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UNIVERSITY OF ALBERTA

THE INFLUENCE OF MAXIMAL AEROBIC POWER ON THE RATE OF RECOVERY FROM ANAEROBIC EXERCISE

by

SUZETTE ROSE COOKE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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ABSTRACT

Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) was used to investigate the influence of maximal aerobic power ($\dot{V}O_{2max}$) on the recovery rate of human gastrocnemius muscle from high-intensity, anaerobic exercise. The $\dot{V}O_{2max}$ of 21 males was measured during treadmill exercise and subjects were subsequently assigned to either a low aerobic power (LAP) group (n = 10) or a high aerobic power (HAP) group (n = 11) Mean (± S.E) $\dot{V}O_{2max}$ of the LAP and HAP groups were 46.6 (± 3.6) and 64.4 (± 4.5) $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. A work capacity test of pedal ergometry was used to familiarize subjects with the exercise pattern and to determine the maximal cumulative load required to assign the same relative exercise intensity to each subject for the MRS protocol. One week after the work capacity test, subjects performed the rest (4 minutes), exercise (2 minutes) and recovery (10 minutes) MRS protocol in a Philips 1.5 Telsa whole body MRS scanner. Relative concentrations of phosphocreatine (PCr) and inorganic phosphate (Pi) were measured throughout the protocol and pH was determined from the chemical shift between Pi and PCr. Mean resting pH was identical (7.07) for both the LAP and HAP groups. Exercise produced a similar mean pH of 6.45 for the (HAP) group and 6.38 for the (LAP) group. PCr depletion (75%) was evident in both groups. No differences in the rate of recovery of PCr and pH were found between groups using a non-linear regression model or one-way analysis of variance. Similarities in the rate of metabolic recovery between the two groups suggest that $\dot{V}O_{2max}$ is a poor predictor of the rate of recovery from high-intensity, anaerobic exercise. However, differences in recovery rate clearly existed between individuals with similar $\dot{V}O_{2max}$ implying that other factors may influence recovery. Such factors may include aerobic capacity, fibre type, and/or the type of activity performed following exercise. It is suggested that the elucidation of the influence of each of these factors on recovery rates following anaerobic exercise be considered for future investigations.

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CHAPTER I
INTRODUCTION

Overview of the Problem

Short term, high-intensity exercise has been defined by Sahlin (1992) as exercise of an intensity that would elicit greater than 90% of $\dot{V}O_{2max}$. Several factors have been implicated as the cause of muscular fatigue resulting from exercise of very high intensity and brief duration (< 2 minutes). The factors which appear to cause fatigue during this type of exercise are typically associated with either the "depletion of high energy phosphagens" or "accumulation of metabolites" hypotheses.

The traditional view (Saltin and Karlsson, 1971) has been that the primary causative agent of fatigue from short term, high-intensity exercise is the accumulation of anaerobic metabolites, specifically lactate and/or dissociated hydrogen ions (H^+). Lactic acid, the by-product of anaerobic glycolysis dissociates into lactate and H^+, and it is the accumulation of unbuffered H^+ and consequent decrease in pH which is believed to cause fatigue. Several studies have provided evidence to support this hypothesis (Nakamura and Schwartz, 1972; Orchardson, 1978; Robertson and Kerrick, 1979; Dawson et al., 1980; MacLaren, 1989). Alternatively, other evidence has been presented which suggests that insufficient energy (in the form of high-energy phosphagens) may also be the basis for fatigue (Sahlin, 1992).

It is important to recognize that both of these hypotheses may be correct and interrelated. During high-intensity exercise of a very short duration (up to ~ 20 seconds), ATP and PCr may be rapidly hydrolyzed without significant accumulation of H^+ and inability to maintain peak power may be due to depletion of PCr. During anaerobic exercise of longer duration (~ 20 seconds - 2 minutes), both PCr depletion and accumulation of H^+ appear to cause fatigue. These factors may promote fatigue in an independent and/or coincidental manner. However, some evidence suggests a cause-effect relationship exists. An elevated concentration of H^+ can impair the contraction process indirectly through an inhibitory effect on the ATP resynthesis processes. The decrease in pH associated with the accumulation of H^+ will promote inhibition of the enzyme, phosphofructokinase (PFK) causing a reduction in glycolysis (MacLaren, 1989; Sahlin, 1992). A decline in pH (due to

the accumulation of H^+) can also reduce the ability to resynthesize ATP through a decrease in the PCr level by way of the creatine kinase equilibrium (Sahlin, 1986). In high-intensity exercise, a high level of lactate is associated with a low level of PCr and possible direct effects of the H^+ metabolite on the contractile process cannot be differentiated from possible indirect effects through impairment of energetic processes (Sahlin, 1992).

The ability of muscle to recover sufficiently to permit more high-intensity exercise, depends at least in part, on the resynthesis of PCr and the removal of hydrogen ions (Sahlin et al., 1983). Restoration of the metabolic environment is a complex process, consisting of an initial fast phase and secondary slow phase (Sahlin et al., 1978). The initial fast phase appears to represent removal of sufficient hydrogen ion to reinstate ATP-resynthesis processes (especially PCr) and restore events associated with muscle contraction. Near maximal recovery in force generation parallels restoration of energy substrates (Sargeant and Dolan, 1987). The time course for restoration of energy supply is rapid: PCr half-time about 30 seconds (Sahlin, 1992). In sharp contrast, the second phase representing elimination of remaining hydrogen ion and lactate is much slower (Metzger and Fitts, 1987). The half-time recovery of pH appears to occur within a range of five to ten minutes as demonstrated in studies by Fitts, (1977); Metzger and Fitts, (1987); Pan et al. (1991); Sahlin (1992); Kuno and Itai, (1992). Since removal of hydrogen ion and lactate from the muscle appears to affect both phases of recovery, this process will be examined in greater depth.

Several pathways exist for the removal of hydrogen ion and lactate. Within the muscle, hydrogen ions may be buffered and reduced (Parkhouse and MacKenzie, 1984), while lactate may be converted back to pyruvate to be utilized as an oxidizable substrate (Jansson et al., 1990). Transport mechanisms also exist which serve to remove hydrogen ion from the muscle and transport lactate to other organs to be oxidized (liver, heart, kidney and skeletal muscle). Lactate clearance through oxidation and gluconeogenesis removes both the proton and lactate anion, so that the clearance of lactic acid has an "alkalinizing effect" on acid-base balance, further promoting ATP generation in the face of H^+ accumulation (Roth, 1991). Pan et al. (1991) found that in the early stages of recovery (up to 20 minutes) the "buffering" of hydrogen ions is dominated by mechanisms of transport and metabolism. If these mechanisms of transport and metabolism can be enhanced, recovery of muscle after two minutes of high-intensity exercise should be accelerated.

Confirmation and elucidation of such a theory would be valuable in developing optimal training strategies for athletes. Enhancing this capacity within muscle might serve to speed the recovery process. Such strategies would be particularly beneficial to athletes engaged in sports in which short episodes of high-intensity exercise are interspersed with brief rest periods. In sports such as ice hockey and volleyball the outcome of the overall performance is influenced by the ability to recover quickly from short bouts of repeated, high-intensity exercise.

Aerobic training may constitute one strategy for the purpose of enhancing the ability of muscle to accelerate the rate of recovery from anaerobic exercise. Support for this approach comes from studies in which a systematic program of aerobic training has been shown to produce peripheral adaptations including increased oxidative capacity within the muscle (Abernethy et al., 1990; Kent-Braun et al., 1990), amplified myoglobin and capillary density (Saltin and Rowell, 1980), an enhanced ability both to remove and buffer lactic acid from exercising muscle (Sahlin and Hendriksson, 1984) and an elevated lactate clearance rate (Donovan and Pagliassotti, 1990; Freund et al., 1992). Review articles by Wagner (1991), Sjogaard (1984), Davies et al. (1981) and Saltin and Rowell, (1980) also support these findings. Such adaptations may serve to speed the recovery rate by increasing the oxidative capacity of the muscle and by enhancing the rate of removal of hydrogen ion and lactate from the muscle. Concurrently, central adaptations resulting from aerobic training occur in the oxygen transport system. These adaptations include an increase in stroke volume and cardiac output as well as enhanced oxygen extraction from the blood by skeletal muscles, all combining to produce a significant increase in $\dot{V}O_{2max}$ (Saltin and Rowell, 1980). Based on these central adaptations (improved transport) and peripheral adaptations (enhanced oxidative capacity and removal) in response to aerobic training, it might be expected that individuals with high aerobic power would demonstrate a faster recovery from anaerobic exercise than individuals with low aerobic power.

At the applied level, many coaches of sports which possess a large anaerobic component presently incorporate aerobic conditioning into their training program for the purpose of enhancing recovery. Yet, at present there appears to be little scientific evidence which would support this practice. Some exercise physiologists (for example, Wenger, 1981 and Thoden et al., 1991) mention the possibility of a potential cause-effect relationship between aerobic training and recovery from anaerobic performance, yet only a few

investigators (McCully et al., 1989; Jansson et al., 1990; Oyono-Enguelle et al., 1990; Hakkinen and Myllyla, 1990) appear to have investigated this relationship. Methodological limitations in the aforementioned studies prevent strong conclusions from being made. These methodological limitations include failure to quantify aerobic fitness levels (McCully et al., 1989; Hakkinen and Myllyla, 1990), use of a very small sample size (McCully et al., 1989), acquisition of a limited (transient) view of recovery using muscle biopsies (Jansson et al., 1990) and secondary measurement sites (venous blood lactates) of recovery (Oyono-Enguelle et al., 1990). Despite the limitations, all of these studies found evidence in support of the proposal that aerobic training may enhance the rate of recovery from anaerobic exercise. The aim of this study was to investigate this relationship by utilizing an improved methodology. Measurement of aerobic power, a more accurate and comprehensive measurement of recovery rate and a greater number of subjects were considered to be important as these elements dramatically affect any results and conclusions.

The purpose of this study was to answer the question:

Is there a difference in the rate of recovery from anaerobic exercise between subjects with high or low levels of maximal aerobic power ($\dot{V}O_{2max}$) ?

To assist in the investigation of this question, two hypotheses were tested:

1. Following two minutes of high-intensity exercise, there will be a difference in the rate of PCr resynthesis and restoration of pH between subjects with high and low levels of maximal aerobic power ($\dot{V}O_{2max}$).

2. Following two minutes of high-intensity exercise, the rate of PCr resynthesis and restoration of pH will be faster in subjects with a high level of maximal aerobic power ($\dot{V}O_{2max}$).

Delimitations

To assist with the investigation of the experimental hypotheses the scope of this study was restricted to the following:

1. Twenty-one subjects;
2. Males ranging in age from 19-39 years old;
3. Subjects were selected on the basis of their likelihood of meeting the criteria of the high or low maximal aerobic power groups;
4. All subjects were from the Edmonton area; and
5. Data collection occurred between July, 1992 and October, 1992.

Limitations

Every attempt was been made to control for outside influences which may have affected the validity or reliability of the results. This study was conducted within the context of the following limitations:

1. Subjects who took part in this investigation did so on a voluntary basis and completed all measurements as requested. It was assumed that all subjects exerted a maximal effort during the tests, adhered to instructions regarding rest prior to laboratory measurements, and attempted to maintain their personal fitness level during the data collection period;
2. One limitation of the cross-sectional design is the assumption that changes in the independent variable were caused by the dependent variable. In this study the assumption is made that differences in recovery rate would be due to differences in maximal aerobic power ($\dot{V}O_{2max}$);
3. It was assumed that the test protocols were of sufficient specificity to test the experimental hypotheses. It was also assumed that the dependent variables, PCr and pH, would accurately detect and describe any differences in the rate of recovery of the subjects;
4. The use of a non-linear curve fitting computer program to plot the data collected graphically was assumed to describe adequately the differences in the recovery rate of the subjects;

5. Results are generalizable to individuals who can demonstrate a similar level of strength and power in ankle plantar flexion exercise; and,
6. ^{31}P-MRS does not permit distinction between slow-twitch fibre and fast-twitch fibres. It is possible that differences in recovery rate may simply reflect genetic disposition (i.e: proportion of fast-twitch to slow-twitch) as opposed to any effects derived from the status of maximal aerobic power.

Definitions

Throughout the ensuing text the following terms are used as defined below:

Fatigue: Failure to maintain the required or expected power output. In this study, subjects were exercised to fatigue during two different exercise protocols: a test of maximal aerobic power ($\dot{V}O_{2max}$) and a test of maximal plantar flexion work capacity (MPFWC). Failure was reached in these tests when the subject demonstrated one of the following:

1. inability to maintain the required pace:
   $\dot{V}O_{2max}$ - the inability to sustain the required power output (combination of treadmill belt speed and grade) during a work interval.
   MPFWC - the inability to produce one contraction every two seconds or inability to fully plantar flex at the ankle joint (depress the footplate the full distance).
2. voluntary cessation of exercise.

Maximal Aerobic Power ($\dot{V}O_{2max}$): The maximal rate of oxygen consumption as measured during a progressive exercise test to exhaustion.

Recovery: Restoration of selected metabolic variables (pH and PCr) to within 5% of pre-exercise (resting) conditions.

CHAPTER II
LITERATURE REVIEW

Introduction

Many sports activities rely heavily on anaerobic metabolism to provide the energy necessary for maximal muscular power. Some sport performances such as weight-lifting may require only one maximal contraction and allow for complete recovery. Other "intermittent" sports such as hockey, require the athlete to perform repeated maximal muscular contractions. During these intermittent sports, recovery between high-intensity bursts within a shift may not be complete or recovery time between shifts may be insufficient. For example, in hockey, one athlete might skate full-out to make the break-away while another will be caught by the rushing defender. The recovery status of the athlete may markedly limit or enhance his or her ability to perform optimally. As such the role of "recovery" becomes critical in intermittent sport performances. Acquiring an understanding of processes associated with fatigue and recovery may offer practical benefits to sport and may also contribute to a better understanding of muscle metabolism.

What factor(s) cause fatigue? What factors influence recovery? What training and performance strategies maximize the recovery process? The purpose of this literature review is to achieve the following:

1. Identify the limiting factors which contribute to peripheral muscular fatigue within the anaerobic alactic and lactic energy systems.
2. Ascertain factors that influence the recovery process.
3. Outline the central and peripheral adaptations which occur as a consequence of aerobic training.
4. Examine the potential role for aerobic training as a method of enhancing recovery from anaerobic exercise.
5. Describe magnetic resonance spectroscopy, the primary tool by which this question will be examined.

Causes of Peripheral Muscle Fatigue

Over the past several years, many definitions for fatigue have been suggested. The definition of fatigue proposed by the organizers of the Fifth International Symposium on Biochemistry of Exercise (Edwards, 1982) was: " the inability of a physiological process to continue functioning at a particular level and/or the inability of the total organism to maintain a pre-determined exercise intensity." A more recent definition which specifically addresses muscular fatigue will be used for the purposes of this review: "failure to maintain the required or expected power output " (Hultman and Sjoholm, 1986).

Location of Fatigue

Researchers have broadly organized fatigue into central and peripheral categories (Roberts and Smith, 1989; Kirkendall, 1990). Central fatigue refers to poor motivation, altered central nervous system transmission from the brain to the spinal cord and recruitment of muscle fibres via peripheral nerves. The focus of this literature review, however, will be on peripheral fatigue, also known as local fatigue. Peripheral fatigue refers to an interruption of the events occurring within the muscle itself, distal to the neuromuscular junction. These events are associated with contraction coupling and resynthesis of ATP (Brooks and Fahey, 1985; Vollestad and Sejersted, 1988).

Fatigue: The Potential Causes

Fatigue used to be easy to define. A muscle was stimulated, lactic acid was produced, and the tension level declined. It was hypothesized that a fixed level of lactate resulted in a fixed reduction of tension (Hill and Kupalov, 1929). The relationship appeared direct and lactic acid was considered to be the cause of fatigue. However, after several years of research and with the advent of more sophisticated measuring instruments, recent studies have implicated new limiting factors. Currently, a great deal of controversy continues to exist with regards to the physiological causes of local muscular fatigue. As Roberts and Smith (1989) state, " ... there does not appear to be a single definitive cause, but rather a spectrum of events which occur ". These events contribute to the development of local muscular fatigue with varying degrees of influence depending on the nature of the work task. The work task may be characterized by the following variables: 1) the duration

and the intensity of the work, 2) the form of the elicited contraction, 3) the predominantly recruited muscle fibre type, 4) the environmental conditions, and 5) the capacity of the individual (degree of training), Fitts et al., (1982) and Roberts and Smith (1989). Contemporary exercise physiologists now realize the etiology of fatigue is more complex than originally thought. Currently, researchers are searching to identify the individual factors and their degree of influence and interaction to delineate the causes of fatigue in various conditions.

Despite the complexity of fatigue, two fundamental principles of muscle metabolism provide direction in discerning potential causes of fatigue resulting from short term, high-intensity exercise. First, muscular contraction requires energy. Regardless of the intensity of exercise, energy supply and production must equal the energy consumption of the muscle, otherwise, the depletion of stored energy will force the intensity of exercise to be lowered or to stop altogether. Since ATP is the only direct source of energy available to the contractile process in skeletal muscle, a decrease or depletion in the pool of ATP available for contraction will severely limit the capacity of the muscle to work (Wenger and Reed, 1976). It is the responsibility of anaerobic metabolic pathways in skeletal muscle to provide enough energy in the form of ATP to meet the metabolic demands of muscle performing work of a high-intensity, short duration nature. Therefore, any factors which adversely alter the efficiency of the metabolic machinery in supplying ATP energy can lead to fatigue. Lack of sufficient energy and the factors which cause this deficiency constitutes the first primary hypothesis of muscle fatigue.

A second fundamental principle of muscle metabolism is that energy breakdown produces metabolic by-products. A series of metabolic events are required to produce a muscular contraction. Each event entails a biochemical reaction in which metabolic products and reactants are generated. Certain by-products stimulate metabolic processes to re-establish ATP levels. For example, inorganic phosphate is a by-product of the hydrolysis of ATP. It serves as a substrate for the production of energy in a number of other reactions. Yet, during intensive exercise high inorganic phosphate levels can interfere with the metabolic processes. Similarly, pyruvate may be converted to lactate when sufficient oxygen is not available. An accumulation of lactate and the associated release of H^+ ions also interferes with metabolism. In either case, by-products of energy metabolism (metabolites) may result in an interruption of continued muscle contraction (Kirkendall, 1990). These

metabolites may act directly on the metabolic machinery or may act indirectly by obstructing the resynthesis of the energy supply, ATP. The accumulation of metabolites constitutes the second primary hypothesis of muscle fatigue.

Energy Supply

The energy for muscular work comes from the high, free energy of hydrolysis of adenosine 5'-triphosphate (ATP) (Sahlin, 1978):

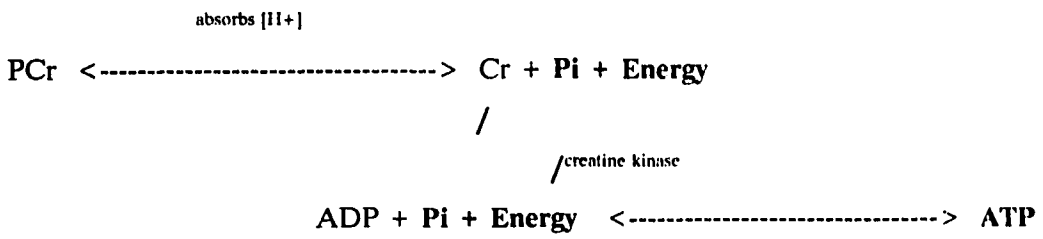
$$ATP + H_2O \text{ ----------> } ADP + Pi + Energy$$

A small amount of ATP is stored directly within the muscle. Additional ATP is supplied by: 1) Reduction of creatine phosphate (PCr), 2) transphosphorylation, 3) anaerobic glycolysis, and 4) oxidative phosphorylation (Yoshida and Watari, 1992). During short term, high-intensity exercise the first three systems are primarily responsible for supplying the energy required for the resynthesis of ATP. Because the focus of this review is on recovery from short term, high-intensity exercise, these energy supply systems will be examined in greater detail.

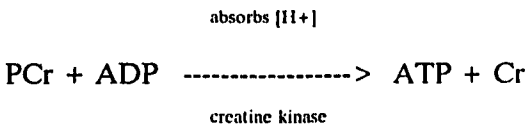
Intramuscular ATP Supply and Resynthesis of ATP by Breakdown of Phosphocreatine

Adenosine triphosphate (ATP) is the primary substrate for muscle contraction, therefore ATP stored directly within the muscle can provide instant energy. This premium fuel has the advantage of being available immediately, however, there is only enough ATP stored in the muscle and available for muscular contraction to last for a few seconds. The capacity of this system is extended by two ATP resynthesis mechanisms.

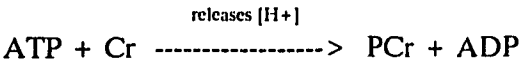
The principal mechanism of ATP resynthesis is through transfer of chemical energy to another high-energy phosphate compound called phosphocreatine, or PCr. Like the ATP molecule, the PCr molecule possesses a phosphate group. When the bond between the creatine and phosphate molecule splits, a large amount of free energy is released. Because PCr has a higher free energy of hydrolysis than ATP, it's phosphate is donated directly to ADP to rebuild ATP as shown in the following reaction (Fox et al., 1988),



This reaction may be summarized as follows (Brooks & Fahey, 1985):



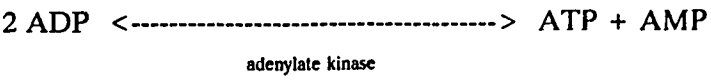
The cell's concentration of PCr is about three to five times greater than that of ATP. As a result of the above reaction, the concentration of ATP during exercise does not fall significantly until nearly all the phosphocreatine has been converted to creatine (Layzer, 1990). For this reason, PCr is considered the high-energy phosphate "reservoir" (Katch et al., 1991). The only means by which PCr can be re-formed from inorganic phosphate and creatine is by the energy released through the breakdown of ATP (Fox et al., 1988). The reaction shown is then reversed:



Normally sufficient energy for this reaction is not available during the exercise interval. The energy demands of the exercise take first priority. Phosphocreatine is only reformed in substantial quantities after the demands of the high-intensity exercise are reduced.

Transphosphorylation

The second method of anaerobic ATP resynthesis is by way of a transphosphorylation reaction where by two free ADP molecules join together:



This reaction contributes to ATP resynthesis once there is a high concentration of ADP in the cell. However due to the build-up of the toxic by-product AMP (adenosine monophosphate) and the necessary deamination of this molecule, this reaction will only proceed for a limited time.

Since the supply of ATP and PCr is brief, it would be logical to expect that the depletion of these sources of energy would lead to inhibition of cross-bridge cycling and eventually lead to a state of fatigue within the muscle. Several studies in the early 1970's, utilizing the needle biopsy technique indicated large inverse correlations between the concentrations of high energy phosphagens (ATP and PCr) and local muscular fatigue (Karlsson, 1971). It was noted however that ATP and PCr are never fully depleted. Certain baseline values are maintained as a protective mechanism to maintain cellular integrity. Using the needle biopsy technique, minimum values at exhaustion were observed to be 70% of resting ATP and 10% of resting PCr (Bergstrom et al., 1971; Karlsson, 1971; Gollnick and Hermansen, 1973). Recently, studies using the more sophisticated MRS (Magnetic Resonance Spectroscopy) measuring technique have demonstrated that during exhaustive exercise, the maximal decrease in muscle ATP content is about 10 to 40% although in certain fibres ATP decreases up to 80% (Soderlund and Hultman, 1990). The baseline for PCr is approximately 10-12% of resting levels (Taylor et al., 1986).

The response of high-energy phosphagens is quite different between progressive fatiguing exercise and short term, maximal exercise. Studies utilizing MRS have demonstrated that during progressive fatiguing exercise, little to no change is seen in ATP concentrations whereas PCr drops markedly with a concurrent increase in P_i (Weiner et al., 1990; Baker et al., 1989; Miller et al., 1988; Taylor et al., 1983). During very brief maximal exercise (1-30 seconds), Hultman et al.(1987) reported that there was no immediate decrease in the ATP stores as rephosphorylation of ADP accelerated at the same rate as the ATP utilization by degradation of phosphocreatine and anaerobic glycolysis. This intensity of exercise resulted in decreased PCr content and an accumulation of lactate. However, if the exercise was prolonged beyond 30 seconds, a decrease of 30-40% (Gollnick and Hermansen, 1973; Bergstrom et al., 1971) and up to 60% (Hultman and Sjoholm, 1986) of the ATP content was also observed. Maximal or near maximal forces could be generated up to 20 seconds, thereafter the force began to decrease and subjects experienced fatigue. These studies indicate that the magnitude of observed depletion in high energy phosphagens

is proportional to the intensity of exercise performed. At low to moderate relative intensities of exercise (between ~ 60 and 90% $\dot{V}O_{2max}$) fatigue is associated with depleted stores of muscle glycogen (Saltin and Karlsson, 1971) and endurance is closely related to the pre-exercise muscle level of glycogen (Hermansen et al., 1967) and to the rate of glycogen depletion. As the level of intensity increases (beyond ~ 90% $\dot{V}O_{2max}$) fatigue progressively becomes more associated with a marked depletion of PCr and to a lesser extent ATP. At these elevated intensities, part of the energy requirement is covered by anaerobic processes and metabolic end-products (lactate, H^+, Pi , ADP) will accumulate (Sahlin, 1992).

The question is raised as to whether a reduction in high energy phosphagens (ATP and PCr) cause fatigue or whether accumulated H^+ ion inhibits further exercise either by direct inhibition of the contractile machinery or interference with phosphagen resynthesis? Sahlin (1992) contends that even with as much as 80% depletion of ATP, remaining levels of ATP are still far above what is necessary for muscle contraction. Several authors suggest that these minimal levels are required for maintenance of cellular integrity and that interference by metabolites may occur in order to protect the muscle to avoid depletion of ATP to the point of rigor (Kirkendall, 1990; Sahlin, 1992). The initial depletion of ATP may "direct" this activity itself as even a small decline in ATP will result in large relative increases in ADP and Pi (due to the creatine kinase equilibrium) which are potential fatiguing agents (Sahlin 1992). Furthermore, when the rate of ATP turnover is as high as seen in high intensity exercise, temporal and spatial concentration gradients will exist within the cell and these local and transient increases in ADP will interfere with the contraction process (Funk et al., 1989). These results suggest some interaction between the leading hypotheses of fatigue: insufficient energy supply and accumulation of metabolites.

Resynthesis of ATP by Anaerobic Glycolysis

Anaerobic glycolysis plays the predominant role in supplying energy for medium to high-intensity bouts of activities lasting longer than 10 seconds but less than 2 minutes. This pathway also comes into play at the onset of exercise and when the transition is being made from one workload to a higher workload. In these situations, the working muscles are in great need of energy in the form of ATP. However, total energy requirements cannot be supplied by either PCr (because it is depleted) or the aerobic system (because the oxygen supply is unavailable).

Anaerobic glycolysis provides ATP from the partial degradation of glycogen derived from carbohydrate food stuffs. The largest stores of glycogen may be found in the liver and skeletal muscle. Glycogen utilized during anaerobic glycolysis is derived from the immediate source, skeletal muscle. While glycogen is a plentiful source of energy, it can not provide "immediate" energy like ATP or PCr can. When necessary, glycogen must first be broken down to units of glucose. Glucose molecules then enter a more complex series of ten chemical reactions collectively known as glycolysis (Appendix A). During glycolysis the six carbon glucose molecule is broken down to two, 3-carbon molecules of pyruvic acid (Katch et al., 1991):

$$[C_6H_{12}O_6]_n \xrightarrow{\text{Energy}} 2\,C_3H_4O_3 + 2\,H_2 + \textbf{Energy}$$

(Glycogen) (Pyruvic Acid)

During glycolysis, two pairs of hydrogen atoms are stripped from the substrate and their electrons are passed to NAD^+ to form NADH. Normally, during moderate exercise and when sufficient oxygen is available, these electrons are processed directly through the respiratory chain with the final products being H_2O, CO_2, heat and energy. In this situation, the hydrogen electrons are "accepted" by oxygen. However in situations of intense exercise, when the energy demands exceed either the rate of utilization or the oxygen supply, all of the hydrogen joined to NADH can not be processed through the respiratory chain. The NAD^+ complex can act as an acceptor of hydrogen however the continued release of anaerobic energy in glycolysis depends on the availability of NAD^+ for the oxidation of 3-phosphoglyceraldehyde, otherwise, glycolysis would come to a standstill. Fortunately another "acceptor" for hydrogen (electrons) exists. Under conditions of anaerobic glycolysis, NAD^+ is "freed" as pairs of "excess" hydrogens combine with pyruvic acid in an additional step, catalyzed by the enzyme lactic dehydrogenase, to form lactate in the cytoplasm. This reversible reaction is illustrated below:

$$C_3H_4O_3 + NADH + H^+ \xleftrightarrow{\text{(Lactic Dehydrogenase)}} C_3H_6O_3 + NAD^+$$

(Pyruvic Acid) (Lactic Acid)

The storage of hydrogen with pyruvic acid is a unique aspect of energy metabolism because it provides a temporary depot for the disappearance of the end products of anaerobic glycolysis (Katch et al., 1991). These circumstances allow glycolysis to proceed to
supply additional energy for the resynthesis of ATP.

Accumulation of Metabolites

ATP is hydrolysed during muscle contraction and specific products result from this reaction:

$$ATP + H_2O \text{ ----------> } \mathbf{ADP + Pi + H^+} + \text{Energy}$$

The "accumulation of metabolites" theory of fatigue centers around the build-up of products associated with this reaction and other anaerobic ATP-liberating reactions. This theory also considers the effect of increasing concentration of H^+ (one of the products) on three ATPase enzymes that are ultimately required for the hydrolysis of ATP in the above reaction: actomyosin ATPase, Ca^{2+} transport (sarcoplasmic reticulum) ATPase and Na^+ - K^+ ATPase (Hultman, Spriet, and Sodelund, 1987).

Lactate, Hydrogen Ion (H^+) and pH

During anaerobic glycolysis, energy requirements can be supplied by glycogen without the presence of oxygen with the end product being lactic acid. Once lactic acid is formed in the muscle it dissociates rapidly into lactate and hydrogen ion. The lactate may be metabolized by the muscle (in the presence of oxygen) or carried in the blood to other organs (liver and heart) or muscles which can oxidize it and use it as an energy source. During high-intensity exercise when oxygen is not available and ATP and PCr cannot provide all the energy required, lactate accumulates in the muscle and blood. Substantial evidence has been presented which supports a high correlation between the concentration of lactate and local muscular fatigue (Karlsson and Saltin, 1970; Tesch, 1978). Tesch et al. (1978) found that after 30 seconds of repeated isokinetic exercise, the decrease in force output was proportional to lactate concentration present in individual muscle fibres. Karlsson and Saltin (1970) proposed that lactate accumulation in muscle, with it's concurrent

decrease in pH was the reason for exhaustion in moderate to heavy exercise. It has also been shown that muscle pH will decrease after dynamic exercise (Hermansen and Ones, 1972). This acidification was suggested as the limiting factor for physical exercise of high-intensity and short duration (McCartney et al., 1983). The implication of lactate as a cause of fatigue has received additional support from studies which show that with training, lactate accumulation at a given workload is reduced (Holloszy, 1973) and the buffering capacity of muscle is increased (Sahlin and Hendriksson, 1984; Sharp et al., 1986). These adaptations resulted in a delay in the onset of fatigue.

More recently, Mainwood et al. (1987) found that the development of, or recovery from local muscular fatigue is proportional to the concentration of H^+ in the fatigued muscle. It is now recognized that the increase in concentration of H^+ (or lowered pH) is created by the dissociation of lactic acid into lactate and H^+ and it is unbuffered hydrogen ion that precipitates most of the effects of lactate in the development of local muscular fatigue. Lactic acid contributes over 85% of the free H^+ generated in exercise induced acidosis (Sahlin, 1982). Most of the H^+ ions are buffered and therefore pH changes are a result of the approximately 0.001% of unbuffered H^+ (Sahlin, 1982). Following continuous high exercise intensity to exhaustion, muscle pH drops from a resting value of approximately 7.0, to a value in the range of 6.3 to 6.6 (Hermanssen and Osnes, 1972; Sahlin et al., 1978; Metzger and Fitts, 1987). It has been postulated that fatigue is probably mediated by increased acidity that inactivates various enzymes involved in energy transfer as well as the muscle's contractile properties (Katch et al., 1991). There are numerous ways that an accumulation of H^+ ions and the associated decrease in pH can affect tension development and ATP production (Appendix B):

Action Potential: (Step 1)

As pH decreases so does the membrane's excitability (Orchardson, 1978). Decreased pH could affect the permeability of the membrane to sodium (Na^+) and potassium (K^+) resulting in a hyperpolarized state (Sjogaard, 1986).

Transmission of Signal from T-Tubule to Sarcoplasmic Reticulum: (Step 2)

Skinned fibre studies have shown that, at reduced pH, there is an increased calcium requirement for similar tension requirements (Fabiato and Fabiato, 1978).

Release of Calcium from Sarcoplasmic Reticulum: (Step 3)

At a reduced pH, the sarcoplasmic reticulum release of calcium is diminished. (MacLaren et al., 1989).

Cross-bridge Formation: (Step 4)

The increased concentration of H^+ from the lactate competes with Ca^{2+} for binding sites on actomyosin crossbridges. As a result there is a decrease in effective crossbridges and the ability to maintain intensive work diminishes (Dawson et al.,1980; Inesi and Hill, 1983).

Reduced Uptake of Calcium: (Step 5)

Once cross-bridge formation is complete, Ca^{2+} must be returned to the sarcoplasmic reticulum, via the enzyme, sarcoplasmic reticulum ATPase. When energy is not available, this enzyme can not function properly and there is a decrease in net Ca^{2+} uptake (Dawson et al., 1980; Inesi and Hill, 1983).

Resynthesis of ATP/ Role of Muscle Enzymes: (Step 6)

A reduction of ATP synthesis will occur if an enzyme of glycolysis is inhibited by a declining pH. Chasiotis (1983) found that inhibition of glycolysis occurs at the level of glycogenolysis in a three-fold manner: 1) At the first level the conversion of phosphorylase b into the more active form phosphorylase a, by phosphorylase kinase, is inhibited by H^+; 2) The second level of inhibition occurs at the level of the formation of cAMP, H^+ is a potent inhibitor of adenylcyclase; 3) The third and strongest level of inhibition of glycogenolysis occurs with a decrease in substrate availability. Phosphorylase will only accept the dianonic form of inorganic phosphate HPO_4 as substrate, and inorganic phosphate is largely present as H_2PO_4 in the presence of excess H^+.

Other enzymes may also be affected. Phosphofructokinase (PFK) has been suggested as a rate limiting enzyme of glycolysis and is considered to be inactivated at a pH of 6.5 (Trevidi and Danforth, 1966; Ui, 1966). The glycolytic intermediates previous to PFK would back up, especially glucose-6-phosphate. This compound inhibits hexokinase and phosphorylase (Newsholme and Leech, 1983). Sahlin et al. (1983) subjected rat muscle to carbon-dioxide-induced acidosis and discovered that acidosis has a marked influence on intermediary metabolism. Results of the induced-acidosis indicated a four-fold increase in

glucose-6-phosphate, a 14% increase in ADP and a decrease in phosphocreatine to 44% of the control value. Sahlin et al. (1983) suggest that the observed metabolic changes can be explained by an effect of H^+ on the activity of phosphofructokinase and on the creatine kinase equilibrium. Newsholme and Leech (1983) also discovered that LDH and ATPase are inhibited at physiological concentrations of lactic acid. As MacLaren et al. (1989) state: "... it is clear that an accumulation of H^+ ions can affect numerous enzymes, any of which could limit ATP synthesis".

The accumulation of lactic acid and lowered pH may also have other effects including changes in the cellular osmolarity. These effects will be considered in the section titled: "Electrolytes and Water".

Inorganic Phosphate (Pi) and the Acidic Form of Phosphate $H_2PO_4^-$)

Recently, it has been proposed that fatigue is not due directly to a high H^+ concentration, but rather is primarily due to production of the diprotonated acidic form of inoganic phosphate or (P_i): H_2PO_4 (Wilkie, 1986; Nosek et al., 1987). This theory has been supported by the presence of a close relationship between $H_2PO_4^-$ and force development in frog skeletal muscle (Wilkie, 1986) and in skinned rabbit muscle fibres (Nosek et al., 1987). Wilson et al. (1985) conducted a study to examine the role of H^+ and $H_2PO_4^-$ in fatigue using ^{31}P-MRS. Results from three different exercise protocols suggest that:
1) Linear relationships exists between developed force and pH and between developed force and $H_2PO_4^-$, 2) Two minutes of submaximal exercise before maximal exercise significantly reduces pH and increases $H_2PO_4^-$, and 3) During subsequent maximal exercise, the relationship between developed force and $H_2PO_4^-$ remains unchanged. In contrast, the relationship between developed force and pH shifts to the left; muscle pH remains lower during submaximal exercise, and developed force remains comparable to that noted during control exercise. The authors concluded that muscle fatigue during intense, short-term exercise is primarily caused by an increase in intramuscular $H_2PO_4^-$ rather than by a decrease in intramuscular pH.

Expanding on the work of Nosek and Wilkie (1987), Weiner et al. (1990) attempted to determine whether the relationship between $H_2PO_4^-$ and fatigue was a causal one or merely a correlative one. To strengthen the generality of this relationship, they tested both fast and slow-twitch muscle, recognizing that fast-twitch fibres fatigue more rapidly, while

slow-twitch fibres exhibit greater endurance. Utilizing ^{31}P-MRS, Weiner et al. (1990) observed similar relationships between the decline in muscle force during fatigue and changes in both phosphate and hydrogen ion concentrations during both anaerobic and aerobic exercise in tibialis anterior (slow-contraction, fatigue resistant muscle). These relationships were observed to be similar to those obtained testing the adductor pollicus muscle (fast-contraction, fatigue susceptible muscle). Weiner et al. (1990) concluded that the data demonstrate constant relationships between diminished muscle force and both [H^+] and [$H_2PO_4^-$] in muscles of different fatigability during both aerobic and anaerobic exercise. They found their data consistent with previous reports that both [$H_2PO_4^-$] and [H^+] have separate and additive inhibitory effects on the contractile proteins. This data also points to the possibility that *in vivo*, the separate changes in [P_i] and [H^+] may combine to generate the single inhibitory species [$H_2PO_4^-$] and that it is this species which causes the down regulation of the ATPase and the force developed by the contractile proteins.

Ammonia

Intense exercise causes a large amount of ammonia to be released from muscle. This occurs as a result of the myokinase reaction: ADP + ADP ------> ATP + AMP. The AMP is converted to IMP and ammonia: AMP + H_20 ------> NH_3 + IMP (Kirkendall,1990). Mutch and Bannister (1983) reported that the concentration of ammonia is higher in fast twitch fibres than slow twitch fibres following exercise and is proportional to the intensity of the work performed. There are several local effects associated with NH_3 accumulation including a reduction in the number of active fibres by limiting membrane function (Heald, 1975), increasing phosphofructokinase enzyme activity (Lowenstein, 1972), inhibition of the Kreb's cycle by inhibition of the enzymes isocitrate dehydrogenase and pyruvate dehydrogenase (Aragon et al., 1981; Newsholme and Leech, 1983; Mutch and Bannister, 1983), and, inhibition of gluconeogenesis (MacLaren, 1989), or inhibition of mitochondrial oxidation (Newsholme and Leech, 1983). Any of the aforementioned effects would result in increased lactic acid production and glycogen depletion (Mutch and Bannister, 1983). It has been suggested that the production of ammonia may precede the decline in pH (MacLaren, 1989; Mutch and Bannister, 1983).

Magnesium

During very high-intensity, short duration exercise the concentration of ATP decreases. As a consequence of the decrease in ATP, a substantial increase in free Mg^{2+} occurs in the cytoplasm (Hultman and Sjoholm, 1986). The concentration of free Mg^{2+} at rest is suggested to be 1 $mmol \cdot l^{-1}$ (Veloso et al. 1973), and is expected to reach approximately 5 $mmol \cdot l^{-1}$ during high-intensity exercise. It is known that Mg^{2+} competes with Ca^{2+} for binding sites of troponin (Donaldson and Kerrick, 1975) and Mg^{2+} is known to depress Ca^{2+} sensitivity of skinned muscle fibres (Donaldson et al., 1978).

Electrolytes and Water

Fluid and ion shifts can be observed at the onset of local muscular fatigue. Increased concentration of Ca^{2+}, Na^+, K^+ and P_i have been seen as well as increases in the enzymes creatine phosphokinase, lactate dehydrogenase and aspartate aminotransferase (Gunderson et al., 1982). Evidence indicates that at exhaustion, there is an increase in the permeability of the sarcolemma (Gunderson et al. 1982; Simonson 1981). Increased concentrations of lactic acid have a direct effect on cellular osmolarity, causing a shift of water into the muscle. This shift may affect the cell in several and possibly, in opposing ways: i) The accompanying increase in intramuscular pressure may lead to a restriction of local circulation (Bergstrom et al., 1971; Sahlin et al., 1978), ii) the shift of water into the muscle may dilute accumulating ions (Roberts and Smith, 1989), and iii) the dilution effect may serve to decrease the ionic potential of the cell through decreased K^+ and increased Na^+ thus inhibiting the development of an action potential (Sahlin et al., 1978) and may disturb the membrane potential sufficiently to decrease the excitability of the sarcolemma (Sembrowich et al., 1982; Gibson and Edwards 1985; Sjogaard 1986). It appears that during high-intensity exercise, electrolytes and the resulting shift of water into the muscle poses a definite threat to the development of the action potential and this in turn can inhibit further muscle contraction and contribute to a state of fatigue.

Fatigue in Review

The factors currently known to influence the fatigue process have been presented. Events which interfere with the ability of the body to supply sufficient energy to meet the energy demand or to rid itself of metabolic waste will lead to the development of fatigue. Fatigue resulting from anaerobic exercise appears to be influenced primarily by the accumulation of unbuffered hydrogen ions which can impair the contraction process directly, or can impair the contraction process indirectly through it's inhibitory effect on the ATP-generating processes. Under anaerobic conditions, the rate of ATP generation is determined by the combined rates of PCr breakdown, phosphorylation and anaerobic glycolysis (Sahlin and Ren, 1989). An increased concentration of H^+ can decrease the capacity to generate ATP both through an inhibitory effect on glycolysis (Ui, 1966) and through a decrease in the PCr level [H^+ is linked to PCr through the creatine kinase equilibrium] (Sahlin, 1986). An accumulation of H^+ ions causes decreased calcium-binding affinity with troponin by way of competitive inhibition. Increased acidity also acts to inhibit ATPase enzymes required for the release and uptake of calcium. H^+ metabolites in concert with Pi (H_2PO_4) may also combine to inhibit the ATPase enzymes required for energy and ultimately contraction. To determine the magnitude of the role of the other factors will require more investigation. The production of excess ammonia may precede H^+ build-up and the effects of the ammonia may act to accelerate the production of lactic acid directly. Magnesium competes with Ca^{2+} (and H^+) for binding sites with troponin. Accumulating ions may cause a shift of water into the muscle, inhibiting the development of an action potential. Currently, however, the build-up of H^+ ions and their affect on the contraction process (directly) and on energy resynthesis (indirectly), appears to the primary cause of fatigue resulting from short term, high-intensity exercise.

Recovery From Exercise

Exercise may be considered a disruption of homeostasis and recovery, the restoration of homeostasis. The recovery process depends, at least in part, on the buffering or removal of hydrogen ions to permit replenishment of energy stores and restoration of the metabolic environment. How is this achieved? Which processes occur first? What factors influence the recovery process? What methods of recovery are most beneficial? Each of these questions will be addressed individually in an attempt to delineate the recovery process.

Phases of the Recovery Process

Several studies have focused on the identification and order of events which constitute the recovery process (Hultman et al., 1967; Piiper, 1970; Harris et al., 1976; Fitts, 1977; Tesch, 1980). Most authors support a biphasic pattern of recovery with an initial fast phase (10 seconds - 5 minutes) representing replenishment of energy, followed by a much slower, second phase (2 - 20 minutes) representing removal of hydrogen ion and lactate (Sahlin, Harris and Hultman, 1978; Pan et al., 1991; Sahlin, 1992). Significant overlap between the two phases exists due to variations in the degree and duration of fatigue experienced.

During examination of the first phase, Hultman et al. (1967), found phosphagen replenishment (PCr and ATP) to be very rapid at first, then somewhat slower, with 70% restoration within 30 seconds and 100% restored within 3 to 5 minutes. Harris et al. (1976) confirmed this time frame but found that the replenishment of PCr only occurred when the circulation was intact. No restoration of PCr took place when the blood flow was occluded, indicating that oxygen is required for this process. The recovery of PCr is also affected by the status of ATP. Taylor et al. (1986) found that the recovery of PCr is slower in the presence of a depleted pool of ATP as occurs in exhaustive anaerobic exercise. Associated with the depleted pool of ATP is the accumulation of H^+. Sahlin and Ren (1989) explain that during the first phase, lactate remains high and pH continues to be suppressed despite lactate removal because PCr is being resynthesized and continues to produce H^+. Now,

many authors support the view that the resynthesis of ATP and PCr is an H^+ mediated event and the rate at which ATP and PCr recover is partially dependent on the metabolic environment of the muscle, specifically the concentration of H^+ (Arnold et al., 1983; Iotti et al., 1991) and the rate of oxidative phosphorylation within the muscle (Sahlin et al., 1979; Taylor et al., 1983; Tesch and Wright, 1983; McCully et al., 1988; Jansson et al., 1990).

The second, slow phase of recovery, representing removal of hydrogen ion and lactate and restoration of pH appears to occur with a half-time of approximately 5 -10 minutes (Fitts, 1977; Metzger and Fitts, 1987; Pan et al., 1991, Sahlin, 1992). During the second phase hydrogen ion and lactate are cleared slowly following muscular exercise, but export of hydrogen ion from the cell is accomplished more quickly than lactate (Sutton et al., 1981).

Factors Which Influence the Recovery Process

Recovery from high intensity, short duration exercise is influenced by several factors which affect the quantity of hydrogen ion produced and the rate of removal of hydrogen ion and lactic acid from the muscle.

Intensity and Duration of the Exercise

Intensity and duration are two factors which play key roles in determining the amount of fatigue or "insult" experienced by the muscle. As the intensity of exercise increases, the metabolic response is to activate energy systems which can provide energy at an accelerated rate. During high-intensity exercise, anaerobic glycolysis is activated and produces ATP, but in doing so produces additional quantities of H^+. Furthermore, as the intensity of exercise increases there is also greater recruitment of fast-twitch fibres. Fast-twitch fibres produce approximately twice as much lactate and H^+ as slow-twitch fibres. The hydrolysis of phosphocreatine absorbs some of the H^+ but does not have the capacity to absorb such large quantities as seen with high-intensity exercise. Consequently, as intensity increases, $[H^+]$ and lactate increases. Extending the duration of high-intensity exercise only contributes to a greater accumulation of H^+ and lactate in the muscle and blood. Once the capacity of the system is overwhelmed by excessive H^+ and lactate, it takes existing transport pathways longer to remove it and the slows the rate of recovery from exercise.

Oxidative Potential of the Muscle

The oxidative potential of the muscle can affect the rate of recovery in two ways. Oxygen is required for PCr resynthesis. The rate of oxidative metabolism within the muscle controls the rate of PCr resynthesis (Taylor et. al., 1983). Secondly, once lactic acid is converted to pyruvic acid, oxygen is required to convert the pyruvic acid into usable energy [lactic acid removed from blood and muscle during recovery can be converted to glucose, protein, glycogen, or to CO_2 and H_2O (via pyruvic acid) (Fox et.al, 1988)]. The vast majority of lactate removed during the recovery process is converted to pyruvic acid and oxidized in active skeletal muscle (~50%) while the heart, liver, brain, kidneys and inactive skeletal muscle oxidize the remainder.

Jansson et al. (1990) stress the importance of the oxidative potential of the muscle during recovery from intense exercise. Results from their study suggest that the recovery of force and the "normalization" of metabolite contents after short-term, intense exercise are dependent on the aerobic-oxidative potential of skeletal muscle. The enzyme, citrate synthase (CS) is an enzyme which converts acetyl Co-A and oxaloacetic acid to give citric acid in the Krebs Cycle (Brooks and Fahey, 1985). Jansson et al. (1990) showed that trained muscles exhibited higher CS activity resulting in marked decreases in lactate and increases in PCr after recovery.

Buffering Capacity Within the Muscle

As opposed to being converted to pyruvic acid and oxidized directly within muscle, lactic acid may combine with another compound and leave the muscle, as occurs in "buffering". Lactic acid can combine with a weak salt such as sodium bicarbonate to form sodium lactate and carbonic acid:

$$LA + NaHCO_3 \text{ -------> } NaLA + H_2CO_3$$

In the blood, carbonic acid readily dissociates into CO_2 and H_2O. Parkhouse and McKenzie (1984) postulated that the major buffering components in human skeletal muscle include the bicarbonate buffer system, creatine phosphate, inorganic phosphate, protein-bound histidine residues and carnosine. They suggest that intracellular buffering plays an important role in the regulation of intracellular pH. They also offer the possibility of an enhanced proton-sequestering capability within sprint-trained subjects possessing high anaerobic capacity. Mainwood et al. (1972), found that external bicarbonate can modulate intracellular proton

balance and limit lactate efflux. An increase in extracellular pH, causing an increase in internal pH was found to increase lactate efflux leading to almost 100% recovery of tension in their frog sartorius muscle preparations. These studies suggest that buffering capacity plays an important role both during exercise and in recovery from exercise. Piiper (1972) and Jones (1980) caution however that although increases in hydrogen ion concentration are reduced by the buffering capacity of the muscle, the metabolic flux during high-intensity exercise may be so great that the role of intramuscular buffering in recovery may be limited.

Increased Local Circulation

Recovery from high intensity exercise depends on the transport of various substances to and from the muscle. Since blood flow is the medium by which most of the oxygen and glucose reach the muscle and by which carbon dioxide, hydrogen ion and lactate are removed, anything that alters blood flow will have an effect on the rate at which these substances will be delivered or removed (Hermansen and Vaage, 1977., Tesch and Wright, 1983; Fox et al., 1988; Caferelli and Flint, 1992).

Active Recovery

Many researchers have demonstrated that an active recovery can increase the rate of lactate removal following intermediate duration exercise (Belcastro and Bonen, 1975; Bonen and Belcastro, 1976; Bonen et al., 1979; Weltman et al., 1977; Boileau, 1983). The recovery-exercise intensity that produces the fastest or optimal rate of removal of lactate is between 30-45% $\dot{V}O_{2max}$ for untrained subjects and 50-65% $\dot{V}O_{2max}$ for trained subjects (Fox et al., 1988). Gollnick and co-workers (1986) suggest the primary mechanism for the accelerated removal of lactate induced by exercise is most likely through an increased blood flow. It has been suggested that increased blood flow through the muscle may facilitate oxidation within the muscle, offer increased muscle efflux from the muscle, promote excretion of lactate via sweat and urine, transport lactate to other tissues for oxidation (or resynthesis to glucose and/or glycogen), or, a combination of these factors.

Few studies (Weltman et al., 1977; Signorile et al., 1993) have examined the impact of an active vs. passive recovery on high-intensity, short duration exercise. The study by Weltman and colleagues (1977) demonstrated that when an "all-out", 1 minute cycle ergometer ride (5.5-kg resistance) was followed by either a passive or active (1.0 kg, 60 rpm) recovery period, the active recovery yielded significantly higher pedal revolutions in a subsequent effort. This improved performance was accompanied by significant

improvements in both lactate clearance and subsequent work due to active recovery. Signorile and colleagues (1993) suggest that the effects of the low intensity active recovery may involve both oxidative metabolism by neighbouring oxidative fibres and movement of metabolites by the "pumping" action of the active musculature.

It should be noted that although an active recovery may enhance the recovery rate of muscle from high intensity exercise, it is not always feasible (from a practical standpoint) to perform an active recovery. As such it is important to focus on the development of strategies that can enhance a passive recovery.

Fitness and Training Adaptations

Training induces positive physiological changes in almost every system of the body and especially within the skeletal muscles and cardiorespiratory system. These adaptations are influenced by heredity but also by the frequency, duration and intensity of the training program. The effects of training are also specific to the type of exercise performed, the muscle groups involved and the type of training program used.

This section will focus on the specific peripheral and central adaptations which occur in response to aerobic training. In the literature, the term, "aerobic" training is often used synonymously with "endurance" training. Although some physiologists have begun to make a make a distinction between adaptations resulting from aerobic power training and aerobic capacity (endurance) training, others still believe that aerobic power and endurance are analogous. Sutton (1992) maintains that, " a test of maximal aerobic power is the gold standard for assessing endurance performance potential". This review will refer primarily to adaptations to aerobic training as a whole, including those which have been reported to result specifically from endurance training. For the purposes of this review, only adaptations which would theoretically improve anaerobic exercise performance or enhance recovery from anaerobic exercise will be included.

Peripheral Adaptations

Energy Supply

Aerobic training increases the capacity of the ATP-PCr energy system. This occurs as a result of two major biochemical changes. First, there is an increase in levels of muscular stores of ATP and PCr. Following a training program of distance running for 7 months for 2-3 days per week, muscular stores of ATP have been shown to increase approximately 25% (Karlsson et al., 1972). Fox et. al. (1988) suggest that the increased storage of phosphagens correlates well with the improved execution of activities requiring only a few seconds to perform. Another adaptation that increases the capacity of the ATP-PCr system is the increase in the enzymes which help resynthesize ATPase. These enzymes include myokinase (catalyzes resynthesis of ATP from ADP) and creatine kinase (catalyzes resynthesis of ATP from PCr) (Fox et al., 1988).

Aerobic training also increases the capacity of the glycolytic energy system through two adaptations. First, aerobic training stimulates increased storage of intramuscular glycogen. Normally, human skeletal muscle contains between 13 and 15 grams of glycogen per kilogram of muscle. Following training this amount increases up to 2.5 times (Gollnick et al., 1973). Part of this increased storage of glycogen is due to the fact that training raises the level of activity of the enzymes responsible for glycogen synthesis. The second adaptation affects the utilization of this increased energy store. Phosphofructokinase (PFK), a key enzyme of glycolysis has been shown to increase from 80-100% following endurance training (Gollnick et al., 1972). Concentrations of phosphorylase and lactate dehydrogenase enzymes have also been shown to significantly increase with training (Holloszy and Coyle, 1984). These enzymes speed up the rate and quantity of glycogen broken down to lactic acid. This adaptation has two effects. One effect is that greater quantities of lactic acid can be produced during maximal exercise. Secondly, the ATP energy derived from the lactic acid system is increased. These effects allow the improved performance of activities that depend heavily on anaerobic glycolysis for energy. Furthermore, for a given submaximal intensity, lactate production is lower in the endurance trained individual than an untrained individual (Holloszy and Coyle, 1984). This allows the trained individual to prolong aerobic metabolism thereby avoiding the accumulation of lactate. Enzymes associated with the citric acid cycle and the electron transport chain including succinate dehydrogenase, NADH dehydrogenase, NADH cyto-chrome c reductase and cytochrome oxidase all increase in response to aerobic training (Holloszy and Coyle, 1984; Sjogaard, 1984). These enzymes

enhance the quantity of ATP derived from oxidative phosphorylation, a pathway critical for the recovery process.

Another adaptation associated with aerobic training and energy supply is the increased oxidation of fat. This serves as an advantage when performing low to intermediate-intensity exercise where the trained person will oxidize more fat and less carbohydrate than the untrained person (Hermansen, Hultman and Sahlin, 1967; Jansson and Kaijer, 1977; Gollnick, 1977). This adaptation would be advantageous to individuals who engage in intermittent exercise (such as soccer) where the intervals may include both maximal and submaximal workloads. Since a greater oxidation of fat during the submaximal workloads would spare glycogen, this glycogen can be reserved for heavier workloads and therefore extend the time that an athlete can perform optimally over several intervals. The increase in the muscles' capacity to oxidize fat following aerobic training is related to three factors: 1) an increase in the intramuscular stores of triglycerides, 2) an increased release of free fatty acids from adipose tissue (glycogen-sparing effect), and, 3) an increase in the activities of the enzymes involved in the activation, transport, and breakdown of fatty acids.

Buffering Capacity

Although sprint training increases buffering capacity (Parkhouse and McKenzie, 1984; Parkhouse et al., 1985; Sharp et al., 1986), research indicates that the buffering capacity of endurance trained athletes is not different from sedentary individuals. Parkhouse et al. (1985) investigated the buffer capacity, carnosine and histidine levels in 20 men from 4 distinct populations: 5 sprinters, 5 rowers, 5 marathoners and 5 untrained individuals. Significantly ($P < 0.01$) elevated buffer capacities, carnosine levels and high intensity running performances were demonstrated by the sprinters and rowers, but no significant differences existed between these variables for the marathon vs. untrained subjects. Sharp et al. (1986) found similar results when comparing sprint-trained subjects with endurance trained cyclists. These results suggest that while buffering may enhance recovery from high-intensity exercise, it is high-intensity training (as opposed to aerobic training) which enhances buffering capacity (Parkhouse et al., 1985).

Oxidative Capacity

With endurance training their is an increased oxidative capacity of skeletal muscle to breakdown glycogen to CO_2 and H_2O with ATP production (Fox et al., 1988; Abernethy

et al., 1990; Kent-Braun et al., 1990). There are three major subcellular adaptations resulting from endurance training which contribute to this overall change: 1) there is an increase in the number, size, and membrane surface area of skeletal muscle mitochondria; endurance athletes have approximately a two-fold higher volume of mitochondria and rate of oxidative metabolism compared with sprint-trained and control subjects (Gollnick et al., 1973; Holloszy and Coyle, 1984; Bloomstrand et al., 1986), 2) there are amplified myoglobin and capillary densities (Saltin and Rowell, 1980) and, 3) there is an increase in the level of activity or concentration of the enzymes involved in the Kreb's cycle and electron transport system (Saltin and Rowell, 1980; Holloszy and Coyle, 1984). These adaptations enhance the ability to exercise at a higher percentage of VO_{2max} in the trained state, by slowing glycogen depletion and reducing lactate production (Holloszy and Coyle, 1984). These adaptations have also been found to correlate with a measurable increase in $\dot{V}O_{2max}$ (Saltin and Rowell, 1980; Davies et al., 1981; Sjogaard, 1984; Wagner, 1991).

Additional biochemical adaptations which increase the oxidative capacity of skeletal muscle that occur primarily as a result of endurance training are increases in total blood volume, hemoglobin, myoglobin and a conversion of fibre type. Both the total blood volume and the total amount of hemoglobin increase with training (Pechar et al., 1974). After endurance training both of these variables have been shown to increase: total blood volume (per kg of body weight) 20%; hemoglobin, the oxygen binding molecule found in red blood cells has been shown to increase 17%. Myoglobin content has been shown to be substantial increase with training (Mole, Oscai and Holloszy, 1971). The main function of myoglobin is to aid in the diffusion of oxygen across the cell membrane to the mitochondria where it is consumed. The response is specific. Myoglobin increases only in those muscles involved in the training program. Hickson (1981) has shown that with rats, myoglobin augmentation is associated with the frequency of training. With exercise at 2 days, 4 days, and 6 days per week, myoglobin levels increased 14, 18, and 26% respectively. Regarding fibre type, endurance training promotes hypertrophy and selective recruitment of slow-twitch (oxidative) muscle fibres (Fox et al., 1988). There is also some evidence to indicate that there is an actual change in fibre composition as a result of endurance training. This evidence indicates that with endurance training there is a gradual conversion of type FTb fibres (fast-glycolytic) to type FTa fibres (fast-oxidative-glycolytic) (Andersen and Henriksson, 1977). The FTa fibres should therefore be able to oxidize lactate within muscle more readily.

Clearance of Lactate

Training can produce increased capillary density (Jansson and Kaijer, 1977; Saltin and Gollnick, 1983; Fox et al., 1988), and the ability to maximally vasodilate (Sinoway et al., 1986). Wagner (1991) suggests that peripheral changes account for the majority of improvement with $\dot{V}O_{2max}$ and the specific reason for this is the increase in capillary surface area available for O_2 diffusion resulting from new capillary formation. This adaptation may play a primary role in explaining the most recent evidence which demonstrates that endurance training produces an increased capacity for lactate clearance from muscle (Tesch and Wright, 1983; Brooks, 1986), and a higher ability to remove lactate from the blood during recovery (Oyono-Enguelle et al., 1990). During and after muscular exercise, lactate is removed from muscle and blood as evidenced by the return to near resting values within 30 to 60 minutes after the cessation of exercise (Sahlin, 1978). Brooks and Donovan (1983) demonstrated that the rate of lactate clearance can be increased with training. Stanley et al. (1988) observed tight relationships between lactate appearance rates (Ra) and metabolic rate ($\dot{V}O_2$) during exercise, but wide differences in lactate metabolic clearance rate in fit and unfit individuals. They found that the lactate disappearance rate was related to the level of fitness of the subjects with the more highly fit subjects demonstrating a significantly higher lactate metabolic clearance rate.

Muscle Blood Flow

Blood flow can be increased at the central and peripheral level. At the central level blood flow can be increased through an increased heartrate and stroke volume to increase cardiac output (Sutton, 1992). At the peripheral level blood flow may be increased through an increase in the number of capillaries which serve the muscle (Jorfeldt et al., 1978; Tesch and Wright, 1983). Saltin and Rowell (1980) state that after 2-3 months of aerobic conditioning, $\dot{V}O_{2max}$, leg blood flow, and muscle capillary density all increase by approximately the same extent (15-20%). The increase in capillary density, expressed as number of capillaries per square millimeter, was attributable to the growth of new capillaries. The size and number of muscle fibers per square millimeter increases less, relative to increases seen in capillary density (Andersen and Henriksson, 1977; Andersen and Pelle, 1978). As a result, each muscle fiber is supplied by more capillaries and diffusion distances from capillaries to muscle fibers diminishes significantly. The increased blood flow, especially at the peripheral level is especially important for the removal of hydrogen ion and lactate, essential for the recovery process.

Central Adaptations

Aerobic training causes certain adaptations to occur at the central level during maximal, anaerobic performances:

No Change or Slight Decrease in Cardiac Output (Resting or Submaximal Conditions)

This occurs as a result of the two opposite effects of stroke volume and heartrate. The stroke volume is increased with endurance training. This effect is mainly due to the increased size of the ventricular cavity and to an increased myocardial contractility that is enhanced by training. During submaximal or resting conditions the effect of endurance training is a decreased heartrate and this is thought to be caused by changes within the heart muscle itself and within the autonomic nervous system, specifically in the form of a decreased sympathetic drive.

Increased Cardiac Output (Maximal Effort Conditions)

This increase is entirely due to an increase in stroke volume since heartrate during exercise is either unchanged or slightly lowered with training.

Increased Maximal Aerobic Power ($\dot{V}O_{2max}$)

The increase in $\dot{V}O_{2max}$ is brought about by two principal changes. Firstly, there is an increased oxygen delivery to the working muscles through an increased cardiac output. Secondly, there is an increase in the oxygen extraction from the blood by skeletal muscles. $\dot{V}O_{2max}$ is highest in athletes who compete and train for endurance type activities.

The Potential Influence of Aerobic Training Adaptations on Recovery from Anaerobic Exercise

In the past two sections the recovery process as well as specific adaptations which result from training have been examined in considerable depth. By linking these processes, it is possible to establish a theoretical connection between training adaptations which may produce the most effective recovery.

After maximal exhaustive exercise, the initial recovery period requires that PCr be resynthesized and pH restored to a level whereby it is not inhibiting muscle contraction or

energy resynthesis. Total recovery (return of metabolic variables to resting values) requires complete restoration of the metabolic environment including the normalization of pH. Several adaptations occur in response to exercise including peripheral and central adaptations. To promote an optimal recovery it would seem logical to search for those training adaptations which could most effectively facilitate the restoration of the metabolic environment. The sequencing of such a chain of events should yield the most direct path to recovery.

One of the first factors to consider in restoration of the metabolic environment is the total quantity of accumulated lactic acid in the muscle. Minimizing the lactic acid production would reduce the amount of lactate that would need to be oxidized. One of the effects of aerobic training is the sparing of glycogen as the primary substrate and the preferential use of fatty acids at submaximal intensities. Although this adaptation does not affect high intensity exercise recovery directly, it does prevent premature accumulation of lactic acid at moderate intensities which usually precede bouts of maximal intensity during intermittent exercise.

Once a given level of lactic acid is produced it must be removed quickly to enhance recovery. Training adaptations which can assist in this process include overlapping events related to the oxidation of lactate (via pyruvate) within muscle and removal of lactate from muscle. Removal rates might be enhanced by increased blood flow which could be achieved through additional blood volume and increased capillary density. Removal rates could also be enhanced by an increase in the buffering capacity of the muscle and blood. Oxidation of the lactate and continued oxidative energy production could be facilitated by all of the adaptations which maximize the oxidative capacity of the muscle including increases in myoglobin, the size and number of mitochondria, the level of oxidative enzymes and an enhanced delivery of oxygen to the muscle through an increased cardiac output and ability to extract oxygen. All of these adaptations occur in response to aerobic training. Therefore, it is postulated that aerobic training adaptations can enhance recovery from maximal exercise by increased oxidative capacity of the muscle and improved removal and transport of lactic acid from the active muscle.

Some researchers have attempted to confirm specific aspects of this theory. Medbo and Sejersted (1985) found that sprint-trained subjects were capable of accumulating more lactate in blood compared with aerobic-endurance trained subjects, but at the expense of a lower pH. Hakkinen and Myllyla (1990) have shown that among aerobic-endurance, power and strength athletes, aerobic-endurance athletes demonstrate the smallest relative

decreases in maximal strength and maximal rates of force production during anaerobic exercise. Furthermore, after a three minute rest period, the relative recovery of maximal force of the endurance athletes was highest while the anaerobic power group demonstrated the lowest relative values in force production and relaxation. The results of Jansson et al. (1990) suggest that the recovery of force and the normalization of metabolic contents after short-term, intense exercise are dependent on the aerobic-oxidative potential of skeletal muscle. Tesch and Wright (1983) support the view that slow-twitch fibre favours a higher potential to recover from previous exercise since slow-twitch muscle is more efficient than fast-twitch fibre in oxidizing lactate due to differences in LDH enzyme and isozyme patterns. They also noted the work of Folkow and Halicka (1968) and Anderson and Henriksson (1977) who suggest that a higher rate of lactate release to the blood stream may be expected from slow-twitch fibres due to a more developed capillary network. The experimental findings of Tesch and Wright (1983) demonstrate that lactate elimination from the exercising muscle is partly dependent upon the capillary supply and influences the rate of muscle force recovery. Oyono-Enguelle et al. (1990) observed that during graded exercise, subjects with a high $\dot{V}O_{2max}$ (range = 58 $ml \cdot kg^{-1} \cdot min^{-1}$ to 75.3 $ml \cdot kg^{-1} \cdot min^{-1}$) were able to shift to higher work rates with an increase in blood lactate concentration but with an associated higher ability to remove lactate during the recovery. Yoshida et al. (1991) showed that after intense exercise there is a transient increase in the ratio of muscle blood flow to oxygen uptake during early recovery. Freund et al. (1992) discovered that highly trained individuals were able to clear lactate from working muscle faster than sedentary or diseased individuals.

Some research using magnetic resonance spectroscopy measuring techniques has proven extremely useful in evaluating muscle metabolism and the effects of training. McCully et al. (1988) have shown that the ratio of Pi to PCr reflects the relative metabolic rate of mitochondrial respiration (V) and the extrapolated maximum capacity of oxidative metabolism (V_m). Differences in human metabolic response to exercise are reflected in the subjects V_ms (oxidative capacity in skeletal muscle) and the degree of acidosis during exercise. McCully et al. (1988) found that human subjects vary considerably in their response to exercise. Some subjects have steep transfer functions with high calculated V_ms while others show lower V_ms with considerable acidosis. Steeper transfer functions reflect greater concentrations of mitochondria with each mitochondrial unit working at a lower proportion of its maximum (V_m). Early acidosis reflects greater reliance on glycolytic pathways for energy metabolism. McCully and Boden (1987) compared the muscle in the wrist flexors of elite rowers and sedentary controls. The wrist flexor muscle of elite rowers

(aerobic - endurance trained athletes) had a slope of the transfer function of 180% of controls as well as faster rates of recovery from exercise (4.39 ± 0.78 compared with 2.31 ± 0.22 PCr/Pi/min) than normal controls. These results indicated a higher capacity for oxidative synthesis of ATP by the rowers compared to the controls. Subsequently, Kuno et al. (1991) followed up this work by conducting a training study to determine whether the increased oxidative capacity was directly related to aerobic-endurance training. They subjected rats to 6 months of aerobic-endurance training and evaluated the hindlimb by ^{31} P-MRS. They found a 40% increase in rate of oxidative ATP synthesis compared with the control group.

The findings of these studies support the theory that aerobic training produces physiological adaptations which facilitate optimal recovery from anaerobic exercise. However, much work needs to be done to confirm and further delineate this relationship. Fortunately, the arrival of magnetic resonance spectroscopy greatly extends the power of the investigator to examine this potential link.

Methods of Studying Muscle Metabolism

The study of muscle metabolism has been an area of keen interest, especially in the areas of medical research and exercise physiology. Professionals from both disciplines have been able to contribute significantly to the understanding of the fatigue and recovery processes in diseased, normal and highly trained individuals. Often the advancement of knowledge in this area has been limited by the methods and available technology. As the equipment and methods have become more advanced, muscle metabolism in a variety of conditions has become more clearly understood. The purpose of this section is to present briefly, the most current method available for the study of muscle metabolism: magnetic resonance spectroscopy.

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a technique which utilizes electromagnetic energy to selectively interact with the magnetic fields of specific atomic nuclei. This energy can be employed in such a way that the elemental content and

molecular structure of chemical compounds within a sample material can be determined. This phenomenon was discovered in 1946 and was first used in applications within the physical sciences. Through the efforts of many others who later refined the technology, the technique has now been refined, enabling the study of living organisms. Today, magnetic resonance spectroscopy is considered one of the most significant discoveries since the medical x-ray (Sepega et al., 1987). As applied to living tissue, ^{31}P-MRS has become a powerful, non-invasive tool for the analysis of energy metabolites within the cell.

MRS offers several advantages over traditional methods. It can provide a direct and continuous assessment of relative concentrations of ATP, phosphocreatine (PCr), inorganic phosphate (Pi) as well as intracellular pH. This single advantage as applied to the study of high-energy phosphates and glycolytic metabolism in exercise, makes MRS a very attractive tool. Another advantage of MRS is that the measurements are repeatable. This is partly due to the non-invasive nature of the method. The safety of the technique for human research is well-documented. MRS does not involve any form of ionizing radiation, and extensive studies have not revealed any deleterious effects (Sepega, et al., 1987).

MRS is not without it's disadvantages however, at least for the time being. The whole body, superconducting magnet scanner is extremely expensive. Generally, only well-equipped, urban hospitals own such a unit. The demand for it's use is very high as it has infinite applications in medicine. This can make the accessability of MRS difficult for the researcher wishing to utilize the technique for normal, healthy subjects. Due to the complexity of the technology and the equipment, only a highly specialized MRS technologist can conduct the testing procedure. Interpretation and analysis of the data also requires a high level of expertise.

From an exercise "mode" perspective, ^{31}P-MRS has certain limitations related to the existence of a magnetic field. Metal apparatus, as is conventionally used for exercise testing can not be used. Therefore, other testing apparatus must be designed which is non-metallic, yet durable enough to permit maximal exercise. The sheer size and shape of the magnet also makes it difficult to simulate many common types of concentric exercise and some isometric activities.

Overall, ^{31}P-MRS provides the most advanced method of studying muscle metabolism. It is highly likely that the information amassed from future bioenergetic studies utilizing MRS will contribute significantly to the resolution of the remaining mysteries surrounding muscle fatigue and recovery.

CHAPTER III
METHODOLOGY

Design

A cross-sectional design was used to investigate the relationship between aerobic power and rate of recovery from anaerobic exercise. This design incorporated a five-stage process as illustrated in Figure 1.

Stage 1: Recruitment of Subjects

Twenty-one male subjects were selected from a larger pool of 34 volunteers. This selection was based on established criteria for a maximal aerobic power test (see Stage 2). Volunteers were recruited from university physical education and science classes, the track and football teams and a local triathlon club. Physical and performance characteristics are shown in Table I.

Consent

Each subject was provided with a written document outlining the research procedures, principal investigators, potential risks and benefits, and the conditions of the study (Informed Consent - Appendix C). The investigator checked with each subject to ensure understanding and agreement for compliance with the procedures before requesting a voluntary written consent. Prior to data collection the study was approved by the University of Alberta, Faculty of Physical Education and Recreation Ethics Committee.

Stage 2: Measurement of Aerobic Fitness

Rationale

The purpose of measuring aerobic power was to identify subjects who would qualify for one of two distinct groups based on maximal aerobic power. Maximal aerobic power may be defined as the maximal rate of oxygen consumption attained by an individual, as measured during a progressive exercise test to exhaustion. $\dot{V}O_{2max}$ is considered to be "the gold standard" for assessing endurance performance potential (Sutton, 1992). With modern equipment and standardized procedures, this test demonstrates good reliability. Katch et al. (1982) reported that biological variability may influence $\dot{V}O_{2max}$ by up to ± 5.6% of the variability seen between two tests on the same subject. Therefore, for an individual with a $\dot{V}O_{2max}$ of 70 $ml \cdot kg^{-1} \cdot min^{-1}$, biological variability may account for differences of up to

Stage 1 ---------->	Stage 2 ---------->	Stage 3 ---------->	Stage 4 ---------->	Stage 5
Recruitment	Aerobic Fitness $\dot{V}O_{2max}$ | | | | Stage 2A Exclusion	Anaerobic Fitness Strength and Power in Ankle Plantar Flexion	Assignment to Groups of High and Low Aerobic Power	Investigation a) Determine MPFWC b) Measure Recovery after Exercise at an intensity of 120% MPFWC

Timelines: (For 1 subject)

May and June	Day 1	Day 2 - 7	Continuous	Within 14 days of $\dot{V}O_{2max}$ test

FIGURE 1 - EXPERIMENTAL DESIGN

± 3.9 $ml \cdot kg^{-1} \cdot min^{-1}$. To measure the reliability of the test administrator regarding consistency in methods and application of criteria, this investigator administered three separate $\dot{V}O_{2max}$ tests on a subject not involved in the study. Each test was separated by a three day period. Differences between tests did not exceed ± 2 $ml \cdot kg^{-1} \cdot min^{-1}$.

Each subject performed an exercise test on a motor driven treadmill to determine $\dot{V}O_{2max}$. Running was chosen as the mode of exercise because this activity places a significant demand on the muscles of the leg, especially the gastrocnemius. The gastrocnemius is also a convenient muscle to study because the size and location permits accurate measurement with ^{31}P-MRS. For these reasons the gastrocnemius muscle served as the primary muscle for the measurement of metabolic flux during rest, exercise and recovery in this study. To further maximize the recruitment of the gastrocnemius muscles during the $\dot{V}O_{2max}$ test, uphill running was incorporated into the final stages of the protocol (Kasch et al., 1976).

Laboratory Measurement of Aerobic Fitness

Each subject performed an exercise test on a Quinton motorized treadmill (Model 1860). This test incorporated progressive increases in intensity by increasing speed (0.22 $m \cdot s^{-1}$ increments) and eventually grade (2% increments). $\dot{V}O_{2max}$ was measured using a Sensormedics 2900Z Metabolic Measurement System. Respiratory gas exchange data were analyzed and reported every 20s throughout the test. The highest $\dot{V}O_2$ value was accepted as $\dot{V}O_{2max}$ provided that certain criteria were met. These criteria included evidence of a plateau or decrease in $\dot{V}O_2$ with an increase in load, and one or more of the following: a) respiratory exchange ratio (R.E.R) > 1.10, b) volitional cessation by the subject, c) a heart rate within 20 beats of the subject's age predicted maximum heart rate (220 - age). All subjects satisfied these criteria. The detailed laboratory procedure used to conduct this test is outlined in Appendix D.

Stage 2A: Exclusion

Subjects (n=11) with $\dot{V}O_{2max}$ values in the middle range (50.1 - 59.9 $ml \cdot kg^{-1} \cdot min^{-1}$) discontinued the study. This strategy of creating low and high aerobic fitness groups based on $\dot{V}O_{2max}$ was employed to create a large statistical and physiological difference between the two groups so that the potential effects of aerobic power on recovery rate could be assessed more clearly. Subjects who were excluded were provided with a copy and an interpretation of their results to use for direction in their personal training programs.

Stage 3: Measurement of Strength and Power in Plantar Flexion Exercise

Rationale

Intense intermittent exercise requires strength and anaerobic power. To make the final results (recovery rates) generalizable to other athletes who compete in intermittent sport, it was necessary to restrict this study to subjects who could demonstrate a level of muscular strength and power that is representative of athletes who engage in intermittent sport. Sexsmith (1985) found that athletes who engaged in five weeks of high-velocity resistance training attained peak torques during ankle plantar flexion in the range of 1.3 - 2.1 $N \cdot m \cdot kg^{-1}$. For the purposes of this study, the minimal level of peak torque was set at 1.3 $N \cdot m \cdot kg^{-1}$. Muscle strength and power was measured during plantar flexion exercise on a Cybex 340 isokinetic dynamometer. Although other muscles contribute to produce plantar flexion at the ankle joint, the gastrocnemius is considered to be the predominant contributor (Weineck, 1986). All subjects were able to meet or exceed a peak torque of 1.3 $N \cdot m \cdot kg^{-1}$. Therefore no subjects were excluded from the study (Table 1).

Laboratory Measurement of Strength and Power in Plantar Flexion

Plantar flexion exercise was performed on a Cybex 340 isokinetic dynamometer system by depressing a footplate attached to a lever system. Detailed procedures are outlined in Appendix E.

From these data, absolute strength and strength relative to body mass were determined from the Best Work Repetition (BWR) attained during exercise (four test repetitions) at an angular velocity of 30 degrees per second. Peak power was determined from the greatest power produced during a single repetition at 180 degrees per second. Average power was calculated from all four repetitions.

Stage 4: Assignment to Groups

Continuing subjects were divided accordingly into one of two groups. Subjects with a $\dot{V}O_{2max}$ of 50 $ml \cdot kg^{-1} \cdot min^{-1}$ or less were assigned to the low aerobic power (LAP) group. Subjects with a $\dot{V}O_{2max}$ of 60 $ml \cdot kg^{-1} \cdot min^{-1}$ or greater were assigned to the high aerobic power (HAP) group. Volunteers were recruited until a minimum of 10 subjects were found for each group. A $\dot{V}O_{2max}$ below 50 $ml \cdot kg^{-1} \cdot min^{-1}$ on a treadmill is considered representative of relatively sedentary to moderately active men, whereas a $\dot{V}O_{2max}$ of 60 $ml \cdot kg^{-1} \cdot min^{-1}$ or

greater (treadmill) is characteristic of well-trained athletes who engage in aerobic power or endurance activities such as intermediate to long distance running, rowing, cross-country skiing, etc (Katch et al., 1991).

Thirty-four individuals were tested before a minimum of 10 subjects were found for each group. The mean (± SE) maximal aerobic power of the LAP and HAP groups were 46.6 (± 3.6) and 64.6 (± 4.5), respectively, demonstrating a mean difference between groups of 18 $ml \cdot kg^{-1} \cdot min^{-1}$.

Stage 5: Measurement of Maximal Plantar Flexion Work Capacity (MPFWC) and ^{31}P-Magnetic Resonance Spectroscopy (^{31}P-MRS)

Prior to the completion of the ^{31}P-MRS protocol, the Maximal Plantar Flexion Work Capacity (MPFWC) was determined for each subject. The purpose of this measurement was to be able to identify the maximal cumulative load that the subject could perform during continuous plantar flexion exercise under specified conditions. The load assigned to each individual for the ^{31}P-MRS rest, exercise and recovery protocol was set at 120% of MPFWC. Thus, each subject was working at the same relative intensity and the recovery rate was a reflection of a similar degree of fatigue for each subject.

Laboratory Measurement of MPFWC

The MPFWC for each subject was measured with a progressive exercise test using the same foot pedal ergometer system used during the exercise protocol measured by ^{31}P-MRS. Detailed procedures for the MPFWC test may be found in Appendix F.

Rationale for ^{31}P-Magnetic Resonance Spectroscopy (^{31}P-MRS)

Magnetic resonance spectroscopy is a technique which utilizes electromagnetic energy to interact selectively with the magnetic fields of specific atomic nuclei (in this case phosphorous-31). This energy can be employed in such a way that the elemental content and molecular structure of chemical compounds within a sample material can be determined. As applied to living tissue, ^{31}P-MRS has become a powerful non-invasive tool for the analysis of energy metabolites. For recent reviews of this technique with specific applications in exercise physiology, articles by Sapega et al. (1987) and McCully et al. (1988) are recommended.

For the purposes of this study, ^{31}P-MRS was used to measure relative concentrations

of PCr, Pi and intracellular pH in the right gastrocnemius muscle during rest, exercise and recovery. The distinct advantage of ^{31}P-MRS is that it can provide a direct and continuous measurement, which is particularly valuable for observation and analysis of the recovery process. Subjects exercised at 120% MPFWC utilizing the same exercise apparatus as that for determining MPFWC. High intensity exercise has been shown to elicit decreases in PCr and pH and increases in P_i (Taylor et al., 1986; McCully, 1988; Soderlund and Hultman, 1990; Pan et al., 1991). Pilot work using 120% MPFWC has produced similar results.

Measurement of Recovery Rate with ^{31}P - Magnetic Resonance Spectroscopy

In vivo muscle metabolism was measured using a Philips 1.5 Tesla (S15 Gyroscan) whole body scanner. The resonance frequency of the ^{31}P nuclei was 25.86 MHz. A 6 cm diameter surface coil was used as a transmitter and receiver. The surface coil was placed 0.6 cm below the belly of the right calf muscle and the excitation band of the radio frequency (RF) field was positioned such that it was centred at the maximum circumference of the calf muscle. The procedure for subject set-up was identical to the set-up for measurement of MPFWC. The complete protocol is reported in Appendix G. The coil was carefully tuned and matched for each subject. The power of the RF electromagnetic wave to the surface coil was adjusted to generate a 180 degree flip angle for ^{31}P nuclei at the centre of the coil. According to Bendall (1984), the most sensitive region under interrogation of gastrocnemius is about 2-3 cm below the skin surface. The ^{31}P-MRS data were collected with an optimal pulse width (90 μs), at a rate of one pulse every second throughout the experiment. The one second scans were combined to provide an optimal number of spectra for each phase of the protocol. A trade-off exists between obtaining an optimal signal-to-noise ratio and acquiring short interval data which display rapid metabolic changes in the muscle. Table 1 illustrates the bin size (scans per spectrum) used during various phases of data collection as well as the number of bins and total time engaged in each phase. The areas under the PCr and Pi peaks of the ^{31}P-MRS spectra were determined by curve fitting using an MRS software processing program and Ramtek computer system. The chemical shift from the centre of the Pi peak to the PCr peak was used to determine intracellular pH according to the following form of the Hendersen-Hasselbach equation:

$$pH = 6.75 + \log_{10} [(a - 3.27)/(5.69 - a)]$$

where "a" is the chemical shift between Pi and PCr. Radda and Gadian (1986) suggest this form of the equation when pH of skeletal muscle is being measured.

TABLE 1

Data Collection Intervals During Measurement of pH and PCr During Rest, Exercise and Recovery

Phase	Scans Per Spectrum	# of Spectra (Bins)	Time (min)
Rest	60	4	4
Exercise	30	4	2
Early Recovery	10	12	2
Middle Recovery	20	12	4
Late Recovery	60	4	4
Total		36	16

Rationale for Timelines

To ensure full recovery between each of the exercise tests, a minimum of 24 hours was provided between the measurement of maximal aerobic power (Stage 2) and the measurement of strength and power in ankle plantar flexion (Stage 3). Immediately after Stage 3, each subject completed the test of MPFWC (Stage 5A). To avoid any consequences of residual muscle soreness, a minimum of one week of recovery was provided prior to the ^{31}P-MRS protocol (Stage 5B). Most subjects reported some degree of soreness the day after the test of MPFWC. However, within 72 hours all subjects reported normal sensation and function. The ^{31}P-MRS protocol (Stage 5B) occurred within two weeks of measuring $\dot{V}O_{2max}$ (since beyond two weeks changes may begin to occur in aerobic power). An attempt was made to minimize any potential changes in aerobic power levels even within the two week period by requesting that each subject maintain their personal fitness level by following a similar pattern of activity, prior to and during the data collection period. If it became impossible to test a subject within the two week period, another $\dot{V}O_{2max}$ test was performed to verify the aerobic fitness status of the subject. One such case occurred in this study. The subject's $\dot{V}O_{2max}$ scores were 48.2 and 49.6 ml·kg^{-1}·min^{-1} on week one and week three, respectively. The value obtained closest to the MRS protocol (49.6 ml·kg^{-1}·min^{-1}) was taken as the $\dot{V}O_{2max}$ for this subject.

Statistical Analysis

Subjects were measured on each of seven variables to determine physical characteristics and performance on specific fitness parameters. These variables included age, height, weight, $\dot{V}O_{2max}$, peak torque and average power of the right calf muscle during ankle plantar flexion and MPFWC. Results for each subject are shown in Appendix H. The mean and standard error for the respective LAP and HAP groups are reported in Table 1. The mean and standard deviations on each variable were determined and statistical analysis between groups was performed using a one-way analysis of variance (ANOVA).

The experimental results of the exercise and recovery protocol were expressed as changes which occur in PCr and pH over time. The PCr and pH results were analyzed using a curve-fitting model derived from non-linear regression to analyze the variance between groups. A line of "best fit" was determined for the LAP and HAP groups on each of these variables. From these models of pH and PCr, the experimental data were used to answer the following three questions:

1. Is there an overall difference in pH or PCr response over time between the two groups?
2. Is there a difference in the slope of the recovery (6 - 16 minutes) of pH or PCr between the two groups?
3. Is there a difference in the 1/2 time of recovery of pH or PCr between the two groups?

A one-way ANOVA was used to determine if the 1/2 time on each of these variables was different between groups. The level of probability considered sufficient to reject the experimental hypotheses was set at $p < 0.05$.

CHAPTER IV
RESULTS

Subject Characteristics

A total of 34 male volunteers were recruited and screened to identify 10 subjects in each of the LAP and HAP groups. The mean aerobic power (± S.E) of the LAP and HAP groups were 46.6 (3.6) and 64.6 (4.5), respectively, resulting in a mean difference ($p < 0.05$) of 18 $ml \cdot kg^{-1} \cdot min^{-1}$ (Table 2). The two groups were also different ($p < 0.05$) with respect to weight. These differences were expected given the fitness level and type of training associated with each group. The LAP group consisted of sedentary (n=7) and anaerobically trained (n=3) individuals. The HAP group (n=11) was more homogenous and consisted of individuals who were all well-trained aerobically. The additional weight of the LAP group was likely due to a higher percentage of fat in the sedentary individuals and/or a higher percentage of muscle mass in the anaerobically trained individuals.

Primary Findings

Magnetic resonance spectroscopy measurements of PCr and pH obtained during the rest-exercise-recovery protocol did not indicate significant differences in the rate of recovery between the LAP and HAP groups.

PCr Response

Figure 2 illustrates the changes in PCr which occur during rest, exercise and recovery phases for LAP and HAP groups (a list of values is reported in Appendix J). For clarity, standard error bars are shown in Figure 3. Throughout each phase, mean PCr values were differe * ($p < 0.05$) between the two groups and the degree of difference was relatively constant throughout the 16 minute protocol. During the resting phase mean PCr values were 9.65 for the LAP group and 11.24 for the HAP group. These PCr values represent the relative concentration of PCr in the sample after curve fitting (Appendix I). Both groups experienced a dramatic drop in PCr during exercise. The lowest mean PCr values for the groups were 2.50 (LAP) and 2.77 (HAP), representing hydrolysis of 74.1% and 75.4% of

resting PCr stores by the LAP and HAP groups, respectively. Both of these values were obtained during the last 30 seconds of the exercise phase. The early recovery period (first 2 minutes) was marked by a rapid recovery for both groups. After 2 minutes, PCr replenishment was 91.8 and 87.7% complete for the LAP and HAP groups, respectively; at 6 minutes of recovery, 98.8% (LAP) and 100% (HAP). Although the LAP group did not reach 100% replenishment until the last minute of recovery (10 minutes), [PCr] can be considered functionally replenished at 6 minutes, especially since the final 0.7% of the PCr value was well within the range of variation seen during the resting phase (PCr is not static even during rest). These data suggest a fast and slow component of recovery for PCr.

An analysis of the PCr response across time was conducted using non-linear regression models (Appendix L). Given all the data (PCr vs. time) for the LAP group, a model was created which represented how well PCr could be predicted by various points in time during the experimental protocol. This procedure was repeated using data from the HAP group. The slope of the regression lines (best fit) were then compared (LAP group vs. HAP group). Three specific questions were asked:

1) Is there an overall difference in PCr response across time between LAP and HAP groups?
2) Is there a difference in the slope of the recovery (6 - 16 minutes) of PCr between the LAP and HAP groups?
3) Is there a difference in the 1/2 time of recovery of PCr (time required to resynthesize 1/2 the relative concentration of PCr lost during exercise) between the LAP and HAP groups?

The non-linear regression model for PCr accounted for 71.7% of the individual variation in pH response across rest, exercise and recovery. The relative concentration of PCr throughout the entire protocol was significantly different ($p < 0.05$) between the two groups. The relative concentration of PCr for the HAP group was consistently higher than the LAP group. The slope of the recovery of PCr was not different between the two groups. There was a high degree of individual variation in the time required for PCr resynthesis (20 to 70 seconds) (Figure 3). The mean 1/2 times for recovery of PCr were 35 seconds (LAP) and 42 seconds (HAP). These values were not significantly different.

pH Response

Figure 4 illustrates the mean values for pH during rest, exercise and recovery for the LAP and HAP groups (list of values reported in Appendix K). For clarity, standard error bars are shown separately in Figure 5. pH values were calculated from the chemical shift (in parts per million units) between the inorganic phosphate (Pi) peak relative to the phosphocreatine (PCr) peak (Appendix I). During the resting phase, mean pH values for both groups were 7.07. The initial pH response to exercise displayed a transient shift toward alkalinity (LAP: 7.10; HAP: 7.13) before a rapid drop followed in both groups for the duration of the exercise period. Although it may appear that the HAP group experienced an earlier rate of decline in pH during exercise, these differences are not significant. During the early recovery period (first 2 minutes), pH continued to drop. The lowest mean pH value after exercise was 6.38 (80 seconds into recovery) for the LAP group and 6.45 (40 seconds into recovery) for the HAP group. After this point, pH began to recover very rapidly for approximately 5 minutes, followed by a slower rate of recovery for the remaining 4 minutes. At the end of the 10 minute recovery period, the LAP and HAP groups had recovered to 100% and 98.6 % of their resting pH values, respectively.

An analysis of the pH response across time was conducted using non-linear regression models (Appendix M). Given all the data (pH vs. time) for the LAP group, a model was created which represented how well pH could be predicted by various points in time during the experimental protocol. This procedure was repeated using data from the HAP group. The slope of the regression lines (best fit) were then compared (LAP group vs. HAP group). As with PCr three specific questions were asked:

1) Is there an overall difference in pH response across time between LAP and HAP groups?
2) Is there a difference in the slope of the recovery (6 - 16 minutes) of pH between LAP and HAP groups ?
3) Is there a difference in the 1/2 time of recovery (time required to restore 1/2 of the value of the decline of pH from resting values) of pH between the LAP and HAP groups ?

The non-linear regression model for pH accounted for 60.4% of the individual variation in pH response across rest, exercise and recovery. The overall response of pH was not

significantly different between LAP and HAP groups. No significant differences in the slope of recovery of pH between the LAP and HAP groups were found. The apparent differences in slope during early recovery are statistically negated by the variation in individual pH values during this phase (Figure 5). Mean 1/2 times for recovery of pH were 3:35 minutes (LAP) and 3:28 minutes (HAP), indicating similarity in pH recovery between groups.

While group responses were not different, it is interesting to note that individuals within each group demonstrated very different responses. Individual plots for pH from two LAP subjects and two HAP subjects are shown in Figures 6 and 7. In Figure 6, LAP subject #5 ($\dot{V}O_{2max}$ = 41.1 $ml \cdot kg^{-1} \cdot min^{-1}$) demonstrates a more severe drop in pH (0.3 units) in response to exercise and a delayed recovery (more than four minutes longer) than subject #5 ($\dot{V}O_{2max}$ = 49.5 $ml \cdot kg^{-1} \cdot min^{-1}$). Figure 7 indicates that HAP subject #5 ($\dot{V}O_{2max}$ = 60.6 $ml \cdot kg^{-1} \cdot min^{-1}$) also required more than four additional minutes to restore pH as compared to subject #8 ($\dot{V}O_{2max}$ = 66.8 $ml \cdot kg^{-1} \cdot min^{-1}$). Further examination of other individual pH responses show a wide degree of variation in rate and pattern of response during the recovery period.

TABLE 2

Physical and Performance Characteristics of Low and High Aerobic Power Groups

Variable	Low Aerobic Power (n = 10)	High Aerobic Power (n = 11)
Age (years)	27.8 (6.7)	24.0 (4.5)
Height (cm)	178.1 (6.2)	179.4 (6.1)
Weight (kg)	88.8 (16.3)*	73.0 (6.4)*
$\dot{V}O_{2max}$ ($l \cdot min^{-1}$)	4.17 (0.97)	4.69 (0.28)
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	46.6 (3.6)*	64.6 (4.5)*
Peak Torque ($N \cdot m$)	136 (27.8)	120 (11.8)
Peak Torque ($N \cdot m \cdot kg^{-1}$)	1.5 (0.2)	1.6 (0.1)
Average Power (W)	120 (41.8)	97 (24.6)
Average Power ($W \cdot kg^{-1}$)	1.3 (0.3)	1.3 (0.3)
MPFWC (kg)	24 (7)	20 (4)
MPFWC ($kg \cdot kg\ bw^{-1}$)	0.3 (0.04)	0.3 (0.04)

Values are Mean ± (S.E.)

* Significant Difference ($p \leq 0.05$)

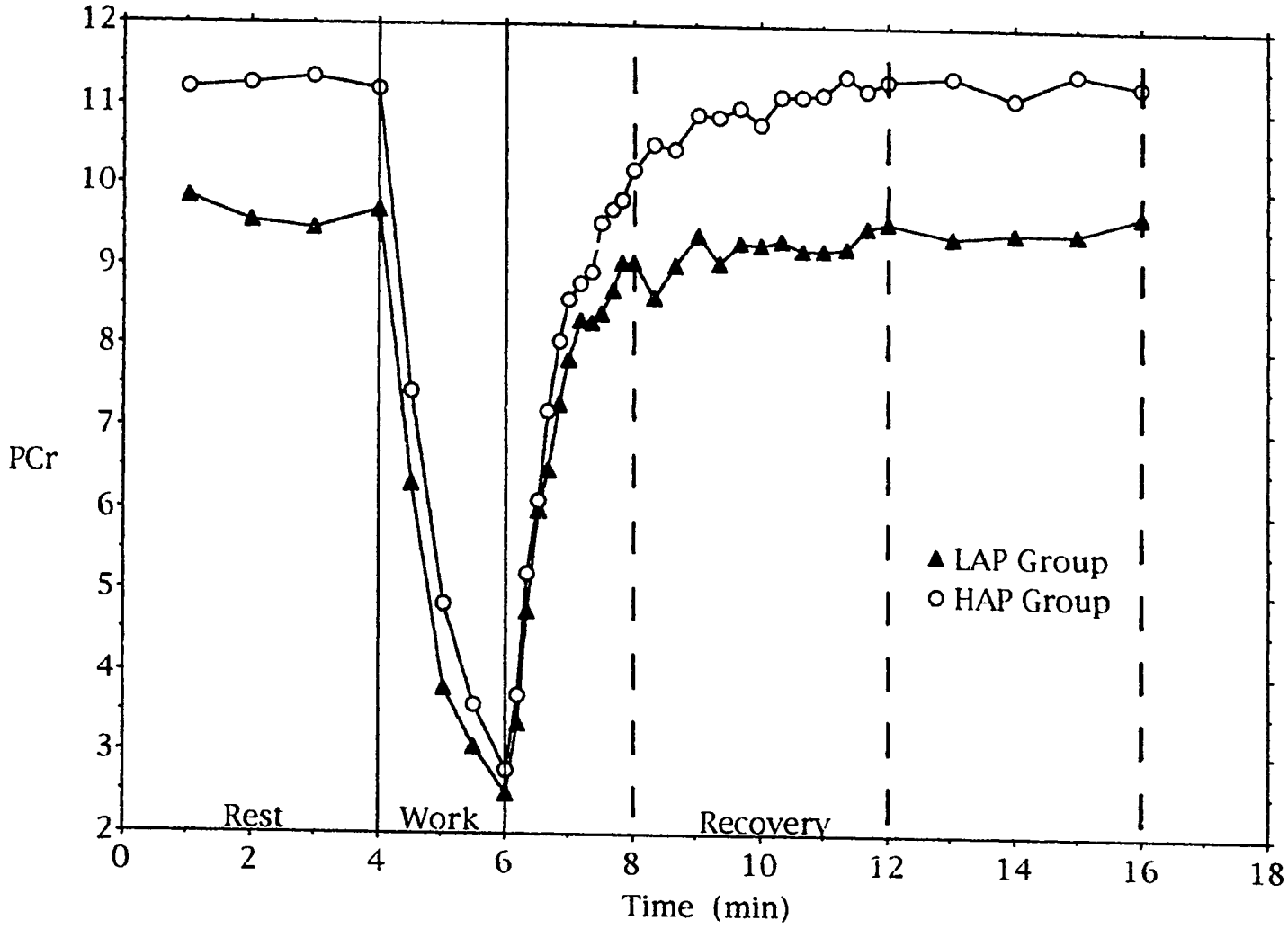


Figure 2: Mean PCr of Gastrocnemius Muscle During Rest, Exercise and Recovery for Low Aerobic Power (LAP) and High Aerobic Power (HAP) Groups

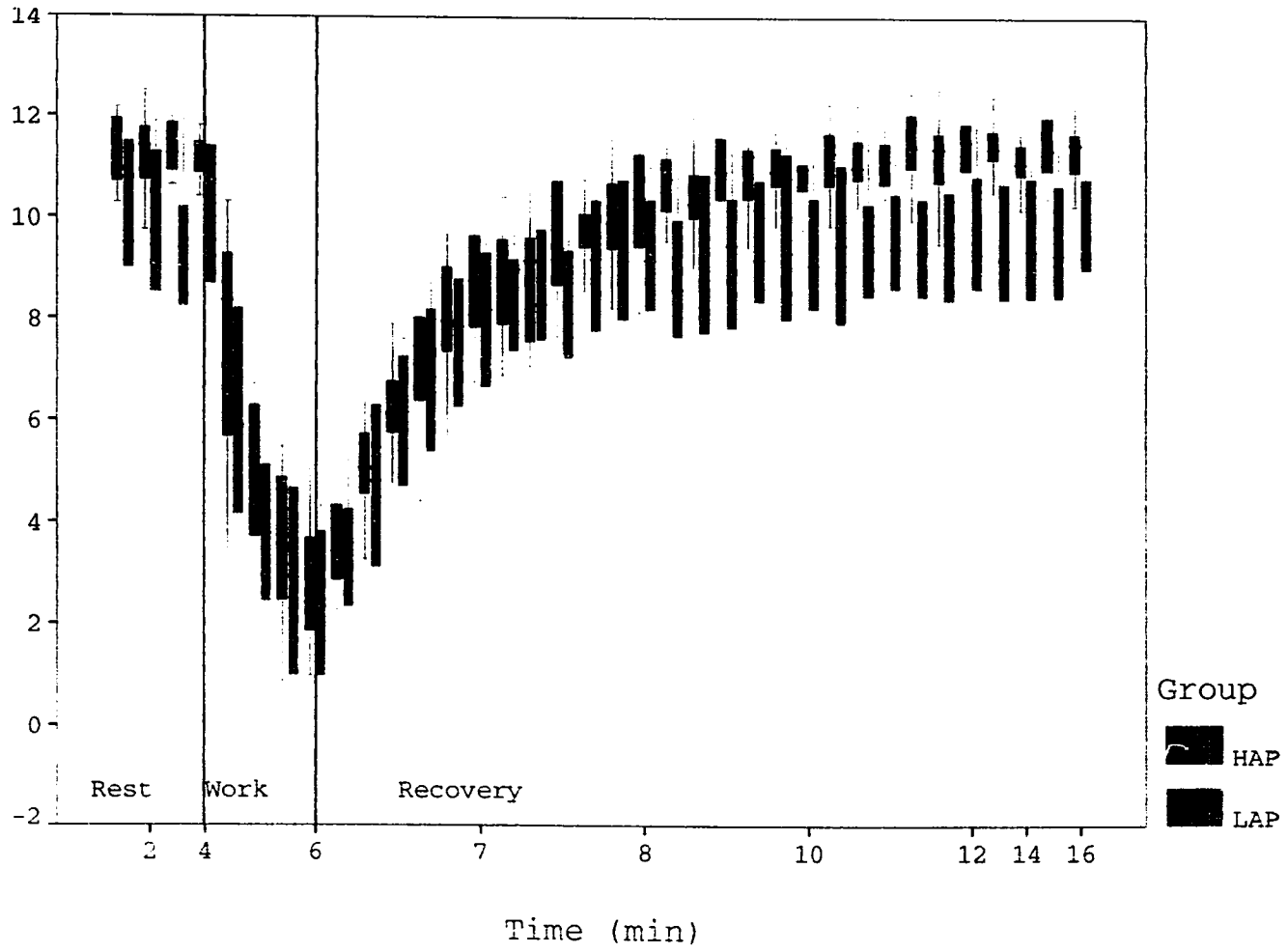


Figure 3: Standard Error Bars for Mean PCr of Human Gastrocnemius Muscle During Rest, Exercise and Recovery for LAP and HAP Groups

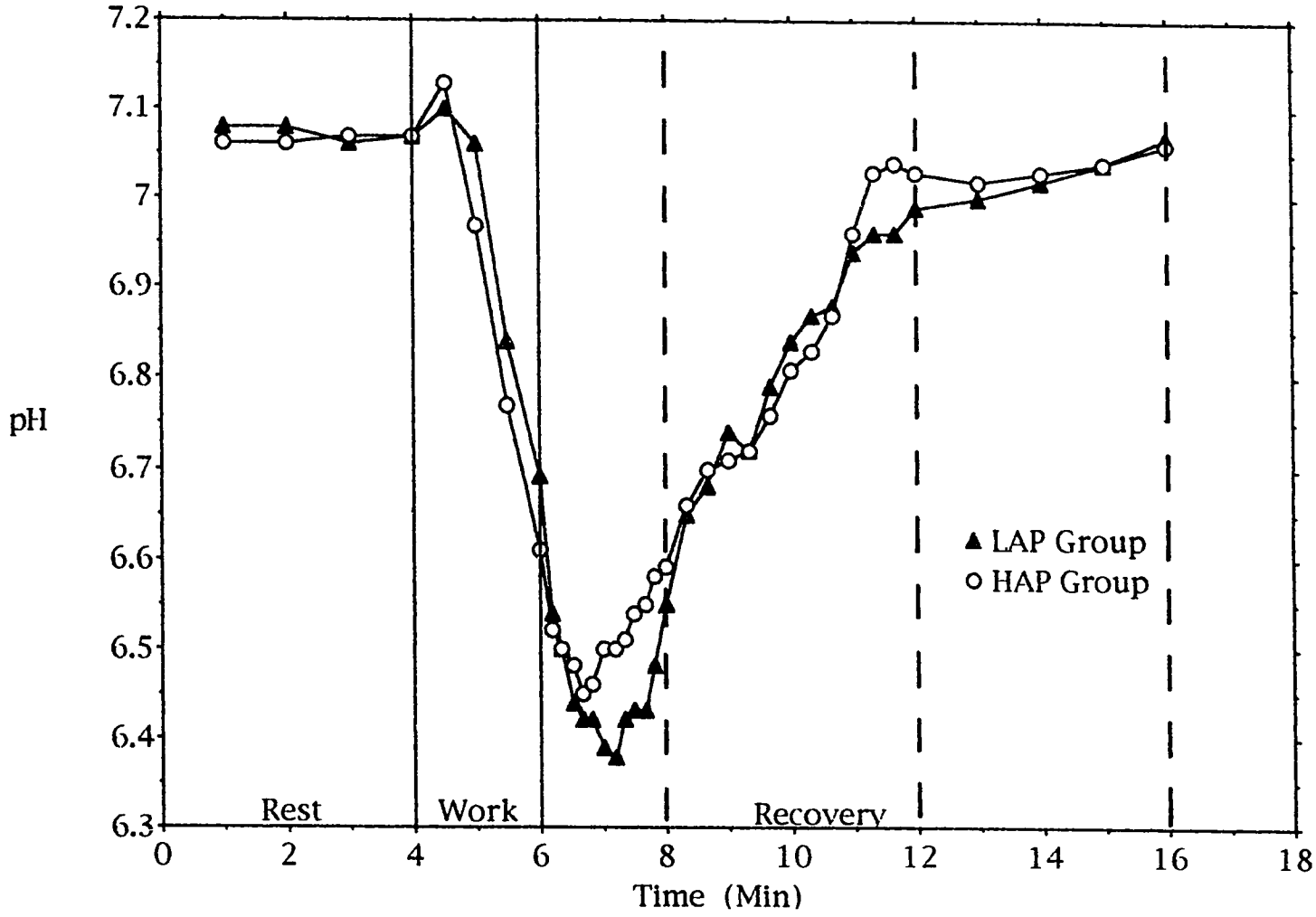


Figure 4: Mean pH of Gastrocnemius Muscle During Rest, Exercise and Recovery for Low Aerobic Power (LAP) and High Aerobic Power (HAP) Groups

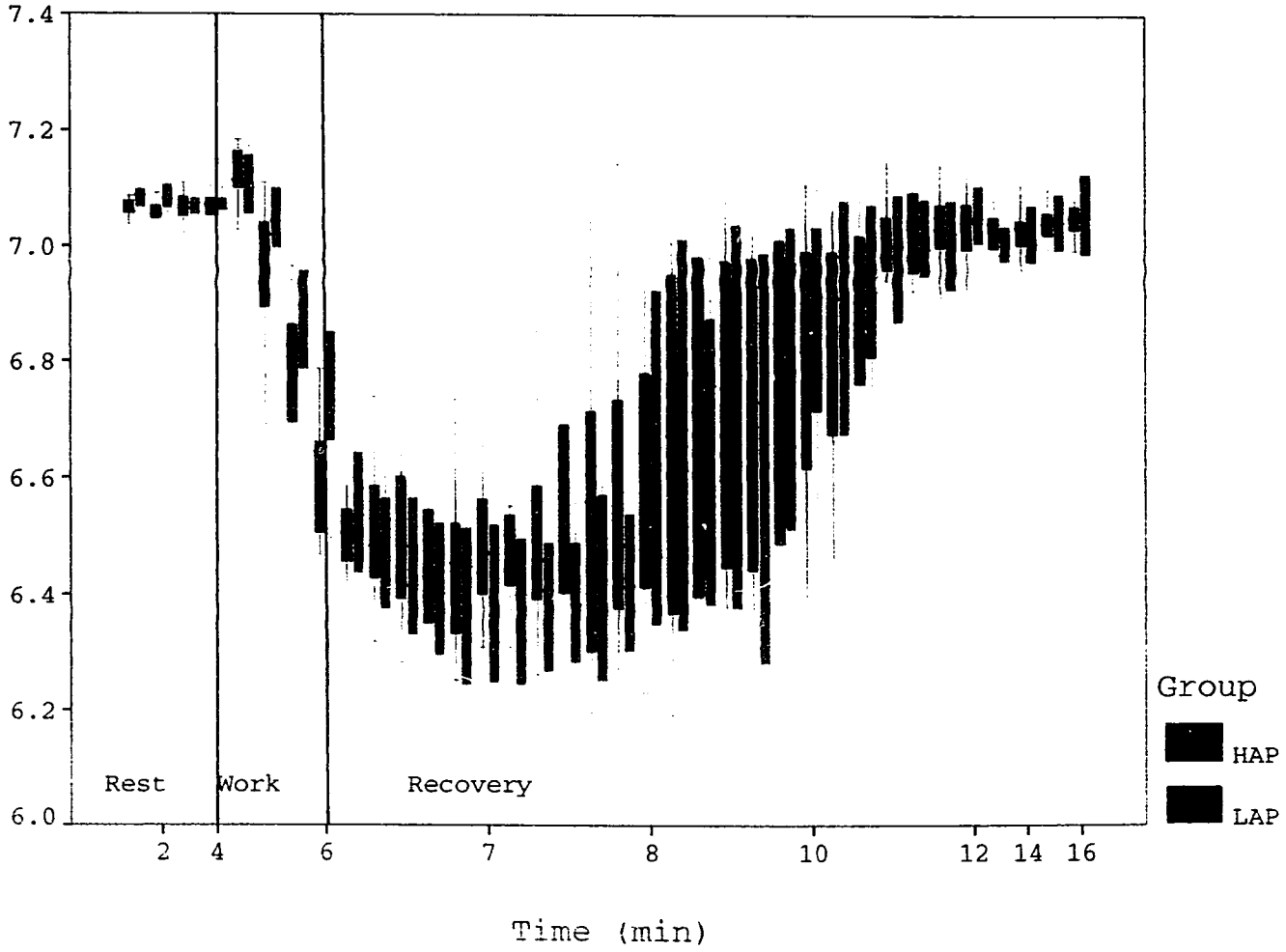


Figure 5: Standard Error Bars for Mean pH of Human Gastrocnemius Muscle During Rest, Exercise and Recovery for HAP and LAP Groups

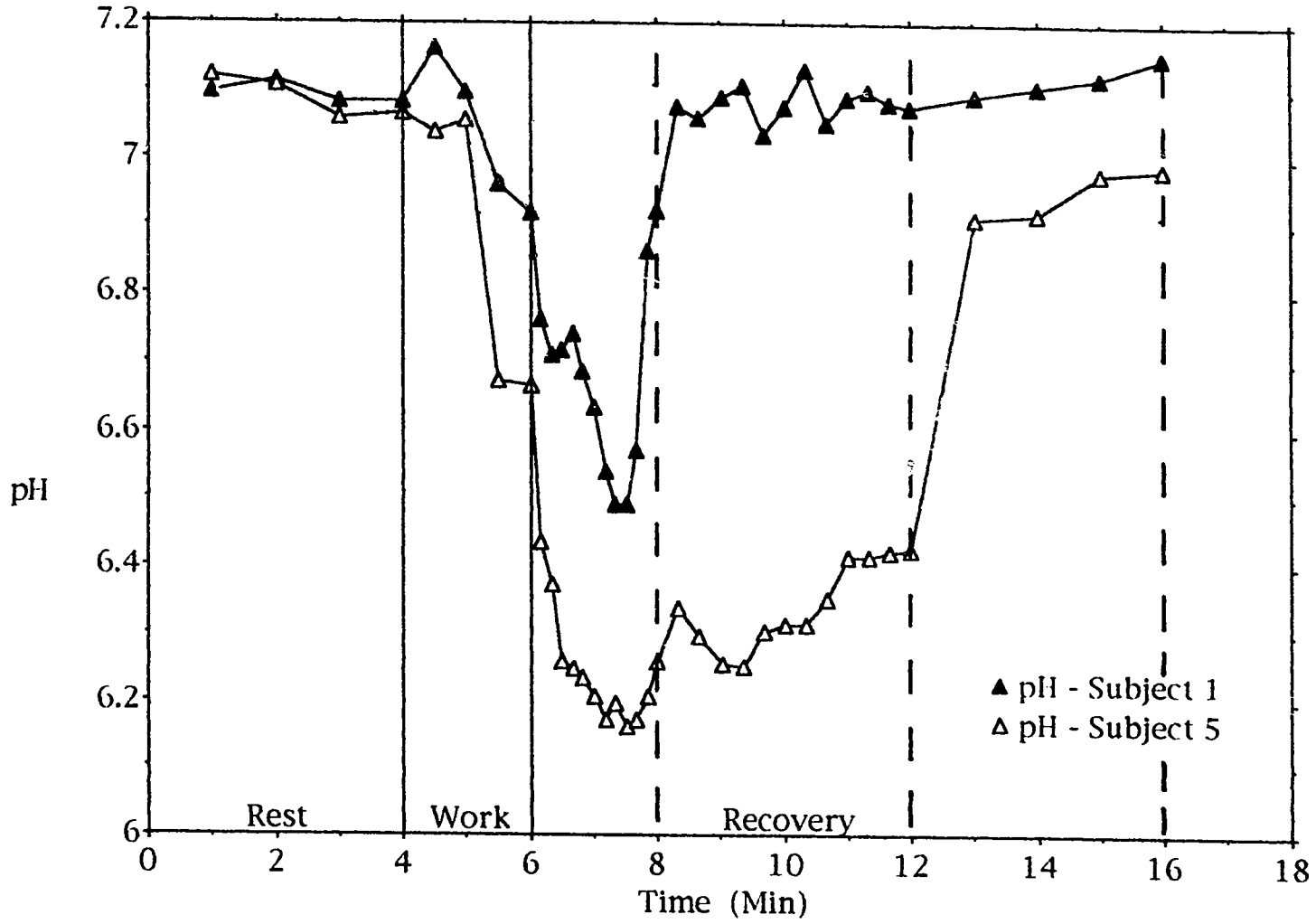


Figure 6: Comparison of pH Responses from 2 Subjects in the Low Aerobic Power (LAP) Group

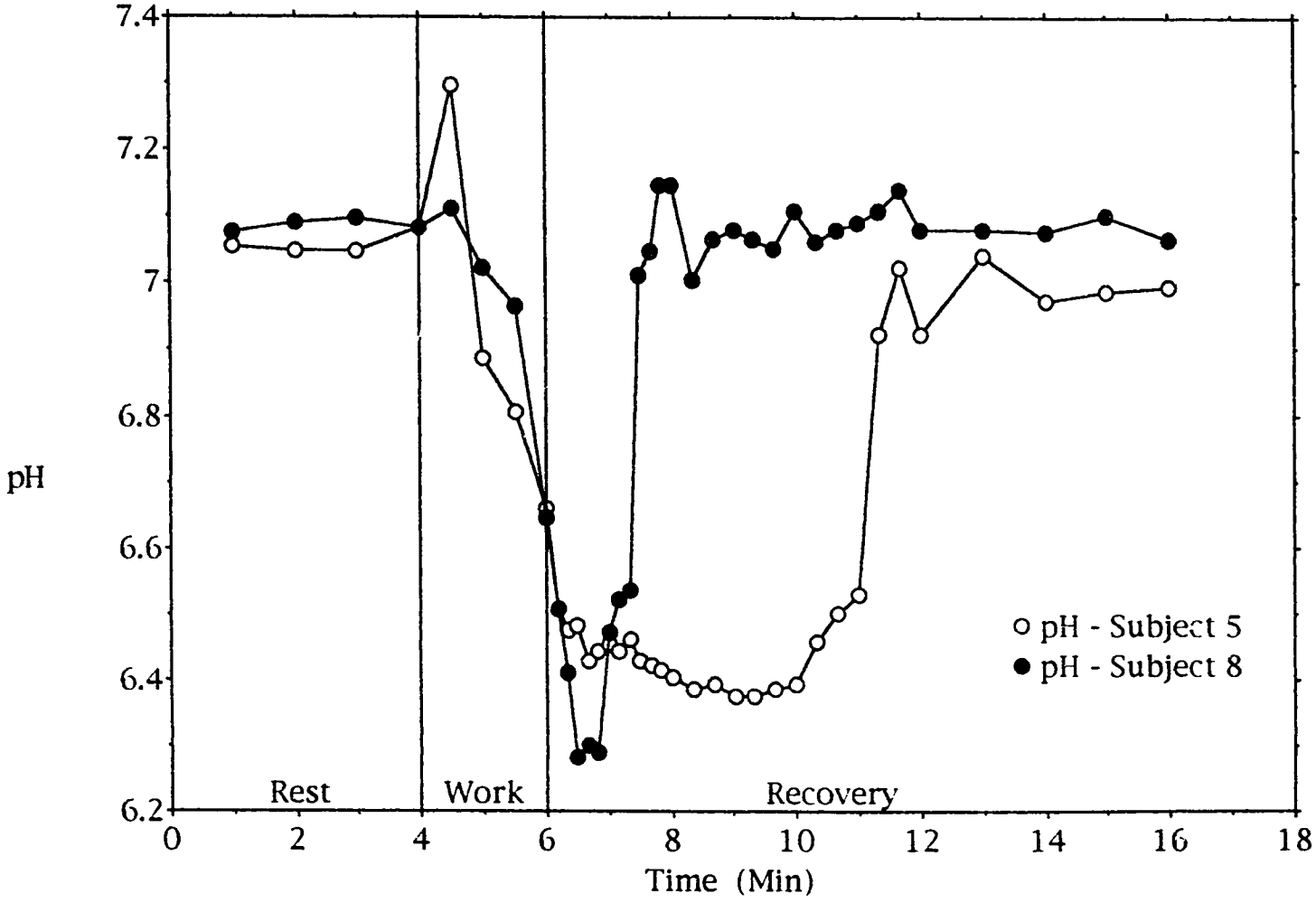


Figure 7: Comparison of pH Responses from 2 Subjects in the High Aerobic Power (HAP) Group

CHAPTER V
DISCUSSION

The major finding of this study was that following two minutes of high-intensity exercise, there were no significant differences in the rate of PCr resynthesis or restoration of pH between subjects with high and low levels of maximal aerobic power ($\dot{V}O_{2max}$). PCr (an indicator of energy supply) and pH (an indicator of the metabolic environment of the muscle) were monitored during rest, exercise and recovery. This discussion is organized to reflect the metabolic responses of the LAP and HAP group over the duration of the protocol (rest, exercise and recovery) with particular emphasis on the three phases of the recovery period (early, middle and late).

Response of PCr and pH During Rest and Exercise

Resting levels of PCr were significantly higher for the HAP group ($p < 0.05$). High resting levels of PCr are normally found in the muscles of trained subjects (Park et al., 1988). Both groups in this study included trained athletes [LAP group (3); HAP group (11)] and 11/14 of these individuals demonstrated resting PCr values in excess of 10.5 (high levels). It appears that the influence of the seven sedentary individuals in the low group (low resting PCr values) contributed to a significantly lower mean resting value for PCr for the LAP group. These differences should have no effect on the rate of recovery from exercise since each subject must return to his own resting level of PCr. Intracellular pH at rest was very similar for both groups. The mean value obtained during the resting phase (7.07) was in agreement with resting intracellular (^{31}P-MRS) pH values obtained from human calf muscle in other studies (Kuno and Itai, 1992; Yoshida and Watari, 1992; Achten et al., 1990; McCully et al., 1988).

The LAP and HAP groups demonstrated a similar response to exercise. Both groups demonstrated a transient shift toward alkalinity during the first 30 seconds of exercise (see Figure 2, data point #5). This temporary alkaline state reflects the role of PCr buffering during early exercise. Within the first few seconds of intensive exercise, the rate of PCr hydrolysis increases rapidly without the formation of lactate. This initial breakdown of PCr will absorb H^+ ions (Degroot et al., 1993):

$$PCr + ADP + [H^+] \quad \text{----------}> \quad ATP + Cr$$

The result may be an increase in pH. This alkalinization facilitates the activation of phosphofructokinase (PFK) and the glycogen phosphorylase system (Danforth, 1965), subsequently accelerating glycolysis and the formation of lactate. Given the higher resting levels of PCr in the HAP group it might have been expected that they would demonstrate more PCr buffering. However, the degree of shift in alkalinity was not different between groups. Furthermore, Jones (1980) suggests that the metabolic flux (and production of protons) is so great during high intensity exercise, buffering capacity is likely to have only a minor effect on pH. After the transient alkaline period (beyond first 10 - 20 seconds of exercise), the rate of lactic acid production by anaerobic glycolysis exceeds the rate of the creatine kinase H^+ consumption, increasing $[H^+]$, producing a rapid decline in pH for both groups. pH values were not statistically different between the groups at the end of exercise. PCr responses to exercise were also similar between the LAP and HAP groups. During the exercise period both groups continued to consume PCr at a relatively equal rate in an effort to rephosphorylate ADP to ATP. At the end of 2 minutes of high-intensity exercise the HAP group had consumed a greater absolute amount of PCr (8.47 HAP compared to 7.15 LAP) but an equal percentage of pre-exercise PCr stores (75.3% HAP and 74% LAP). The equal utilization of a significant amount of PCr indicates that both groups relied heavily on PCr hydrolysis to provide energy for the 2 minutes of high intensity exercise. In summary, the overall metabolic response (slope of PCr and pH) of each group to exercise was not statistically different.

Response of PCr and pH During Recovery

The results of this study confirm a biphasic pattern of recovery as demonstrated in previous studies (Sahlin et al., 1978; Pan et al., 1991; Sahlin, 1992). Energy stores, in the form of PCr were replenished first. An initial rapid phase (first 2 minutes) of recovery was characterized by resynthesis of phosphocreatine followed by a second, much slower phase (2 - 10 minutes) representing the restoration of pH.

Comparisons between the LAP and HAP groups during the recovery period illustrate the most important results with respect to the research hypotheses. Given the similarities in exercise responses, the metabolic responses during recovery could be used to test the hypothesis that differences would exist between the two groups and that the HAP group

would recover more quickly. Considering the metabolic and circulatory characteristics usually associated with high levels of $\dot{V}O_{2max}$, it was expected that the HAP group would recover more quickly from the anaerobic exercise than the LAP group. The results of this study do not support this hypothesis. During the early phase of recovery (first 2 minutes), PCr began to be resynthesized immediately and at a similar rate for both groups while pH continued to decline. The post-exercise drop in pH was expected as it has been reported by others (Wong et al., 1990; Achten et al., 1990; Yoshida and Watari, 1992). The major reason for the continued decline in pH is because the resynthesis of PCr (through breakdown of ATP) may release H^+ ions:

$$\text{Creatine} + \text{ATP} \text{ -----> } \text{PCr} + \text{ADP} + [H^+]$$

ATP may also be resynthesized via anaerobic glycolysis (which also produces H^+), or by oxidative phosphorylation. Phosphocreatine began to recover immediately and rapidly for both groups, especially during the first 45 seconds of recovery. The half-time (50% replenishment) for PCr was 35 seconds (LAP) and 42 seconds (HAP). These values are in general agreement with the literature as Sahlin (1992) reported the 1/2t of PCr to be approximately 30 seconds. The rate of PCr resynthesis is influenced by the metabolic environment of the muscle, specifically the status of ATP and the rate of oxidative phosphorylation within the muscle (Sahlin et al., 1979; Taylor et al., 1983; Tesch and Wright, 1983; McCully et al., 1988; Jansson et al., 1990) as well as the concentration of H^+ (Arnold et al., 1983; Iotti et al., 1991). It appears that these factors (either individually or in combination) were not sufficiently different to produce a significant difference in the 1/2t recovery of PCr between the LAP and HAP groups. Differences between the 1/2t recovery of PCr reported by Sahlin (1992) compared to the 1/2t reported in this study may have been caused by differences in the intensity of exercise protocols (which may affect PCr depletion and/or H^+ ion accumulation), or may have been caused by differences in oxidative capacity. A higher intensity of exercise in this study appeared to have caused more extensive depletion of PCr (>74% compared to <33% in other studies) and subsequently may have contributed to the slower rate for 1/2t of recovery of PCr. Arnold et al. (1983) found that the average rate of resynthesis of phosphocreatine was slower after a heavy-exercise protocol compared to a light exercise protocol.

The initial resynthesis of PCr corresponded with a continued decline in the pH of the muscle until 40 and 70 seconds (HAP and LAP, respectively) post-exercise. After this point, pH began to increase for both groups. Although the LAP group seems to have reached a lower pH and did not begin to recover until 30 seconds after the HAP group, the 1/2t of recovery of pH and PCr and the overall recovery of each group are not statistically different. It is interesting however to consider the possible reasons for the small but apparent variations between the groups in early recovery. It may be noted that at the end of the exercise phase, the HAP group appears to have accumulated more lactate. Given these conditions, it might be expected that they would eventually achieve the lowest pH. Yet, it is at this point in the protocol where the greatest variation between the two groups may be seen. The pH did not drop as low and began to recover sooner than the LAP group. There are several possible reasons for this difference. One possibility is that the increased absolute amount of PCr utilized by the HAP group (1.26) created an increased "buffering" capacity in early recovery. A second possibility is that the LAP group may produce more H^+ ion based on an increased reliance on anaerobic metabolism (glycolysis) to replenish phosphagen stores. In comparison, the HAP group may utilize oxidative phosphorylation to a greater extent to restore the high energy phosphagens. A third, and perhaps, more likely theory is that the LAP group may not be able to clear the accumulated H^+ (in the form of lactic acid) from the muscle at the same rate as the HAP group. Therefore, a potential reason that the HAP group resists a similar drop in pH and demonstrates a quicker "turn-around" time is that the HAP group transports the extruding hydrogen ion away from the muscle more quickly. While it is tempting to speculate on possibilities for these variations, it must be remembered that none of these apparent differences were statistically significant.

The middle phase of the recovery period (2nd to 6th minute) is characterized by a much slower rate of PCr resynthesis as [PCr] approaches resting levels for each group. With PCr resynthesis almost complete, recovery of pH depends primarily on the oxidation or removal of lactate from the muscle. The 1/2t restoration of pH appears to occur more rapidly than has been previously reported in other studies. The half-time for recovery of pH in this study was 3:35 minutes for the (LAP) group and 3:28 for the (HAP) group compared with 1/2 times in the range of 5-10 minutes in studies by Fitts, (1977); Metzger and Fitts, (1987); Pan et al. (1991); Sahlin, (1992); and Kuno and Itai, (1992). The reasons

for this apparent discrepancy are unknown. Small differences in the exercise protocols and/or the influence of a higher proportion of trained subjects in this study compared to other studies may account for the differences. It is worth noting that the drop in pH reported in the above studies was similar in magnitude to the decline in pH found in this study (↓ of 0.5 - 1.0 pH units). Despite theoretical evidence which would support a faster recovery for the HAP group, no differences are seen in rate of recovery of pH between the groups during this phase. It is interesting to note the high degree of individual variability with regards to the response of pH during the middle recovery period as shown in Figure 5. This variability may reflect actual differences between subjects, but may also be influenced by the difficulties experienced while measuring the Pi peak in the middle recovery period. Despite this variation, the mean values for pH converge to a similar slope for each group during this period.

A separate phenomena, not known to have been previously reported, was observed while exploring individual pH responses during the early and middle recovery periods. Of 21 subjects in this study, 17 subjects demonstrated sudden increases in pH (0.35-0.75 pH units within an 80 second period). This event appeared to represent some "critical individual threshold" which, once reached, permitted a more rapid recovery of pH. It was interesting to note that this event consistently occurred at a pH between 6.25 and 6.55 and the magnitude of pH increase was greatest in subjects who had reached very low pH values (pH of 6.2-6.3). The cause of this phenomenon is unknown, however it is possible that it may represent the activation (removal of inhibition) of various enzymes required for resynthesis of energy. For example, phosphofructokinase (PFK) is the rate limiting enzyme of glycolysis and is inhibited below a pH of 6.5 (Ui, 1966).

Finally, the late phase of recovery (6th to 10th minute) shows a "plateau" effect as both pH and PCr return to pre-exercise levels by the 10th minute. These results are similar to the results of Degroot et al. (1993) who found that complete PCr resynthesis required 8 minutes and Kuno and Itai (1992), who found that pH recovery required approximately 11 minutes.

The individual data presented in Figures 6 and 7 clearly show that metabolic responses to an exercise challenge of the same relative magnitude may be different between subjects with similar levels of $\dot{V}O_{2max}$. Since both groups exhibited this variation, it appears that the metabolic and circulatory characteristics associated with $\dot{V}O_{2max}$ do not significantly

influence metabolic recovery following short-term, high intensity exercise. These results suggest that $\dot{V}O_{2max}$ is a poor predictor of recovery rate. While $\dot{V}O_{2max}$ does not appear to accurately predict recovery rates, substantial variation in metabolic response among individuals suggests that other factors may be better predictors of recovery rate following anaerobic exercise. It is plausible that recovery rate may be influenced to a greater extent by aerobic capacity training. Aerobic power training may not produce the same degree of peripheral adaptation as aerobic capacity training. Much of the literature related to the adaptations associated with long duration exercise fail to distinguish between changes which occur as a result of aerobic power training vs. aerobic capacity training. Although aerobic power training produces many cardiovascular adaptations, the magnitude of the peripheral changes may be limited. Aerobic capacity training may specifically enhance the peripheral changes which facilitate increased oxidative capacity (Minotti et al., 1990; Kuno et al., 1992) and/or lactate clearance (Hatta and Soma, 1992; Fukuba et al., 1992). Boulay et al. (1984) suggest that while maximal aerobic power is widely utilized as a criterion of cardiorespiratory fitness, it is a poor predictor of endurance performance. McCully et al. (1992a) found that the metabolic capacity, as measured by the rate of phosphocreatine recovery, was significantly higher in endurance trained athletes compared with middle-distance athletes, sprinters and controls after 5 minutes of intermediate-high intensity exercise. In a subsequent study, McCully et al. (1992b) found that 2 weeks of muscle specific endurance training produced relatively small changes (14% increase) in the rate of PCr resynthesis compared to previously trained endurance athletes. McCully and co-workers suggest that these results are consistent with the idea that genetic endowment as well as endurance training are major factors in determining muscle metabolism. Other research (Park et al.,1988; Kuno et al.,1992) has shown that after endurance training, fatiguing exercise produces significantly higher (more alkaline) pH values as compared to the values seen prior to endurance training. In light of these findings, a test which describes the ability for maximal endurance performance may serve as a better predictor of rate of recovery from anaerobic exercise. Two tests which may best predict maximal endurance performance include the 90 minute capacity test proposed by Boulay et al. (1984) and measurement of ventilatory threshold (VT) suggested by Reybrouck et al. (1986).

A second proposal is related to the role of heredity and endurance training suggested by McCully et al. (1992a and b). The metabolic profile of the predominant muscle fibre

type may be the single most important factor in determining the quantity of lactate produced and the speed of the recovery process. Achten et al. (1990) state that slow-twitch fibres are recruited solely when low-intensity exercise is performed. With increasing work load there is a progressive recruitment of fast-twitch fibres until all motor units are recruited during high intensity dynamic work above maximal oxygen uptake. Slow-twitch (oxidative) fibres have little glycolytic activity and therefore produce only small amounts of protons during exercise. In contrast, fast-twitch (glycolytic) fibres have low aerobic capacity and utilize glycolysis extensively to produce ATP. In the process many protons are produced contributing to a lower pH. Colliander et al. (1988) suggest that skeletal muscle composed of mainly fast-twitch fibres has a lower content of aerobic-oxidative enzymes of energy supply, lower capillary density and slower rate of blood flow than skeletal muscle consisting of mainly slow-twitch fibre. These metabolic characteristics of fast-twitch muscle explain why performance during repeated bouts of maximal concentric contractions is different in individuals with "high" and "low" fast-twitch fibre compositions and further explains why individuals with a higher composition of slow-twitch fibre experience a lesser decrease in force during exercise and greater restoration of such during recovery between bouts (Colliander et al., 1988). While human gastrocnemius muscles are reported to contain roughly the same number of fast-twitch fibres and slow-twitch fibres (Saltin and Gollnick, 1983), it is believed that the metabolic profile of these fibres may change and that endurance training may promote the conversion of fast-glycolytic fibre to fast-oxidative fibre, thereby enhancing the overall oxidative capacity of the muscle. Since fibre type, distribution and/or enzymatic characteristics of muscle were not measured in this study, it is impossible to determine whether the fibre type or metabolic profile of subjects in the LAP and HAP group were different and whether potential differences in these variables may have contributed to the rate of recovery from anaerobic exercise. Since it is clear that fibre type and especially the metabolic profile of the muscle may influence the rate of recovery, it is recommended that these variables be measured in further studies.

Another possibility which may help to explain the similarity seen in the rate of recovery between the LAP and HAP groups is related to the type of activity performed during the recovery period. Despite the large mean difference in aerobic power between the two groups, the proposed ability of the HAP group to demonstrate a faster recovery may have been suppressed by a passive recovery. That is, aerobic training (be it power or

capacity) may enhance development of peripheral vascularization which may facilitate removal of lactate. However, if the rate of blood flow declines (as might be expected with a passive compared to active recovery), the potential of the lactate removal system may never be expressed.

The goal of this study was to investigate the influence of maximal aerobic power on recovery from anaerobic exercise. To view the results in proper context it should be remembered that a test of "whole-body" maximal aerobic power ($\dot{V}O_{2max}$) was used to predict recovery from "local" muscular fatigue (plantar flexion exercise with one joint of one limb) and this comparison may possess inherent limitations. While anaerobic exercise may induce a certain level of local muscular fatigue, it is probable that fatigue resulting from most sports involving high-intensity exercise is more "distributed" throughout the body than that seen in the present study. How the exercise tests and the conditions of those tests has influenced the results and the ability to predict and/or apply these results can be ascertained only when it is possible to accurately and precisely monitor recovery under true exercise conditions.

Conclusions

The present results suggest that following high intensity local muscular exercise, there are no significant differences in the rate of PCr resynthesis and pH restoration between groups with high and low levels of maximal aerobic power. Many sport scientists and coaches currently use a $\dot{V}O_{2max}$ test to predict recovery rates following anaerobic exercise. Contrary to this practice, the results of this research indicate that $\dot{V}O_{2max}$ alone appears to be a poor predictor of recovery ability. However, observation of differences between individuals in this study clearly suggest that differences in recovery rate do exist. In addition, the results of new research (McCully et al., 1992a and b) demonstrate that endurance trained athletes have greater metabolic capacity and recover PCr stores more rapidly than middle-distance athletes, sprinters or controls. Factors which may more significantly influence the rate of recovery from anaerobic exercise may include aerobic capacity, the metabolic profile of the predominant fibre type of the muscle, and the type of activity performed during the recovery period following exercise. It is suggested that the elucidation of the role of these factors on recovery rates following anaerobic exercise be considered for future investigations.

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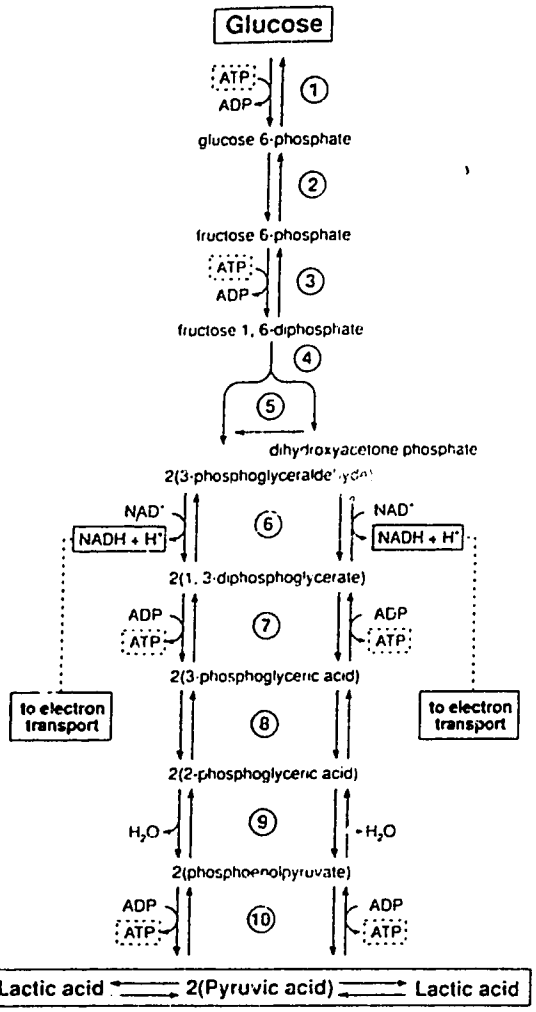
APPENDIX A

APPENDIX A

GLYCOLYSIS



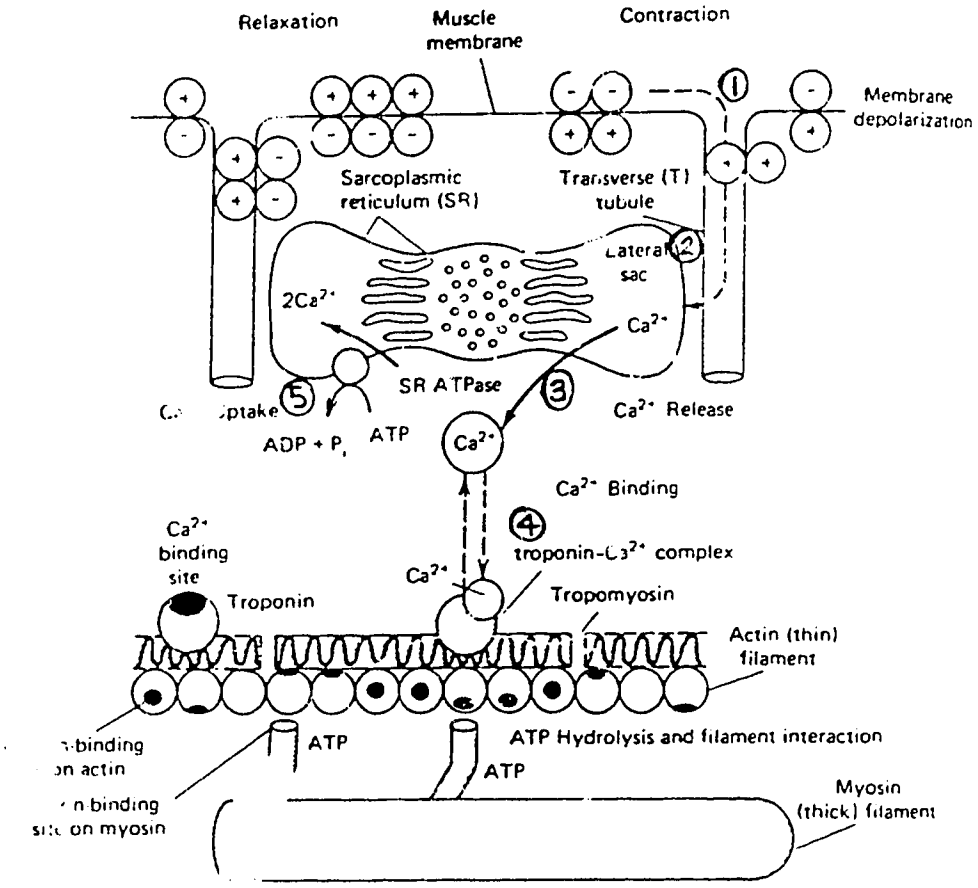
In the first reaction, ATP acts as a phosphate donor to phosphorylate glucose to glucose 6-phosphate. During energy metabolism, glucose 6-phosphate is changed to an isomer, fructose 6-phosphate. At this stage, no energy has been removed but energy has been invested into the original glucose molecule at the cost of one molecule of ATP. The fructose 6 phosphate molecule is then changed to fructose 1, 6-diphosphate. This reaction is controlled by the enzyme phosphofructokinase (PFK). Fructose 1, 6-diphosphate then splits into two phosphorylated molecules with three carbon chains. Through five successive reactions these carbon chains are further degraded to pyruvic acid.

(From Katch, Katch and McArdle, 1991)

APPENDIX B

APPENDIX B

EXCITATION AND CONTRACTION COUPLING



(1) The depolarization and transmission of action potentials over the entire sarcolemma and along the transverse tubules.
(2) The transmission of the signal from the T-tubules to the sarcoplasmic reticulum membrane.
(3) The subsequent release of calcium ions from the sarcoplasmic reticulum.
(4) The binding of the calcium ions to troponin (crossbridge formation).
(5) The uptake (pumping) of calcium ions back into the sarcoplasmic reticulum.
(6) The resynthesis of ATP.

(Adapted from Brooks and Fahey, 1985; Vollestad and Sejersted, 1988).

APPENDIX C

University of Alberta
Edmonton
Canada T6G 2H9

Department of
Physical Education and Sport Studies
P-421 Universiade Pavilion
Van Vliet Physical Education and Recreation Centre

INFORMED CONSENT FOR PARTICIPATION IN THE RESEARCH PROJECT:

"MUSCLE FATIGUE AND RECOVERY: THE INFLUENCE OF AEROBIC POWER ON THE RATE OF RECOVERY FROM ANAEROBIC EXERCISE"

I, _____ (please print name) agree to participate in a research project conducted by Mrs. Suzi Cooke and Dr. S.R. Petersen for the purpose of studying factors influencing muscle fatigue and recovery. I agree to participate in the exercise testing procedures to the best of my ability. I understand that I may withdraw at any time without prejudice. I also understand that the staff conducting these tests will discontinue the procedure if any indications of abnormal responses become apparent. I understand that prior to performing any test listed below, I will have the opportunity to question and discuss the exact procedures to be followed.

Physiological Assessments:

1. Anthropometry: Measurement of height and weight.
2. Submaximal and maximal cardio-respiratory function will be assessed during a progressive exercise test on a treadmill. During the test, expired gases will be collected and analyzed with an automated Metabolic Measurement system and heartrate will be monitored with an electrocardiograph.
3. Strength and power of the right ankle plantar flexors will be assessed using a Cybex 340 Isokinetic Dynamometer system.
4. Bioenergetic characteristics ofthe gastrocnemius muscle of the right leg will be studied at rest, during dynamic plantar flexion exercise and subsequent recovery periods using Phosphorus Nuclear Magnetic Resonance Spectroscopy (^{31}PNMRS).

Risks

The exercise tests will require intense concentration and physical effort however, these will be no more rigorous than might be encountered in normal sport or training situations. They present virtually no risk to healthy and physically active young adults.

There are no known risks associated with NMR spectrospcopy procedures.

Benefits

The information gained through completion of this study is important to the field of exercise physiology. An increased awareness of the factors which influence muscle function will contribute to an enhanced understanding of muscle function in normal, healthy individuals and elite athletes. Subsequently, this information will assist in the optimal preparation of athletes.

Subjects will receive a detailed report of their performance on the physiological assessments. This information can be used to describe personal fitness levels and serve as a basis for further training, if desired.

Consent

I acknowledge that I have read this form and I understand test procedures to be performed and the inherent risks and benefits associated with participation in this study. I consent to participate, understanding that I may withdraw at any time without prejudice. I may expect a report of my personal test results after the study is complete. I understand that the data will be collected and maintained in a confidential fashion and will be used for research purposes in a form that will not allow personal identification. I acknowledge that I have received a copy of this document.

Subject

Name: _____ Signature: _____
(please print)

Address: _____ Date: _____

_____ Phone: _____

_____

Principle Investigators

Mrs. Suzi Cooke and Dr. S.R. Petersen

Department of Physical Education and Sport Studies

University of Alberta

Office Telephone Numbers: 492-7394 (SRC) / 492-1026 (SRP)

Signature: _____ Date: _____

APPENDIX D

APPENDIX D

Measurement of Aerobic Power

1) The purpose and procedures of the test are explained to the subject.
2) The subject is asked to read and sign the consent form.
3) Measurement of height and weight are taken.
4) The subject is allowed to stretch for 7 minutes.
   - 4 minutes of stretching using 4 exercises provided by the investigator
     * Straight leg calf stretch
     * Bent knee calf stretch
     * Quadriceps stretch
     * Hamstring (hurdler's) stretch
   - 3 minutes of stretching exercises of the subject's choice.
5) The Sensormedics 2900Z Metabolic Measuring System was calibrated for volume using a 3L cylinder and calibrated for composition of gases (O_2 and CO_2) using known compositions of sample gases (O_2 and CO_2).
6) Preparations are made for the placement of electrocardiograph (E.C.G) electrodes and the electrodes are attached.
7) Treadmill Warm-Up
   - The subject is provided with instructions regarding how to begin on the treadmill, where to run and the hand signals to be used during the test.
   - 5 minute warm-up on the treadmill consisting of a walk-jog-run progression.
   - Introduction to treadmill walking begins at 3.0 mph and is gradually increased to the subject's preferred warm-up speed of 5.0 - 7.0 mph.
8) A five minute recovery period is allowed.
9) The headgear and mouthpiece are fitted to the subject and the air collection hose is attached to the metabolic cart.
10) The subject begins by walking and within two minutes graduates to the starting speed for the test. Starting running speed (Between 5 - 7 mph) is dependent on running skill and activity pattern for the subject. Starting grade is 0%. Heart-rate is recorded every minute using an Hewlett-Packard 43200A Electrogardiogram Heart-rate Monitor. Strips are run during the last ten seconds of each minute of the test and

heart rate measurements calculated using the distance between 2 full cardiac cycles.

11) Measurement of gas variables is made continuously and displayed as an average over 20 seconds.

12) Progressive increases in speed (usually 1/2 mph every 2 minutes) are made until Ventilatory Threshold (VT) is reached. Criteria for reaching VT will include the following:

    1) A systematic increase in the ratio of V_E/VO_2 without a concomitant increase in V_E/CO_2.

    2) R.E.R > 1.0

    Once this state is achieved further increases in intensity are made by increasing the treadmill grade by 2% every minute.

13) The subject is asked to communicate with the test administrator by the use of hand signals. Prior to each increase in intensity (usually every 2 minutes for speed, 1 minute for grade), the subject indicates whether he is able to begin the next exercise load. If he can not achieve the next intensity the subject may terminate the test by grabbing on to the handrail (subject terminates test). If the subject begins to lose position or coordination on the treadmill, the test administrator terminates the test by stopping the treadmill.

14) Criteria for reaching $\dot{V}O_{2max}$ includes evidence of a plateau or decrease in $\dot{V}O_2$ with an increase in load, and one or more of the following:

    1) Respiratory exchange ratio (R.E.R) > 1.10

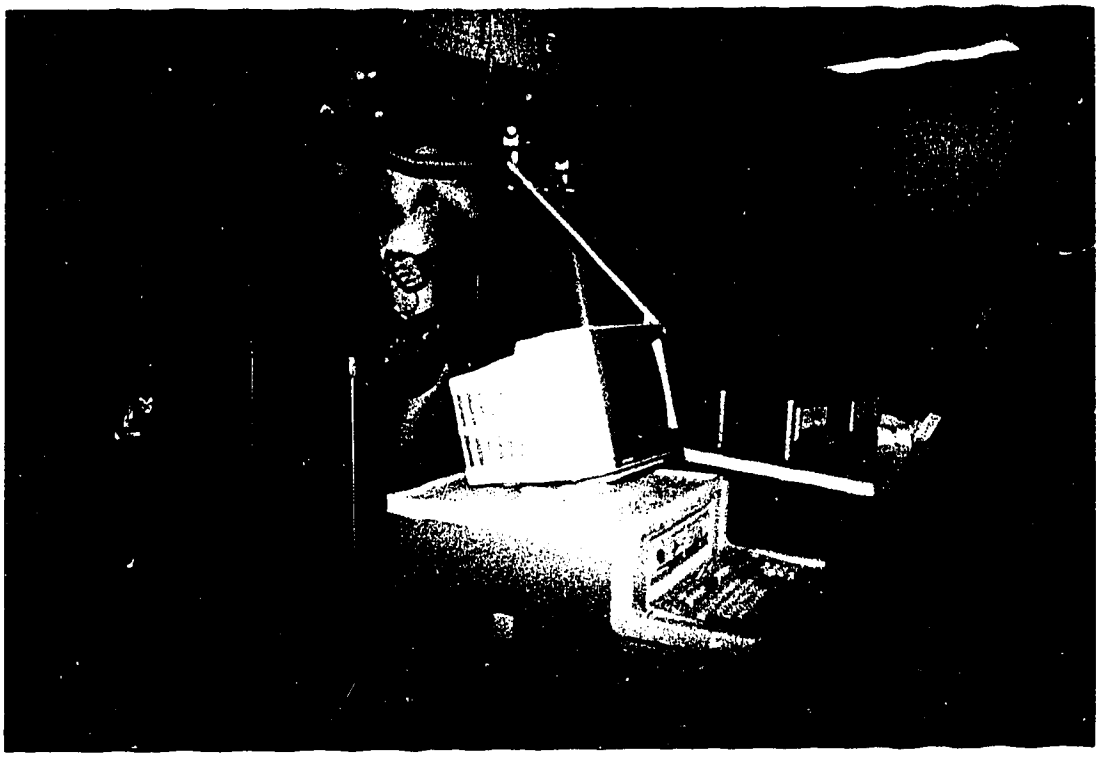
    2) A heartrate within 20 beats of the subject's age predicted maximum heartrate (220 - age), and

    3) Volitional cessation be the subject.

15) The subject is encouraged to "cool-down" by resuming moderate exercise at 0% grade and a self-selected speed for approximately 5 minutes.

16) The subject is then allowed to recover to a heartrate of 120 mph (or longer if requested). This cool-down may take place on or off the treadmill.

17) Following each $\dot{V}O_{2max}$ test a gas calibration check was performed on the Sensormedics 2900Z metabolic measuring system. A manual correction of the highest VO_2 was done when inconsistencies in composition of O_2 and CO_2 gases (pre-test to post-test) were beyond 0.05.



Measurement of Maximal Aerobic Power

APPENDIX E

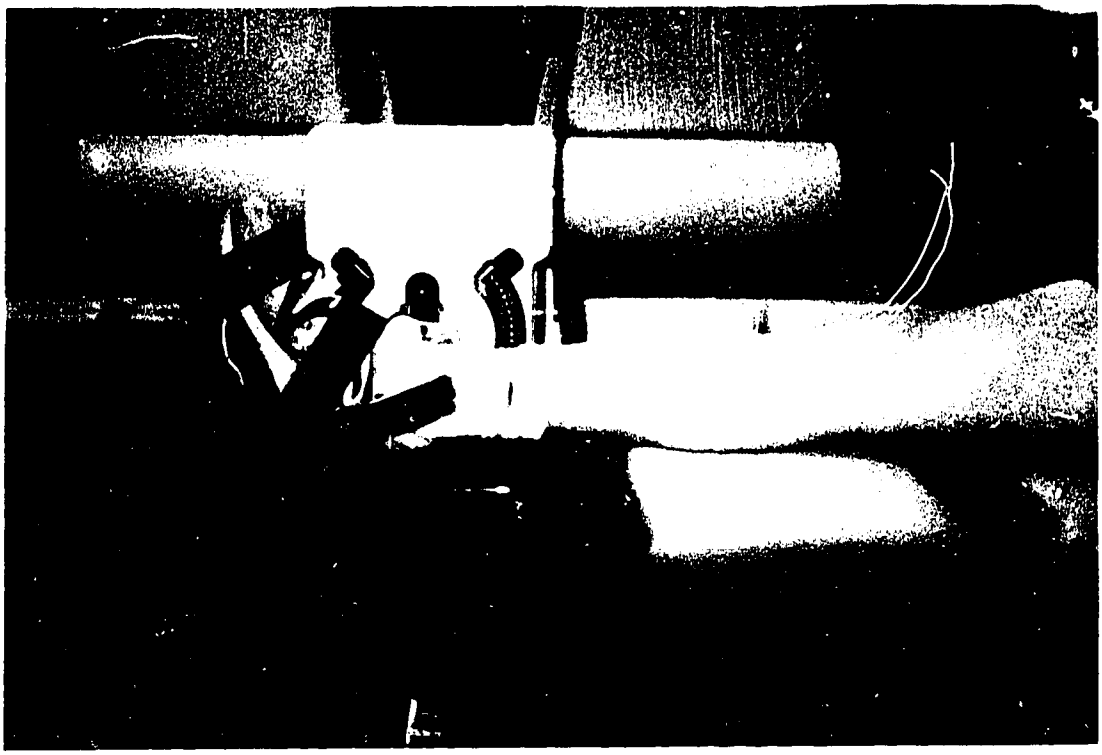
APPENDIX E

Measurement of Strength and Power in Ankle Plantar Flexion (Cybex)

1) The purpose and procedures of the test are explained to the subject.
2) The subject is allowed to stretch for 7 minutes.
   - 4 minutes of stretching using exercises provided by the investigator; emphasis on leg muscles.
     * Straight leg calf stretch
     * Bent knee calf stretch
     * Quadriceps stretch
     * Hamstring (hurdler's) stretch
   - 3 minutes of stretching exercises of the subject's choice.
3) The Cybex 340 was calibrated for torque (using known weights dropped at selected speeds) prior to each session (day) of testing. Verification of torque was performed after each test. Manual correction of peak torque and average torque were done when torque showed inconsistencies (pre-test to post-test) beyond ± 2 ft-lbs.
4) The subject is positioned on the "Upper Body Exercise and Testing Table" (U.B.X.T) as follows:
   - The dynamometer faces forward and is set at 0 degrees tilt.
   - The height of the dynamometer is set to align with the height of the subject's leg resting on the U.B.X.T.
   - The subject lies in a supine position on the U.B.X.T, then places his right foot into the footplate and slides back enough to fully extend the knee.
   - The foot is positioned in the footplate so that the hip is internally rotated (about 15 degrees).
   - The right foot should be flat on the footplate with the ankle neither everted or inverted.
   - The subject moves (as required) in order to ensure that the malleoli of the ankle are perpendicular to the dynamometer input shaft.
   - Ankle plantar flexion/ dorsiflexion footplates are secured.
   - The right thigh is also secured just above the knee with the U.B.X.T thigh belt.

4) The subject is instructed to keep the right knee locked in full extension throughout the movement to maximize the role of the gastrocnemius muscle (as opposed to knee flexion where soleus is more heavily recruited).
5) The test is started in the neutral position (anatomical zero). The footplate will be depressed through a range of motion of approximately 50 degrees of plantar flexion. The subject will then return the footplate back to anatomical zero using dorsiflexion exercise.
6) Subjects complete a warm-up consisting of 4 submaximal repetitions of ankle plantar flexion and dorsiflexion at a velocity of 90 degrees per second. A 20 second rest period follows. Next, the subject performs 4 maximal repetitions at the same velocity. This exercise is followed by a 90 second recovery period. Using the exact same procedure (4 warm-up repetitions, 20 seconds rest, 4 maximal repetitions, 90 seconds rest) the subject performs 2 more exercise sets using velocities of 30 degrees and 180 degrees per second. The complete protocol is illustrated below:

	Set 1	Set 2	Set 3	Set 4
Velocity	90	30	180	180
Warm-up Repetitions	4	4	4	4
Rest	30 sec	30 sec	30 sec	30 sec
Test Repetitions	4	4	4	4
Rest Before Next Set	90 sec	90 sec	90 sec	90 sec



Measurement of Strength and Power in Ankle Plantar Flexion

APPENDIX F

APPENDIX F

Laboratory Measurement of Maximal Plantar Flexion Work Capacity (MPFWC)

1) The purpose and procedures of the test are explained to the subject.
2) The subject is allowed to stretch for 7 minutes.
   - 4 minutes of stretching using exercises provided by the investigator; emphasis on leg muscles:
     * Straight leg calf stretch
     * Bent knee calf stretch
     * Quadriceps stretch
     * Hamstring (hurdler's) stretch
   - 3 minutes of stretching exercises of the subject's choice.
3) The subject lies down on the MPFWC sled so that the widest aspect of the right leg is centred over the M.R.S coil. The coil box is moved as required to achieve this position. The subject positions his right foot on the footplate of the MPFWC sled. His right shoelace is untied and the shoe loosened slightly to ensure normal blood flow during exercise. A towel is placed over the top of the right foot and the two straps firmly secured.
4) The right thigh (just proximal to the knee) and the hips are also secured with straps to avoid extraneous movements.
5) The subject is provided with two handles secured to a fixed rope to provide additional stabilization.
6) The subject is asked to begin the test by depressing the footplate the full 4 centimetres, raising a basket a pre-determined amount of weight. The starting weight of the basket is 4 kg for each subject. This weight will pull the footplate towards the original position. The subject controls the speed of the return and continues to depress the footplate at a rate of 1 contraction every 2 seconds utilizing a metronome (30 contractions per minute) until he reaches his MPFWC. Emphasis is placed on completing the full range of motion and holding the footplate down for 1/2 second before returning it to the starting position.
7) MPFWC was defined as the last load in which the subject could perform 30 repetitions moving the resistance the full 4 cm or until volitional exhaustion.

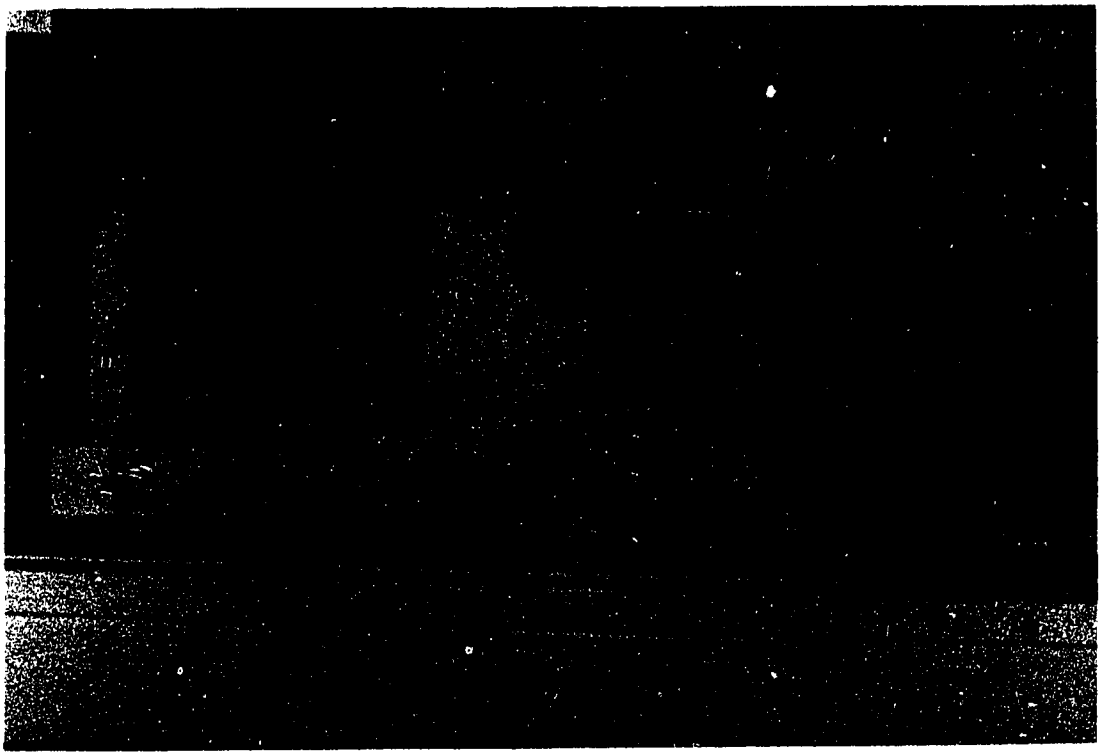
APPENDIX G

APPENDIX G

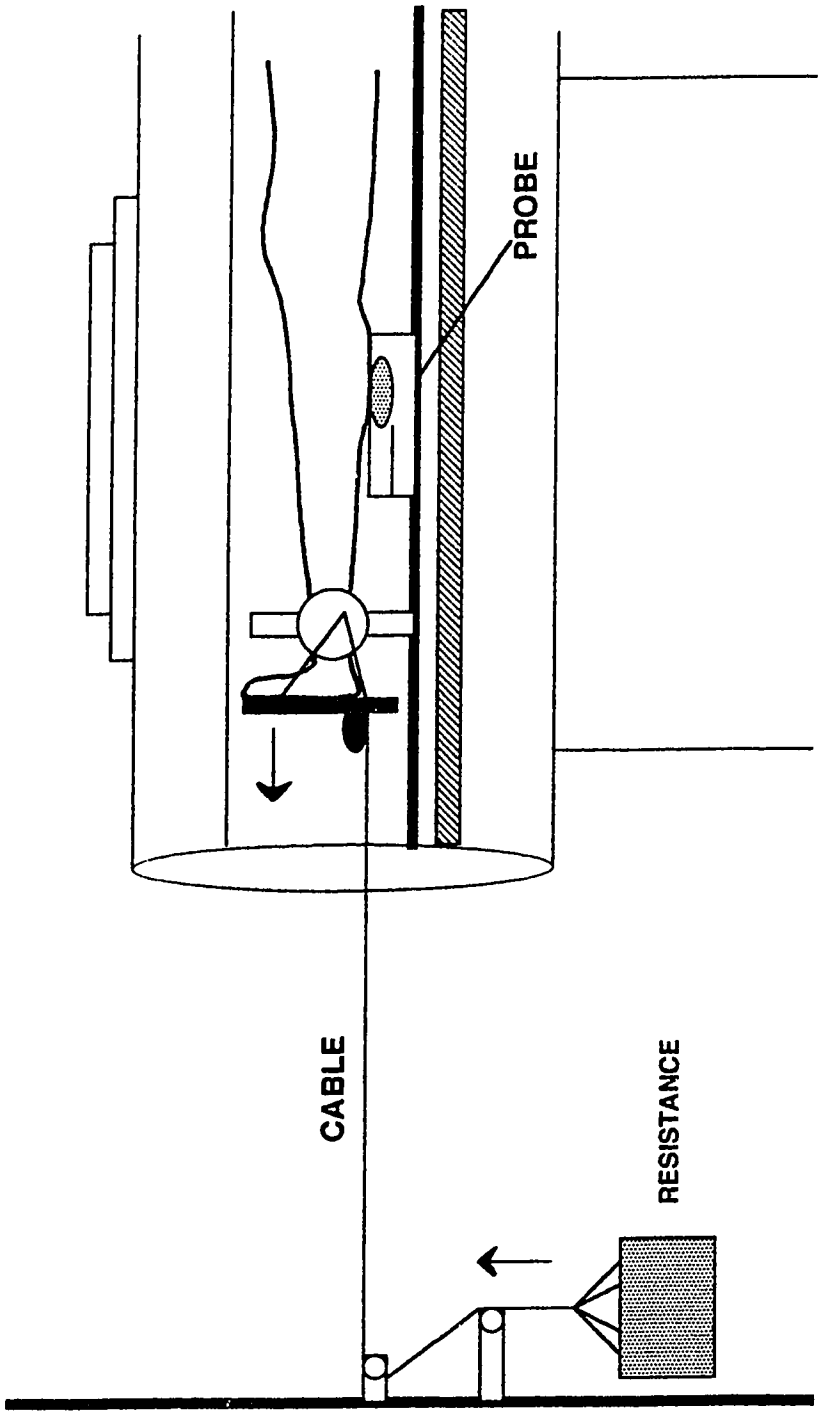
Measurement of Recovery Rate With Magnetic Resonance Spectroscopy

1) The purpose and procedures of the test are explained to the subject.
2) The subject is asked to read and sign the consent form.
3) The subject is allowed to stretch for 7 minutes.
   - 4 minutes of stretching using exercises provided by the investigator; emphasis on leg muscles.
     * Straight leg calf stretch
     * Bent knee calf stretch
     * Quadriceps stretch
     * Hamstring (hurdler's) stretch
   - 3 minutes of stretching exercises of the subject's choice.
4) The subject lies down on the MPFWC sled so that the widest aspect of the right leg is centred over the MRS coil. The coil box is moved as required to achieve this position. The subject positions his right foot on the footplate of the MPFWC sled. The shoelace of his shoe is untied and the shoe loosened slightly to ensure normal blood flow during exercise. A towel is placed over the top of the right foot and the two straps firmly secured.
5) The right thigh (just proximal to the knee) and the hips are also secured with straps to avoid extraneous movements.
6) The subject is provided with two handles secured to a fixed rope to provide additional stabilization.
7) The subject enters the bore of the magnet. Preparations are made by the test administrator to secure the sled straps, pull the head cover, secure the magnet screen, attach the rope to the basket, attach the rope required for the distance transducer and prepare the correct amount of weight for the basket (120% of Subject's MPFWC).

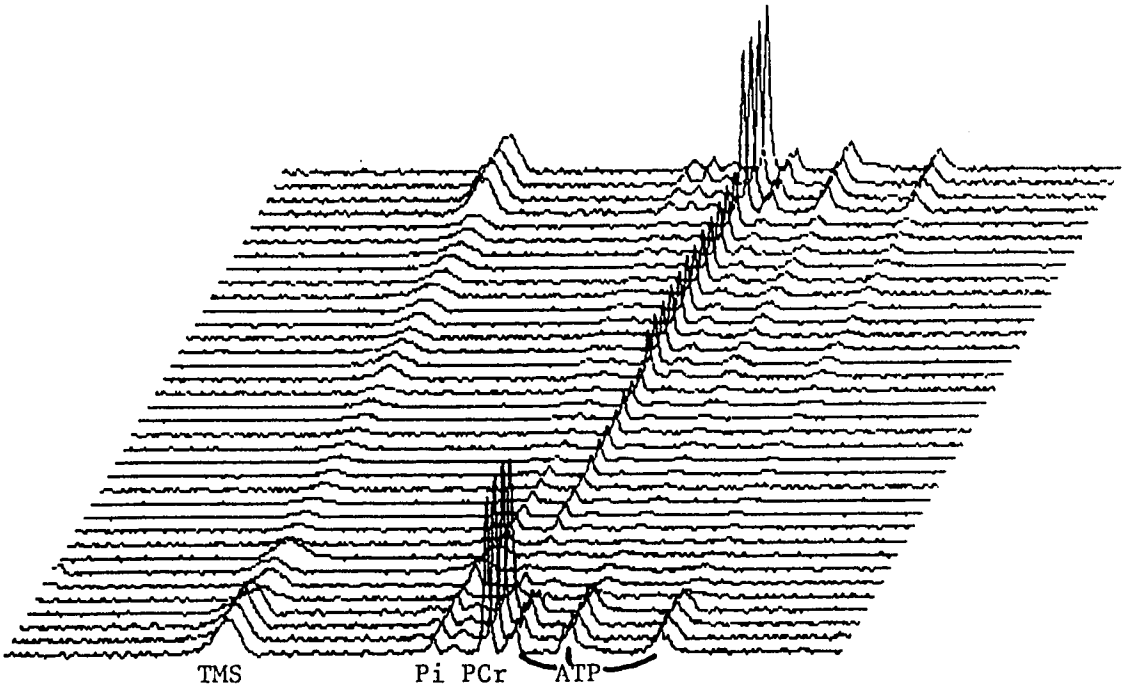
8) During the first 4 minute period the subject rests. At the 3 minute mark the basket containing 120% of the subject's MPFWC is attached. At the end of the 4th minute the subject is asked to begin the test by depressing the footplate the full 4 centimetres. The subject continues to depress the footplate at a rate of 1 contraction every 2 seconds (30 contractions per minute) for 2 minutes.
9) The 2 minute exercise period was followed by 10 minutes of passive recovery.
10) The intracellular MRS spectra of inorganic phosphate (P_i), phosphocreatine (PCr) and pH (derived from the ratio of inorganic phosphate to PCr) was measured continuously during 4 minutes of rest, 2 minutes of plantar flexion exercise at 120% MPFWC and 10 minutes of passive recovery following the exercise. Phosphorus spectra was collected and displayed each minute of rest and every 30 seconds during exercise. For the recovery segment, phosphorus spectra was collected and displayed every 10 seconds for the first 2 minutes, every 20 seconds for the next 4 minutes and each minute of the final four minutes of recovery. End values of pH as well as the percentage of PCr utilized were used as an index of overall metabolic response to the exercise test. The rate of recovery was calculated as the time for PCr to return to 1/2 the initial value (t 1/2).



Set-up of the Leg and Ankle Joint in the Pedal Ergometer During the Measurement of Recovery Rate Of the Human Gastocnemius Muscle With ^{31}P-MRS Magnetic Resonance Spectroscopy



Plantar Flexion Exercise Within The Magnet



A Series of Phosphorous-31 Magnetic Resonance Spectroscopy Spectra Obtained From Human Gastrocnemius Muscle, In Vivo, At Rest (lower 4 traces), Exercise (next 4 traces), and Recovery (upper 28 traces). Spectral Assignments: TMS, Tetramethylsilane (reference compound); P_i, inorganic phosphate; PCr, phosphocreatine; γ, α, β, three phosphate groups of adenosine triphosphate. Chemical shift values are in parts per million (ppm) from the resonance of PCr.

APPENDIX H

SUBJECT	AGE (Years)	HEIGHT (cm)	WEIGHT (kg)	VO2 MAX (l/min)	VO2 MAX (ml/kg/min)	PEAK TORQUE (N.m)	PEAK TORQUE (N.m/kg)	AVERAGE POWER (Watts)	AVERAGE POWER (Watts/kg)	MPFWC (kg)	MPFWC (kg/kg bw)
LOW											
1	32	177.0	85.5	4.2	49.5	120.7	1.4	119.0	1.4	18.0	0.21
2	19	176.8	65.7	2.6	39.9	93.6	1.4	68.0	1.0	18.0	0.27
3	22	177.5	75.8	3.4	45.3	116.6	1.5	118.0	1.6	24.0	0.32
4	34	189.0	112.0	5.6	50.0	189.8	1.7	220.0	2.0	34.0	0.30
5	35	172.5	88.5	3.6	41.1	127.5	1.4	120.0	1.4	22.0	0.25
6	39	170.5	64.2	3.0	47.1	137.0	2.1	100.0	1.6	17.0	0.26
7	25	173.0	98.5	4.5	45.6	145.1	1.5	131.0	1.3	28.0	0.28
8	25	176.0	92.6	4.6	49.6	130.2	1.4	83.0	0.9	18.5	0.20
9	22	187.5	106.4	5.2	48.8	172.2	1.6	146.0	1.4	36.0	0.34
10	25	181.4	99.0	4.9	49.5	124.8	1.3	98.0	1.0	26.0	0.26
MEAN	27.8	178.1	* 88.8 *	4.2	* 46.6 *	135.7	1.5	120.3	1.3	24.2	0.27
SD +/-	6.7	6.2	16.3	1.0	3.6	27.8	0.2	41.8	0.3	6.8	0.04
HIGH											
1	23	181.0	62.8	4.2	66.4	111.2	1.8	97.0	1.5	16.0	0.25
2	21	175.6	72.8	4.8	65.4	119.3	1.6	98.0	1.3	20.0	0.27
3	24	178.7	70.9	4.3	60.9	99.0	1.4	92.0	1.3	24.0	0.34
4	21	175.5	80.0	4.8	60.0	135.6	1.7	86.0	1.1	18.5	0.23
5	19	195.0	83.4	5.1	60.6	131.5	1.6	138.0	1.7	26.0	0.31
6	24	181.6	78.4	4.7	60.1	126.1	1.6	98.0	1.3	22.0	0.28
7	25	174.3	68.4	4.9	72.4	127.5	1.9	116.0	1.7	16.0	0.23
8	29	174.0	69.2	4.6	66.8	111.2	1.6	55.0	0.8	16.0	0.23
9	22	182.5	75.0	5.1	67.6	115.3	1.5	91.0	1.2	22.0	0.29
10	35	174.5	65.2	4.6	70.6	109.8	1.7	65.0	1.0	18.5	0.28
11	21	181.0	76.5	4.6	60.0	134.2	1.8	129.0	1.7	24.5	0.32
MEAN	24.0	179.4	* 73.0 *	4.7	* 64.6 *	120.1	1.6	96.8	1.3	20.3	0.28
SD +/-	4.5	6.1	6.4	0.3	4.5	11.8	0.1	24.6	0.3	3.6	0.04

Appendix H: Individual and Performance Characteristics of Subjects Arranged By Low Aerobic Power (LAP) and High Aerobic Power (HAP) Groups

APPENDIX I

APPENDIX I

Determination of pH and PCr Values from MRS Data

The areas under the PCr and Pi peaks of the ^{31}P-MRS spectra were determined by curve fitting using an MRS software processing program and Ramtek computer system.

PCr

PCr peak area represents the total signal received by the surface coil. For the purposes of this investigation, the following mathematical expression can be used to describe the dependence of total signal size:

$$\text{Sig PCr}^{(t)} = \mu\,(1-e^{-TR/T1PCr}) \int_V \rho PCr(\vec{\delta})\,\theta(\vec{\delta})\,\mathrm{Sin}(\theta(\vec{\delta}))\,e^{\,i\,\Delta\phi_p(\vec{\delta})T}\,d\vec{\delta}$$

where SigPCr (t) is the PCr signal size.

$\rho PCr(\vec{\delta})$ is the density (or concentration) of the PCr, which may be a function of r.
TR is the repetition time.
$T1PCr$ is the spin-lattice relaxation time of 31P in PCr of the subject.
$\theta(\vec{\delta})$ is the flip angle of the nuclei (^{31}P) by the RF pulse.
$\Delta\phi(\vec{\delta})$ is the inhomogeneity of the static field.
V is the volume excited.
μ is a constant which is dependent on tuning, matching, etc.
i is the square root of -1.

If the density of the PCr is a constant over the volume under the interrogation, the expression may be simplified as follows:

$$\text{Sig PCr}^{(t)} = \mu\,(1-e^{-TR/T1PCr})\,\rho PCr(\vec{\delta}) \int_V \theta(\vec{\delta})\,\mathrm{Sin}(\theta(\vec{\delta}))\,e^{\,i\,\Delta\phi_p(\vec{\delta})T}$$

From the expression, it is possible to understand that the peak area is not only proportional to the PCr concentration, but also depends on the RF power, RF coil, fat and muscle size and shape, and tuning and matching of the RF coil.

When one takes the ratio of two peak areas, say PCr and Pi, we have:

$$\frac{\text{Sig PCr (t)}}{\text{Sig Pi (t)}} = \frac{\rho PCr(1-e^{-TR/T1PCr})}{\rho Pi\ (1-e^{-TR/T1Pi})}$$

If we assume that T1 of PCr and Pi are constant in muscle or the difference is negligible, we have:

$$\frac{\text{Sig PCr (t)}}{\text{Sig Pi (t)}} = \frac{\rho PCr}{\rho Pi}, \text{ where } \alpha = \frac{(1-e^{-TR/T1PCr})}{(1-e^{-TR/T1Pi})}$$

Therefore, we say that the peak ratio is proportional to the ratio of the concentrations. We can also compare two PCr peak areas from different spectra of the same experiment of the same subject. For example,

$$\text{Sig Pcr } (t_1) / \text{Sig Pcr } (t_2) = \rho PCr(t_1) / \rho PCr(t_2)$$

From the above discussion, we can conclude that the ratios of PCr/Pi are independent of tuning, matching, shimming or any other instrument adjustments. The purpose of tuning, matching and shimming is to obtain the best signal to noise ratio which will minimize the fluctuation of the measured peak areas.

pH

The chemical shift from the centre of the Pi peak to the PCr peak was used to determine intracellular pH according to the following form of the Hendersen-Hasselbach equation (Gadian et al., 1982):

$$pH = 6.75 + \log_{10}[(a - 3.27)/(5.69 - a)]$$

where "a" is the chemical shift between Pi and PCr. Radda and Gadian (1986) suggest this form of the equation when pH of skeletal muscle is being measured.

APPENDIX J

APPENDIX J

Mean PCr of Gastrocnemius Muscle During Rest, Exercise and Recovery for Low (LAP) and High (HAP) Aerobic Power Groups

	Time (min)	Low Aerobic Power (n = 10)	High Aerobic Power (n = 11)
Rest	1:00	9.85 (1.88)	11.19 (0.70)
	2:00	9.53 (1.70)	11.26 (0.75)
	3:00	9.46 (1.61)	11.33 (0.52)
	4:00	9.69 (1.76)	11.19 (0.49)
Work	4:30	6.30 (2.67)	7.43 (2.72)
	5:00	3.79 (2.15)	4.84 (1.73)
	5:30	3.07 (1.96)	3.58 (1.56)
	6:00	2.50 (1.58)	2.77 (1.39)
Early Recovery	6:10	3.35 (1.30)	3.71 (1.34)
	6:20	4.76 (1.60)	5.22 (1.21)
	6:30	6.00 (1.39)	6.10 (1.21)
	6:40	6.49 (2.05)	7.21 (1.62)
	6:50	7.29 (1.74)	8.07 (1.32)
	7:00	7.84 (1.79)	8.60 (1.32)
	7:10	8.34 (1.81)	8.79 (1.20)
	7:20	8.29 (1.80)	8.93 (1.61)
	7:30	8.42 (1.79)	9.54 (1.37)
	7:40	8.70 (1.96)	9.72 (0.89)
	7:50	9.05 (2.04)	9.84 (1.33)
	8:00	9.05 (1.83)	10.20 (1.21)
Middle Recovery	8:20	8.63 (1.86)	10.53 (0.99)
	8:40	9.01 (1.95)	10.47 (0.91)
	9:00	9.39 (1.76)	10.91 (0.75)
	9:20	9.05 (1.89)	10.86 (0.81)
	9:40	9.30 (2.02)	11.00 (0.71)
	10:00	9.29 (2.08)	10.80 (0.70)
	10:20	9.34 (2.12)	11.14 (0.74)
	10:40	9.21 (1.87)	11.13 (0.47)
	11:00	9.24 (1.83)	11.15 (0.47)
	11:20	9.26 (1.92)	11.39 (0.74)
	11:40	9.52 (1.81)	11.21 (0.91)
	12:00	9.57 (2.06)	11.34 (0.74)

Late Recovery	13:00	9.40 (1.97)	11.39 (0.48)
	14:00	9.46 (1.89)	11.15 (0.45)
	15:00	9.47 (1.92)	11.46 (0.65)
	16:00	9.68 (2.02)	11.32 (0.56)

Values are Mean ± (S.E)

APPENDIX K

APPENDIX K

Mean pH of Gastrocnemius Muscle During Rest, Exercise and Recovery for Low (LAP) and High (HAP) Aerobic Power Groups

	Time (min)	Low Aerobic Power (n = 10)	High Aerobic Power (n = 11)
Rest	1:00	7.08 (0.04)	7.06 (0.02)
	2:00	7.08 (0.02)	7.06 (0.02)
	3:00	7.06 (0.03)	7.07 (0.03)
	4:00	7.07 (0.04)	7.07 (0.03)
Work	4:30	7.10 (0.06)	7.13 (0.07)
	5:00	7.06 (0.12)	6.97 (0.12)
	5.30	6.84 (0.16)	6.77 (0.14)
	6:00	6.69 (0.23)	6.61 (0.11)
Early Recovery	6:10	6.54 (0.15)	6.52 (0.11)
	6:20	6.50 (0.15)	6.50 (0.13)
	6:30	6.44 (0.15)	6.48 (0.13)
	6:40	6.42 (0.18)	6.45 (0.13)
	6:50	6.42 (0.15)	6.46 (0.16)
	7:00	6.39 (0.13)	6.50 (0.15)
	7:10	6.38 (0.13)	6.50 (0.19)
	7:20	6.42 (0.17)	6.51 (0.20)
	7:30	6.43 (0.22)	6.54 (0.28)
	7:40	6.43 (0.26)	6.55 (0.29)
	7:50	6.48 (0.25)	6.58 (0.30)
	8:00	6.55 (0.31)	6.59 (0.30)
Middle Recovery	8:20	6.65 (0.33)	6.66 (0.31)
	8:40	6.68 (0.31)	6.70 (0.31)
	9:00	6.74 (0.34)	6.71 (0.28)
	9:20	6.72 (0.36)	6.72 (0.28)
	9:40	6.79 (0.30)	6.76 (0.29)
	10:00	6.84 (0.26)	6.81 (0.24)
	10:20	6.87 (0.26)	6.83 (0.21)
	10:40	6.88 (0.23)	6.87 (0.18)
	11:00	6.94 (0.21)	6.96 (0.17)
	11:20	6.96 (0.20)	7.03 (0.08)
	11:40	6.96 (0.20)	7.04 (0.07)
	12:00	6.99 (0.21)	7.03 (0.06)

Late Recovery	13:00	7.00 (0.06)	7.02 (0.04)
	14:00	7.02 (0.08)	7.03 (0.04)
	15:00	7.04 (0.07)	7.04 (0.05)
	16:00	7.07 (0.09)	7.06 (0.05)

Values are Mean ± (S.E)

APPENDIX L

APPENDIX L

Non-linear Regression Model, Equations, and Summary of Statistics

Dependent Variable: PCr

Model

b_{11} = - 19.26170894 b_{15} = -0.640555781

b_{12} = 2.974493827 b_{21} = -12.46026068

b_{13} = 4.229524964 b_{23} = 1.958816627

b_{14} = 7.592279570 b_{24} = 10.198837211

Equations

1) If Time is < 6 Minutes:

Predicted Value = $[b_{12} + b_{15} * \text{group}] + [b_{14} * (1/ 1+e^{v})]$

$^{v} = b_{11} + b_{13} * \text{Time}$

2) If Time is ≥ 6 Minutes:

Predicted Value = $[b_{15} * \text{group}] + [b_{24} * (1/1+e^{v})]$

$^{v} = b_{21} + b_{23} * \text{Time}$

Non-linear Regression Summary Statistics

Source	DF	Sum of Squares	Mean Square
Regression	8	62101.34504	7762.66813
Residual	748	1699.89198	2.27258
Uncorrected Total	756	63801.23702	
(Corrected Total)	755	6003.35497	

R squared = 1 - Residual SS/ Corrected SS = 0.71684

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95% Confidence Interval	
			Lower	Upper
B11	-19.26170894	3.430381153	-25.99602919	-12.52738870
B12	2.974493827	0.261277910	2.461568574	3.487419080
B13	4.229524964	0.760509382	2.736538179	5.722511749
B14	7.592279570	0.337328399	6.930056521	8.254502620
B15	-0.640555781	0.054889819	-0.748312209	-0.532799353
B21	-12.46026068	0.989781131	-14.40334012	-10.51718123
B23	1.958816627	0.150546807	1.663272089	2.254361165
B24	10.198837211	0.084109784	10.033717886	10.363956536

APPENDIX M

APPENDIX M

Non-linear Regression Model, Equations, and Summary of Statistics

Dependent Variable: pH

Model

b_{11} = 26.810703584 b_{14} = -0.602140318

b_{12} = 7.060763141 b_{21} = 36.524471888

b_{13} = - 4.823262377 b_{23} = -5.838985515

Equations

1) If Time is < 6 Minutes:

Predicted Value = $b_{12} + [b_{14} * (1/ 1+e^{v})]$

$^{v} = b_{11} + b_{13} * \text{Time}$

2) If Time is ≥ 6 Minutes:

Predicted Value = $b_{12} + [b_{14} * (1/ 1+e^{v})]$

$^{v} = (b_{11} - b_{21}) + (b_{13} - b_{23}) * \text{Time}$

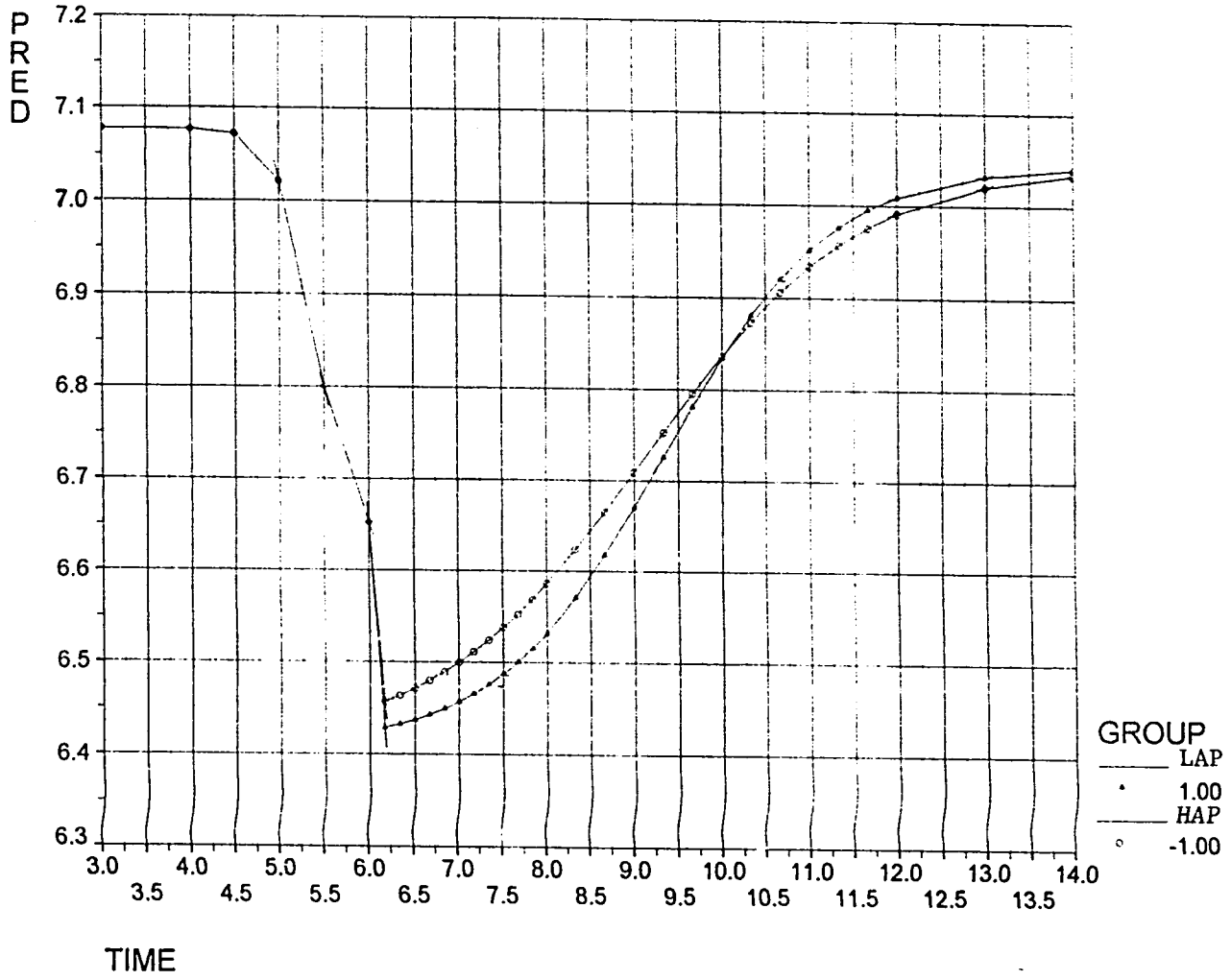
Non-linear Regression Summary Statistics

Source	DF	Sum of Squares	Mean Square
Regression	6	34735.02287	5789.17048
Residual	750	27.41037	0.03655
Uncorrected Total	756	34762.43324	
(Corrected Total)	755	69.33936	

R squared = 1 - Residual SS/ Corrected SS = 0.60469

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95% Confidence Interval	
			Lower	Upper
B11	26.810703584	13.686705226	-0.058105921	53.679513089
B12	7.060763141	0.014700560	7.031904002	7.089622281
B13	-4.823262377	2.497325109	-9.725841305	0.079316550
B14	-0.602140318	0.031498347	-0.663975733	-0.540304903
B21	36.524471888	13.957670907	9.123720972	63.925222803
B23	5.8389855515	2.523617264	-10.79317941	-0.884791614

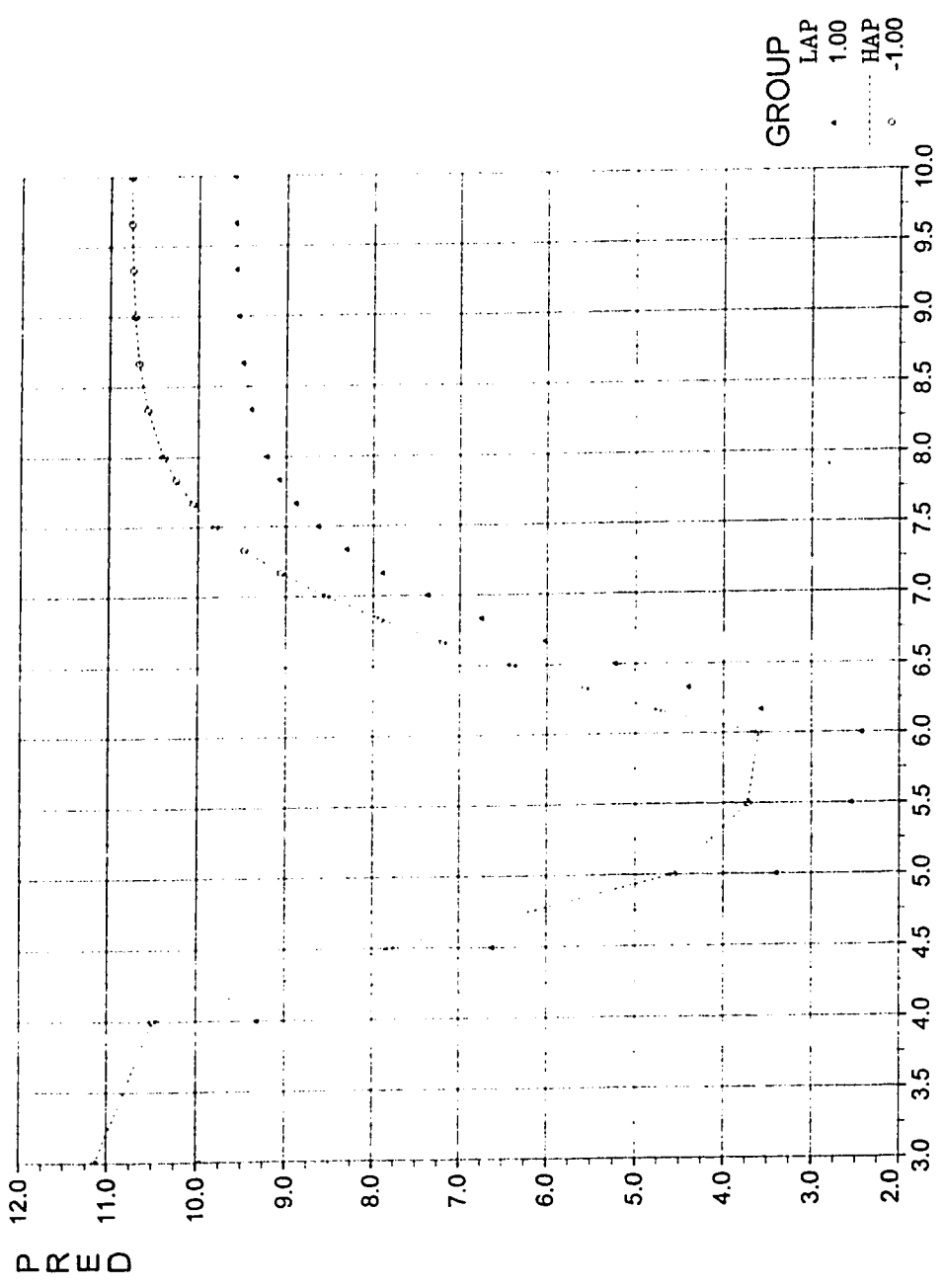
APPENDIX N



APPENDIX N

Predicted Values for PCr Using the Non-linear Regression Model

APPENDIX O



APPENDIX O

Predicted Values for pH Using the Non-linear Regression Model