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31-phosphorous, Nuclear Magnetic Resonance Spectroscopy Studies Of Exercising Human Muscle

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By

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ABSTRACT

Phosphorous 31, nuclear magnetic resonance spectroscopy (^{31}P NMRS) was used as a non-invasive probe of forearm muscle metabolism in three studies of healthy men and women. Specific objectives of the studies were to: 1) observe the changes in high energy phosphates and intracellular pH in muscle, during a ramp exercise protocol, 2) study the effect of endurance training on muscle metabolism and evaluate the utility of the forearm ramp test as a means to assess muscle oxidative capacity, and 3) describe the kinetics of phosphocreatine (PCr) and inorganic phosphate (Pi) metabolism during the on and off transient response to moderate exercise.

In the first study, 18 subjects performed a forearm ramp exercise to fatigue. Exercise caused a biphasic increase in the Pi/PCr ratio of the muscle. Change in Pi/PCr was initially slow, followed by a rapid phase. The transition or threshold between the two rates corresponded to the onset of intracellular acidosis. Repeated testing of 6 subjects showed that this threshold was reproducible. These findings demonstrated the existence of a threshold in intracellular metabolism (IT), which was related to aerobic capacity.

In the second study, 4 older subjects trained the dominant forearm daily for 12 weeks, using a light weight. Muscle metabolism was evaluated using the ramp protocol and ^{31}P NMRS before training and after 6 and 12 weeks of

training. The onset of the IT was delayed 14%, and submaximal exercise endurance time was increased 58% by training. Muscle blood flow was not altered by training. The results indicated that endurance training improved forearm muscle oxidative capacity, and that the IT could be used to assess this change.

Finally, the kinetics of forearm muscle PCr metabolism were studied during the transition to, and the recovery from, moderate intensity work. Five young men completed 6 square wave exercise tests each. The results of these tests were combined and the changes in PCr and Pi modelled using an exponential growth or decay function. The time constants (τ) calculated for both metabolites were about 30 s, which is similar to the τ reported for oxygen uptake during whole body exercise. These data suggest a first-order relationship between muscle oxygen consumption and substrate utilization during exercise transition states.

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GLOSSARY OF TERMS AND ABBREVIATIONS

ADP adenosine diphosphate

ATP adenosine triphosphate

Chemical shift The position of a molecular compound in an NMR spectra. This shift is due to the shielding of the applied magnetic field by the electrons surrounding a particular nucleus. Chemical shift makes it possible to differentiate compounds containing the same nucleus (ie ^{31}P) such as ATP and PCr.

Deconvolution (convolution difference) A method of improving the appearance of the spectrum. A smooth curve is subtracted from the baseline to remove broad resonances arising from immobile nuclei in the sample, for example ^{31}P in bone.

Exponential function A function of the type e^{At}, where e is the base of the natural logarithm and A is the characteristic time constant of the particular growth or decay process.

Fourier transform A mathematical procedure that separates out the inherent frequency components of a signal. The Fourier transform is used to generate the spectrum from the FID.

Free Induction Decay (FID) The transient signal induced in the NMR coil after an radiofrequency pulse has excited the system. It will decay toward zero with a characteristic time constant.

Hertz (Hz) The standard SI unit of frequency equivalent to one cycle per second.

Least Squares (method of) A way of minimizing the sum of the squares of the residuals as a criterion for best fit.

Line Broadening (apodization) A means of improving the signal to noise ratio by multiplying the spectrum by a decreasing exponential function. Line broadening also has the undesirable effect of decreasing the resolution.

NMR Nuclear magnetic resonance

NMRS Nuclear magnetic resonance spectroscopy

PCr Phosphocreatine

Pi Inorganic phosphate

Probe Part of the NMR spectrometer containing the radiofrequency coil and associated electronics. The probe may consist of separate receiver and transmitter coils or a single coil which performs both functions.

$\dot{Q}O_2$ muscle oxygen utilization

Radiofrequency (rf) Intermediate frequency between auditory and infrared. The rf used in NMR experiments is commonly in the megahertz (MHz) range.

Residuals The distance from a data point to a regression line. The residuals represent the unexplained variation after the fitting of a model.

Resonance An increase in the amplitude of a vibration caused by a small periodic stimulus with a frequency at or near the natural frequency of the system.

Shimming Correction of the disruption of the main magnetic field of an NMR system caused by the presence of the sample being studied.

Spectrometer Portions of the NMR apparatus that actually produce the NMR phenomena and acquire the signals, for example the magnet, probe, etc.

Spectrum The frequency components of the NMR signal. After Fourier transformation, the different compounds (ATP, PCr, etc.) will appear as series of peaks called the spectrum.

Stoichiometry The proportions in which elements form compounds. In NMR, PCr and Pi have been shown to change in equal, but opposite proportions. For example, a one unit decrease in PCr is accompanied by a one unit increase in Pi and vice versa.

Tesla (T) Si unit of magnetic flux. A magnetic field of one Tesla is about 20,000 times stronger than the earth's magnetic field.

Truncation Loss of data points from the beginning or end of the FID causing distortion of the transformed spectra. Truncation can also refer to the underestimation of the base width of a peak during spectral analysis. This will result in errors in the determination of the peak's area.

Tuning The process of adjusting the components of the spectrometer for optimal NMR signal strength.

$\dot{V}O_2$ Oxygen consumption

White Noise Noise which is randomly distributed over all frequencies of the spectrum. It is called "white" because white light contains an equal distribution of all colours or frequencies.

Zero filling Spectral resolution depends on the separation of the data points. The appearance or apparent resolution of the spectra can be improved by interpolating smoothly between existing data points.

CHAPTER 1

INTRODUCTION

Much of the current exercise physiology literature is concerned with identifying the factors which control metabolism in working muscle. There are several important problems in this area which have yet to be resolved, including: the role of aerobic and anaerobic metabolism in graded exercise, whether the rate of oxygen uptake is limited by the supply of oxygen (O_2) to the muscle or, by the utilization of available O_2, and how the creatine kinase reaction affects mitochondrial respiration. Answers to these questions have been sought using the traditional techniques of indirect calorimetry and invasive muscle sampling. Occasionally these procedures are combined, but more often, cardio-respiratory physiology and muscle biochemistry are considered separate domains. Clearly, much could be learned if it were possible to continuously monitor muscle metabolism as well as pulmonary gas exchange throughout exercise. The technology to do both these procedures is now available: breath by breath analyses of gas exchange and phosphorous nuclear magnetic resonance (^{31}P NMR) spectroscopy. Originally the intent of the thesis was to combine these two techniques, but it soon became apparent that with the apparatus available, this was not practical. Only the exercising forearm could be accommodated in the NMR magnet and this was too small a muscle mass to yield valid measures of gas exchange. Still it would be useful to study muscle metabolism with ^{31}P NMR only, if suitable exercise protocols could be

developed. The results of these metabolic studies could then be compared with existing studies of whole body metabolism and perhaps some inferences could be made. Most NMR exercise protocols currently found in the literature, evaluate muscle oxidative capacity during high intensity interval work. The results of these studies may not be directly relevent to continuous aerobic exercise.

1.1 ^{31}P NMR and Muscle Energetics

The first NMR experiments were conducted nearly 50 years ago (Purcell et al., 1946; Bloch et al., 1946) and since then, the technique has progressed from a research tool for analytical chemistry to a routine medical imaging modality. In the past decade, NMR has also become increasingly important in the biological and health sciences, as a non-destructive method of monitoring metabolism in cells and tissues. This application of NMR was made possible by the development of large bore superconducting magnets that could accommodate larger animals, human limbs or even entire human bodies.

The NMR phenomenon is due to the unique behavioural characteristics exhibited by the nuclei of certain atoms. Those that possess an odd number of particles, either protons or neutrons, have an intrinsic angular momentum or spin. The rotational movement of these charged particles generates a magnetic field and causes the nucleus to behave like a bar magnet. Under normal conditions, these tiny nuclear magnets are randomly oriented in space, or more specifically in the sample being analyzed (for example a beaker of water, or a

human arm muscle). However, when an external magnetic field is applied to the sample, the nuclear magnets will align either with the external field (parallel) or against it (antiparallel). More energy is required to maintain the antiparallel orientation, therefore more of the atoms in the sample are found in the parallel alignment. If the sample is then irradiated with an appropriate, or resonant, radio frequency (rf), the nuclei will absorb this energy and make the transition to the antiparallel orientation or higher energy state. After a very brief period of excitation (micro-seconds), the nuclei lose the absorbed rf energy and return to the lower energy condition. How much energy is released depends, at least in part, on the number of nuclei in the sample or the concentration of a specific metabolite containing the nuclei. This energy can be detected by a receiver and is recorded as a decay curve called a free induction decay (FID). The FID is then manipulated mathematically to produce a frequency spectra with the area under the spectral peak proportional to the concentration of the metabolite in the sample. This is of course a very brief and elementary discussion of the physical basis of NMR. A more comprehensive discussion (for non-physicists) of the magnetic resonance phenomenon can be found in the texts of Gadian (1982), and Cady (1990).

Not all atoms possess the property of spin, but fortunately many of those that do are of considerable biological significance. For studies of muscle metabolism 31 phosphorous (^{31}P) is the most important element because it is contained in the metabolites involved in energy transfer; phosphocreatine (PCr),

inorganic phosphate (Pi) and adenosine triphosphate (ATP).

NMR has several definite advantages when compared with traditional methods of assessing muscle metabolism. Safety is one of the most important advantages of NMR for human research. NMR does not involve any ionizing radiation, and to date no harmful effects of magnetism have been shown (Eiselein et al., 1961; Cook and Morris, 1981). In addition, unlike a biopsy, the procedure is totally non-invasive and therefore does not disrupt the tissue under study. This allows serial measures to be obtained ***in vivo***, with a temporal resolution of seconds. In this way the time course of metabolic reactions can be determined under physiological conditions. Another important advantage is that to a certain extent, NMR is a non-specific assay. This means that the concentration of all the phosphate compounds present in a sample can be determined simultaneously and their interactions observed. In addition, ^{31}P NMR provides a means of continuously monitoring intracellular pH with an accuracy of about 0.05 units (Gadian, 1982).

While NMR has some very obvious advantages, there are disadvantages to this technique as well. Spatial and magnetic constraints associated with NMR limit the type of exercise that can be performed and the size of the limb that can be studied. In most cases, this means that a small muscle mass model must be used. However, the impact of this limitation may be minimized if the muscle is exercised appropriately.

1.2 Thesis Outline

The general purpose of this thesis was to study the metabolic response of human muscle to dynamic exercise using ^{31}P NMR spectroscopy. To achieve this goal a series of 3 studies was devised in which different "whole body" exercise protocols were adapted for use with ^{31}P NMR spectroscopy.

In the first study, a progressive resistance or ramp exercise test was used in conjunction with ^{31}P NMR spectroscopy to assess oxidative metabolism in the forearm muscles of 18 volunteers of various ages. Ramp exercise on a cycle ergometer is known to elicit threshold phenomena in pulmonary ventilation and blood lactate concentration. These observations have been taken as evidence for the anaerobic threshold hypothesis. If this is true, then it seemed reasonable that intracellular correlates of the ventilation and lactate threshold could exist.

The subsequent experiments, described in Chapter 3, were designed to test the results of the prior study. A threshold in intracellular metabolism was observed during the forearm ramp test, suggesting that there was some point during progressive exercise when the rate of aerobic metabolism could no longer keep pace with the increasing energetic demands of the task. If the intracellular threshold was a measure of the aerobic capacity, exercise training should delay the onset of the threshold. To test the hypothesis, 4 sedentary, older individuals were trained for 12 weeks with an exercise protocol which would stress only the forearm muscles without significantly stressing the

cardiovascular system. Older individuals were chosen as subjects because there is limited data regarding the metabolic response to exercise, in the elderly.

The final study described the changes in the muscle high energy phosphates during the transition from rest to moderate exercise and recovery from exercise. Pulmonary oxygen consumption ($\dot{V}O_2$) is known to increase exponentially during the onset of exercise, but the pattern of change of PCr, Pi and ATP is not clear. These PCr and Pi transients were modeled as simple first order reactions. The time constants calculated for the on- and off-transients were compared with the $\dot{V}O_2$ data.

CHAPTER 2.

COINCIDENT THRESHOLDS IN INTRACELLULAR PHOSPHORYLATION POTENTIAL AND pH DURING PROGRESSIVE EXERCISE.

2.1 Abstract

Dynamic changes in intracellular phosphocreatine (PCr), inorganic phosphate (Pi) and pH in human forearm muscle were studied from rest through heavy exercise using a ramp exercise protocol and ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Eighteen healthy volunteers performed an isotonic wrist flexion exercise of repeated contractions at a frequency of 0.5 Hz. Work rate was increased continuously (ramped) at approximately 0.13 Watts each minute from 0.34 W to 1.5 W, or until fatigue. The ratio of Pi/PCr was used as an estimate of the cellular phosphorylation potential of the muscle. Exercise caused a progressive increase in Pi/PCr with an initial slow component followed by a later fast component. The transition between these components was distinct and corresponded to the onset of pH decline in all subjects. These changes in Pi/PCr and pH were fit with a piecewise linear regression model. The ramp exercise protocol elicited changes in PCr, Pi and pH which were not significantly different from a 5 min steady-state protocol. Repeated ramp testing of 6 subjects showed that the threshold was reproducible ($r = 0.98$). The results of this study demonstrated the existence of an intracellular metabolic threshold, and suggested that indirect threshold measures (i.e. the

lactate and ventilatory thresholds) may reflect events at the cellular level.

2.2 Introduction

The anaerobic threshold (AT) has been defined as the level of oxygen consumption or power output immediately preceding the onset of metabolic acidosis (Wasserman, 1986). This concept implies that at a certain exercise intensity, aerobic metabolism is no longer capable of supplying the required energy, and consequently the rate of glycolysis is abruptly increased to provide additional ATP. Since the AT was first introduced, (Wasserman and McIlroy, 1964) this hypothesis and the subsequent variations of it have been highly controversial. Considerable disagreement exists as to the appropriateness of the terminology, the validity of the measurements, and the causal mechanism(s) associated with the AT. Still other authors question the existence of any true metabolic threshold (Brooks, 1986; Hughson et al., 1987).

Much of the controversy is related to the indirect methods used in determining the AT such as, changes in blood lactate, ventilation and expired gases during progressive exercise. Clearly, if these indirect threshold measures are valid representations of tissue respiration, then similar trends in intracellular metabolism should occur. Such corresponding thresholds have not yet been identified, primarily due to the technical difficulty of observing the sequence of intracellular events during exercise. ^{31}P nuclear magnetic resonance (NMR)

spectroscopy, however, is a technique that permits continuous, non-invasive monitoring of muscle metabolism and is therefore ideally suited to this type of work. Recently, Matheson and co-workers (1989) observed a non-linear decline in muscle pH during electrical stimulation, using ^{31}P NMRS. Non-linear changes in other intracellular metabolites were not apparent, possibly due to the type of exercise used in the study. Whipp et al. (1981) have shown that exercise protocols with a continuously increasing, or ramp work rate are most effective in demonstrating the threshold phenomenon. In the present study, a ramp exercise protocol for a small muscle mass model (wrist flexors) was used in conjunction with ^{31}P NMRS to determine if intracellular thresholds in high energy phosphate compounds and pH occur during progressive exercise.

2.3 Methods

Subjects: A total of 18 volunteers (16 males, 2 females) agreed to participate in the study. All of the subjects were healthy and active, although none were specifically trained in sports which involved extensive use of the forearm musculature, such as squash or tennis. Informed consent was obtained from all subjects prior to their participation in the study. Twelve of the subjects (mean age 31 ± 7 years) performed a single forearm ramp exercise using the dominant limb. Three of these individuals (mean age 31 ± 9 years), completed a graded steady state test in addition to the ramp protocol. The two tests were conducted on different days. To determine the reproducibility of the threshold

measures, a separate group of 6 volunteers (mean age 47 ± 19 years) underwent repeated ramp testing with 6 weeks separating the two trials.

NMR Spectroscopy: ^{31}P NMRS data were accumulated using a 30 cm bore, 1.89 Tesla superconducting magnet and an Oxford Research Systems TMR-32/20 spectrometer. While in the supine position, the subjects inserted the arm into the bore of the magnet with the flexor muscles of the forearm situated over a 4 cm diameter surface coil. The subjects then grasped the lever of the exercise ergometer which was adjustable to facilitate various limb lengths. Ideal placement of the forearm aligned the pivot of the lever at the centre of the wrist joint and located the coil approximately 7 to 9 cm distal to the medial epicondyle of the humerus. In this position, the NMR signal obtained was primarily from the flexor digitorum superficialis muscle. A diagram of the exercise apparatus is shown in Figure 1.

Each spectrum consisted of the average of 32 scans obtained over 32 s and these were collected sequentially throughout the rest, exercise and recovery periods. Several seconds were required to store the acquired data making the minimum time resolution of the protocol approximately 35 seconds. At the pulse repetition rate used (1 s), the signals associated with the high e ıergy phosphate (HEP) compounds were significantly saturated. However, since only ratios of metabolites were used, correction factors were not applied.

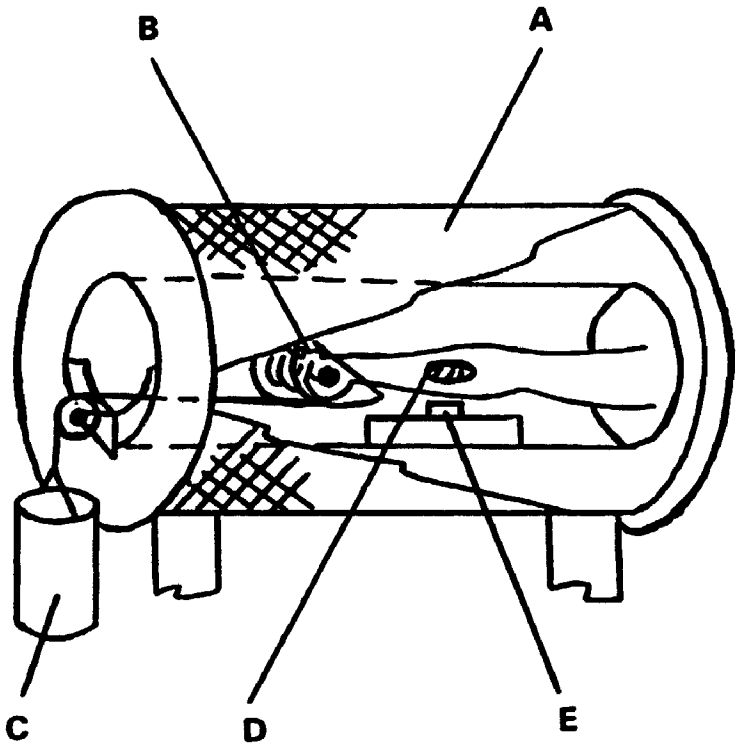

Figure #1: A simplified diagram of the apparatus used in the exercise studies. A: 1.89 Tesla superconducting magnet; B: lever of the exercise ergometer; C: water reservoir (resistance); D: region of interest of the forearm; E: coil and transceiver probe. Depression of the lever assembly caused the water reservoir to be raised via a cable and pulley arrangement.

^{31}P data were collected using a spectral width of 4000 Hz and 2048 data points. Before Fourier transformation (Bracewell, 1986), the data were zero filled to 4096 points, and the free induction decay (FID) was multiplied by a 10 Hz exponential line broadening factor to improve the signal to noise ratio. Any broad phosphorous resonances in the FID were eliminated using standard deconvolution techniques. Typical rest and exercise spectra are shown in Figure 2.

Exercise Protocol: Both the ramp and the steady state protocols were done using an isotonic wrist flexion ergometer built by the investigators. The exercise consisted of repeatedly depressing a lever at a frequency of 0.5 Hz through a range of motion of 70°. This action raised and lowered a variable resistance located outside the bore of the magnet via a cable and pulley arrangement (see Figure 1). The resistance was changed by adjusting the level of water in a suspended reservoir. Work done by the subject was calculated from the known repetition rate, the arc distance of the lever and the weight of the water and reservoir using standard physical relationships.

The ramp exercise protocol was similar to that used previously by Massie et al. (1987). Following a 2 minute warm-up period of zero-load wrist flexion, water was continuously added to the reservoir at a rate of 250 $mL \cdot min^{-1}$ using a roller pump (Cole-Parmer Instruments, Chicago). This produced a ramp slope of approximately 0.13 Watts each minute. Work was initiated at 0.34 W (the empty reservoir) and continued until 1.5 W, or until the subject was fatigued.

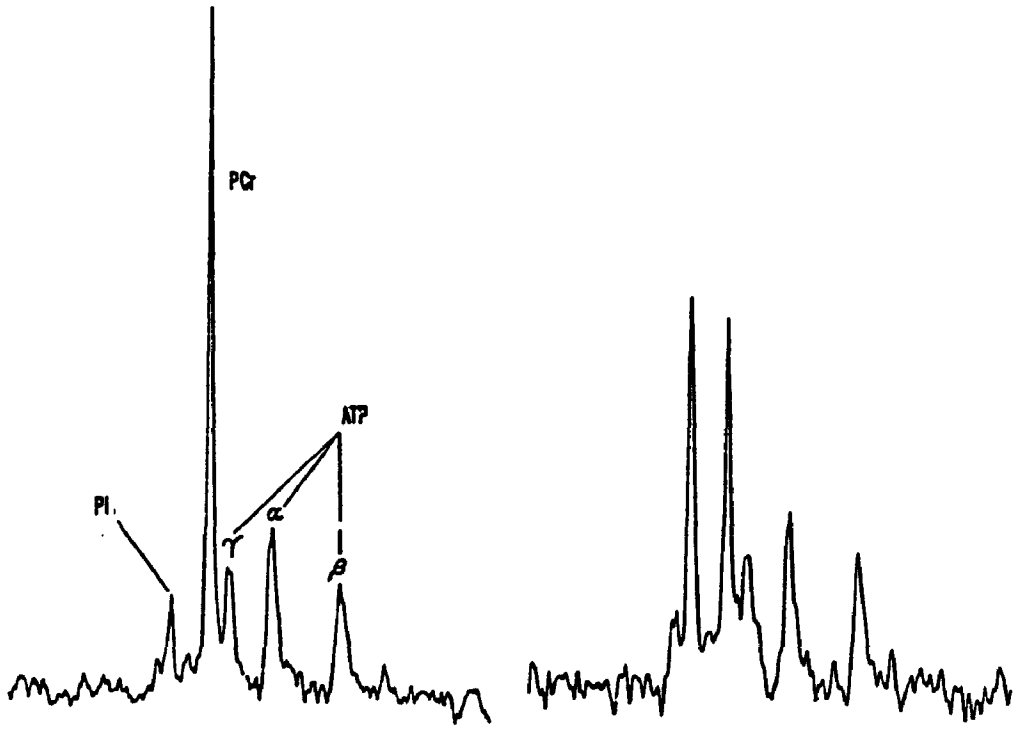

Figure #2. ^{31}P NMR spectra of the wrist flexor muscles at rest (left) and during exercise (right). Peak assignments are: Pi, inorganic phosphate; PCr, phosphocreatine; ATP, adenosine triphosphate (alpha, beta and gamma phosphate).

The exercise time of the test ranged from about 8 to 15 minutes, depending on the individual.

For the steady-state protocol, subjects completed 5 work increments each of 5 minute duration. The work rate was increased in steps of 0.13 W from a minimum of 0.40 W to a maximum of about 1.1 W. NMR spectra were obtained during the final minute of each interval with the same acquisition parameters used in the ramp protocol.

Data Analysis: Fourier transformed (Bracewell, 1986) spectra were analyzed using a non-linear least squares fitting routine. The computer software used in the spectral analysis was developed in our laboratory. Relative contributions of individual phosphate metabolites, beta-adenosine triphosphate (β-ATP), phosphocreatine (PCr) and inorganic phosphate (Pi) were determined from the area under the fitted curve and the ratio Pi/PCr was calculated from these areas. Intracellular pH was determined from the chemical shift of Pi with respect to PCr (Taylor et al., 1983).

The ratio of Pi/PCr has been used to estimate the phosphorylation potential of cells (Chance et al., 1986a, 1986b) since this ratio approximates changes in muscle oxygen consumption that occur with increasing workloads. In the present study, the logarithm of the relationship (log Pi/PCr) was plotted against power output to facilitate modelling (Beaver et al., 1985). Piecewise linear regression analysis was applied to these plots using a computer program based on the algorithm of Vieth (1989). The program estimated the parameters

of two regression functions and determined a break point (threshold) at which the slopes of the 2 lines diverged. An F test was used to evaluate whether a single or multiple regression provided the optimal fit of the data. Intracellular pH data were also plotted as a function of power and analyzed in a similar manner.

A comparison of the ramp and steady-state conditions was made using a paired t-test. The test-retest reliability of the threshold measures was evaluated using a one way analysis of variance (ANOVA) with repeated measures. Correlation coefficients were calculated to determine the association between the Pi/PCr (PiT) and pH (pHT) breakpoints. A p-value of <0.05 was considered to be significant for all forms of statistical analysis.

2.4 Results

Both test conditions elicited a decrease in the relative concentration of PCr and an increase in the relative concentration of Pi. The magnitude of this effect was dependent on the subject's final power output. Small changes of less than 10% occurred in the concentration of β-ATP during exercise. Intracellular pH values declined only at higher workloads. Variations in the total visible phosphate (PCr,Pi,ATP) signal were typically less than 10%.

The ramp exercise caused an increase in the log Pi/PCr with an initial slow component followed by a later fast component. The transition from the slow to the more rapid rate was distinct and coincided with the point at which pH began to decline. Typical results for an individual subject are shown in

Figure 3. In this case, both the log Pi/PCr and pH showed a significant ($p < 0.05$) non-linear response to increasing work rate. The data were best fit by the piecewise regression model with a threshold point evident at approximately 1.2 W. While, these findings were similar for all subjects, the slopes of the two regression lines and the power output at which the transition occurred varied considerably (Figure 4). Table 1 shows the threshold power values for each subject as determined by the log Pi/PCr and also by pH. A high intrasubject correlation ($r = 0.99$) was found between the two inflection points.

Steady-state exercise produced changes in PCr, Pi and pH which were quantitatively similar to those found with the ramp protocol at low and moderate work intensities. The results of the two procedures for a single subject are shown in Figure 5. A comparison between the ramp and steady state data showed no significant differences ($p > 0.05$).

Repeat ramp testing of a separate group of subjects ($n = 6$) after six weeks produced no statistically significant ($p > 0.05$) change over time in the work rate at which the threshold in metabolism occurred. Test-retest reliability was evident by a correlation of 0.92 and 0.98 for the threshold determined by log Pi/PCr and pH, respectively (Table 2). As was found in the original ramp test group, the breakpoint was the same whether it was identified by phosphorylation potential or pH.

Twelve members of the total subject population completed the ramp test to volitional fatigue or were stopped when they could no longer maintain the

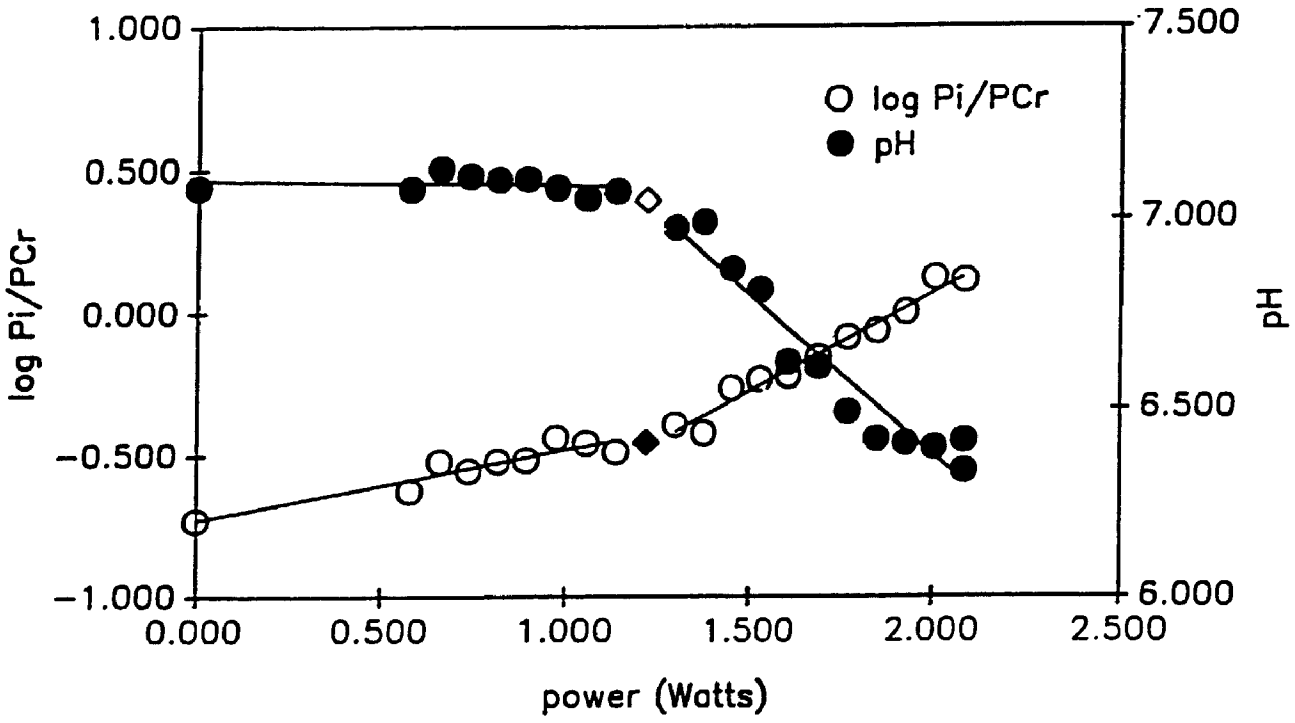

Figure 3: Metabolic response of the wrist flexor muscles to the ramp exercise protocol (subject #12). Changes in both Pi/PCr and pH showed a non-linear trend with a threshold evident at approximately 1.2 Watts (◇ ◆).

Table 1: Power output at the threshold, determined by log Pi/PCr (PiT) and pH (pHT).

Subject	PiT (W)	pHT (W)
1	1.00	0.94
2	1.31	1.30
3	1.02	1.02
4	1.30	1.32
5	0.95	0.95
6	1.78	1.70
7	1.51	1.54
8	1.55	1.53
9	1.09	1.02
10	1.59	1.68
11	0.95	0.88
12	1.23	1.13
Mean	**1.27***	**1.25***
± SE	**0.08**	**0.08**

* $r = 0.99$, significant correlation ($p < 0.05$) between threshold measures.

SE = Standard error

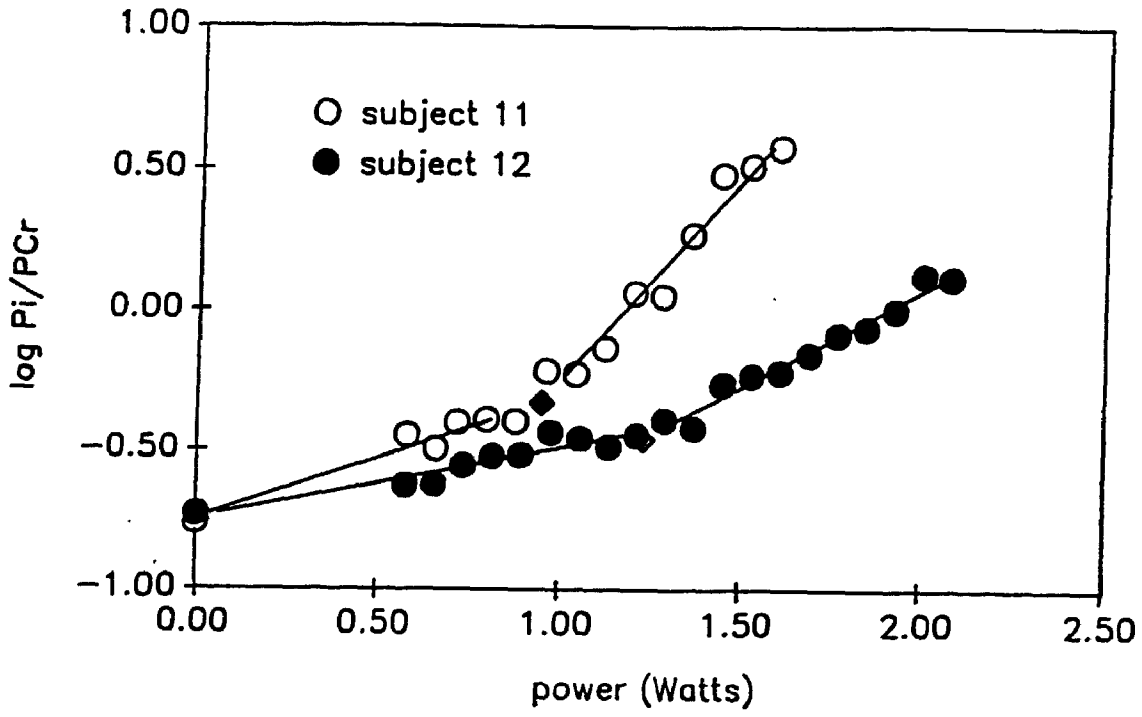

Figure #4: Intersubject differences in the metabolic response to progressive exercise. Test data for all subjects showed a threshold in Pi/PCr (◆, ◇). The power output at the threshold varied considerably and appeared to be related to the individuals total work capacity.

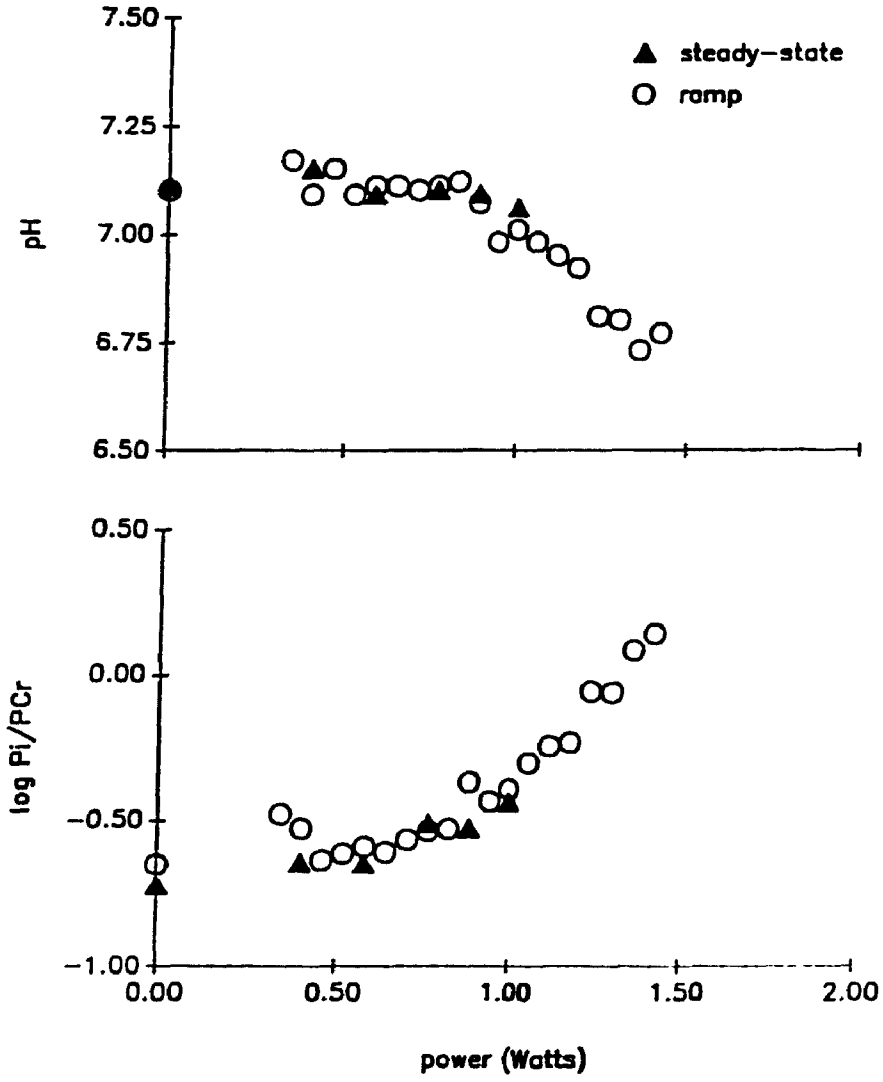

Figure #5: A comparison of the metabolic response to the ramp and steady state exercise protocols for a representative subject (#1). Changes in pH and Pi/PCr were similar during both tests.

Table 2: Reliability and reproducibility of the threshold (IT), determined by log Pi/PCr (PiT) and pH (pHT).

	Test#1	Test#2	r
PiT	1.11	1.13	0.92*
± SE	0.05	0.06	
pHT	1.08	1.12	0.98*
± SE	0.05	0.06	
r	0.87†	0.94†	

* significant correlation ($p < 0.05$) between tests 1 and 2.

† significant correlation ($p < 0.05$) between threshold measures.

SE = Standard error

required contraction frequency. The remaining 6 subjects did not achieve a true maximal power because of a limitation of the exercise apparatus. Mean maximal power output attained by the group of 12 subjects was 2.06 ± 0.34 Watts. The threshold occurred at 1.25 ± 0.08 Watts or 61 ± 2.7 % of the maximum power (Table 3).

2.5 Discussion

Phosphocreatine is known to provide a source of energy for the resynthesis of ATP broken down during muscle contraction, but, it may also play an important role in the control of metabolism. Several possible mechanisms, either directly or indirectly involving PCr have been proposed for the control of mitochondrial respiration. These include cytosolic ADP concentration (Moreadith and Jacobus, 1982), the creatine kinase reaction (Bessman and Carpenter, 1985; Mahler, 1985) and the creatine phosphate mole fraction (Seraydarian and Artaza, 1976). Work by Erecinska and Wilson (1982) suggests that the phosphorylation potential (PP), which is defined by the relationship ATP/ADP•Pi, is a key metabolic regulator. A decrease in this ratio acts to increase the rate of mitochondrial respiration while simultaneously stimulating glycolysis through higher levels of Pi (Chasiotis, 1983). The PP can be conveniently estimated ***in vivo*** using ^{31}P NMR spectroscopy by the ratio of PCr/Pi or the inverse of the relationship, Pi/PCr, as was done in this study (Chance et al., 1986a,1986b). Intracellular concentrations of PCr and Pi reflect

Table 3: Intracellular Threshold* as a percent of maximal power output.

Subject #	Threshold (W)	Max Power (W)	%
5	0.95	1.62	58
6	1.78	2.34	76
7	1.51	2.35	64
8	1.55	2.26	68
9	1.09	2.02	54
10	1.59	2.07	77
11	0.95	1.60	59
12	1.23	2.09	59
13	1.05	1.70	62
14	1.08	2.06	52
16	1.02	1.81	56
18	1.23	2.76	46
Mean	1.25	2.06	61
± SE	0.08	0.10	2.7

* IT determined by Pi/PCr inflection.

SE = Standard error

the metabolic activity of the muscle; thus, stable values of Pi/PCr indicate an adequate energy status while rapid changes in this ratio show that energy homeostasis cannot be maintained.

During the ramp type exercise, muscle pH remained constant until a critical power output was attained and then began to decline. The change in pH can be attributed mainly (but not exclusively) to an increased formation of lactate from pyruvate, because the dissociation of lactic acid produces most of the H^+ liberated during exercise (Sahlin, 1983). The increase in metabolic proton production may be greater than it appears at the IT because of the simultaneous increase in PCr hydrolysis. The creatine kinase reaction consumes protons and it has been shown that this reaction is an important intracellular buffer system (Adams et al., 1990). Chance et al. (1986a), have suggested that control of oxidative phosphorylation is largely pH independent, but, the value of Pi/PCr is proportional to pH and this factor may explain the coincidence of the breakpoints. While the rate of PCr hydrolysis may be influenced by $[H^+]$, the IT does not appear to be solely a function of pH. Evidence that the rapid phase of the Pi/PCr-work relationship is not strictly pH dependent has come from work with patients having M^{c}Ardle's Disease, a muscle phosphorylase deficiency (Driedger et al., 1990). Individuals with the disease do not produce lactate, or experience metabolic acidosis; rather their muscles become alkaline during exercise. Nevertheless, these patients still showed a rapid transition in

Pi/PCr during the ramp exercise similar to normals, although at a much lower work intensity. Lewis and Haller (1986) have attributed the exercise intolerance found in M^{c}Ardle's patients to their extremely low $\dot{V}O_2$max values. It would seem then, that the rapid change in the Pi/PCr ratio elicited by progressive exercise, is more closely related to inadequate ADP phosphorylation, than to a change in pH.

Caton et al. (1987) have questioned the use of the Pi/PCr ratio to describe the energetics of muscular work, maintaining that a lack of stoichiometry between decreases in PCr and increases in Pi during exercise invalidates the technique. Whether or not this is a legitimate concern is controversial. In the present study, these compounds did change proportionately. Similarly, Meyer and Adams (1990) have examined the stoichiometric relationship between phosphates during electrical stimulation of the rat gastrocnemius and found no selective loss of NMR observable Pi signal. They did however, find a small decrease in the total phosphate (PCr,Pi,ATP) concentration, as was the case in the present study. The loss was attributed to deshimming or detuning during the stimulation protocol, and it is likely that limb movements in human studies would have the same effect. Several possibilities exist to explain the apparent loss of Pi signal, including truncation errors associated with integration of the spectra and broadening of the Pi peak due to pH heterogeneity within the tissue sampled. Therefore, it remains to be

seen if the selective loss of NMR visible phosphates represents a real physiological process or simply an artifact induced by methods of peak analysis.

The IT occurred at about 61% of maximal power in those subjects (n = 12) who continued the ramp test to fatigue. Although there was no objective criterion to determine a true maximal capacity and muscle $\dot{V}O_2$ was not measured, the onset of the threshold was consistent with the findings of Knuttgen and Saltin (1972) and Chwalbinska-Moneta et al. (1989) who used muscle biopsies to study the lactate threshold. These authors found that muscle and blood lactate concentrations increased suddenly at an exercise intensity of about 60% $\dot{V}O_2$ max.

Several studies using ^{31}P NMR spectroscopy have shown that both the muscle PP and pH are sensitive to oxygen delivery. With a perfused animal hindlimb model, Idstrom et al. (1985) demonstrated an inverse relationship between the Pi/PCr ratio and the oxygen concentration of the perfusate. A study of impaired muscle blood flow in humans by Wiener et al. (1986) gave similar results. Reductions in muscle blood flow of 40 to 60% caused significant increases in Pi/PCr and decreases in pH during exercise when compared to control conditions. These previous studies and the current finding of the intracellular threshold appear to support the hypothesis of Wasserman (1986) that muscle oxygen demand may exceed supply at some time during

progressive exercise. Nevertheless, other investigators (Connett et al, 1984; Brooks, 1986) have made observations which are incompatible with this theory and doubt the possibility of hypoxic conditions within working muscle. The minimum oxygen pressure (PO_2) required to support aerobic ATP production has been calculated to be less than 0.5 torr (Chance and Quistorff, 1978). Connett et al. (1984) have found mitochondrial PO_2 to be between 1.0 and 2.0 torr in dog gracilis muscle, during supermaximal exercise. Thus, it seems unlikely that hypoxic conditions exist in muscle during exercise. However, hypoxia is not necessarily required for a metabolic threshold to exist, and there are several alternative mechanisms which could explain this phenomenon.

The ability of muscle to oxidize fat may be responsible for the threshold phenomenon rather than limitations in oxygen supply. This explanation was suggested by Holloszy (1976) and was based mainly on the increased capacity for fat oxidation found in muscle following training. The availability of fat as a substrate may also have an effect on the threshold. Increasing the level of plasma free fatty acids (FFA) has been shown to delay the onset of blood lactate accumulation during exercise (Ivy et al., 1981). Studies of normal volunteers taking the methylzanthine compound theophylline, have shown that the onset of the intracellular threshold was delayed significantly compared to control conditions (Marsh, et al., 1990). Theophylline, like caffeine, can increase plasma FFA concentration significantly (Vestal et al., 1983).

Muscle fibre recruitment is known to occur in an orderly fashion during

incremental exercise (Henneman et al., 1965) with the fast twitch (FT) motor units recruited at exercise intensities of about 60% of $\dot{V}O_2$ max. Since these higher threshold units have a limited oxidative capacity and produce more lactate than the slow twitch (ST) motor units, recruitment has been suggested as the cause of the lactate threshold (Ivy et al., 1980). Kushmerick and Meyer (1985) found that cat biceps, a mixed muscle, had a greater increase in Pi/PCr and acidified more during exercise than did the soleus, a predominately ST muscle. It is possible then that activation of FT motor units could account for the intracellular threshold observed in the present study. A study by Connett et al. (1986), however, showed that a lactate threshold could be elicited from ***in vivo*** muscle preparations that contain no FT fibres.

The comparison of the ramp and steady-state protocols confirmed that the observed changes in muscle metabolites were not simply time dependent, but were a function of the intensity of the exercise (Figure 5). Moonen et al. (1987) also compared steady-state changes in high energy phosphate and pH with those induced by continuously increasing dynamic work. Although the rate of the ramp increase used was substantially less than in the present study (100 g•min^{-1} and 250 g•min^{-1}, respectively), the results were identical. Thus, it is reasonable to assume that pH, PCr and Pi levels do provide a valid indicator of the metabolic activity of the exercising muscle. The magnitude of the changes in Pi/PCr ratio at a given power output were considerably less than those found in a number of studies using a steady-state exercise protocol

developed by Chance and co-workers (1981, 1986b; Wiener et al., 1986). In these experiments, subjects performed a single isokinetic contraction of 0.5 s duration, every 5 s to yield average power values of 0.2, 0.4 and 0.6 W. In a group of normal subjects exercising at 0.6 W, Pi/PCr was 2.0 while pH decreased to 6.74 (Wiener et al., 1986). In contrast, during the ramp protocol Pi/PCr ratio at a similar work rate was approximately 0.4, and pH was not significantly different from resting values. These discrepancies may be due to the intermittent isokinetic exercise used and the very intense contractions necessary to produce an average power of 0.6 W using a work to rest interval of 1:9. With intense contractions, nearly all of the fibres in the muscle would be recruited simultaneously, and thus the resulting metabolic stress observed by ^{31}P NMR would be substantially greater than with the ramp exercise. The total work completed in these two exercise routines may have been comparable, but the metabolic means to achieve each task was likely different.

The results of the repeated ramp tests demonstrated that the intracellular threshold was highly reproducible (Table 2). The effect of varying the ramp slope on the threshold was not examined, but it is unlikely that changes in work rate would have a significant effect. Whipp et al. (1981) have shown that during cycling the onset of the threshold was essentially independent of the duration of each work increment in a multi-stage test or the slope in a ramp test. The results of the ramp/steady-state comparison in the present study support this conclusion.

In summary, the findings of this study establish the existence of a threshold in skeletal muscle metabolism during incremental exercise that can be identified from changes in Pi/PCr and/or pH. The finding of the IT further suggests that the lactate and possibly the ventilatory thresholds observed during whole body exercise may be reflections of changes in respiration occurring at the cellular level. Further work is needed to determine the relationship between the intracellular and extracelluar threshold measures.

CHAPTER 3

METABOLIC ADAPTATIONS TO ENDURANCE TRAINING IN OLDER INDIVIDUALS

3.1 Abstract

Previous work with ^{31}P Nuclear Magnetic Resonance Spectroscopy (NMRS) established the existence of a threshold in intracellular metabolism (IT) during progressive exercise. In this study, the effect of moderate intensity endurance training on human forearm muscle metabolism was examined using ^{31}P NMRS. Changes in physical performance were also monitored. Four sedentary older subjects (58 ± 4 yrs) trained the dominant arm daily for 12 weeks, using a hand held weight. The IT was identified from changes in the intracellular phosphorylation potential (Pi/PCr) and pH during a progressive ramp exercise test in both forearms. Mean power output at the IT increased by 14% ($p<0.05$) after 6 weeks of training (1.18 ± 0.06 to 1.35 ± 0.05 Watts), but, was unchanged in the untrained arm. An additional 6 weeks of exercise did not increase the IT power further. Endurance time for a submaximal wrist flexion test was increased 34% ($p<0.05$) after 6 weeks and 58% ($p<0.05$) following 12 weeks of training. Maximal voluntary strength was not effected by training. These findings demonstrate that endurance training of the forearm was effective in altering muscle energetics and that the IT could be used as a parameter to identify such changes.

3.2 Introduction

The adaptation of skeletal muscle to continuous aerobic exercise training has been well documented. Among the changes that occur are increases in mitochondrial density (Holloszy and Booth, 1976) and the activity of enzymes involved with fatty acid oxidation (Molé et al., 1971), the citric acid cycle (Hickson et al., 1976) and the electron transport chain (Davies et al., 1981). These factors result in a considerable increase in the oxidative capacity of the muscle, thereby contributing to improvements in submaximal endurance and perhaps to maximal exercise capacity as well.

Skeletal muscle metabolism has usually been evaluated using traditional biochemical analysis of tissue samples obtained by surgical or needle biopsy. The biopsy is an essential tool in the study of met bolism , but, there are some obvious limitations associated with the procedure because it is both invasive and destructive. For this reason, the majority of information regarding muscle metabolism has corne from studies of animals or in young healthy males. The technique may not be suitable for some protocols which require repeated sampling or for certain populations such as children or the elderly. As an alternative, ^{31}P Nuclear Magnetic Resonance Spectroscopy (NMRS) while also being limited in some respects, has the distinct advantage of allowing continuous non-invasive measurement of muscle metabolism throughout exercise and recovery. Recently, several authors (Kent-Braun et al., 1990; Minotti et al., 1990) have used ^{31}P NMRS to demonstrate the effects of training

on intramuscular phosphate and intramuscular pH. They found lower Pi/PCr ratios and higher pH values for several different intensities of isokinetic contractions.

Results from previous ^{31}P NMRS studies in this laboratory have established the existence of a threshold in intracellular metabolism (IT) during progressive ramp type exercise which may be a useful parameter to assess muscle oxidative capacity (Marsh et al., 1991). The IT is characterized by an abrupt decrease in intracellular pH and a corresponding increase in the rate of phosphocreatine (PCr) hydrolysis. The finding of the IT suggests that there is a point during progressive exercise when aerobic metabolism cannot provide the necessary energy to perform the task, and additional ATP must be supplied by anaerobic metabolism. If this is so, moderate intensity, endurance training would be expected to delay the onset of the IT. Therefore, the objectives of the current study were: a) to observe changes in muscle metabolism and physical performance following 6 and 12 weeks of exercise training, and b) to test the utility of the IT as a parameter to evaluate muscle oxidative capacity in ^{31}P NMRS studies of human skeletal muscle.

3.3 METHODS

Subjects and Experimental Design

Four sedentary older individuals (3 male, 1 female) volunteered to participate in the study. Mean age of the group was 58 ± 3.7 years. The

subjects were in good health and had no previous history of metabolic or cardiovascular illness. Informed consent was obtained from all subjects before acceptance into the study.

Each participant underwent testing at three intervals: upon entry into the study (T0), and following 6 (T6) and 12 (T12) weeks of exercise training. Testing was conducted on two separate days at each interval. On the first day, maximal voluntary strength and endurance of the wrist flexor muscles were determined. Forty-eight hours later, forearm muscle metabolism was evaluated at rest and during progressive exercise to fatigue using ^{31}P NMRS. Following the initial consultation, subjects trained the dominant arm daily for a period of 12 weeks. The non-dominant arm was not trained during this period and served as a within-subject control.

Strength and Endurance Measures

Maximal voluntary strength (MVS) and muscular endurance time (ET_M) were evaluated in both the dominant and non-dominant forearms using an isokinetic wrist flexion dynamometer (Baltimore Therapeutic Instruments, Baltimore, Maryland). Subjects were seated during testing, with the elbow flexed at 90^0 and the forearm supported in pronation. The dynamometer was set to the static mode for the determination of MVS. Each subject attempted three maximal isometric contractions, with the highest torque attained deemed to be MVS. The criteria used to evaluate muscular endurance was the length of time that repeated wrist flexion could be maintained at a frequency of 0.5

Hz and at an intensity of 25% of MVS. Range of motion of the wrist movement was about 70^0 (35% each side of neutral). Subjects continued the ET_M test until they could no longer perform the exercise or until they were unable to maintain the required rate of contraction.

Ramp Exercise Test

A ramp type exercise was used to provide a metabolic stress of the forearm muscles during the ^{31}P NMRS studies (Marsh et al., 1991). This protocol required the subjects to work against a progressively increasing resistance until fatigue. The test was done with the subjects in the supine position. The subjects pronated their forearm and inserted it into the horizontal bore of the NMR magnet, grasping the lever of the exercise ergometer. Flexion of the wrist depressed the lever, and this action raised a variable resistance (a water filled container) located outside the magnet via a cable and pulley system. Wrist flexion was repeated at a frequency of 0.5 Hz, and the resistance was continuously increased by adding water to the container at a rate of 250 $mL \cdot min^{-1}$, using a roller pump (Cole-Parmer Instruments, Chicago). Work rate (power, in Watts [W]) was calculated from the frequency, distance and mass variables. The exercise began at an intensity of 0.50 W (the empty container) and continued until the subjects were fatigued.

Exercise Training

Training was done daily for a period of 12 weeks in the subjects' homes at a time of their choice. The exercise consisted of repeated wrist flexion of

the dominant limb using a hand held weight with the forearm supported. Workloads were different for each individual and were based on 25% of MVS. The subjects lifted the weight at a frequency of 0.5 Hz until they were fatigued. They were provided with a log book and asked to record the duration of the exercise period, the number of repetitions completed, and to rate the extent of their fatigue following the exercise on a scale of 1 to 10. (Unlike the Borg scale which involves descriptions associated with a numerical scale, this was simply a numerical scale.) The intensity of the exercise (i.e. weight lifted) was not changed throughout the 12 week training period. The subjects were however, encouraged to increase the duration of the exercise during the first few weeks of the study as they became accustomed to the exercise. The participants were consulted bi-weekly to ensure that the training program was progressing satisfactorily.

NMR Spectroscopy

Phosphorous spectra were acquired using a 30 cm bore, 1.89 Tesla, superconducting magnet and a TMR-32/20 spectrometer (Oxford Research Systems, Oxford). The forearm was positioned over a 4 cm surface coil so that the signal obtained was from the wrist flexor muscles, primarily the flexor digitorum superficialis (see Chapter #2, pg 10). A pulse repetition rate of 1 second was used, with 32 scans averaged for each spectrum. Spectra were acquired sequentially throughout rest and exercise. Before Fourier transformation, the data were zero filled to enhance resolution and multiplied

by a 10 Hz exponential line function to improve the signal to noise ratio.

Fourier transformed spectra were analyzed using a non-linear least squares fitting routine developed in this laboratory. The relative contributions of the phosphate metabolites, beta-adenosine triphosphate (β-ATP), phosphocreatine (PCr) and inorganic phosphate (Pi) were determined from the area under the fitted curve and the ratio of Pi/PCr was calculated using these areas. Intracellular pH was determined using the chemical shift of Pi in relation to PCr (Taylor et al., 1983). Both the logarithm of the Pi/PCr relationship and intracellular pH were plotted as a function of the exercise intensity or power output. Piecewise linear regression analysis was applied to these plots (Vieth, 1989). With this technique, the threshold in intracellular metabolism (IT) could be identified (Marsh et al., 1991).

Statistical Analysis

An F-test was used to determine if the piecewise regression provided a significantly better fit of the data than simple linear regression. The effects of time and training on muscle metabolism and exercise performance were evaluated using an analysis of variance with repeated measures (ANOVA) and Tukey's test where appropriate. Comparisons between the trained and control limbs were made using paired t-tests. Differences between groups were considered significant if $p<0.05$. All data were expressed as means $\pm$ SE.

3.4 Results

Subject compliance during the 12 weeks of training was high, with 99% of the daily exercise sessions being completed. The resistance and frequency of the wrist flexion exercise remained constant throughout the 12 week period of the study. The average duration of the training sessions was prolonged approximately 30% during the first 2 weeks of training as the subjects quickly became familiar with the exercise. In subsequent weeks, the duration of each session changed little. Improvements in the duration of exercise over the final 6 weeks of the study were typically less than 10% greater than the duration at T6. The most frequently recorded rating of exertion following the training sessions was 7, indicating the subjects were exercising at moderate intensity.

Before training, static MVS was similar in both forearms. As shown in Figure 6, strength was unaffected by the training protocol. The group ET_M was 47% longer in the dominant (757 ± 457 min), compared to the non-dominant forearm (515 ± 177 min) at T0. Although this difference was large, it was not statistically significant because of the extensive variability within the group. Training prolonged the group ET_M of the dominant limb only (see Figure 7). This effect was slight at T6 (783 ± 287 min) and increased to 25% by T12 (943 ± 489 min), but, these changes were again not significant. When changes in ET_M were calculated as a percentage of individual baseline (T0) values; the mean increase was 34% at T6 and 58% at T12. The percentage change from T6 to T12 was not statistically different.

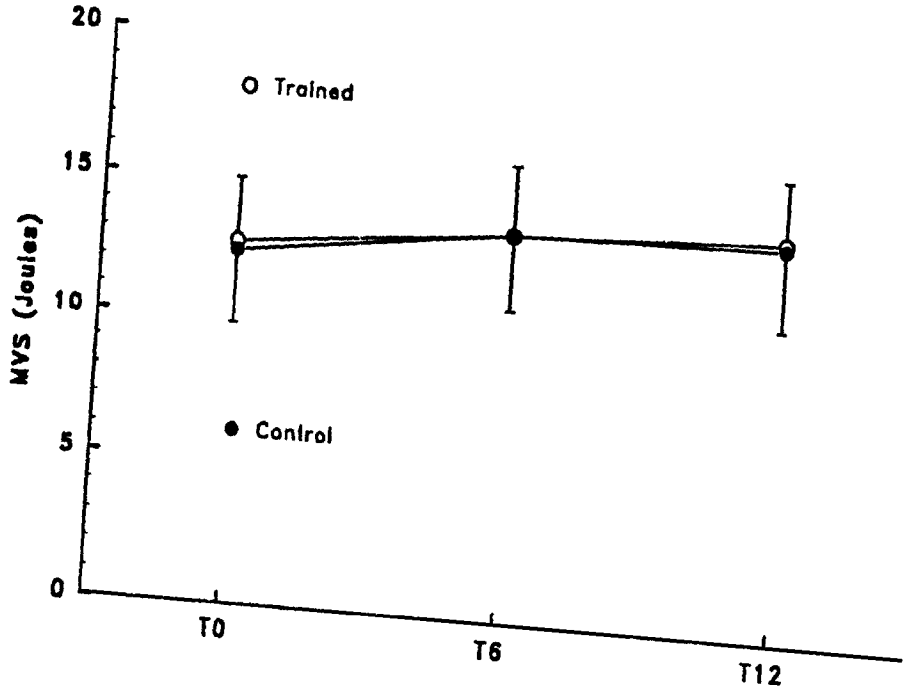

Figure #6: Static maximal voluntary strength (MVS) of the wrist flexor muscles before (T0) and after 6 (T6) and 12 (T12) weeks of endurance training. MVS was similar for both forearms and was unaffected by training. Values are group means ± SE.

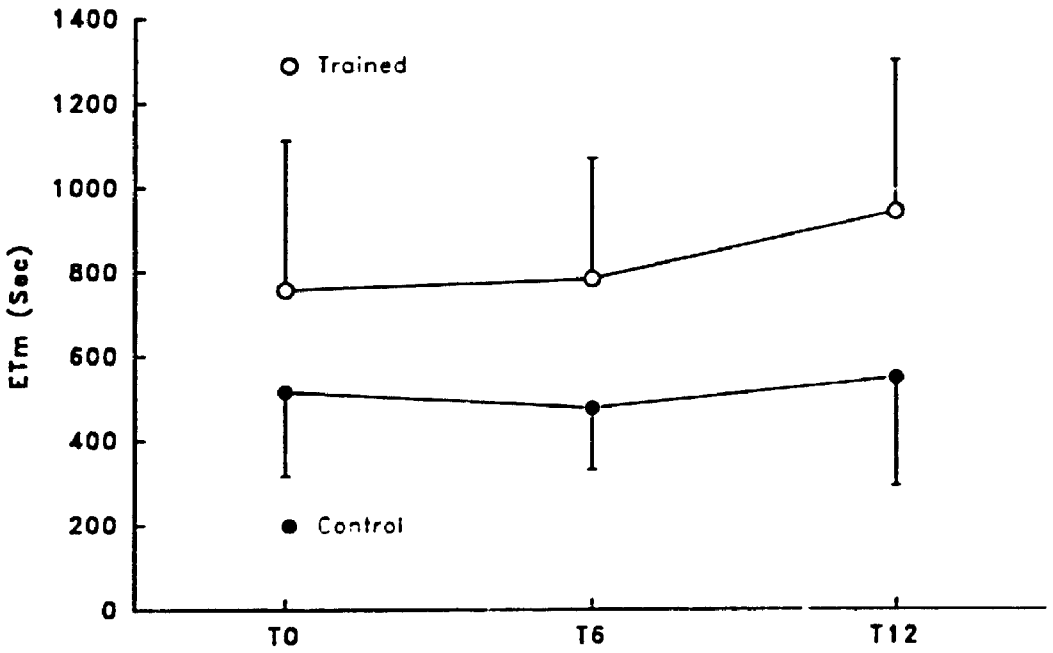

Figure 7: Maximal endurance time (ET_M) of the wrist flexor muscles for a submaximal performance test. Intensity of the test was fixed at 25% of MVS. ET_M was about 47 % greater in the dominant forearm (Trained) before training and was increased about 25% by T12. Differences were not significant. Values are group means ± SE.

The onset of the IT was found to be reproducible in the non-dominant arm, regardless of the variable used to determine it. Mean power values for the IT when determined from the Pi/PCr ratio (PiT) were 1.06±0.09, 1.09±0.14 and 1.05±0.15 Watts (W) for T0, T6 and T12, respectively. Similarly, no change in the onset of metabolic acidosis (pHT) was observed over 12 weeks of the study (Figure 8).

The training protocol delayed the onset of the IT in the muscles of the dominant forearm. Figure 9 shows the effect of 6 weeks of training on the dominant forearm of a single subject. Both PiT and the pHT were increased by about 20%. Six weeks of wrist flexion exercise increased the mean IT power for the group by 14% ($P<0.05$), from 1.18 ± 0.11 W to 1.35 ± 0.09 W. No further increase in this parameter occurred following an additional 6 weeks of training (Figure 8).

Peak power for the group attained during the ramp test was significantly ($P<0.05$) higher at T0 in the dominant forearm (1.93 ± 0.16W) than in the non-dominant limb (1.68 ± 0.08W). The lower peak power of the non-dominant forearm at T0 was due in part to a limitation of the apparatus during the testing of 2 subjects. The maximal resistance for these tests could not be increased beyond that which would produce a maximal power of 1.5 W, and this factor may have been responsible for the observed difference. Over the 12 weeks of training, peak power of the dominant forearm did not change. Peak power of the untrained limb increased 13% at T6, eliminating any

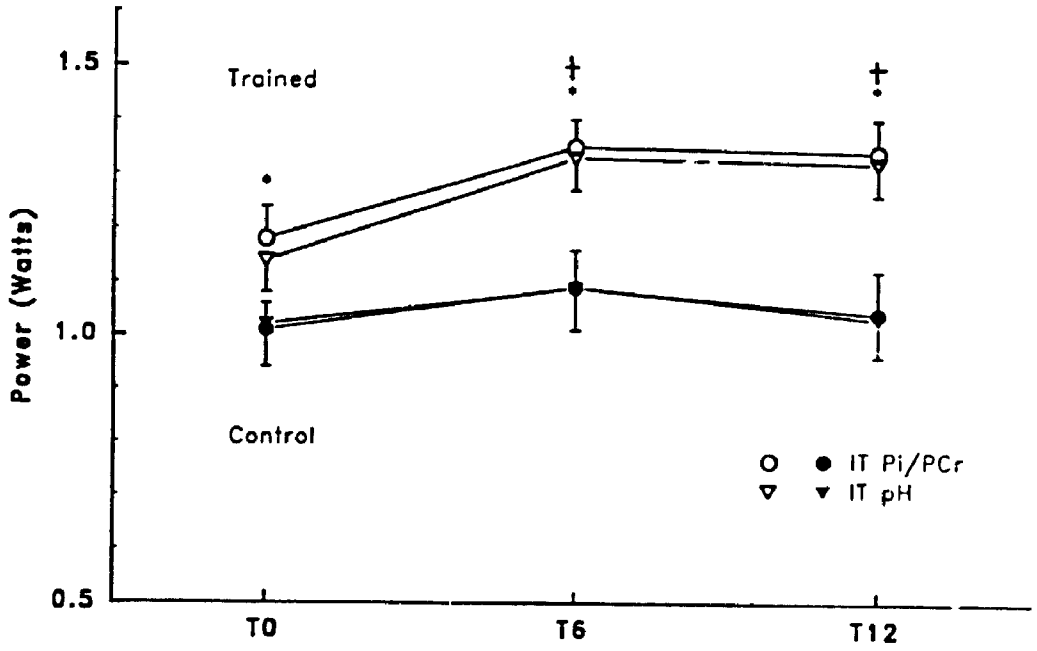

Figure 8: Effect of training on mean IT power. There was a significant ($p<0.05$) difference in IT power between the forearms at T0. A 14% ($p<0.05$) increase in IT power was evident at T6 in the trained limb. Training for a further 6 weeks (T12) did not produce an additional increase in the IT. * indicates a significant difference ($p<0.05$) between the trained and control forearms. + indicates a significant difference ($p<0.05$) from T0 for the trained forearm only.

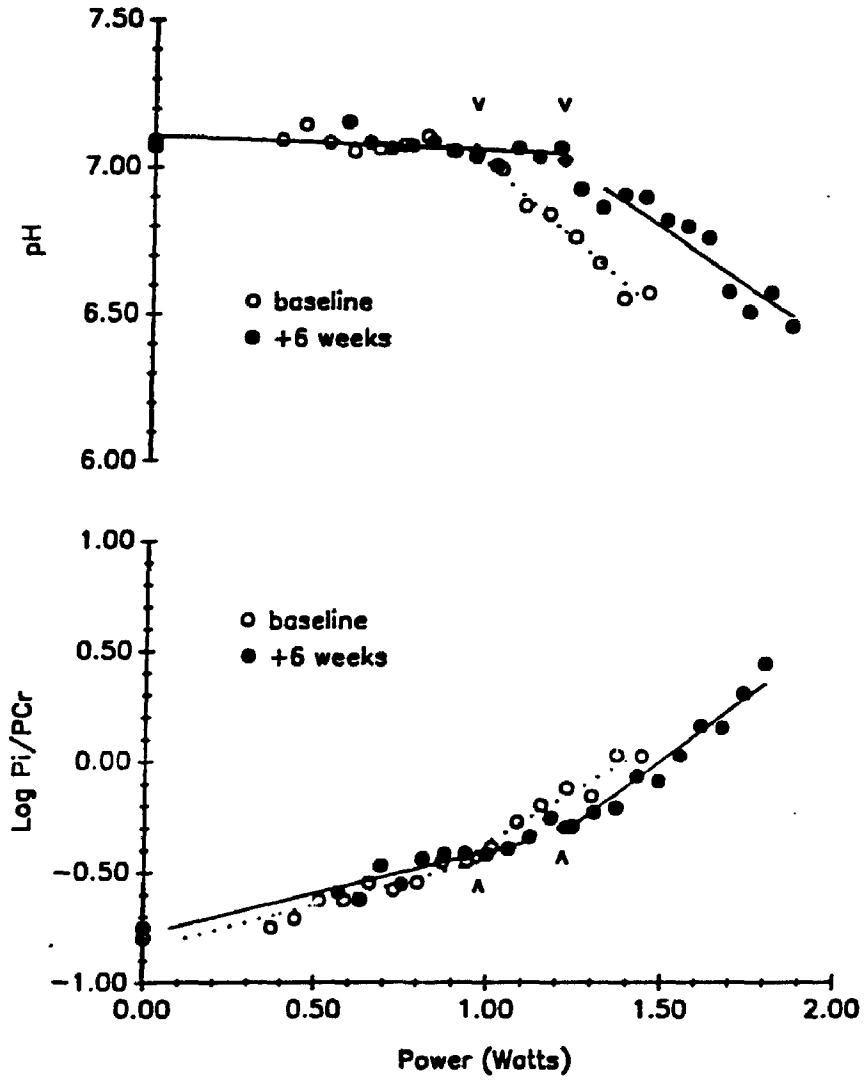

Figure 9: Changes in intramuscular pH (top) and PCr metabolism (bottom) during the ramp exercise protocol after 6 weeks of training. Data are for an individual subject. The intracellular threshold (IT) is indicated by the arrows. The onset of the IT was delayed by training.

significant differences between the two forearms. The additional 6 weeks training period elicited no further changes in maximal work capacity.

3.5 DISCUSSION

The results of this study confirm that changes in skeletal muscle function induced by exercise, can be demonstrated using the non-invasive technique of ^{31}P NMR spectroscopy. The benefits of training were shown by an increase in endurance performance (ET_M) and a delay in the onset of intracellular acidosis (IT). Although the peripheral response to training has been documented extensively, the previous exercise protocols involved large muscle groups or whole body exercise and invasive biopsy techniques. To interpret the results of the present study, comparisons must be made with the earlier work, but these comparisons must be viewed with caution because the small and large muscle mass models may not be identical.

Minotti and co-workers (1989) have reported differences in the metabolic response to exercise between the dominant and non-dominant forearms. They used ^{31}P NMRS to study muscle metabolism and found that at a given submaximal workload, the non-dominant forearm showed a greater increase in the Pi/PCr ratio and lower pH. Asymmetry of the limbs was also evident in the present study in both the biochemical and submaximal performance measures before training. ET_M of the dominant limb was 47% longer at T0 (Figure 7), even though muscular strength in both arms was the same (Figure 6). Power

output at the IT during the ramp exercise test was also significantly greater in the dominant forearm before training; 1.18 ±0.11 W compared to 1.07 ±0.09 W. This suggests that daily activities are of sufficient intensity to provide a training stimulus for the dominant limb, and further that the training stimulus primarily affects aerobic metabolism.

The percentage improvement in aerobic capacity induced by training is inversely related to the individual's initial level of fitness (Pollock,1973). Therefore, researchers typically use the non-dominant, or the most unfit limb as a model in unilateral training studies (Minotti,1990), so that the greatest possible improvement in aerobic capacity will be attained. In the present study, however, the dominant forearm was selected as the training limb and the non-dominant as the control. This was done for several reasons. First, the wrist flexion exercise was more easily done with the dominant forearm. It was believed that a greater compliance over the 12 weeks of the program would be obtained if the subjects did not have problems performing the task. The strategy was apparently successful, since compliance over the 12 weeks was nearly 100%. Secondly, central nervous system (CNS) adaptations to endurance training are particularly important when the exercise requires the acquisition of coordinated movements associated with new skills. Improvements in coordination (and thus efficiency) may be partially responsible for the increases in endurance performance which occur following training, since fewer motor units are needed to maintain a given submaximal force

(Sale,1987). Minotti et al. (1990) observed neural adaptations in the wrist flexor muscles following an endurance training program similar to that used in the present study. They found lower Pi/Pcr ratios in the muscles of the untrained forearm during submaximal exercise indicating a cross training or "cross transfer" phenomenon (Hardman et al., 1987; Sale, 1987). Cross transfer was not observed in the current study. Use of the dominant forearm may have decreased the significance of neural adaptations to the training protocol.

The maximal power (MP) attained during the ramp exercise protocol was similar in both arms and was unchanged by training, contrary to the expected outcome. There are several possible explanations for the absence of any effect. With whole body exercise such as running or cycling, the most significant factor which determines $\dot{V}O_2max$ is the increased ability of the cardiovascular system to deliver oxygen to the working muscles (Holloszy and Coyle, 1984). Enhancement of the oxidative capacity of the muscle seems to be of lesser importance. Furthermore, improvements in the respiratory capacity of muscle can occur without accompanying changes in $\dot{V}O_2max$ or vice versa (Henriksson and Reitman, 1977; Klausen et al., 1981, Sjodin et al., 1982)). Alternatively, failure of the exercise program to increase maximal power may simply be a reflection of the validity of MP as a measure of muscle oxidative metabolism. While the IT does seem to have a direct relationship to aerobic capacity, MP of the wrist flexors may not. No objective criteria were assessed

to establish that MP represents an intracellular correlate of $\dot{V}O_2$max. Maximal oxygen consumption of the forearm muscle was likely exceeded during the ramp test, but, MP may also depend on other factors including muscle strength, subject motivation, or the ability to tolerate discomfort. If MP is not a valid criterion (and there does seem to be some doubt), then the importance of the IT as an objective parameter to assess aerobic function is emphasized.

The most significant result of the study was the delay in the IT with training. An improvement in the maximal (or peak) power attained during the ramp test was not a consistent finding in the study, however an increase in IT power was observed for all subjects. The relationship between the IT and extracelluar threshold measures has yet to be defined, but increases in the ventilation threshold (Sady et al., 1980; Ready and Quinney, 1982; Poole and Gaesser, 1985;) and the lactate threshold (Sjodin et al., 1982; Yoshida et al. 1982; Poole and Gaesser, 1985) following training have previously been reported. The magnitude of these changes varies considerably from approximately 5% (Sjodin et al., 1982) up to 70% (Ready and Quinney, 1982), and is apparently related to the threshold parameter measured, and how the training variables are manipulated. Therefore, the 14% increase in IT power observed in the present study is consistent with the current literature values.

There are several mechanisms by which the onset of the IT could have been delayed. Biochemical alterations may have taken place that would increase oxidative capacity of the muscle so that PCr stores were maintained

and lactate production reduced. Alternatively, the delay in the onset of the IT could have been related to changes in blood flow to the working muscle. Intramuscular pH and the Pi/PCr ratio are quite sensitive to blood supply (Wiener et al., 1986), so an increase in flow would produce a lower Pi/PCr ratio and $[H^+]$ at any submaximal exercise intensity. There is some evidence that muscle blood flow is increased following endurance exercise (Leinonen, 1980; Klausen et al., 1982), although, other studies have shown that flow is unaffected by training (Grimby et al., 1967). Minotti et al. (1990) found that training did not alter forearm muscle blood flow during a submaximal wrist flexion exercise test, similar to the one used in the present study. Even though total muscle perfusion may not be increased with training, the possibility of a more effective redistribution and utilization of the existing flow cannot be excluded (Mackie and Terjung, 1983). Other peripheral vascular adaptations may also occur. A prolonged transit time of the blood due to an increased capillarization or a decreased oxygen diffusion distance associated with a greater mitochondrial density would enhance oxygen extraction from the blood (Terjung et al., 1988). Studies demonstrating these changes, however, have involved whole body exercise, so it is not known whether these adaptations will occur following training of a small muscle mass. The forearm exercise protocol does not stress the central component of the cardiovascular system.

While the intensity of the training stimulus appears to be the primary factor in determining the extent of changes in $\dot{V}O_2max$ (Gaesser and Rich,

1984; Thomas et al., 1984), improvements in submaximal exercise capacity are not as dependant on intensity (Seals et al., 1984). Fox et al. (1975) showed that the frequency and duration of training sessions were most influential in reducing heart rate during exercise at a fixed submaximal workload. These findings are of practical significance, particularly for the elderly and people with physical limitations due to an illness. These individuals are more likely to participate in a daily walking program in which the distance travelled increases, rather than a training regimen requiring more intense effort. We attempted to duplicate these exercise conditions with the small muscle mass model by having the subjects exercise daily at a fixed intensity for a duration selected by each individual. This procedure was successful to a degree, with training having the greatest effect on the submaximal parameters, the IT and ET_M, as anticipated. Such programs, however, do appear to have limitations. The training was only effective over the first 6 week period, with little or no further improvements in the IT power or ET_M observed at T12. Frequency of training was maximized by having the subjects exercise daily, but the duration of the sessions did not continue to increase over the final 9 weeks of the study as it had initially. Therefore, the subjects were exercising with the intensity, frequency and duration variables constant. Although encouraged to do so, subjects did not increase the time of the training sessions beyond about 20 minutes. Boredom was likely a factor in the subjects failure to increase the exercise duration, because the task was a very simple and repetitive one.

Hickson et al. (1981) studied the kinetics of changes in aerobic fitness in young males and suggested that when the training variables were constant, most of the adaptation in $\dot{V}O_2max$ was completed by 3 weeks. In the present study of older individuals, muscle metabolic changes were completed in 6 weeks or less which is consistent with the findings of Hickson et al. (1981). Evidently, the time course for the peripheral adaptations to training are similar to those occurring in aerobic power.

In summary, continuous moderate-intensity exercise training was effective in delaying the onset of intracellular metabolic acidosis and increasing the subjects' capacity for submaximal work. These findings suggested that the oxidative capacity of the forearm muscles had been increased with training, although the possibility of a greater perfusion of the working tissues can not be excluded. Regardless of the precise mechanism responsible, NMR spectroscopy and the ramp exercise protocol were shown to be an effective means of monitoring training induced changes in human muscle metabolism.

CHAPTER 4

TRANSIENT CHANGES IN MUSCLE HIGH ENERGY PHOSPHATE DURING MODERATE EXERCISE

4.1 Abstract

Analysis of the response of muscle high energy phosphate compounds to various exercise perturbations may provide information about the physiological control of respiration. The purpose of this study was to use ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy to examine changes in wrist flexor muscle [PCr] and [Pi] during the transition from rest to steady state exercise (on-transient) and back to rest (off-transient). Following a brief warm-up, five young males (25 ± 2 yrs) performed a square wave exercise test for 5 min followed by a 5 min recovery period. The intensity of the exercise was set below each individual's threshold for intracellular metabolic acidosis. The subjects repeated the protocol 6 times. ^{31}P NMR spectra were collected throughout the exercise and recovery periods. The relative concentration of the phosphate metabolites and intramuscular pH were determined from the spectra and plotted as a function of time. The results of the 6 tests were pooled for each individual before analysis. ATP and intracellular pH did not change significantly during exercise or recovery. The PCr and Pi data were fit with a first order exponential growth or decay model as appropriate. The time constants for the on transient were 33.1 ± 11.8 s for PCr and 32.7 ± 9.4 s

for Pi. Similarly, the time constants for the off transients were 33.2 ± 4.3 s and 31.1 ± 8.1 s for PCr and Pi, respectively. These values are nearly identical to the time constants for oxygen consumption during submaximal exercise which have been reported previously by several authors. The results of this study show that the metabolism of muscle PCr during steady state exercise and recovery can be accurately described by a mono-exponential model and further suggest that a first order proportionality exists between metabolic substrate utilization and oxygen consumption.

4.2 Introduction

An exponential rise in pulmonary oxygen uptake ($\dot{V}O_2$) occurs following the onset of physical work in humans. After the exercise is terminated, $\dot{V}O_2$ decreases in a similar manner. It is assumed that these changes in $\dot{V}O_2$ reflect the rate of oxygen utilization by the muscle ($\dot{Q}O_2$), but the precise coupling of external and internal respiration remains controversial. Several different models have been proposed which describe the relationship between $\dot{V}O_2$ and $\dot{Q}O_2$ as first-order exponential. However, one scheme suggests that the kinetics of $\dot{V}O_2$ and $\dot{Q}O_2$ are equal (Whipp et al., 1982) while another implies that $\dot{Q}O_2$ kinetics are significantly faster than $\dot{V}O_2$ kinetics (di Prampero, 1981). Hughson and Morrissey (1983) and others (Molé et al., 1985) believe that the first order model may be an oversimplification.

Since direct measurement of $\dot{Q}O_2$ in humans is not possible, most of

what is known about muscle oxidative metabolism has been obtained from in-vitro preparations (see for example Chance and Williams, 1955), or from research involving animals (see for example Mahler, 1985). Intramuscular phosphocreatine (PCr) is frequently used as an indirect measure of oxidative metabolism since PCr decreases proportionally with $\dot{Q}O_2$ and $\dot{V}O_2$ (Karlsson and Saltin, 1970; Knuttgen and Saltin, 1972). Moreover, Whipp and Mahler (1980) have suggested that the creatine kinase reaction is a major mechanism controlling mitochondrial respiration. Other authors (Seraydin and Artaza, 1976; Connett, 1984; Meyer, 1988) have also proposed control systems involving a first-order relationship between PCr and $\dot{Q}O_2$.

There has been very little published information regarding the pattern of PCr metabolism in human muscle during the transition from rest to exercise. Bergstrom (1967) used muscle biopsy techniques to study muscle metabolism at an exercise intensity equivalent to 60% of $\dot{V}O_2$max and found that PCr declined exponentially at the onset of exercise and then plateaued. Molé and co-workers (1985) were the first to use ^{31}P NMR spectroscopy to monitor non-invasively the transient changes in intracellular high energy phosphates during exercise. The results of the study have cast some doubt on the validity of the first-order model of $\dot{Q}O_2$ kinetics. They found that while Pi appeared to increase exponentially, PCr changed in a biphasic manner, and further, that the rates of the two processes were not the same. ^{31}P NMRS studies of the recovery from exercise have also shown the resynthesis of PCr to be biphasic

(Arnold et al., 1984). However, both of these studies involved exercise at absolute work rates sufficiently intense to cause a decline of intramuscular pH, which may be a confounding variable. The kinetics of PCr metabolism have not yet been carefully analyzed for exercise of moderate intensity, or more specifically, for work rates below the intracellular threshold for metabolic acidosis (Whipp and Ward, 1990).

The purpose of this study therefore, was to use ^{31}P NMR spectroscopy to assess non-invasively, the pattern of change of muscle high energy phosphates (PCr, Pi, ATP) in human skeletal muscle during the transition from rest to moderate exercise and back to rest. The kinetics of PCr and Pi were determined for both the on- and the off-transient response to exercise using a first order exponential model.

4.3 Methods

Subjects and Experimental Design. Five young males (mean age 25 ± 2 yrs), were recruited as subjects for the study. While all of the volunteers were healthy and active, although none could be considered highly trained. Subjects reported to the lab on 2 different occasions. The initial session was used to familiarize the participants with the experimental procedures and the NMR apparatus. At this time, the subjects also completed a forearm ramp exercise test to fatigue. The results of this test were used to determine the individual work rates for the subsequent steady state tests. During the second session,

the subjects performed a series of 6 rest-work-rest transitions at a fixed work rate (square wave test).

Exercise Protocols. The ramp exercise protocol for the wrist flexors, used in conjunction with ^{31}P NMR spectroscopy, has been described in detail previously (Marsh et al., 1991). Only a brief summary of the protocol will be given here. Following a 2 minute warm-up of unloaded wrist flexion, the subjects continued to flex the wrist at a frequency of 0.5 Hz against a continuously increasing resistance. Work was initiated at a rate of 0.50 Watts (W) and was increased continuously by approximately 0.13 $W \cdot min^{-1}$ until fatigue. Changes in intracellular pH and the relative concentrations of phosphocreatine (PCr) and inorganic phosphate (Pi) were monitored throughout the exercise using ^{31}P NMR spectroscopy.

Work rates for the square wave studies were calculated from the results of the ramp tests. The intensity of the exercise was equivalent to 90% of the power output (W) at each individual subject's intracellular threshold (IT). The IT is the point during the ramp test where a sustained metabolic acidemia and a simultaneous increase in the rate of PCr metabolism occur (Marsh et al., 1991). The range of work rates below the IT were considered to be of moderate intensity. The square wave tests consisted of 3 phases. Following a 2 min warm-up of unloaded wrist flexion, the square wave workload was done for about 5 min, and was followed by 5 min of recovery. Intramuscular pH and the relative concentrations of phosphate metabolites were monitored

throughout the rest, exercise and recovery phases of the square wave tests using ^{31}P NMR spectroscopy. Each subject performed 6 repeats of the square wave test.

NMRS Data Acquisition. ^{31}P NMR data from the flexor muscles of the forearm were acquired using a 4 cm (diameter) single turn surface coil and an Oxford Research Systems (Oxford, England) TMR-32/20 spectrometer operating at 1.89 Tesla. Thirty-two free induction decays (FID) were collected and averaged for each spectra using a repetition rate of 1 s. Several additional seconds were needed to store the data in the computer, making the minimum time resolution of the experiment about 36 s. Before Fourier Transformation, the FID data were zero filled to enhance resolution and multiplied by a 10 Hz exponential line function to improve the signal to noise ratio. The transformed spectra were analyzed using a computerized, non-linear, least squares fitting routine. Relative concentrations of beta-adenosine triphosphate (βATP), phosphocreatine (PCr) and inorganic phosphate (Pi) were determined from the area under the fitted curve, and the ratio of Pi/PCr was calculated using these areas. Intracellular pH was determined using the chemical shift of Pi relative to PCr (Taylor et al., 1983). Data from the ramp test, the logarithm of the Pi/PCr ratio and intracellular pH were plotted as a function of the exercise intensity in Watts. Piecewise linear regression analysis was applied to these plots to identify the IT (Vieth, 1989).

The sensitivity of the NMR technique is dependent on the signal to noise

ratio which is greatly influenced by the length of time over which the spectra is accumulated. The 36 s, which were used to obtain each spectra did not permit adequate resolution of the changes in PCr and Pi during the onset of the square wave work function and during recovery. Shorter data acquisition times (hence an increased frequency of sampling) were possible if fewer FID's were averaged for each spectra. However, with only 16 or 8 FID's, the signal to noise ratio of the spectra was not suitable. An alternative solution to this problem was to use the longer 36 s acquisition time, but, repeat the test several times, staggering the timing of the data accumulation. The results of the repeated tests could then be combined for each individual. This procedure has the same effect as increasing the sampling frequency, without compromising the signal to noise ratio. At the onset of exercise, the concentration of PCr continuously decreases and Pi continuously increases until a steady state condition is reached. The reverse is true when exercise is stopped. Unlike a muscle biopsy which samples metabolite concentrations at a single moment in time, the transformed spectra represents the average concentration of the metabolite over the 36 s acquisition period. Therefore, the midpoint of the acquisition period was taken to be the time for each spectra (see Figure 10). For example, the first 3 spectra were assigned times of 18, 54 and 90 s rather than 36, 72 and 108 s. For the square wave studies, the timing of the data acquisition with respect to the onset of exercise was varied for each of the 6 repeats of the test. Data accumulation commenced 10 and

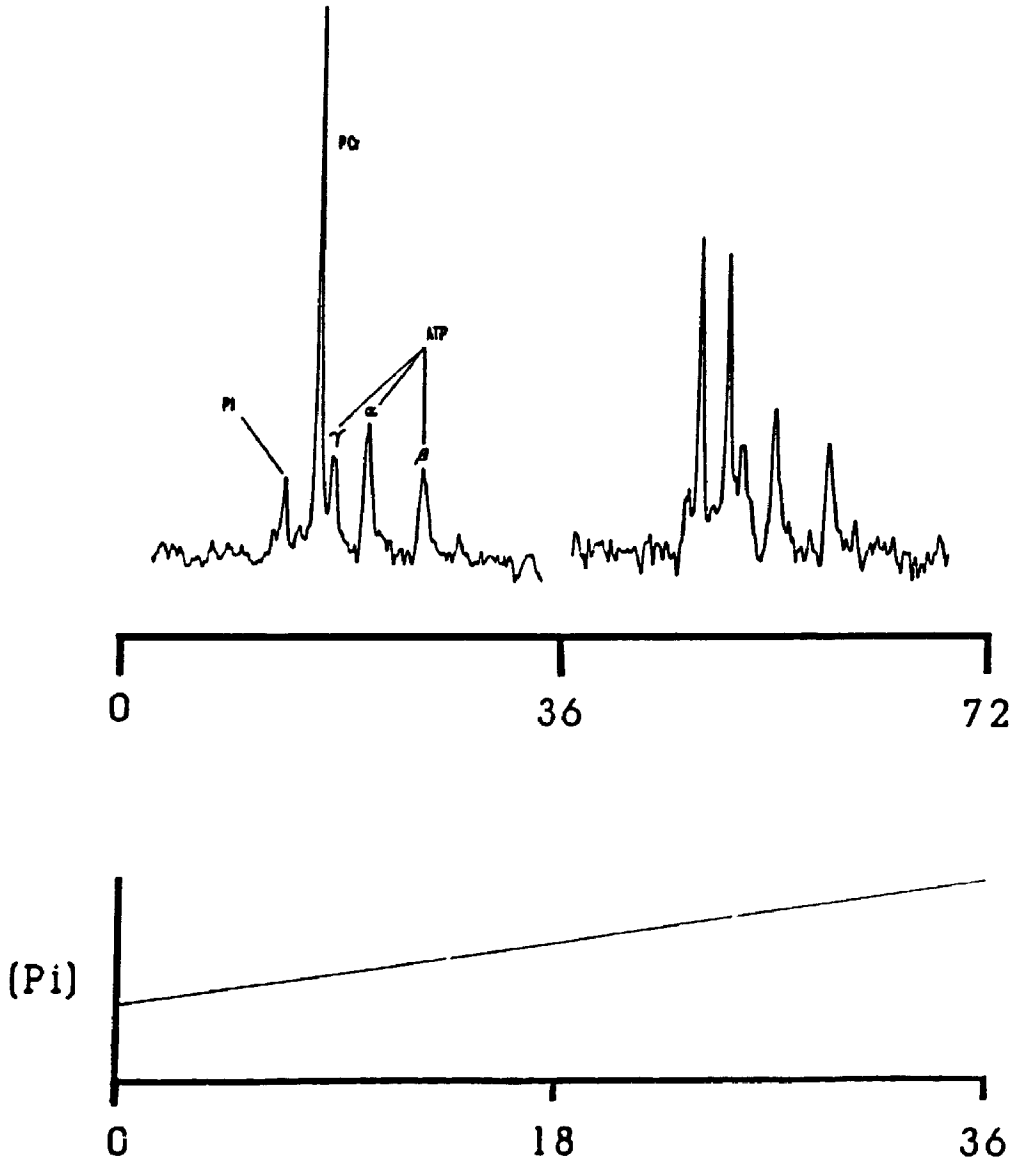

Figure 10: Consecutive resting (left) and exercise (right) spectra are shown in the upper portion of the figure. Each spectrum represents the average concentration in metabolites over the aquisition period (36 s). This is shown graphically in the lower part of the figure. The midpoint of the aquisition period was considered the time of the spectra (18 s) rather than the end (36 s). The rest and exercise spectra were assigned times of 18s and 54s respectively.

5 s prior to the start of exercise, coincident with the onset of exercise and 5, 10 and 15 s after exercise had begun. This procedure is shown graphically in Figure 11. The concentrations of PCr and Pi were determined from the fitted spectra as described above. PCr and Pi were expressed as a fraction of the sum of the total phosphate signal (ie. ATP + PCR + Pi), and plotted as a function of time for the on- and off-transient response to the square wave exercise. These curves were then fit using a first order, exponential growth or decay model (depending on the metabolite and the exercise phase) using an iterative optimization routine on a personal computer. The form of the model was:

$$y(t) = a\{1-e^{-t/r}\}$$

where y represents PCr or Pi concentration (relative) at a given time (t), a is the amplitude of the response under steady state conditions, and *r* is the time constant of the response. Time constants were determined for PCr and Pi for both the on- and the off-transient for each subject individually.

4.4 Results

The forearm ramp exercise caused a progressive decline of intramuscular PCr and an increase in Pi, but little change was observed in the relative concentration of ATP. When the logarithm of the Pi/PCr ratio was plotted as a function of the exercise intensity it was evident that the metabolism of PCr

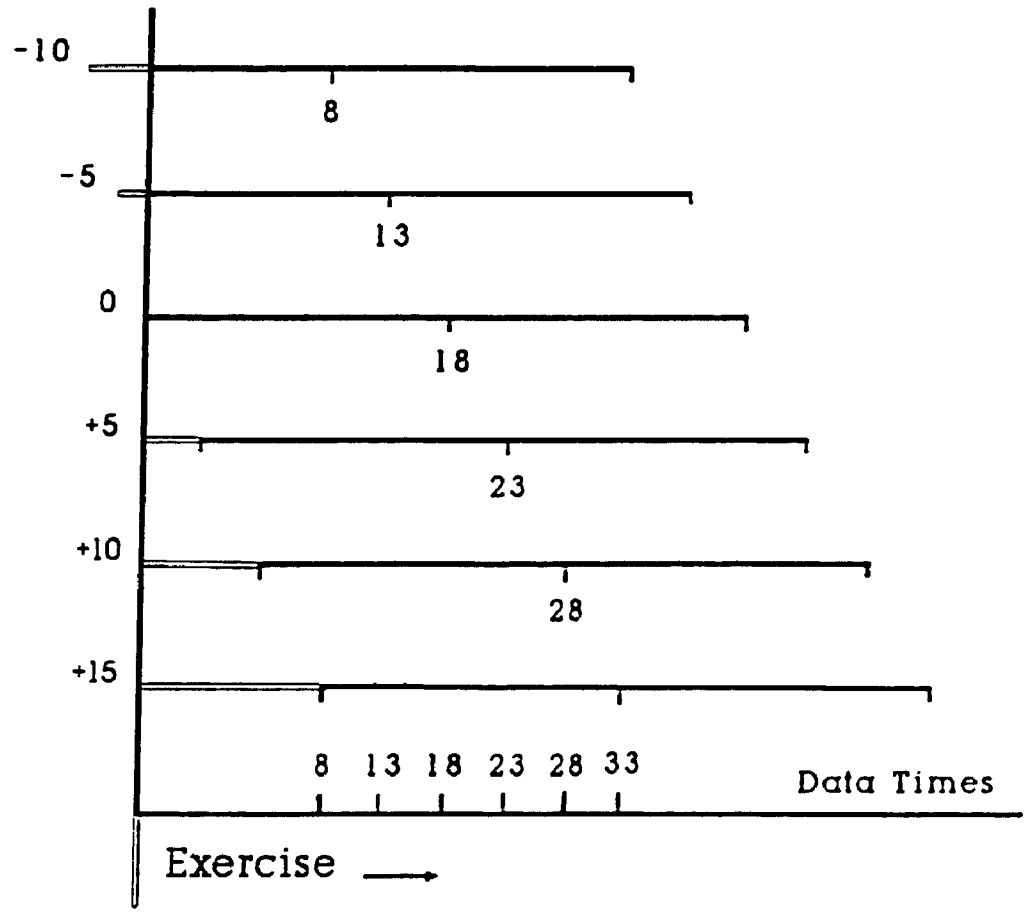

Figure 11: A schematic representation of the accumulation of data in each of the 6 square wave exercise tests. Acquisition of ^{31}P NMR spectra was staggered at 5 s intervals, relative to the onset of exercise (vertical axis). This technique allowed a greater sampling frequency while maintaining the quality of the spectra.

was biphasic. The Pi/PCr data were best fit by a piecewise linear regression model (Vieth, 1989), with the breakpoint or threshold between the two regression lines coinciding with the onset of intracellular acidosis. This relationship was shown previously by Marsh et al., (1991) and termed the intracellular threshold (IT). One of the subjects was unable to reach a true maximal power during the ramp test due to a limitation of the exercise apparatus. For the subjects who continued the exercise to fatigue (n = 4), the threshold occurred at about 59% of the maximal power attained. The square wave work rates, which were calculated from the ramp tests, were equivalent to 90% of the IT for each individual, and varied from 0.92 Watts (W) to 1.32 W. The results of the ramp testing are summarized in Table 4.

The square wave exercise did not effect the relative concentration of ATP significantly. Table 5 shows the mean values for ATP expressed as a percent of the total NMR visible phosphate signal (ie: PCr + Pi + ATP) for each subject and for the group as a whole. These means of ATP were averaged throughout the rest, exercise and recovery phases of the six square wave tests. ATP contributed about 24% of the total phosphate signal (P). The percent coefficient of error (%CV) within subjects was approximately 10% and was only slightly higher between subjects (Table 5). Intracellular pH was also unchanged by the square wave protocol. Since the logarithmic transformation used to calculate pH attenuates changes which occur in the proton concentration ($[H^+]$), the values of pH obtained from the fitted spectra were

Table 4: Summary of the results of the ramp testing.

Subject	Max Power	IT	% Pk Power	Sq Workrate
TD	2.42	1.46	61	1.32
RE	2.04	1.10	54	0.99
MP	**	1.31	**	1.18
DG	2.09	1.18	56	1.06
JP	1.61	1.02	63	0.92
GROUP	**2.04**	**1.21**	**59**	**1.09**
±SE	**0.17**	**0.09**	**2.0**	**0.07**

Max Power, IT and SQ. Workrate are expressed in Watts (W)

IT = Intracellular Threshold, value is the mean of Pi/PCr and pH threshold determinations.

SQ Workrate = 90% of IT

** = Subject unable to reach maximal power due to a limitation of the exercise apparatus.

SE = Standard error

Table 5: Relative concentration of ATP during the rest, exercise and recovery phases of the 6 square wave tests.

Subject	n	ATP/Px100	%CV
TD	89	21.9	10.3
RE	87	24.6	10.2
MP	85	26.8	10.7
DG	78	23.6	10.0
JP	73	23.9	10.7
GROUP	**412**	**24.1**	**12.3**

n = number of spectra

%Cv = percent coefficient of variation

Table 6: Hydrogen ion concentration during the rest, exercise and recovery phases of the 6 square wave tests.

Subject	n	pH	$[H^+]$	%CV
TD	89	7.04	90.5	9.2
RE	87	7.05	88.6	9.8
MP	85	7.06	86.6	11.9
DG	78	7.05	88.8	10.0
JP	73	7.07	84.5	7.5
GROUP	**412**	**7.05**	**87.7**	**10.6**

n = number of spectra

$[H^+]$ = nM

%CV = percent coefficient of variation

converted to $[H^+]$. Still the $[H^+]$ was consistent both within and between subjects (see Table 6). About 10% variation was evident in $[H^+]$ which corresponds to a pH change of about 0.05 units.

A progressive decrease in the relative concentration of PCr (PCr/Px100) and an increase in Pi (Pi/Px100) were observed following the onset of the square wave exercise. When exercise was terminated, PCr was resynthesized, thus relative PCr concentration increased, while Pi declined toward resting levels. Fluctuations in these metabolites throughout the work and recovery phases of the 6 different square wave tests are shown in Figure 12. When these data were temporally aligned and combined, the pattern of change appeared to be exponential. Regardless of which metabolite was monitored, or the phase of the exercise protocol, steady-state conditions were reached by about 2 min during both exercise and recovery. The PCr and the Pi responses were well fit by the first order models. The fitted data for the on-transient response of a single subject (RE), are shown in Figure 13 (PCr) and Figure 14 (Pi). The off-transient data for the same individual are shown in Figure 15 (PCr) and Figure 16 (Pi). The residuals for each model are included in the figures.

Individual time constants (τ) determined for the on-transient response ranged from approximately 18 s to 49 s. Mean τ values were 33.5 ± 11.8 s for PCr and 32.7 ± 9.4 s for Pi (Table 7). The range of the individual τ's for the off-transient were not as great (22 s to 40 s). Means (Table 8) were similar to the on-response, 33.2 ± 4.3 s and 31.1 ± 8.1 s for PCr and Pi respectively.

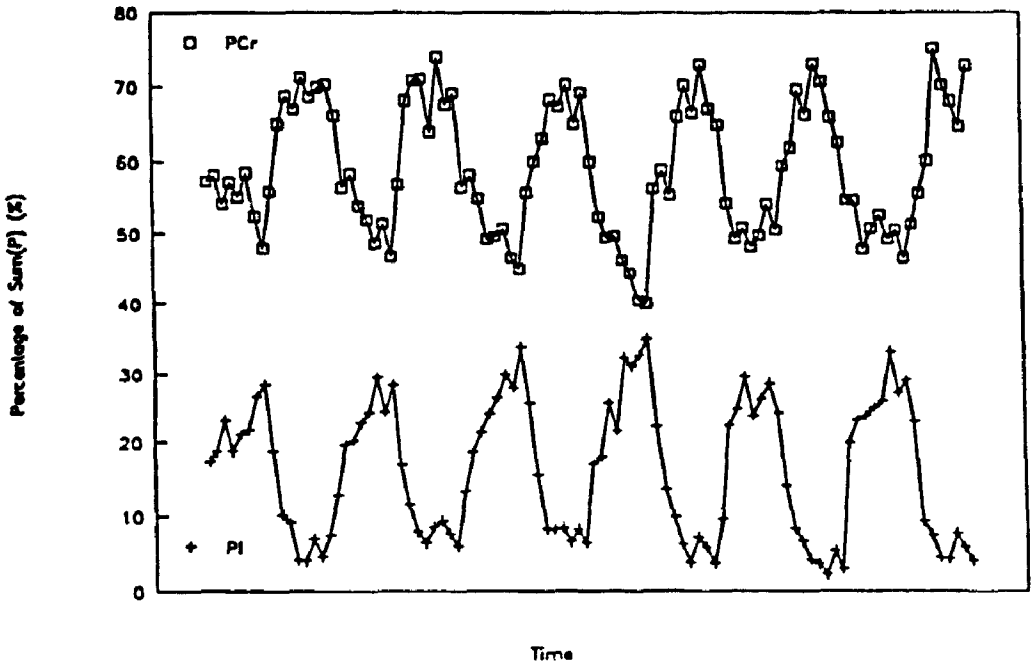

Figure 12: Changes in the relative concentration of PCr (squares) and Pi (crosses) during 6 consecutive square wave exercise tests. Data shown are for a single subject (RE). For each test, data acquisition was staggered with respect to the onset of exercise. The results were combined and then fit using a first-order exponential model.

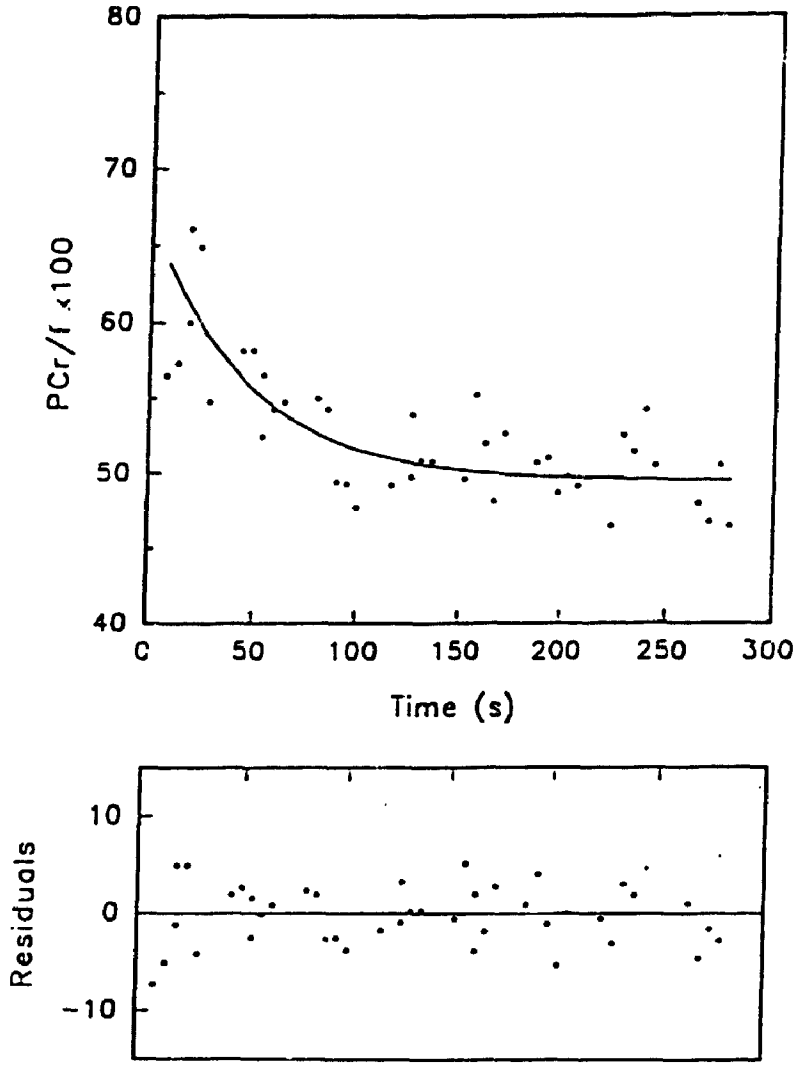

Figure 13: Changes in the relative concentration of PCr during the transition from rest to moderate exercise. Data shown are for a single subject (RE). The solid line is first-order fit of the data. Residuais for the model are shown in the lower part of the figure.

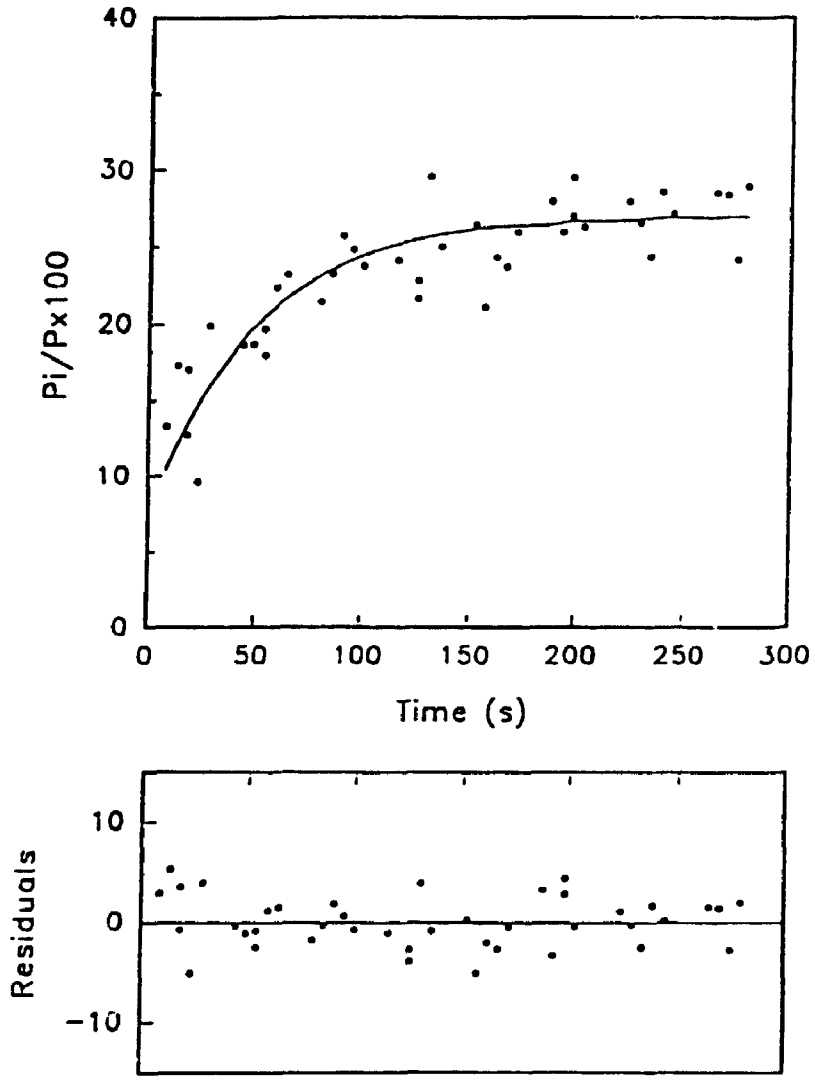

Figure 14: Changes in the relative concentration of Pi during the transition from rest to moderate exercise. Data shown are for a single subject (RE). The solid line is a first-order fit of the data. Residuals of the model are shown in the lower part of the figure.

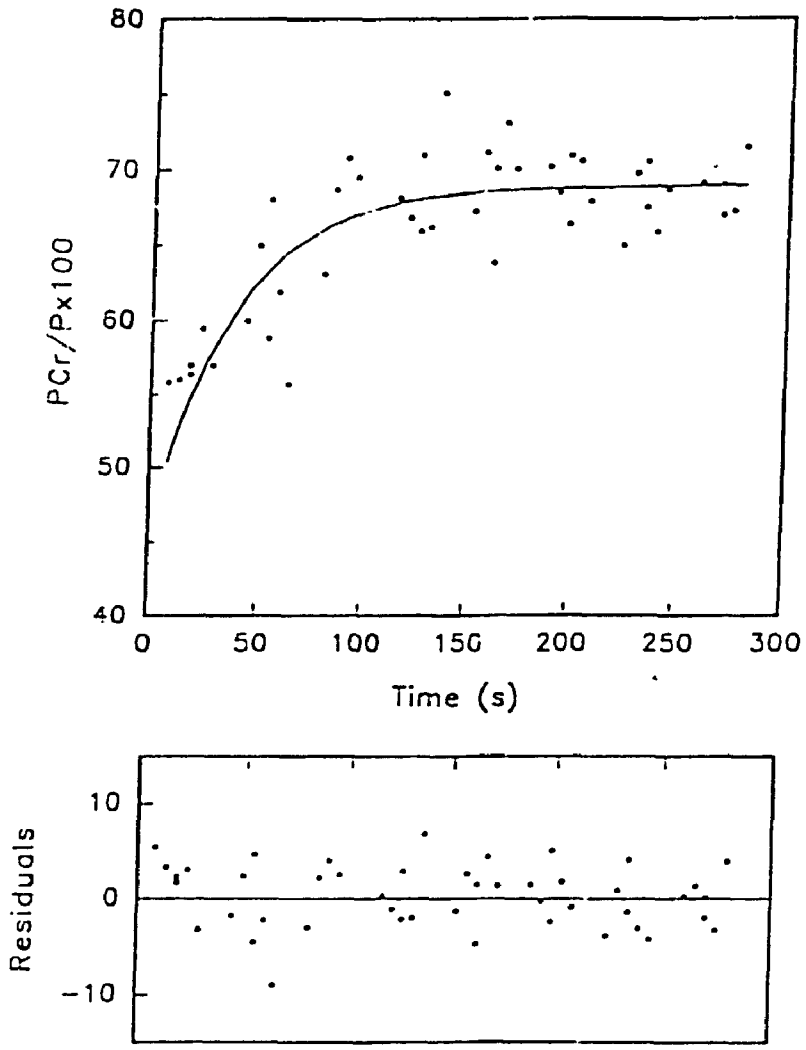

Figure 15. Changes in the relative concentration of PCr during the transition from moderate exercise to rest. Data shown are for a single subject (RE). The solid line is a first-order fit of the data. Residuals of the model are shown in the lower part of the figure.

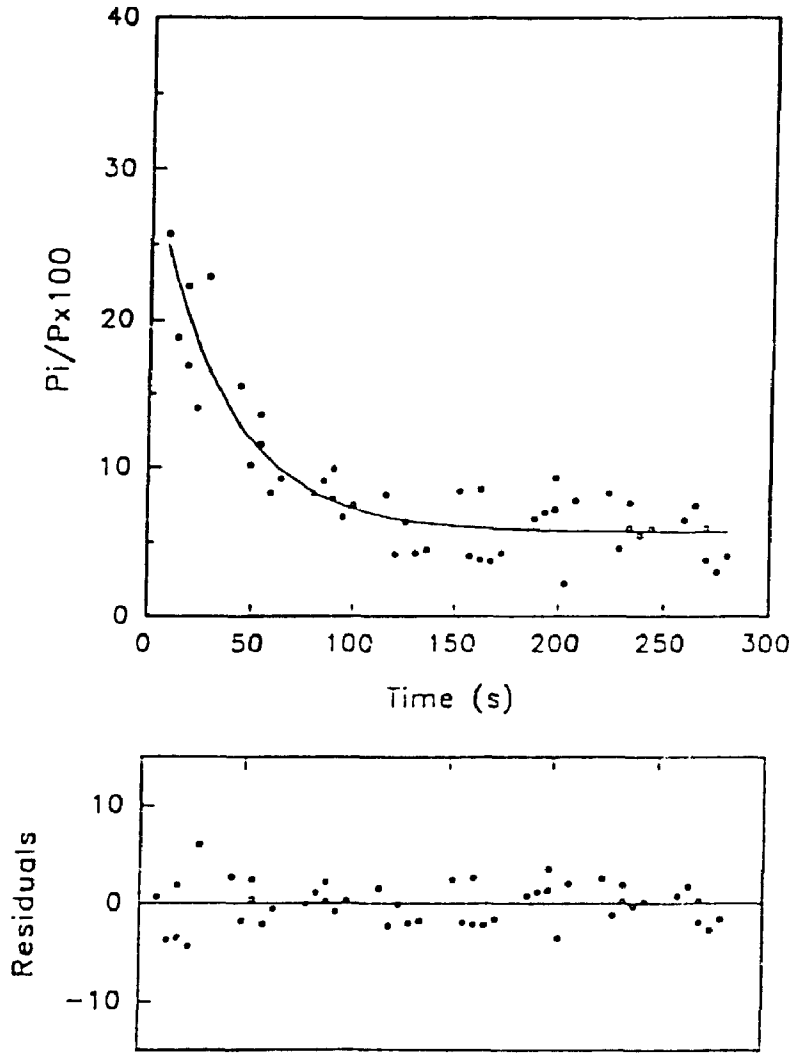

Figure 16: Changes in the relative concentration of Pi during the transition from moderate exercise to rest. Data shown are for a single subject (RE). The solid line is a first-order fit of the data. Residuals of the model are shown in the lower part of the figure.

Table 7: Time constants (τ) the on-transient response of PCr and Pi to moderate exercise.

Subject	PCr	SD	Pi	SD
TD	17.9	6.9	18.8	5.4
RE	49.6	9.3	47.8	7.1
MP	30.0	10.3	32.9	6.3
DG	30.9	10.1	28.9	9.6
JP	39.5	10.7	34.9	11.8
GROUP	**33.5**	**11.8**	**32.7**	**9.4**

SD = standard deviation

all values are expressed in seconds

Table 8: Time constants (τ) for the off-transient response of PCr and Pi to moderate exercise.

Subject	PCr	SD	Pi	SD
TD	30.4	7.4	29.0	5.4
RE	36.4	5.9	38.3	3.3
MP	37.5	7.5	40.7	3.9
DG	27.2	4.9	24.9	4.3
JP	34.3	6.8	22.4	9.8
GROUP	**33.2**	**4.3**	**31.1**	**8.1**

SD = standard deviation

all values are expressed in seconds

4.5 Discussion

The constancy of muscle ATP under various exercise conditions, has been shown in both animals (Dawson, et al., 1978; Kushmerick and Meyer, 1985) and humans (Gollnick and Hermansen, 1973; Chance et al., 1982; Newham and Cady, 1990). However, information regarding ATP changes during the transition from rest to steady state exercise and vice versa is limited. Molé and coworkers (1985) observed intramuscular ATP to change biphasically following the onset of exercise. They concluded that since the change in ATP (an important intermediate in the creatine kinase reaction) was biphasic, it was unlikely that PCr metabolism could be a monoexponential process. The results of the present study do not support this conclusion. The relative concentration of ATP was not altered appreciably during the different phases of the square test (rest, exercise, recovery), between tests, or even between subjects. As shown in Table 5, the variance in ATP levels for an individual subject throughout the 6 tests (about 80 spectra) was about 10%. For the group as a whole (over 400 spectra) the coefficient of variation was only slightly higher at 12.3% (Table 5). These values agree favorably with the %CV from ^{31}P the NMR estimates (12.6%) of resting muscle ATP reported by Molé et al. (1985), and with data from human muscle biopsy samples (15.2%) published by Karlsson (1971). Similarly, no change was found in the H^+ concentration of the forearm muscle either within the square wave tests, between tests, or between individuals. Mean H^+ concentration of the group was 87.7 nM ± 9.2

nM, corresponding to a pH of 7.06 ± 0.06 units. The variance in the pH measure is simply a reflection of the sensitivity of the NMR technique. Given the digital resolution of NMR, changes in intracellular pH can only be measured by NMR with a precision of 0.05 units (Gadian, 1982; Thompson et al., 1991).

Kinetic changes in ATP concentration such as those found by Molé et al., (1985) may be related to the intensity of the exercise being performed. The handgrip protocol used in that study was sufficiently intense to bring about a progressive decline in intramuscular pH. A ^{31}P NMR study by Newham and Cady (1990) showed that handgrip exercise at 50% of maximal voluntary contraction (MVC) caused a decline in ATP concentration and in intramuscular pH, while the same exercise at 25% MVC produced no change in ATP. The purpose of the present study was to model the kinetics of PCr metabolism specifically at sub-threshold exercise intensities. Workrates for the square wave tests were determined from prior ramp testing of the subjects, thus ensuring stable ATP and pH values.

Both the on-transient and off-transient responses of PCr and Pi to the square wave exercise were well described by the first-order model (see Figures 13 to 16). The adequacy of this model is evident from an analysis of the residuals shown in the lower portion of Figures 13 through 16. It can be seen that the residuals do not systematically deviate from zero, and can therefore be considered as white noise. The first order model is consistent with the dynamics of PCr metabolism observed in a number of animal muscle

preparations (Kushmerick and Paul, 1976; Mahler, 1985). Muscle O_2 consumption paralled the monoexponential kinetics of PCr in these preparations, with time constants ranging from seconds to minutes. The results of these (and similar) studies have prompted many investigators to postulate control models for muscle oxygen consumption which involve the mitochondrial enzyme creatine phosphokinase (CPK) (Seraydarian and Artaza, 1976; Saks et al., 1977; Whipp and Mahler, 1980; Bessman and Carpenter, 1985). However, the pattern of change in PCr during non-steady state exercise in humans has not been as well defined.

In an early study, Bergstrom (1967) used muscle biopsies from the quadriceps, to show that a rapid decrease in PCr concentration occurs immediately following the onset of cycling exercise. After this initial decline, PCr concentration remains constant if the intensity of the exercise is moderate. The magnitude of the steady state concentration of PCr is inversely proportional to whole body $\dot{V}O_2$ (Karlsson, 1971) and also to work rate (Hultman et al., 1967). The limited data from these studies would seem to imply first-order kinetics for PCr. Molé et al., (1985) published the first report describing a detailed study of the on-transient response of intracellular high energy phosphates. Although they concluded that PCr metabolism could not be a first-order reaction because of a biphasic change in ATP, both PCr and Pi did appear to change in an exponential manner. They also stated that the rates at which PCr fell and Pi rose to be dissimilar. The validity of this observation is difficult

to assess with any certainty, because the data were not modelled, nor were any time constants determined. In the present study, curve fitting analysis showed that not only were the changes in PCr and Pi exponential, but the rates of the responses were identical. Mean values for the time constants were 33.5 s and 32.7 s for PCr and Pi, respectively (see Table 7). This is about the same as the time constant for $\dot{V}O_2$ measured during bicycling exercise at subthreshold intensities (Whipp et al., 1982; Lamarra et al., 1987).

Other more complex models have been used to assess dynamic changes in gas exchange parameters. For example, Whipp et al., (1982) used a single exponential model with a delay to describe the kinetics of pulmonary $\dot{V}O_2$ from rest to steady state exercise. In this case, they believed the delay was neccessary to account for the transit delay from the site of the increased metabolic activity to the lungs, or the so-called period of "cardiodynamic hyperpnea". More complex models were not used to fit the phosphate data because there was no physiological rational to justify their use.

Curve fitting of the off-transient gave time constants which were the same as the on-reponse. Table 8 shows that the time constant for PCr resynthesis was 33.2 s and 31.1 s for Pi. This similarity is significant because it indicates that the metabolism of PCr demonstrates dynamic linearity. Dynamic linearity means that the time constant is independent of any proir conditions such as, the initial concentration of the metabolites, or the amplitude of the response. This is an essential characteristic of any first order system

and therefore, there can be no differences between the on-kinetics and the off-kinetics if the model is correct (Lamarra, 1990).

Variations in the time constants determined for each individual subject were about 20 to 25%. This is a large degree of uncertainty, but not inconsistent with the variation found in the modelling of cardio-respiratory dynamics (Lamarra et al., 1987). One way to reduce the effect of noise and improve the accuracy of the fit would be to average the results of additional square wave tests (Lamarra, 1990). The number of tests which could be averaged is limited only by practical considerations. Because the analysis of NMR spectra is a very time consuming procedure (at least at this point), the present study was limited to 6 repeats of the square wave test.

In summary, the results of this study have shown that the pattern of change of PCr metabolism in exercising human muscle is exponential. The time course of this change is about 30 s which is the same as the time constant for $\dot{V}O_2$ measured during moderate cycling exercise. The current findings, when combined with existing evidence of coupled changes in muscle PCr and O_2 consumption in animals, make it highly probable that the time course of human skeletal muscle O_2 uptake is monoexponential.

CHAPTER 5

GENERAL SUMMARY AND IMPLICATIONS FOR FURTHER RESEARCH

The primary objective of the thesis was to study the metabolic response of human muscle to dynamic exercise. To achieve this objective, several different "whole body" exercise protocols were adapted for use with ^{31}P NMR spectroscopy, a non-invasive probe of muscle energetics. Spatial and magnetic constraints of the NMR technique limited the type of exercise that could be done and necessitated the use of a small muscle mass model. Nevertheless, these experiments proved successful.

The results of the first study showed that the ramp test could be used effectively, in conjunction with NMR spectroscopy to study exercising human muscle. The ramp test has several distinct advantages over the majority of the experimental protocols appearing in the literature. This is particularly true of those using isokinetic contractions based on a percentage of MVC, which involve a brief period of work followed by a very long rest interval. Determining oxidative capacity under these conditions is somewhat artificial, and the findings of such studies may not be directly relevant to continuous muscular exercise, like running or cycling.

The ramp testing demonstrated the existence of a threshold in intracellular metabolism (IT) during progressive exercise. Identification of the threshold is significant, because it provides an objective biochemical marker

which can be used to assess aerobic metabolism in a number of different situations. Illness, drug therapy and exercise training are some examples of conditions which may be studied. This parameter is quantitative and far more easily reproduced than other less specific criteria which are currently used in NMR studies, such as a change in the slope of the Pi/PCr relationship.

The utility of the IT as a parameter to assess the aerobic power of muscle was shown by the training study described in Chapter #3. Continuous, moderate intensity training increased the IT power of the wrist flexor muscles by 14%, and produced a parallel improvement in submaximal exercise capacity. The magnitude of these enhancements in metabolism and performance were similar to previously observed increases in the ventilation and lactate thresholds following training. So it seems, that the IT is indeed an indicator of aerobic capacity.

Probably of greater importance are the theoretical implications of the IT. The finding of the IT suggests that there may be some validity to the concepts of the ventilation and lactate thresholds, and thus offers many possibilities for further inquiry. A variety of interventions could be used to determine the mechanism responsible for the threshold. In addition, NMR spectroscopy could be combined with other techniques to examine the relationship (if any) between these intracellular and extracelluar phenomena.

In the final study, the dynamic changes in muscle PCr and Pi were monitored during the transition from rest to moderate exercise and during the

recovery from exercise back to resting conditions. Results of this study indicated that muscle metabolism during these transient phases occurred in a simple exponential fashion. Such a relationship had long been assumed by many authors, but, was previously unproven. The time constants calculated for the changes in PCr and Pi during exercise were essentially the same as those determined for O_2 uptake. These findings argue strongly in favour of the hypothesis that the rate of O_2 uptake is controlled by the biochemical utilization of O_2, rather than any limitation due to gas transport. However, O_2 consumption during whole body exercise may not be limited by the same factors as work with small muscle groups. With the recent developments in magnet technology and large bore magnets becoming more commonplace, it should soon be possible to adapt both the ramp and square wave exercise tests described in this thesis for use with larger muscle groups. By combining these exercise protocols with breath by breath measures of pulmonary O_2 uptake, it should be possible to determine the rate limiting step for $\dot{V}O_2$ kinetics and perhaps, the factors controlling mitochondrial respiration.

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