mixture was kept constant at 37 degrees C. in a water bath.

Activity of ASAT was calculated as for CPK and LDH. The absorbancy coefficient for NADH at 340 nm is 6.3 cm2/umole, the specimen volume is 0.5 ml, the assay volume is 3.0 ml and the light path is 1 cm, giving the final equation:

$$\text{ASAT (U/l)} = \text{A/min} \times 952$$

According to the instructions accompanying the kit, hemolysis interferes with ASAT analysis, presumably due to the addition of red blood cell enzymes to the serum. The degree of interference is not discussed.

ASAT is not stable over a long period of time in the serum, and for this reason the test, as for the others, was performed on the same day the blood sample was obtained.

STATISTICAL ANALYSIS

In order to compare the control week values with the experimental period values of each of the three enzymes and of hemoglobin, it was desired that the effects of dehydration that can sometimes be seen in runners was eliminated. The need for taking this into account was confirmed by the observation that hematocrit values showed a fluctuation of between 2% and 5% for individual subjects during the control week alone. For this reason enzyme and hemoglobin concentrations during the experimental period were adjusted to discount any effects of hemoconcentration.

Based on the assumption that all changes in hematocrit were due entirely to fluid shifts, and that the total cellular mass of the blood remained constant, the enzyme activity levels of the experimental period were adjusted by a factor of:

$$\frac{HCTc}{HCTe} \times \frac{(100 - HCTe)}{(100 - HCTc)}$$

where HCTc and HCTe represent control period and experimental period hematocrit values, respectively. The hemoglobin concentrations of the experimental period were adjusted by a factor of:

$$\frac{HCTc}{HCTe}$$

Derivations of both factors can be found in Appendix D. These adjusted values were then used to make the comparisons between control and experimental period data.

The training performed by the subjects was analyzed descriptively. Training volume was determined in terms of the total time for running. The weekly volume for each

subject was calculated and the week's percentage increase in training volume was calculated based on the total volume for each week.

The results of the blood analysis and the corresponding training were analyzed descriptively for each subject and on a group basis. The variation in the length of the experimental period for the subjects and the number of subjects able to maintain the protocol for the minimum experimental period of three days precluded the application of significance testing. As the intention of the study was to observe and describe the effects of the increased training volume on serum enzymes and hemoglobin and hematocrit, a descriptive analysis of the results was considered the best approach.

CHAPTER IV

RESULTS AND DISCUSSION

It was the purpose of this study to observe and describe the effects of selected increases in training volume in competitive middle- and long-distance runners on serum activity levels of the enzymes creatine phosphokinase (CPK), aspartate aminotransferase (ASAT) and lactate dehydrogenase (LDH), and on hemoglobin concentration and hematocrit levels. This chapter discusses the results of the study with sections on the individual subjects, the actual training they performed and their blood components over the two week study period.

A major obstacle over the course of this study was the high rate of subject attrition both prior to and during the two week study period. This is the topic of the first section of this chapter.

Following the completion of the study period the subjects were ranked in ascending order according to the percentage increase in volume that their training showed from the control to the experimental week. Subjects were numbered S1 to S9 according to their ranking. The second section of this chapter is an overview of the training performed by the group of subjects.

After the overview of the general data on the group each of the subjects is examined on an individual basis. Progressing from S1 through to S9 the training and blood measurements are discussed. In the text, when discussing changes in enzyme, hemoglobin and hematocrit levels, increases and decreases on a given day refer to changes from the control to the experimental period.

The results of the study for the nine individual subjects are then compared and possible groupings of subjects in terms of similar data are proposed.

SUBJECT ATTRITION

Nine subjects completed the experimental period of three days or more, making their data useful for further analysis. A number of additional athletes were willing and scheduled to participate but had to opt out due to various circumstances.

Subject attrition was a significant obstacle throughout the course of the study. The high demands placed on the subjects, especially in terms of the length of time over which their activities were controlled and monitored, was perhaps the major factor which contributed to this situation. Athletes scheduled to act as subjects for this study had to opt out at various stages - some prior to even the initial two weeks of recording their training data, some during this two week period and some during the control week during which blood samples were taken. Factors which contributed to subject attrition, both prior to and during the study period, were beyond the control of the researcher. Injury and illness contributed to a high degree. Unforeseen job and personal commitments including out-of-town travel and inability to be present for blood sampling were other factors. Excessive fatigue during the experimental period caused one subject to be unable to continue with the prescribed training protocol and to withdraw voluntarily. Circumstances influencing the subjects are discussed on an individual basis in the discussion of the training data.

TRAINING DATA

A tabular summary of data on the nine subjects is given in Table III. Included is information on sex and age of subjects, and their overall training during the control and experimental periods. Daily training volume for the study period can be found in the individual subject tables along with blood analysis data.

The percentage change in training volume from the control to the experimental period of the study varied over the nine subjects from an increase of 9% in subject S1 to a maximum of a 26% increase in subject S9. The average increase for the group was 13.7%. The actual percentage increase was determined using the total number of minutes of training during the experimental period and the total number of minutes of training in the corresponding days of the control week.

In labelling the days of the week, Day 1 of the experimental week was the first day of the experimental period. Days were then numbered in chronological order. Day 1 of the control week was the same day of the week as Day 1 of the experimental week.

TABLE III: SUBJECT DATA

SUBJECT	SEX	AGE (YRS)	EXPERIMENTAL PERIOD (DAYS)	PERCENT INCREASES (CONTROL TO EXPERIMENTAL PERIOD)				
				TRAINING VOLUME	CPK	ASAT	LDH	HB
S1	F	26	3	9	91	9	-10	0
S2	M	41	5	10	13	-8	8	1
S3	F	23	6	10	-14	6	6	1
S4	F	22	7	14	69	22	11	-1
S5	F	19	5	19	-19	1	0	2
S6	M	29	7	19	105	52	9	2
S7	M	28	7	20	2	5	11	1
S8	M	27	7	22	56	19	0	1
S9	M	23	7	26	0	11	4	0

Table III: SUBJECT DATA Subjects were numbered S1 through S9 by ranking them in ascending order according to their percentage increase in training volume from control to experimental period. Values for percent increase were determined as the percent increase in the mean daily values from the control to the experimental period for each of the parameters. Experimental period enzyme and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations).

SUBJECT S1

Subject S1 completed three days of increased training during the experimental week. On the fourth day she incurred a minor injury which necessitated the abortion of that day's training followed by a short-term lay-off to prevent further damage to the injured foot.

On the two days of blood sampling during the experimental period, Days 3 and 4, this subject showed a large increase in CPK values from control to experimental week. ASAT responded to a lesser degree, although an increase was still apparent. A decrease was seen in LDH on Day 4 while the change on Day 3 was too small to be of importance as the magnitude of the change was within the limits of measurement error.

It is interesting to note the drop in CPK from control to experimental week on Day 5, the day following the aborted training, when the training volume was cut to almost 50% of the control value. Although ASAT was not lower than control week on the same day, it showed no increase and thus a return to control values after an elevation on the previous two days. Hematocrit also showed a substantial decrease on Day 5.

TABLE IV(A): SUBJECT S1: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/1) C	E	%INC	ASAT (U/1) C	E	%INC	LDH (U/1) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	130	140	8															
2	*	44	47	8															
3	*	57	65	14	170	350	+105	28	31	+11	290	280	-7	13.6	13.8	+1	40	39	-3
4		59	31	-47	170	300	+76	27	29	+7	320	270	-16	13.6	13.5	-1	40	41	+3
5					340	190	-44	30	29	-3	310	320	+3	13.8	14.2	+3	42	39	-7
6																			
7																			
8																			

Table IV(a): SUBJECT S1: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT S2

Subject S2 completed five days of training during the experimental week after which he was required to be out of town for a number of days and therefore was unable to participate in the blood sampling on the remaining two days.

The change in training volume was somewhat variable from day to day for this subject. On Day 6 of the experimental week, however, he was unable to complete his normal training due entirely to fatigue. The decrease seen in his training volume was thus 100%. His training log indicates an increasing fatigue level throughout the experimental week leading to exhaustion on Day 6. Over four days of blood sampling, Days 3 through 6 during the experimental week an increase of 10 to 29% was seen in CPK except on Day 3 where no change was observed. ASAT also showed an increase of 13 to 25% on three of the four days. LDH appeared to increase on two of the four days by 7 to 19%. A definite increase in hemoglobin was seen on Days 5 and 6 while no definite pattern of change was noted for hematocrit.

TABLE IV(B): SUBJECT S2: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/1) C	E	%INC	ASAT (U/1) C	E	%INC	LDH (U/1) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	29	32	+11															
2	*	102	116	+14															
3	*	69	68	- 1	200	190	- 5	20	24	+20	260	270	+ 4	16.4	16.2	-1	47	47	0
4	*	16	20	+25	170	220	+29	20	25	+25	270	320	+19	17.3	17.0	-2	48	47	-2
5	*	67	73	+ 9	180	220	+22	21	22	+ 5	270	280	+ 4	16.2	16.9	+4	48	48	0
6		32	0	-100	210	230	+10	23	26	+13	280	300	+ 7	16.5	17.2	+4	47	45	-4
7																			
8																			

Table IV(b): SUBJECT S2: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT S3

Subject S3 completed six days of training during the experimental week. Due to the recurrence of a minor injury she was unable to complete the seventh day of training. Blood samples were taken on Days 3 through 8.

An increase in CPK was noted on Day 4 while a large decrease was observed on Days 6 and 7. It should be noted that in addition to training this subject played strenuous tennis on Day 6 of both weeks, a sport to which she was unaccustomed. She complained of muscular soreness the following day, especially during the control week. This exertion could account for the sudden large increase in CPK on Day 7 of both weeks. As the volume and intensity of the play were not monitored specifically, differences between the two weeks could have accounted for the drop in CPK from control to experimental week on the following day.

ASAT is seen to increase on Days 3 and 4. Again a drop is noted on Day 6. Although the levels on Day 7 are the highest values of the entire week, as for CPK, the difference between Day 7 and the rest of the week is not as outstanding as for CPK.

Throughout the study period LDH showed no large changes, except on Day 4 where an increase of 23% was observed. Hemoglobin increased on only one day of the week; the same observation was made for hematocrit.

TABLE IV(C): SUBJECT S3: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/1) C	E	%INC	ASAT (U/1) C	E	%INC	LDH (U/1) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	28	32	+11															
2	*	75	82	+ 8															
3	*	27	30	+10	160	170	+ 6	22	28	+27	300	330	+10	13.8	14.0	+1	41	38	-7
4	*	0	0	—	110	130	+18	19	24	+26	260	320	+23	13.7	13.8	+1	40	39	-3
5	*	56	62	+11	90	100	+11	17	17	0	270	270	0	13.7	14.1	+3	40	41	+3
6	*	28	31	+11	170	120	-29	24	19	-21	290	280	- 3	14.1	14.1	0	40	41	+3
7		54	0	-100	500	370	-23	30	31	+ 3	290	290	0	14.3	14.4	+1	40	40	0
8					300	210	-30	29	23	-21	280	260	- 7	13.3	13.2	-1	38	40	+5

Table IV(c): SUBJECT S3: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT S4

Subject S4 completed seven days of training during the experimental week. However, on two intermediate days, Days 4 and 5, the training was reversed during the experimental week as compared to the control week. During the control week the subject ran for 27 minutes on Day 4 and did not run on Day 5. Due to commitments at work she was unable to run on Day 4 of the experimental week and ran for 33 minutes (22% more than 27 minutes) on day 5. Blood values for Days 5 and 6 have been disregarded for the purpose of this discussion.

An increase in CPK was seen at both the beginning and the end of the study period for this subject. The comparison of control and experimental weeks on Days 5 and 6 is not valid due to the minor change in training as described above. The increase on Days 7 and 8 may be affected to some extent by this deviation from protocol as well.

Large increases in ASAT were seen on Days 3 and 4. It is interesting to note that no change in ASAT was seen on Day 7 though a 50% increase was again observed on the following day.

LDH followed the pattern of ASAT for subject S4. An increase was seen on Days 3 and 8, disregarding as invalid Days 5 and 6. Both hematocrit and hemoglobin appeared relatively consistent from control to experimental week with the exception of a decrease in hemoglobin from 14.7 to 14.1 on Day 4.

TABLE IV(D): SUBJECT S4: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/1) C	E	%INC	ASAT (U/1) C	E	%INC	LDH (U/1) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	60	67	+12															
2	*	80	88	+10															
3	*	30	33	+10	230	530	+130	19	31	+63	290	380	+31	14.4	14.3	-1	42	41	-2
4	*	27	0	(+22)	150	270	+80	18	24	+33	310	350	+11	14.7	14.1	-4	40	39	-3
5	*	0	33	(+22)	160	110	-31	19	14	-26	300	280	- 7	12.8	12.8	0	37	39	-3
6	*	52	62	+19	120	160	+33	17	20	+18	270	320	+19	14.0	14.5	+4	40	39	-3
7	*	30	34	+13	130	230	+77	17	17	0	300	290	- 3	13.5	13.4	-1	39	41	+5
8					100	200	+100	14	21	+50	290	330	+14	13.8	13.7	-1	40	40	0

Table IV(d): SUBJECT S4: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT S5

Due to minor illness, the initial day of the study period was postponed by two days for S5. Five days of training were therefore completed during the experimental week.

On the first three days of blood analysis, Days 3 through 5, a decrease or no signifciant change was seen in all enzyme levels, CPK showing the largest negative change, ASAT showing a decrease on Day 3 and LDH showing no changes. Hemoglobin, on the other hand, showed an increase on the first two of these days while hematocrit showed no change. It is possible that the two days of decreased activity and training due to illness prior to the initial study were contributing factors to the results seen. By Day 6 of the experimental week subject S5 did show positive changes in both CPK and ASAT. On this day and the previous day as well, a 7% decrease in hematocrit was observed.

TABLE IV(E): SUBJECT S5: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/l) C	E	%INC	ASAT (U/l) C	E	%INC	LDH (U/l) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	44	52	+18															
2	*	0	0	—															
3	*	40	47	+18	100	60	-40	17	14	-18	220	210	- 5	13.6	14.3	+5	41	41	0
4	*	14	18	+29	120	80	-33	19	18	- 5	230	240	+ 4	14.6	15.0	+3	42	41	-2
5	*	36	43	+19	170	110	-35	22	22	0	260	250	- 4	15.0	14.8	-1	43	40	-7
6					130	170	+31	17	22	+29	230	240	+ 4	14.1	14.4	+2	41	38	-7
7																			
8																			

Table IV(e): SUBJECT S5: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT S6

Subject S6 completed the full seven days of both the control and the experimental week with no deviation from the desired protocol. Changes in training volume on a daily basis were reasonably consistent over the study period, ranging from 16 to 20%.

Increases in CPK were seen on all six days during which blood was sampled. The change in CPK increased from 19 to 167% over the initial three days. In the final days there may have been a tendency towards a lesser increase from control to experimental week.

ASAT would appear to have followed a pattern similar to that of CPK though the magnitude of the relative changes are only one third to one half those of CPK. The peak increase in ASAT occurred on Day 6 with a 67% increase while CPK showed a peak of 167% on Day 5.

LDH showed an increase of 13 to 22% on Days 5 through 8 with no changes earlier in the week. No trend can be established from these data.

Large variations were seen in hematocrit, especially on Days 3, 4 and 5. As these changes are not consistent in their direction, no conclusions can be drawn. Hemoglobin changes also showed a wide variation over the week as did hemoglobin values themselves. No consistency or pattern was seen for these results.

TABLE IV(F): SUBJECT S6: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/l) C	E	%INC	ASAT (U/l) C	E	%INC	LDH (U/l) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	22	26	+18															
2	*	78	92	+18															
3	*	59	71	+20	210	250	+19	24	30	+25	350	330	- 6	17.4	17.2	-2	51	48	-6
4	*	50	59	+18	210	370	+76	24	29	+21	270	270	0	16.2	16.5	+2	46	49	+7
5	*	44	51	+16	240	640	+167	24	49	+104	300	350	+17	16.1	17.1	+6	48	45	-6
6	*	65	78	+20	230	490	+113	27	45	+67	320	360	+13	17.1	16.7	-2	49	49	0
7	*	37	44	+19	200	500	+150	27	42	+56	270	330	+22	16.5	16.8	+2	49	48	-2
8					220	430	+95	28	40	+43	270	310	+15	16.3	16.8	+3	48	46	-4

Table IV(f): SUBJECT S6: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT S7

Subject S7 completed the full seven days of the experimental week's training. This subject was unique in his training regime in that he trained only four days per week. It is interesting to note the pattern in the absolute values of CPK during both weeks. A significantly lower value was seen on each of the days following a non-training day.

The only days on which an increase in CPK from control to experimental week is seen was on Days 3 and 4. A decrease was seen on Day 7.

ASAT showed increases on Days 3, 6 and 8, the largest being recorded on Day 8. LDH registered increases on Days 3, 6, and 8, matching the pattern of ASAT. It is possible that the unusual pattern of changes seen in this subject was the result of the different training regime he was following.

Hemoglobin values increased on Days 3 through 5 and again on Day 8 with decreases on both other days. Hematocrit showed a large increase on Day 7, a decrease on Day 3 and little change on the other days.

TABLE IV(G): SUBJECT S7: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/l) C	E	%INC	ASAT (U/l) C	E	%INC	LDH (U/l) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	65	78	+20															
2	*	86	102	+20															
3	*	0	0	—	250	300	+20	27	30	+11	340	430	+26	15.8	16.5	+4	46	43	-6
4	*	47	53	+14	150	170	+13	24	24	0	300	330	+10	15.6	15.9	+2	45	44	-2
5	*	0	0	—	210	200	- 5	23	22	- 4	330	330	0	15.1	15.5	+3	44	43	-2
6	*	47	60	+28	140	140	0	21	23	+10	310	370	+19	16.4	15.9	-3	45	45	0
7	*	0	0	—	210	180	-14	24	22	- 8	330	320	- 3	15.4	14.8	-4	43	48	+10
8					160	150	- 6	23	29	+26	300	340	+13	15.6	15.9	+2	45	43	-4

Table IV(g): SUBJECT S7: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT S8

Seven days of training were completed by subject S8 in the experimental study period. He showed a relatively consistent increase in CPK over the entire six day blood sampling period. A similar pattern of consistent change was seen in ASAT over the entire study period. LDH showed little or no change throughout the week.

Hemoglobin and hematocrit were unaffected by the change in training although an increase of 3% was seen in hemoglobin on Day 5 and a decrease of 6% was seen in hematocrit on Day 4.

TABLE IV(H): SUBJECT S8: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/l) C	E	%INC	ASAT (U/l) C	E	%INC	LDH (U/l) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	118	151	+28															
2	*	55	69	+25															
3	*	48	51	+ 6	130	210	+62	25	34	+36	280	280	0	16.0	16.1	+1	49	48	-2
4	*	94	116	+23	120	200	+67	25	28	+11	290	290	0	16.1	16.2	+1	49	46	-6
5	*	49	55	+12	140	220	+57	24	29	+21	280	280	0	15.5	15.9	+3	48	49	+2
6	*	30	40	+33	160	240	+50	27	30	+11	290	280	- 3	15.6	15.5	-1	47	48	+2
7	*	0	0	—	150	220	+47	25	31	+24	280	290	+ 3	15.1	15.3	+1	47	47	0
8					130	200	+54	25	28	+12	270	270	0	15.4	15.4	0	46	45	-2

Table IV(h): SUBJECT S8: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT S9

Although seven days of training were completed by subject S9 in the experimental week, he was unable to be present for the blood sampling on the final day of the study due to the onset of illness the previous evening. Thus five days of blood analysis were used.

A small increase in CPK was seen on Day 6 while no changes were seen on the other days of the week. ASAT, on the other hand, showed an increase on four days of the week, the largest being on Days 4 and 6.

LDH appeared to show a trend towards increasing positive changes over the study period. A decrease of 19% was observed on day 3, progressing to an increase of 19% on Days 6 and 7.

Hemoglobin appeared largely unaffected by the changes in training volume while hematocrit showed a decrease on two of the five days, the largest being a drop of 12% on Day 6.

TABLE IV(I): SUBJECT S9: TRAINING AND BLOOD ANALYSIS DATA

DAY		TRAIN (MIN) C	E	%INC	CPK (U/l) C	E	%INC	ASAT (U/l) C	E	%INC	LDH (U/l) C	E	%INC	HB(mg/100ml) C	E	%INC	HCT (%) C	E	%INC
1	*	70	85	+21															
2	*	32	36	+12															
3	*	0	0	—	160	150	- 6	18	20	+11	330	300	- 9	16.3	16.0	-2	47	48	+2
4	*	50	67	+34	140	140	0	18	22	+22	330	290	-12	17.2	17.1	-1	50	49	-2
5	*	60	74	+23	180	180	0	21	20	- 5	340	350	+ 3	17.0	17.3	+2	50	47	-6
6	*	41	57	+39	150	170	+13	21	25	+19	320	380	+19	17.8	17.9	+1	51	45	-12
7	*	56	70	+25	160	150	- 6	19	21	+11	310	370	+19	17.1	17.1	0	50	49	-2
8																			

Table IV(i): SUBJECT S9: TRAINING AND BLOOD ANALYSIS DATA

Days marked with an asterisk are included in the experimental analysis. Experimental period enzymes and hemoglobin values are adjusted for variations in hematocrit (see Appendix D for derivations). Raw data is included in Appendix C. [C = control period; E = experimental period; %INC = percentage increase from control to experimental period for that day.]

SUBJECT COMPARISONS

Of the nine subjects, seven can be divided into two groups of approximately equal training volume increases. Subjects S1, S2, and S3 showed 9 to 10% increases and subjects S5, S6, S7 and S8 showed 19 to 22% increases. Subjects S4 and S9 with 14 and 26% increases respectively do not fit into either of these groups of similar increases.

The three subjects with 9 and 10% training volume increases all showed CPK increases initially (excluding Day 3 for S2). Later in the week only S3 has available data and here a decrease in CPK was seen. All three also showed an increase in ASAT paralleling that of CPK. No consistent change in LDH or hemoglobin was seen.

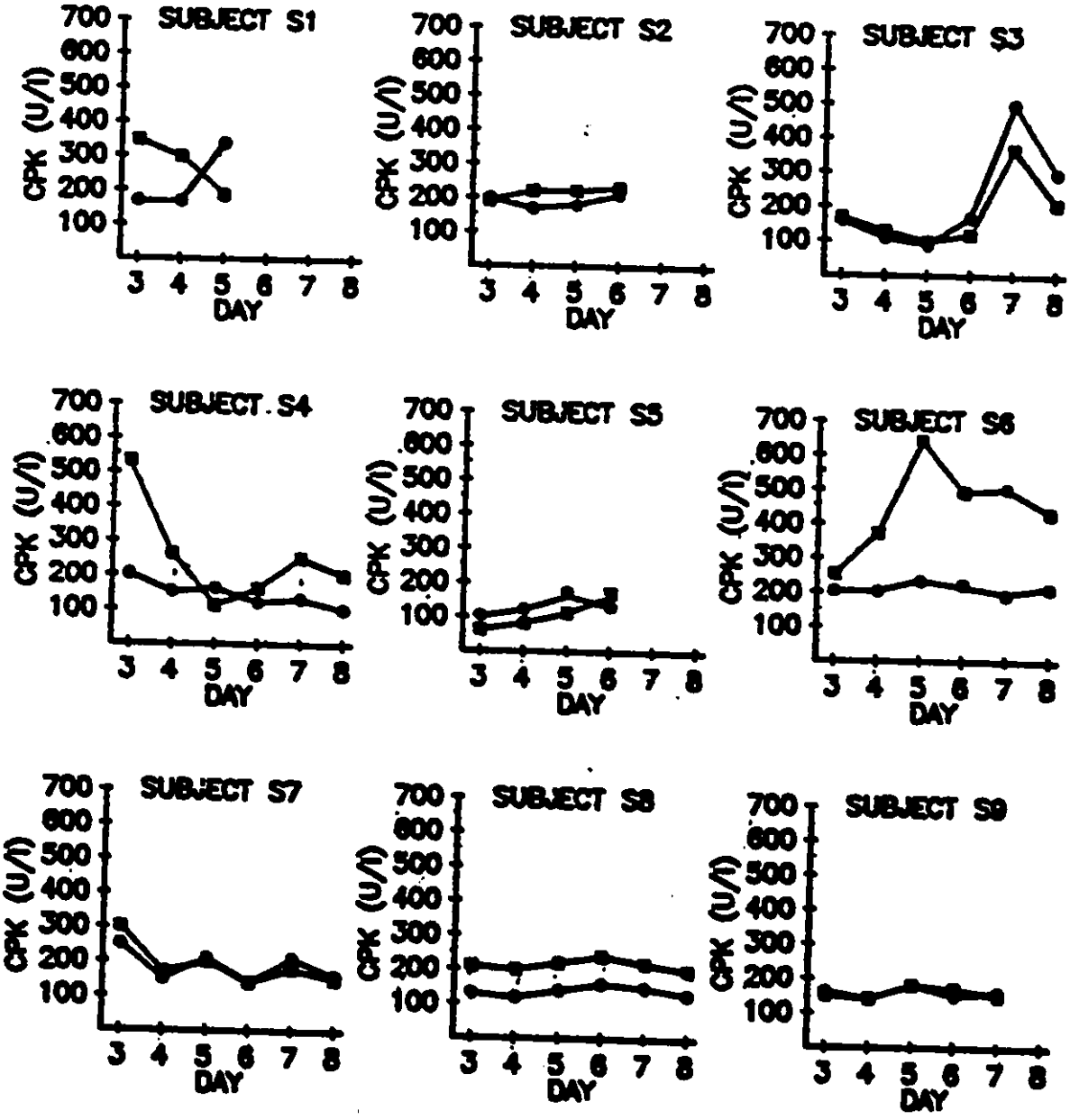
Of the four subjects clustered around a 20% increase, S6 and S8 showed consistent increases throughout the week in both CPK and ASAT but not in LDH or hemoglobin. Neither S5 nor S7 showed the same consistency, however, in the case of S5, the rest period prior to the start of the experimental week may account for the drop seen and in the case of S7 the unusual training regime of running only four days per week may account for the lack of increase.

S9, who maintained the greatest increase in training volume, showed no consistent increase in any of the enzymes, except for a minor trend in ASAT.

As can be noted from the comparison of subjects, the results obtained from this study were not entirely consistent across the sample of athletes. This indicates the presence of a number of cofactors which the researcher was unable to eliminate. It would be possible to make conjectures regarding the reasons for individual variations in the responses between subjects, however such conjectures would only be calculated guesses of the cofactors involved. Parameters that may have effects on the results of this

study are age, sex, previous training epxerience, and performance level. In addition, the type of training presently underway, the total volume, the number of training sessions per week, and the training volume in relation to prior training volumes may affect results. Once the parameters have been examined and their effects on training responses established, the cofactors can be eliminated from future studies on the effects of increases in training volume on serum enzyme activity levels.

FIGURE 1: COMPARISON OF CONTROL AND EXPERIMENTAL CPK VALUES FOR EACH SUBJECT

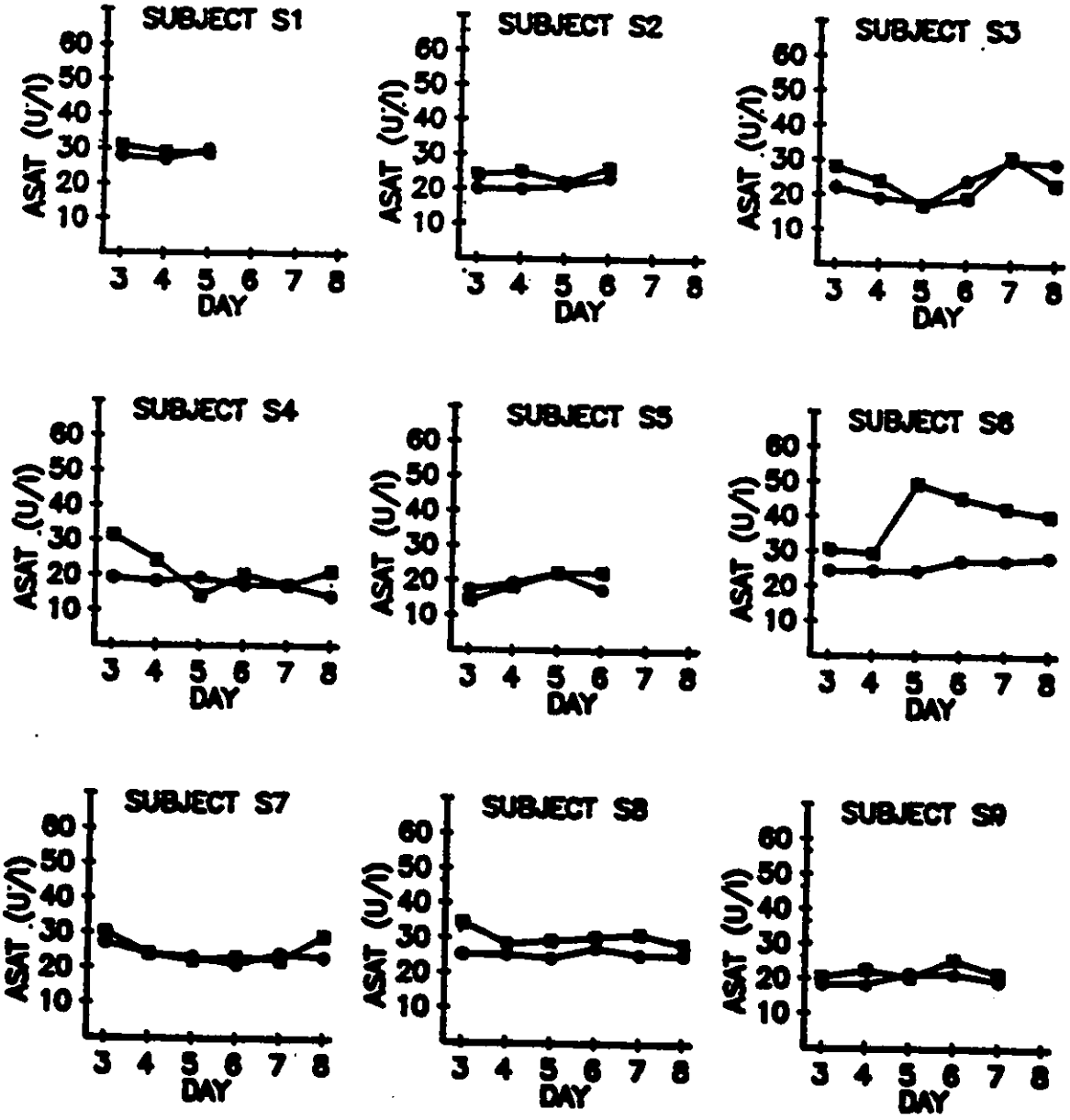


LEGEND:

CONTROL PERIOD VALUES

EXPERIMENTAL PERIOD VALUES

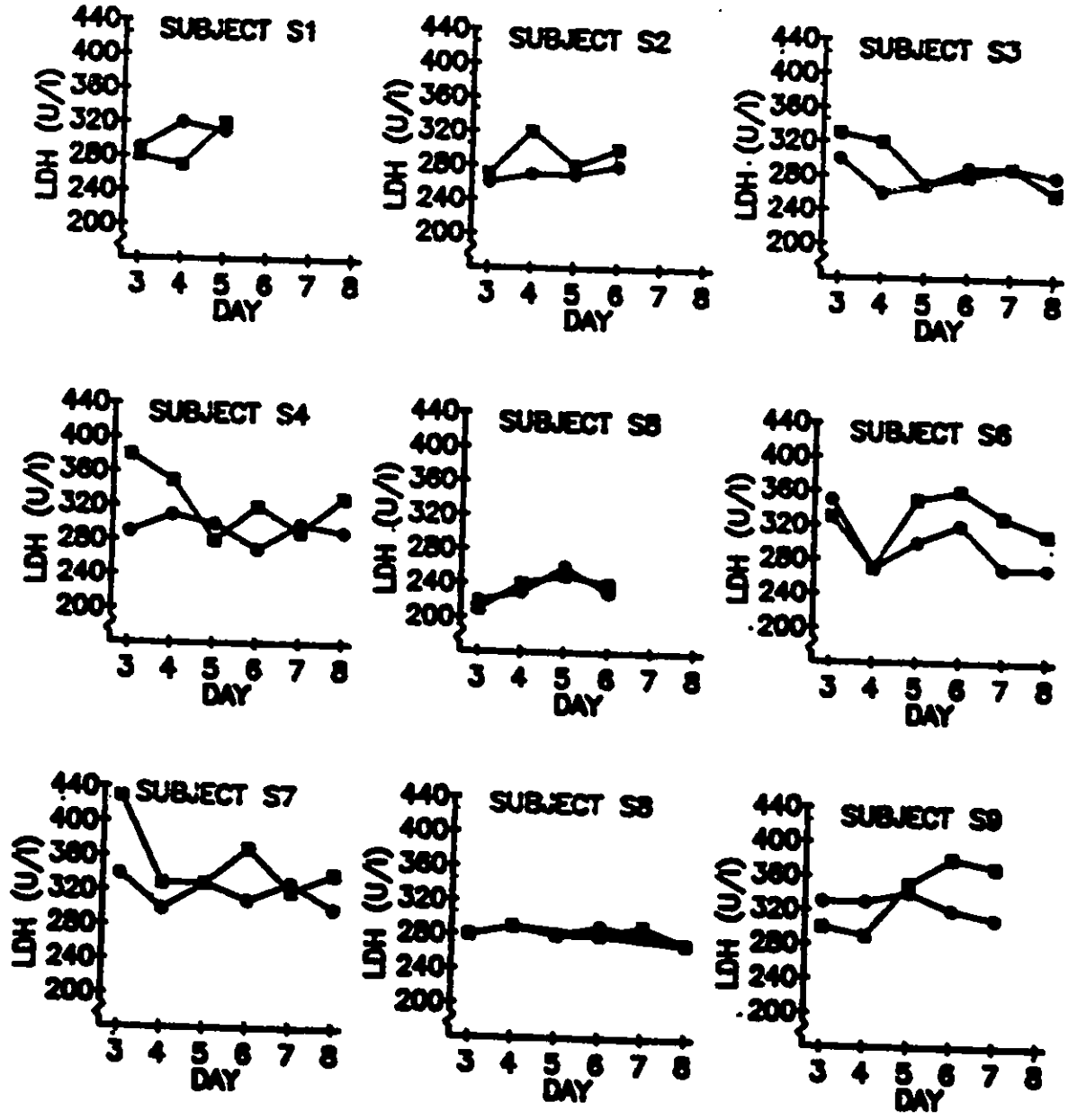
FIGURE II: COMPARISON OF CONTROL AND EXPERIMENTAL ASAT VALUES FOR EACH SUBJECT



LEGEND: CONTROL PERIOD VALUES

EXPERIMENTAL PERIOD VALUES

FIGURE III: COMPARISON OF CONTROL AND EXPERIMENTAL LDH VALUES FOR EACH SUBJECT



LEGEND: CONTROL PERIOD VALUES

EXPERIMENTAL PERIOD VALUES

COMPARISON OF FIVE BLOOD COMPONENTS

The five blood components measured throughout this study, CPK, ASAT, LDH, hemoglobin and hematocrit have been shown to differ in their responses to an increase in training volume. That different responses have been observed suggests a number of interesting points.

CPK was seen throughout the subjects to show a greater response to the increased training during the experimental period than either ASAT or LDH. ASAT, in many cases appeared to follow a response pattern similar to that of CPK, but with proportionately smaller changes. LDH, when considered over the entire study period for all subjects did not register any consistent trend in its response to the increased training volume. The comparison of the responses of the three enzymes in this study is consistent with that observed throughout the literature available on serum enzyme responses to exercise of various types, namely that serum enzyme levels increase with increasing exercise stimuli and that of the three enzymes studied, CPK is the most responsive, LDH is the least responsive, and ASAT is intermediate between the other two in its responsiveness to such stimuli.

Hemoglobin, a protein frequently used in the assessment of training levels in the past, showed no consistent response to the increased training volume experienced by the subjects in this study. In light of the fact that CPK and ASAT showed a consistent trend in their responses to the same training stimulus, the application of hemoglobin measurements in monitoring training levels must be closely examined. It would appear from the results of this study that hemoglobin is much less responsive to increases in training volume than either CPK or ASAT, at least over a

period of a week or less. It may respond to a more extended period of increased training volume. As an early indicator of overtraining its usefulness is questionable.

Hematocrit showed no consistent response to the increased training volume experienced by the subjects in this study. Whether hematocrit responds over a longer period of increased training remains a question to be considered.

The different responses observed for the three serum enzymes CPK, LDH, and ASAT and hemoglobin and hematocrit not only indicate a probable preference for CPK or ASAT as an indicator of increased training volume, but also either contra-indicate a common stimulus for the response or indicate differing mechanisms of response.

COMPARISON OF RESULTS WITH PREVIOUS STUDIES

The results of this sudy reflect an inconsistency in the response of serum enzymes to exercise that is also seen in comparing the studies reviewed previously. The volume of literature isolating specific parameters is small. The trend seen in this study toward an increase in CPK and ASAT values with an increased training volume would be supported by the literature which suggests that longer duration exercise causes greater increases in CPK (King et al. 1976, Ahlborg & Brohult 1967) and ASAT (Allard & Highman 1961, Schnohr 1974).

Discrepancies between findings of previous studies are evident in the review of the literature. It was suggested that these differences may be due to variations in the fitness activities of subjects, the intensity of exercise, or the daily activities of subjects, among other factors. This study was designed in a way that permitted subjects to train following their regular schedule. As such exercise

intensity, exercise volume and non-running activities were not standardized for the subjects, possibly resulting in a wide range of serum enzyme values.

The review of literature generally supports the findings of this study that serum CPK is the most responsive to exercise while LDH is the least responsive of the 3 enzymes studied. King et al. (1976) found CPK to show greater increases after exercise than either ASAT or LDH. Schnohr (1974), in his study of runners in a 100 km event, found CPK to show the greatest increase, and LDH to show the least increase of the three enzymes CPK, ASAT and LDH after completion of the run.

Actual values for CPK, ASAT and LDH seen in this study are not always in concurrence with those seen in previous studies. As Table II shows there has been a wide range of techniques used to analyze serum for these enzymes. Units used to express activity levels are also inconsistent. This fact makes it difficult to compare absolute values observed in this study with values found in the literature. In accordance with this no such comparison is attempted.

EFFECTS OF REST DAYS

Although the observation of the effects of rest days (where training volume is 0) on serum enzymes was not the purpose of this study, rest days are commonly used as a means of recovery, and their effect on serum enzyme activity levels warrants discussion. In particular, rest days were consistently followed by a decrease in CPK values. ASAT and LDH did not always demonstrate the same decrease following a rest day. This lack of response is consistent with the commonly observed lesser response of ASAT and LDH to stimuli, as compared to CPK.

SUMMARY

A trend toward an increase in both CPK and ASAT levels was seen as a result of increased training volume. The degree of the response did not appear to show any consistent relationship to the degree of the increase in training volume. CPK showed the greatest responsiveness while ASAT tended to follow a similar response curve, but proportionately smaller. LDH, the third serum enzyme studied, showed no consistent response to the training changes. These findings are generally supported by the findings of previous studies on serum enzyme responses to exercise. That no relationship between the level of training increase and the serum enzyme response was observed is not consistent with the literature, however a larger sample size and greater variations in the training increases may provide better insight into this matter.

Neither hemoglobin nor hematocrit showed any consistent response to the increased training volume. This would suggest that hemoglobin levels are not a good early indicator of training response, and that their response is not the same as that of the serum enzymes, CPK and ASAT.

The differences in the responses of the five blood components indicate differing mechanisms of response and/or different stimuli for their response to training.

The findings of this initial study on the response of serum enzymes to increases in training volume indicate that further study in the area is warranted. Recommendations for further study follow with the conclusions of this study.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Within the limitations of this study of serum enzymes in middle- and long-distance runners, the following conclusions appear justified:

(1) Of the serum enzymes measured in the present study, CPK was the most responsive to the individual increases in training volume.

(2) ASAT was also responsive to individual increases in training volume but to a lesser degree than CPK.

(3) LDH showed no consistent response to the increases in training volume experienced by the subjects in this study;

(4) Hemoglobin concentrations and hematocrit levels showed no consistent response to the increases in training volume experienced by the subjects in this study;

A number of questions remain to be answered with regard to the response of serum enzymes to changes in training volume.

(1) Is their response subject to differences in (a) age; (b) sex; (c) training background and experience; or (d) performance levels?

(2) Does the response reach a peak, and if so, after how many days? Do enzyme levels return to normal after a certain period of continued increased training, and if so, after how long?

(3) Is there a minimum change in training volume required before a serum enzyme response is noted, and is there any correlation between the size of the change in training volume and the size of the enzyme response?

There is a need for further study of the response of serum enzymes to increases in training volume in middle- and long-distance runners. Based on the results of this study and the many questions arising from it, a number of recommendations can be made at this time for consideration in future studies of serum enzymes during changes in training.

(1) A large population is required from which to draw subjects, and a large subject sample size is required.

(2) Correlations between serum enzyme changes with training volume increase and each of age, sex, training experience, and performance level should be examined.

(3) Future studies may benefit from studying groups of subjects following identical training regimes. Such groups may be found in university teams training together on a daily basis.

(4) The time course of enzyme response to training volume changes should be studied to eliminate the need in future studies for daily blood samples and any unnecessarily extended study period.

APPENDIX A

SERUM ENZYMES IN OVERTRAINING

CONSENT FORM

I,_____, the undersigned, agree to participate in the study of serum enzymes in competitive middle and long distance runners in training and overtraining. I understand that I may be asked to increase my training volume (mileage) by up to 20 percent for one week and that for this week and the control week immmediately preceding, I will be required to give a venous blood sample of approximately 10 ml. each day. I understand that it will be necessary for me to keep a detailed log of my training and other activities for these two weeks and for the two weeks immediately preceding the study.

I realize that I shall be permitted to opt out of the study at any time and that under conditions of overstress I may be asked to discontinue my participation. I understand that there may be some risk involved in the taking of blood samples though these are minimal, and that soreness and bruising of the arm may result from the repeated testing.

By signing this form, I certify that I have read the information package concerning the study and my participation in it and have had the opportunity to have my questions answered by the researchers.

Date: _____ **Subject:** _____
(signature)

Witness:
(signature)

N.B. Form must be signed by parent or guardian as well as by subject, for subjects under 19 years of age.

DAILY LOG

GENERAL INFORMATION

SUBJECT _____

WEEK # _____ DAY _____ DATE _____

SLEEP

Time to bed last night _____

Time up this morning _____

Hours in bed _____

Hours of sleep _____

Sleep during day (list time of day, hours in bed, hours asleep) _____

Number of training sessions today _____

Resting pulse last night _____

Resting pulse this morning _____

Weight this morning _____

DIET

Meal/Snack	Time	Food

PHYSICAL ACTIVITIES

(list type of activity, start and finish time, intensity, effort, distance (eg. swimming, skiing), number of games (eg. squash), weight, reps. (eg. Nautilus workout), etc.)

SUBJECTIVE FEELINGS

COMMENTS

DAILY LOG
TRAINING - DISTANCE RUN

SUBJECT _____

WEEK # _____ DAY _____ DATE _____

ROUTE (describe fully)

Starting Time _____

Time for Run _____

Distance _____

WARM-UP/WARM-DOWN

INTERMEDIATE TIMES (approximately every 15 minutes)

Check Points	Time

WEATHER AND ROAD CONDITIONS
(during run)

SUBJECTIVE FEELINGS

COMMENTS

DAILY LOG

TRAINING - TRACK WORKOUT

SUBJECT _____

WEEK # _____ DAY _____ DATE _____

N.B. Use this form for track, hill, fartlek, circuit workouts

Start Time _____

Finish Time _____

Location _____

WEATHER AND ROAD CONDITIONS
(during workout)

WORKOUT (include warm-up, stretching, warm-down, interval distances, times, recovery times, recovery activity (jog or walk, etc.))

ACTIVITY	TIME	RECOVERY	TIME

SUBJECTIVE FEELINGS

COMMENTS

APPENDIX C

RAW DATA

SUBJECT S1:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	31	220	280	14.3	42
TUES.	28	170	290	13.6	40
WED.	27	170	320	13.6	40
THURS.	30	340	310	13.8	42
FRI.	27	250	300	14.4	42
SAT.					
MON.	29	280	300	13.6	40
TUES.	30	340	270	13.5	39
WED.	30	310	280	13.8	41
THURS.	26	170	290	13.2	39
FRI.					
SAT.					

SUBJECT S2:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	20	200	260	16.4	47
TUES.	20	170	270	17.3	48
WED.	21	180	270	16.2	48
THURS.	23	210	280	16.5	47
FRI.	24	220	290	14.3	46
SAT.	21	150	270	16.0	47
MON.	24	190	270	16.2	47
TUES.	24	210	310	16.6	47
WED.	22	220	280	16.9	48
THURS.	24	210	280	16.5	45
FRI.					
SAT.					

SUBJECT S3:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	22	160	300	13.8	41
TUES.	19	110	260	13.7	40
WED.	17	90	270	13.7	40
THURS.	24	170	290	14.1	40
FRI.	30	500	290	14.3	40
SAT.	29	300	280	13.3	38
MON.	25	150	290	13.0	38
TUES.	23	120	310	13.5	39
WED.	18	100	280	14.5	41
THURS.	20	130	290	14.5	41
FRI.	31	370	290	14.4	40
SAT.	25	230	280	13.9	40

SUBJECT S4:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	19	230	290	14.4	42
TUES.	18	150	310	14.7	40
WED.	19	160	300	12.8	37
THURS.	17	120	270	14.0	40
FRI.	17	130	300	13.5	39
SAT.	14	100	290	13.8	40
MON.	30	510	360	14.0	41
TUES.	23	260	340	13.7	39
WED.	15	120	300	13.5	39
THURS.	19	150	310	14.1	39
FRI.	19	250	320	14.1	41
SAT.	21	200	330	13.7	40

SUBJECT S5:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	20	180	240	14.1	42
TUES.	19	160	230	14.4	42
WED.	17	100	220	13.6	41
THURS.	19	120	230	14.6	42
FRI.	22	170	260	15.0	43
SAT.	17	130	230	14.1	41
MON.	18	100	250	13.9	39
TUES.	18	170	280	14.2	42
WED.	14	60	210	14.3	41
THURS.	17	80	230	14.6	41
FRI.	19	100	220	13.8	40
SAT.	19	150	210	13.3	38

SUBJECT S6:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	24	210	350	17.4	51
TUES.	24	210	270	16.2	46
WED.	24	240	300	16.1	48
THURS.	27	230	320	17.1	49
FRI.	27	200	270	16.5	49
SAT.	28	220	270	16.3	48
MON.	27	220	290	16.2	48
TUES.	33	420	300	17.6	49
WED.	43	570	310	16.0	45
THURS.	45	490	360	16.7	49
FRI.	40	480	320	16.5	48
SAT.	37	400	290	16.1	46

SUBJECT S7:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	27	250	340	15.8	46
TUES.	24	150	300	15.6	45
WED.	23	210	330	15.1	44
THURS.	21	140	310	16.4	45
FRI.	24	210	330	15.4	43
SAT.	23	160	300	15.6	45
MON.	27	270	380	15.4	43
TUES.	23	160	320	15.5	44
WED.	21	190	320	15.1	43
THURS.	23	140	370	15.9	45
FRI.	27	220	390	16.5	48
SAT.	27	140	310	15.2	43

SUBJECT S8:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	25	130	280	16.0	49
TUES.	25	120	290	16.1	49
WED.	24	140	280	15.5	48
THURS.	27	160	290	15.6	47
FRI.	25	150	280	15.1	47
SAT.	25	130	270	15.4	46
MON.	33	200	270	15.8	48
TUES.	25	180	260	15.2	46
WED.	30	230	290	16.2	49
THURS.	31	250	290	15.8	48
FRI.	31	220	290	15.3	47
SAT.	27	190	260	15.1	45

SUBJECT S9:

DAY	ASAT (U/1)	CPK (U/1)	LDH (U/1)	HB mg/100ml	HCT (%)
MON.	18	160	330	16.3	47
TUES.	18	140	330	17.2	50
WED.	21	180	340	17.0	50
THURS.	21	150	320	17.8	51
FRI.	19	160	310	17.1	50
SAT.	15	90	280	16.4	47
MON.	21	160	310	16.3	48
TUES.	21	130	280	16.8	49
WED.	18	160	310	16.3	47
THURS.	20	130	300	15.8	45
FRI.	20	140	360	16.8	49
SAT.					

APPENDIX D

DERIVATION OF ADJUSTMENT FACTORS

FOR HEMOGLOBIN AND ENZYMES

Enzyme and hemoglobin values were adjusted for each subject during the experimental period to discount any changes in fluid volume from consideration in the analysis of the data.

It was assumed that the cellular mass of the blood remained constant and that changes in hematocrit were due entirely to changes in the fluid volume of the blood.

As the comparisons being made in the analysis of the data were only between corresponding days of the control and experimental period, adjustments to permit comparison between days within either period were considered superfluous.

Given the following data for a subject on corresponding days of the control and experimental period:

Control Period, Day X:	Enzyme activity	=	Ec
	HB concentration	=	HBc
	Hematocrit	=	HCTc
Experimental Period, Day X:			
	Enzyme activity	=	Ee
	HB concentration	=	HBe
	Hematocrit	=	HCTe

Adjusted Values, Day X: Enzyme activity = E'e
HB concentration = HB'e

calculations of adjusted values were performed as explained below.

Symbols used:

BV = Blood volume
C = Cellular volume of blood
S = Serum volume
e (as subscript) = experimental period, Day X
c (as subscript) = control period, Day X
' (as superscript)= values after adjustment for fluid shift from control to experimental period
t (as subscript) = total volume

$BV = C + S$

$C = BV \times HCT/100$ $\quad S = BV \times (100 - HCT)/100$

$BV = S \times 100/(100-HCT)$

By definition, serum volume of experimental Day X, Se, is being adjusted to equal serum volume of control Day X, Sc, therefore:

$$Se' = Sc$$

Also, since cellular volume, C, remains constant, by assumption, so:

$$Ce' = Ce = Cc$$

Thus: $BVe = Ce + Se$

$$BVe' = Ce' + Se' = Cc + Sc = BVc$$

And: $Ce = BVe \times HCTe/100$

$Cc = BVc \times HCTc/100$

Therefore: $BVe \times HCTe = BVc \times HCTc$

Or: $BVc = BVe \times HCTe/HCTc$

Also: $HBt = HB \times BV$

Or: $HB = HBt/BV$

So: $$HBe' = \frac{HBte}{BVe'} = \frac{HBte}{BVc} = \frac{HBte}{BVe} \times \frac{HCTc}{HCTe}$$

$$= \frac{HBte}{BVe} \times \frac{HCTc}{HCTe} = HBe \times \frac{HCTc}{HCTe}$$

Therefore the correction factor for determining the adjusted value for hemoglobin is:

$$\frac{HCTc}{HCTe}$$

For enzyme adjustments, since enzyme activity is measured in the serum,

$$Et = E \times S$$

And therefore: $$E = Et/S$$

$$Se' = Sc = \frac{100 - HCTc}{100} \times BVc$$

And: $$BVc = BVe \times \frac{HCTe}{HCTc}$$

Therefore: $$Se' = \frac{100 - HCTc}{100} \times BVe \times \frac{HCTe}{HCTc}$$

$$= \frac{100 - HCTc}{100} \times Se - \frac{100}{100 - HCTe} \times \frac{HCTe}{HCTc}$$

$$= Se \times \frac{HCTe}{HCTc} \times \frac{100 - HCTc}{100 - HCTe}$$

And: $$Ee' = \frac{Ete}{Se'} = \frac{Ee \times Se}{Se'}$$

$$= Ee \times Se \times \frac{1}{Se} \times \frac{HCTc}{HCTe} \times \frac{100 - HCTe}{100 - HCTc}$$

Therefore the correction factor for enzyme concentrations is:

$$\frac{HCTc \times (100 - HCTe)}{HCTe \times (100 - HCTc)}$$

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