SKELETAL MUSCLE METABOLISM DURING EXERCISE: AN IN VIVO ^{31}P NUCLEAR MAGNETIC RESONANCE STUDY

By

GORDON OMAR MATHESON

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Department of INTERDISCIPLINARY STUDIES

The University of British Columbia
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ABSTRACT

The metabolic and biochemical adaptations which set the endurance limit in skeletal muscle and are modified by physical training, and those which set the fatigue limits in conditions of chronic hypoxia, are not completely understood. Therefore, the purpose of this study was to measure the key metabolites involved in the control of oxidative and glycolytic metabolism, during the elevated metabolic demands of exercise, in subject groups which were separated by distinct differences in their training status or by their exposure to chronic hypobaric hypoxia. Since repeated measures of the key metabolites involved in energy metabolism (PCr, Pi, ATP) and intracellular pH (pH_m) would be exceedingly difficult using the conventional muscle needle biopsy technique, ^{31}P NMR was selected as an appropriate, noninvasive method for measuring these metabolites. Two separate exercise models were developed for use within a 1.0 m bore NMR machine. An electrical stimulation model using the rectus femoris muscle was developed and the factors which influenced reliability and reproducibility of the data were determined. In addition, a dynamic exercise model was developed in which the gastrocnemius muscle was exercised in a mechanical calf ergometer.

The results of the experiments using the electrical stimulation model indicate that RF coil geometry, stimulation intensity and duty cycle, electrode placement, and subject tolerance require very close control for the model to be reliable. It is felt that this model is best suited for experiments which require a within-subject design and is ideally suited for experimental or therapeutic intervention studies.

The calf ergometer was used to compare sedentary lowlanders, marathon and ultramarathon runners, power trained athletes, and Quechua Indians, native to altitudes of 4,200 m in the Andes, before and after deacclimation to sea level. It was found that the Andean natives did not possess a standard physiological phenotype with respect to aerobic and anaerobic capacities. In addition, given the Andean's very low anaerobic capacity and intermediate aerobic capacity, this group performed calf work equivalent to that of highly trained endurance and power athletes. Moreover, pH_m, PCr, Pi, and ATP showed equivalent perturbation at fatigue and in recovery compared with the marathon runners but considerably less perturbation than was found in the power trained athletes who possess equivalent aerobic capacities but far greater anaerobic capacities. NMR derivable estimates of the phosphorylation potential in this study support the theory that closer coupling between ATP supply-ATP demand may be responsible for reduced kinetic and thermodynamic activation of mitochondrial metabolism seen in the Andean natives.

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LIST OF ABBREVIATIONS

AMP	adenosine monophosphate
ADP	adenosine diphosphate
ATP	adenosine triphosphate
BMR	basal metabolic rate
Cr	creatine
EMG	electromyography
FID	free induction decay
IMP	inosine monophosphate
MVC	maximum voluntary contraction
NAD	nicotinamide adenine dinucleotide - oxidized
NADH	nicotinamide adenine dinucleotide - reduced
NMR	nuclear magnetic resonance
PCr	phosphocreatine
Pi	inorganic phosphate
pK	dissociation constant
PME	phosphomonoesters
PP	phosphorylation potential
RER	respiratory exchange ratio
RF	radiofrequency
$\dot{V}O_2$	oxygen consumption
$\dot{V}O_{2max}$	maximal oxygen uptake

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Without the support of my wife Brenda, and the understanding of my three children Lisa, Kimberley, and Andrew, this thesis would not have been possible.

To any student contemplating a doctoral degree, I would pass on the words of Calvin Coolidge: "Nothing in the world can take the place of persistence. Talent will not; nothing is more common than unsuccessful men with great talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence, determination alone are omnipotent."

This thesis is dedicated to Elmer Link.

Introduction to the Thesis

Considerable physiological research is currently directed toward gaining an insight into the factors which control energy metabolism in healthy and diseased skeletal muscle. Many good reasons exist to justify this research, not the least of which is the fact that skeletal muscle is by far the greatest oxygen consuming tissue in the body, and disturbances of O_2 delivery or utilization can significantly impair human locomotory ability. In addition, muscle is adaptable, capable of considerably expanding its metabolic rate in response to the demands of exercise. The factors which control energy metabolism in skeletal muscle in health and disease are therefore at the 'heart' of several current research questions including: the role of O_2 as a limiting substrate during muscular exercise, the mechanism of fiber type transformation resulting from chronic electrical stimulation, the factors which control the endurance range and set the fatigue limits of skeletal muscle, and adaptations which control the sustainable metabolic rate of skeletal muscle in the presence of chronic hypoxia (including the lactate paradox and the $\dot{V}O_{2max}$ paradox).

A great number of these research questions are studied using the classical techniques of invasive sampling in animal models. For a variety of research problems, considerable advantages exist in studying the intact human system where metabolic controls are not only set by the tissue under study but also by the integrated network of physiologic organ responses regulated by the competitive demands for O_2 and other substrates. Invasive sampling in humans is limited because it is painful and perturbs the system

under study, making it extremely difficult to obtain serial measures during elevated metabolic demands. Nuclear magnetic resonance (NMR) spectroscopy is a powerful noninvasive tool capable of measuring many of the key metabolites involved in energy metabolism in skeletal muscle. In addition to being able to monitor metabolites continuously, NMR spectroscopy has the advantage of being a global assay technique with no discomfort or harm to the subject or patient.

Since an essential element in the methodology of experiments designed to look at skeletal muscle metabolism is the ability to stress the muscle through a metabolic range, and, since the magnetic and spatial constraints of the NMR working environment pose considerable technical difficulties in accomplishing this goal, until recently, studies of muscle metabolism in man have chiefly been limited to the exercising forearm. By comparison, the lower extremity musculature is required to meet much larger loads associated with weight-bearing and ambulation and consequently possesses greater metabolic capacity.

Thus, the first general purpose of this thesis was to develop techniques and hardware which would be capable of stressing lower limb skeletal muscle simultaneous with NMR data acquisition in human subjects. In particular, a major goal was to develop exercise protocols which simulated continuous whole body exercise tests, eliminating the need for artificially imposed work/rest ratios. The second general purpose of this thesis was to apply these experimental techniques to the study of skeletal muscle metabolism in subject groups separated by distinct differences in their training status or exposure to chronic hypoxia.

In order to accomplish these general objectives, two approaches were developed for studying skeletal muscle metabolism using ^{31}P NMR spectroscopy - an electrical stimulation model used in the study of the quadriceps muscle, and a dynamic exercise ergometer used in the study of the gastrocnemius muscle. As with any thesis, trial and error comprised a significant portion of the early data collection but these preliminary results are not included in this study.

Electrical Stimulation Model

This model is described more fully in Chapter 2. Briefly, it consists of image localized placement of an RF antenna over the rectus femoris muscle of a supine subject. The graded exercise protocol consists of electrical stimulation of the rectus femoris through a range of stimulation frequencies via surface electrodes. The major advantages of this model include: (i) overcoming the need for a cumbersome exercise apparatus, (ii) removing the potentially confounding variable of volitional effort from the subject's control, and (iii) accurately grading the exercise increments. The most important considerations with respect to reliability and reproducibility using this model are RF coil geometry, stimulation frequency and intensity, and stimulation duty cycle.

For reasons discussed in more detail at the end of Chapter 2, this model was not used in further experiments in the thesis. Instead, a dynamic exercise model was developed.

Dynamic Exercise Model

A calf ergometer was developed to enable a between-group experimental design for the study of muscle metabolism in differing subjects. This model is more fully described in Chapters 3 and 4 but briefly consists of a foot pedal device which allows continuous, graded exercise to fatigue, simultaneously with ^{31}P NMR spectroscopy of the gastrocnemius muscle.

Current NMR exercise studies using the forearm flexor muscles suffer from three limitations. Forearm muscles are not usually required to meet repetitive load demands such as those of normal locomotory exercise and, as a result, incremental exercise protocols for the forearm are somewhat artificial. Secondly, most experiments using forearm muscles have been performed at a given percentage of MVC, usually between 20 and 80%. Chapters 3 and 4 of this thesis show that the maximum sustainable metabolic rate of the gastrocnemius muscle during graded exercise to fatigue is less than 20% MVC, even in highly trained athletes. Thus, the work rates used in the vast majority of forearm studies are far beyond the oxidative range of the muscles being studied. Finally, the manipulation of duty cycle to produce steady state exercise by allowing a long recovery or relaxation interval (typically 1:10 on:off) prevents the mapping of metabolites during normal continuous physiologic exercise. Thus, current methods for noninvasively mapping muscle metabolites have been restricted to the use of the relatively inactive forearm muscle and have used exercise protocols which may not be directly applicable to isotonic muscular exercise.

These limitations were circumvented in the present study by using a lower limb (locomotory) muscle and a protocol of continuous exercise to fatigue.

Subjects

All of the subjects used in this thesis were healthy. However, considerable group differences existed with respect to whole body measures of work capacity. Lowlander subject groups included sedentaries, endurance trained athletes (marathon and ultramarathon runners), and power trained athletes (sprinters, ice hockey players). In addition, a group of indigenous altitude adapted natives from the Andes of Peru (barometric pressure in their native homes approximately 460 Torr) were studied.

In Chapter 2, a homogeneous group of trained subjects is used to develop the electrical stimulation model. In Chapter 3, the dynamic exercise model is used to allow a comparison of the levels of high energy phosphates, adenylates and pH_m values during exercise to fatigue and in recovery in trained and untrained athletes, and in the acclimated Andeans (Quechua Indians). Chapter 4 describes the use of the dynamic exercise calf ergometer to study the effects of sea level deacclimation on muscle bioenergetics in the group of Andean natives.

CHAPTER 1

The Application of ^{31}P NMR Spectroscopy to the Study of Skeletal Muscle Metabolism in Humans: Theoretical Considerations

ABSTRACT

In man, skeletal muscle enjoys a very large metabolic range, consuming more than 95% of the O_2 taken up by the lungs during maximal exercise. Adaptations which expand (e.g. training) or shrink (e.g. muscle disease) this metabolic range are reflected by an absolute up-scaling or down-scaling of skeletal fiber oxidative and glycolytic capacities. Experiments designed to study the factors which control the relationships between oxidative and glycolytic metabolic rates, and the onset of muscular fatigue, have, until recently, depended upon invasive tissue sampling. Over the past decade, technical improvements in ^{31}P nuclear magnetic resonance (NMR) spectroscopy have enabled investigators to obtain noninvasively serial measurements of the key metabolites involved in energy metabolism - adenosine triphosphate (ATP), phosphocreatine (PCr), and inorganic phosphate (Pi) as well as intracellular pH. Moreover, NMR spectroscopy during exercise allows measurement of metabolism during elevated energy demands. As a result, a wealth of new information has become available regarding the thermodynamic and biochemical factors which regulate energy metabolism in skeletal muscle. Until now, these measurements were largely obtained from animal preparations or from the small, non-weight bearing muscles of the forearm. To date, virtually all of the

measurements from the human forearm have been obtained at work rates relative to the maximum voluntary contraction of the muscle being studied rather than relative to the maximal sustainable metabolic rate of the muscle. In addition, these measurements have been obtained during steady state by manipulating the duty cycle so as to allow sufficient time for recovery of the muscle prior to the next contraction. As a result, the exercise protocols do not physiologically simulate the normal pattern of voluntary exercise.

The present review briefly discusses practical and theoretical considerations regarding the application of ^{31}P NMR to human studies and describes the development of two new exercise models to study skeletal muscle metabolism in the weight-bearing lower limb. Both models use continuous, graded protocols to fatigue which simulate normal voluntary exercise and both allow real-time mapping of the bioenergetics of skeletal muscle. The first model uses electrical stimulation to stress the quadriceps (rectus femoris) muscle through a wide metabolic range. The second model uses a calf ergometer to measure isotonic plantar flexion work rate simultaneously with ^{31}P NMR measurement of the gastrocnemius muscle. Both of these models have been validated and subsequently used in experimental situations (Chapters 2, 3 and 4).

INTRODUCTION

Less than five decades ago, physicians were taught that the rudiments of clinical diagnosis were based on inspection, palpation, percussion and auscultation. Since then, technologic developments related to laboratory medicine ushered in an era of diagnostic medicine which was dominated by blood metabolite measurements and invasive tissue sampling. These measurements, coupled with whole body determinations of ventilation, heart rate, oxygen consumption, and lactate production, have been the mainstay of medical research aimed at determining the factors which control work performance in whole body systems. While further advances in molecular biology and metabolic biochemistry revolutionized our understanding of normal and diseased physiologic mechanisms, application in this sphere unfortunately lagged behind theory since classical technologies here are difficult to apply to human whole body systems because of their invasive nature. Yet the data they yield are undoubtedly fundamental to the interpretation of normal physiologic and disease adaptations: understanding subcellular molecular and biochemical events is vital to our explanation of phenomena observed at the whole body level.

Measurement of work capacity and blood and tissue metabolites have greatly advanced our understanding of the factors which regulate energy metabolism and ATP turnover in skeletal muscle and are presently widely used to assess the results of interventional strategies. However, the data from whole body measures may only be able to infer the underlying cellular mechanisms which control glycolysis and oxidative phosphorylation [39]. Alternative experimental approaches are few.

Animal models have limitations in extrapolating the data based on inter-species differences in fiber capillarity and enzyme function, and circumstances unique to the preparation under study [13, 14]. On the other hand, invasive sampling in humans (e.g. muscle biopsy) is destructive, painful, not easily repeatable, and most importantly, limited in its scope simply because it perturbs the system under study.

The ideal solution would be a research tool which would allow the measurement of important intracellular metabolites in vivo simultaneously with other whole body measures of work capacity. Fortunately, the disciplines of physics and engineering have supplied us with such a tool: nuclear magnetic resonance (NMR) spectroscopy and molecular-based imaging. Over the past 20 years, improvements in electronics, data processing, and increases in magnetic field strengths (with the advent of superconducting magnets), have increased NMR sensitivity by several orders of magnitude. NMR can now be used to study tissues noninvasively (through an invisible window into real time biochemistry) even in whole body situations. NMR spectroscopy has the potential to provide medical scientists with the most powerful tool available for measuring energy coupling mechanisms that are at the 'heart' of metabolic adaptations in physiologic and diseased systems. It is suggested that the concept of "user friendly" molecular diagnosis is a critical point of departure for medicine in the future.

The purpose of this introduction is to briefly review how NMR works, summarize its advantages and disadvantages, provide examples of NMR application to the study of skeletal muscle energy metabolism and fatigue, and outline the rationale for the experiments which form the basis of this

thesis. For more detailed reviews, the reader is referred to the excellent monograph by Gadian [25] and other shorter reviews [45, 51].

APPLICATION OF ^{31}P NMR TO HUMAN SYSTEMS

A Brief History

NMR was discovered simultaneously in 1946 by Nobel Prize winners Felix Bloch (Stanford University) and Edward Purcell (Harvard University). Through the efforts of many workers who have helped to refine its application to living systems, NMR has become one of the most significant discoveries in medical physics since the X-ray. In 1974, the first NMR images were published, and in addition, high resolution NMR spectra were obtained from intact rat skeletal muscle [29]. During the next five years, developments included the interfacing of appropriate physiological techniques with NMR, the design and construction of larger magnets, the introduction of new radiofrequency coils [1], and the demonstration of new methods for localizing ^{31}P NMR measurements. For many years, technical constraints limited the size of the high-field magnets required for high resolution NMR spectroscopy. With technologic advances allowing the construction of super-conducting magnets large enough to accommodate a human limb, Chance et al. [9], and Ross et al. [47] were able to use NMR to study intact healthy and diseased human muscle. Eleff et al. [23] were one of the first groups to measure high energy phosphate flux in human muscle during controlled isokinetic exercise. In the past decade, NMR spectroscopy has become a powerful tool for the measurement of energy metabolites within the living cell and NMR imaging (two- or three-

dimensional tissue proton density representations of anatomical structure) is already revolutionizing medical diagnostic imaging.

1.2 How NMR Works

Although the question of how NMR works is beyond the scope of this thesis, it is important to briefly consider the principles of operation. In effect, NMR is a form of analytical spectroscopy that uses non-ionizing, radio-frequency (RF) energy as a physical probe for identifying the molecular structure of chemical compounds within a given sample. Atomic nuclei such as ^{1}H, ^{13}C, or ^{31}P possess an odd number of either proton or neutron particles, each of which possess intrinsic angular momentum. Since the odd particle imparts a net angular momentum or spin to the nucleus, a nuclear magnetic moment is created. These nuclei behave like tiny dipole magnets and when placed in an external magnetic field, tend to align themselves with that field. When short bursts of electromagnetic energy of the appropriate frequencies are applied to these nuclei, they will absorb energy as they make transitions between magnetic field orientation states.

Following the short RF energy pulse delivered to the sample via an NMR probe, the precessing nuclei relax to their original spatial orientation (ground state energy) and in doing so emit a weak RF signal. Since the amount of energy absorbed is directly proportional to the number of resonating nuclei present in the sample, the re-emitted energy is directly proportional to the quantity of the corresponding metabolite in the tissue being scanned. The re-emitted RF energy is detected by the NMR probe and subjected to Fourier transformation, which converts this multi-

frequency time domain signal to a frequency spectrum (Figure 1) with signal amplitude on the vertical axis and frequency on the horizontal axis. The area under each spectral peak represents the quantity of the metabolite present in the sample and can readily be determined using a variety of computer curve fitting programs.

The resonant frequency required to induce the NMR phenomenon is determined by the type of nucleus and the net magnetic field to which it is exposed. The latter consists of the main external field produced by the superconducting magnet, and the local field effects produced by neighbouring electrons and/or other magnetic nuclei within the sample. Not only will different elemental nuclei have different resonance characteristics, but the same type of nucleus in different molecular environments (e.g. three phosphorus nuclei of ATP) will possess different resonant frequencies. This phenomenon allows NMR spectroscopy to measure the quantity of a given metabolite with great accuracy.

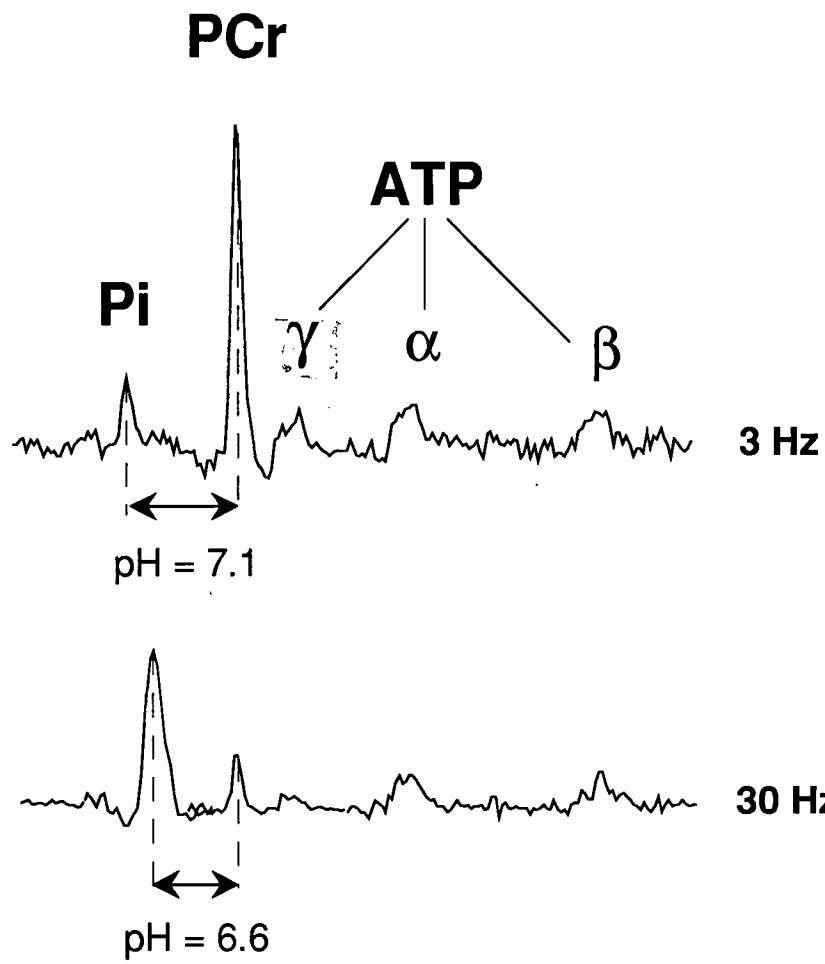
The rate at which emitted signals can be measured depends on the nuclear relaxation time constant, T_1, which in the case of ^{31}P, is approximately two to three seconds [24]. When pulses are delivered faster than three to four times the T_1 (ie. $\leq$ 15 s), nuclear saturation occurs. Most experimental protocols involving limb exercise use shorter pulse intervals (1-5 s) to increase the time resolution of data collection. Since these shorter pulse intervals produce saturation, correction factors are applied to the data. These correction factors are calculated from the difference in metabolite quantities detected when the desired pulse sequence is compared to a non-saturating ($\geq$ 15 s between RF pulses) sequence. The values for the correction factors must be measured with the same NMR transceiver probe

Figure 1

Two typical ^{31}P NMR spectra obtained from the rectus femoris muscle during electrical stimulation. Pi = inorganic phosphate, PCr = phosphocreatine. The upper spectrum is acquired at 3 Hz stimulation frequency and the lower spectrum at 30 Hz stimulation frequency. [Pi] is much higher and [PCr] is much lower at the higher stimulation frequency. pH is calculated by the chemical shift difference between PCr and Pi and in the present example, has dropped from 7.1 at 3 Hz stimulation frequency to 6.6 at 30 Hz stimulation frequency (see text for details).

Details of Spectral Acquisition:

averages = 60, T.R. = 1 sec, samples = 512, sampling frequency = 2,000 Hz, spectral width = 1,000 Hz, t 90° = 100 μs, coil shape is 'racetrack' 20 mm by 80 mm.


PCr
ATP
Pi
γ
α
β
3 Hz
pH = 7.1
30 Hz
pH = 6.6

during a stable baseline interval when metabolite quantities are not changing. Correction values used in the experiments described in the following chapters are Pi/PCr = 1.27, Pi/ATP = 2.86, and ATP/PCr = 0.45.

The use of RF surface coils allows the study of a wide range of skeletal muscles by providing spatial localization of a sample of tissue at the site of the coil placement. The surface coil excites the nuclei in a localized region near the surface of the muscle. Because the ^{31}P signal is weak, signal averaging must be employed with the signal-to-noise ratio improving with $\sqrt{N}$, where N is the number of summed scans. In this thesis, all spectra were acquired with a repetition interval of 1 sec and 60 scans were summed in 1 min bins.

Metabolite Measurements from NMR

In living human muscle, the energy metabolites detectable by NMR in the mM range include ATP, PCr, and the low energy breakdown product inorganic phosphate (Pi). Because the static field homogeneity and therefore the frequency resolution of large sample (human extremity) NMR is currently less than that obtainable with small sample spectroscopy, the cellular phosphate monoesters (PME) in human muscle spectra appear collectively as a single peak. Under most conditions, this almost exclusively represents the phosphorylated glycolytic intermediates (glucose-6-phosphate, fructose-6-phosphate, etc.) but in circumstances of ATP depletion, adenosine monophosphate (AMP) and inosine monophosphate (IMP) may constitute a significant portion of this peak. Compounds normally in the μM range (ADP, AMP) are not present in

sufficient quantity for direct measurement, but their levels can be calculated by using measurable metabolite levels and chemical reaction equilibrium constants [18, 25, 41, 53].

NMR, when practiced in vivo, is designed to detect only those metabolites with rapid molecular motion, i.e. those in solution. For this reason, NMR studies have revealed a higher cell energy state (as measured by PCr/Pi equlibrium ratios) than that determined by classical biochemical assays which necessarily include bound compounds (e.g. myofibrillar ADP) [1, 10, 17, 35]. Likewise, the phosphorus in the crystal lattice of bone does not resolve on ^{31}P NMR spectra because of limited nuclear mobility. Skin and subcutaneous fat do not produce a significant phosphorus signal since the quantity of phosphorylated energy metabolites present in these metabolically inactive tissues is extremely small [56]. For these reasons, ^{31}P NMR is particularly well suited to the study of skeletal muscle metabolism.

The levels of the energy metabolites detected by NMR can be quantified in a variety of ways. In experimental situations where absolute tissue concentration data are required, standardized calibration solutions can be used [17, 18] if the localization technique permits. Alternatively, reference biopsy data (e.g. intracellular ATP concentrations are relatively constant at 8.0 mM [52]) or direct NMR methods [58] can provide accurate calculations of absolute metabolite concentrations. When absolute concentrations are not required, several simple methods of metabolite quantification can be used. If absolute signal strength is held constant, experimentally induced changes in the concentration of a given metabolite over time have been expressed as a percentage of its baseline value [44, 48].

An equally acceptable approach is to express the level of each metabolite as a percentage of the total intracellular phosphate [36, 40, 41], thus quantifying the relative concentrations of each metabolite to the same internal standard.

Many NMR investigators have utilized ratios of the measurable high and low energy phosphate metabolites (e.g. PCr/Pi, PCr/PCr+Pi, Pi/PCr) as indices of the overall bioenergetic state of the cell [9, 10, 17, 53]. Changes in these ratios are (within limits) insensitive to variations in the spectrometer performance [8] and bear a theoretical relationship to the free energy available for muscular contraction from ATP hydrolysis [17].

^{31}P NMR can provide an indirect measurement of glycolysis by measuring intracellular pH. The chemical shift difference between cellular Pi and PCr in a ^{31}P NMR spectrum is pH dependent [43], and this relationship can be used to determine the median cell pH within the tissue volume being scanned. Extracellular Pi is only $\approx 1\%$ of total tissue Pi and therefore not in sufficient quantity to affect these measurements. The accuracy (in terms of absolute pH) of in vivo pH determination by NMR is approximately 0.1 pH unit and relative changes of less than 0.05 pH units are detectable [47].

Inorganic phosphate exists chiefly in two forms that are in rapid exchange at neutral pH: $H_2PO_4^-$ and HPO_4^{-2}. In the absence of chemical exchange between these two species (as occurs in solution), each would give rise to a distinct resonance separated by 2.4 ppm. Since, in solution, the species exchange rapidly, the observed spectrum is a time-averaged single resonance, the frequency of which is proportional to the relative amounts

of the two species. Thus, the frequency of the signal measured as a function of pH produces a typical sigmoid calibration curve which can be used to determine intracellular pH in vivo as a function of the Pi resonance frequency.

For the experiments in this thesis, a standard pH titration curve comparing the chemical shift of Pi with pH was used from previous calibration experiments using solutions of 120mM NaCl, 20mM PCr, 10mM ATP, 5mM Na_2HPO_4, 5mM $MgCl_2$ titrated by addition of small aliquots of 0.1M HCL or 0.1M NaOH (Cynthia Stewart MSc. Thesis, University of Alberta, 1986). The PCr resonance, defined at 0.00 ppm was used as an internal chemical shift reference. An apparent pK for Pi was estimated at 6.80 from the titration curve. The relationship between pH and the measured chemical shift of Pi is described by the following equation:

$$pH = pK - \log (\delta obs - \delta HPO_4^{-2}) / (\delta H_2PO_4^{-} - \delta obs)$$

where δobs is the observed chemical shift of Pi, δHPO_4^{-2} and $\delta H_2PO_4^{-}$ are the limiting chemical shifts at high (pH=10) and low (pH=4) pH (see ref [25]). The values for the acidic and basic endpoints were determined to be δHPO_4^{-2} = 3.19 ppm and $\delta H_2PO_4^{-}$ = 5.72 ppm. Using this technique, it is generally believed that errors due to variations in ionic strength, metal ion binding, temperature shifts, and Pi binding to macromolecules are not greater than 0.1 pH units in absolute terms and 0.05 pH units in relative measurements [25].

In general, spectral resolution of the Pi peak in a ^{31}P NMR spectrum can only be enhanced at the expense of the signal to noise ratio. During

experiments in which the limb is exercising, the signal to noise ratio is often decreased as a result of movement of the RF antenna interrogation volume from the homogeneous center of the spectrometer. Therefore, when the data is processed using line broadening, even though the method of convolution difference [25] is also used, significant peak broadening is usually seen during the latter portion of a graded exercise to fatigue protocol. Another reason for spectral broadening is changes in pH within the skeletal fiber during exercise. For example, the γATP and βATP peaks are moderately sensitive to changes in pH as evidenced by spectral broadening and the disappearance of splitting due to *J* coupling in the γATP peak during exercise requiring glycolytic activation. In contrast, the αATP peak is insensitive to changes in pH and does not broaden significantly with drops in intracellular pH. Similarly, the Pi peak broadens far more than the PCr peak during glycolytic exercise. In certain circumstances, splitting of the Pi signal into two distinct components is observed, implying that the sample contains two environments of differing pH. This has been interpreted as resulting from the measurement of Pi in distinct fiber types (oxidative vs. glycolytic). However, even in the absence of splitting of the Pi peak, broadening may reflect a range of environments within a sample of differing pH values in a muscle of mixed fiber type.

The related techniques of in vivo ^{13}C and ^{1}H NMR are still in the early stages of development with regard to the study of exercising human muscle. Both methods, however, have the potential to monitor glycolytic metabolism through the noninvasive assay of tissue glycogen and lactate, and are likely to assume a significant role in future metabolic studies.

The Advantages of NMR

The ability of low intensity RF energy to penetrate tissue without physically or chemically altering it allows NMR to monitor in vivo cell metabolism in a totally noninvasive manner while exerting no artifactual influence on the biochemical processes observed. Because NMR measurements are noninvasive, they do not perturb the processes being investigated. Serial measurements therefore enable temporal evolutions to be studied in vivo, with precision, and with a temporal resolution of a few minutes. Biopsy techniques require a spatially different sample for each data point. By comparison, as long as the spatial relationship between the transceiver probe and the muscle remains constant, the same tissue can be repeatedly studied by NMR spectroscopy. In addition, NMR is a global assay, providing important information on several energy metabolites and intracellular pH simultaneously.

Biochemical assays necessarily measure bound compounds (such as myofibrillar ADP) as well as those in free, active form in the cytoplasm whereas NMR detects only the latter [9, 42]. Even the best biopsy and assay techniques permit some breakdown of the unstable high energy phosphates during preparation and analysis [17, 41, 42]. The error variance of NMR is, at most, similar to and is often less than that encountered with conventional invasive assays [17, 40, 41].

NMR data acquisition can be gated so that measurements may be made during muscle contraction or relaxation, and muscle bioenergetics can be followed from rest through exercise of all intensities and into recovery. In addition to quantitative measurements of metabolite levels, saturation

transfer and inversion transfer techniques are capable of determining the kinetics of energy-linked reactions such as creatine kinase [26, 30, 46].

With the use of RF surface coils, the volume and spatial localization of the muscle tissue scanned in human exercise studies can be varied by changing the size and location of the surface coil transceiver probe that is held in contact with the extremity and/or altering the regional limits of the homogeneous field area within the center bore of the superconducting magnet. In addition, depth pulse localization techniques, as used in this thesis, provide the measurement of compounds at separate sites within the same sample [50]. The benefit of depth pulse sequencing is that NMR spatial localization is used to ensure that the signal arises from the desired interrogation volume. In the case of the the electrical stimulation experiments in this thesis (Chapter II), depth pulse sequencing was used to limit the area of spectral acquisition to the rectus femoris muscle, excluding contribution to the signal from other surrounding muscles. In the case of the dynamic calf exercise (Chapters III and IV), depth pulse localization was used to center spectral acquisition on the gastrocnemius muscle, thereby avoiding signal contribution from the soleus muscle.

The safety of NMR for human research has been well documented. NMR does not involve any form of ionizing radiation, and extensive studies have not revealed any deleterious effects of the magnetic fields or the RF probes used [16, 22, 54, 57].

The Limitations of NMR

NMR is inherently insensitive, capable of measuring metabolites in the mM range but the measurement of compounds present in μM concentrations

(such as ADP and AMP) is impossible and is likely to remain so. Large bore superconducting magnets are presently expensive to acquire and maintain and good technical support is needed to ensure optimum spectrometer performance. A signal-to-noise ratio high enough for accurate metabolite peak integration can be achieved only when multiple NMR pulse signals are accumulated. As a result, NMR scanning typically offers less time resolution than tissue biopsy and biochemical assay, with 15-30 s as the lower limit for spectral resolution in human exercise studies. NMR provides only volumetrically averaged biochemical data; it cannot noninvasively study individual muscle cells or motor units in a metabolically heterogeneous cell population. Finally, the exercise apparatus designed to stress the muscle through a complete metabolic range must be designed with the significant (both magnetic and physical) constraints of the NMR working environment in mind. As a result, we are presently unaware of any studies which have been performed using human subjects exercising at whole body metabolic rates above 1.0 L $O_2 \cdot kg^{-1} \cdot min^{-1}$ where competitive demands for blood flow and substrate delivery become important determinants of metabolic rate.

^{31}P NMR Measurement of Skeletal Muscle Bioenergetics

Energy metabolism is central to an understanding of metabolic control in healthy and diseased tissues with two elements being necessary in the experimental methodology: (i) experimental designs which stress tissue through a metabolic range, thereby allowing measurement of the type of information that is required and (ii) localization techniques to ensure that the data are acquired from the stressed region under investigation. ^{31}P NMR is particularly useful for the study of tissue energy metabolism

because of the central role played by the high energy phosphates and other phosphorylated metabolites in net cellular energy fluxes:

muscle contraction

$$ATP \rightarrow ADP + Pi$$

creatine kinase reaction

$$ADP + PCr \rightarrow ATP + Cr$$

adenylate kinase reaction

$$ADP + ADP \rightarrow ATP + AMP$$

anaerobic glycolysis

$$Glycogen + 3\ ADP + 3\ Pi \rightarrow PME\ Glycolytic\ Intermediates \rightarrow Glycogen_{n-1} + 3\ ATP + 2\ Lactate$$

oxidative phosphorylation

$$2\ NADH + 2H^{+} + O_2 + 6\ ADP + 6\ Pi \rightarrow 6\ ATP + 2\ NAD^{+} + 2\ H_2O$$

PCr, Pi and ATP levels represent initial values in the contraction/relaxation cycle which permit restitution of resting conditions (state 4) in the mitochondria prior to the next contraction and the continuation of steady state work performance [10]. The capability of the mitochondria to conserve energy can be described by the phosphorylation potential (PP) as [ATP]/[ADP] x [Pi]. Thus, energy expended (measured as work) and the metabolic rate (measured as key substrates in metabolic control, i.e., PP) provide an index of energy flux through the system and the relative energy cost/unit work (energy work transfer function) [11]. The ratio of Pi/PCr reflects the relative metabolic rate of mitochondrial respiration (V/V_m) where V is the observed velocity and V_m is the maximum velocity [10]. Since ADP appears to be a major regulating substrate in skeletal muscle, using the creatine kinase equilibrium reaction [ADP] = [Cr] x

[ATP]/KCrk([PCr] x [H^+]) and a few assumptions (pH = 7.0; Pi , O_2 and NADH are in excess of their respective K_m values), then V/V_m = kPi/PCr. The linear curve predicted from this relationship can also be used to interpret data acquired from non-steady state experiments by taking pH_i into account [10, 11].

It should be pointed out that the above analysis was developed from human data in which the forearm was exercised at various percentages of maximum voluntary contraction (MVC). Since high work rates were often used (20-70% MVC), a duty cycle of 1:9 (work:rest ratio) was used to prevent fatigue and intracellular acidosis. This manipulation of the rest duration allowed the muscle to recover between contractions and the data so obtained is thus not referable to continuous (i.e. normal physiologic) exercise. In the latter, progressive fiber recruitment is the physiological mechanism for increasing work rate (see Chapter II). Consideration should also be given to the fact that any work above 30% MVC requires glycolytic activation since 'aerobic' tests to fatigue using a single muscle group do not achieve work rates greater than 20% MVC (see Chapter III). The fact that the data mentioned above show no change in intracellular pH indicates that the rest duration was long enough for complete recovery between contractions. Finally, the forearm musculature is not normally used in rhythmic exercise unless the subject is athletic or employed in a profession requiring regular contraction of the forearm muscles. Thus, the lower limb musculature is better suited for experiments which use continuous exercise to fatigue since it is adapted for at least the regular physical activity of ambulation.

Using NMR spectroscopy in animal models, investigators have monitored the changes in phosphorus containing metabolites during increasing rates of electrical stimulation and during recovery from muscular exercise, documenting a linear relationship between twitch frequency and Pi/PCr [3, 36]. ^{31}P NMR spectroscopy has also been used to separate fiber types on the basis of function using PCr/Pi ratios [36]. Steady state levels of phosphate metabolites are achieved in a graded fashion with graded exercise intensities and PCr levels have been found to be inversely correlated with [lactate] [19]. Studies of O_2 delivery using ^{31}P NMR spectroscopy have documented positive correlations between O_2 supply and the steady state levels of PCr and PCr/Pi, and inverse correlations with lactate release [31]. Challiss et al. [7] studied blood flow after femoral artery ligation in rats and found a negative correlation between blood flow and PCr and pH_i and a positive correlation with Pi.

Due to the powerful magnetic field and physical constraints imposed by the superconducting magnet, in vivo human studies using NMR during exercise have been limited chiefly to the forearm muscles. These types of experiments show PCr/Pi to be an index of the steady state capability of oxidative phosphorylation in normal and trained muscle [11], and muscle with poor nutritional status [38] or phosphorylase deficiency [47]. At least one study has shown impaired recovery kinetics in the calf muscles of patients with peripheral vascular disease [59].

One of the difficulties encountered in applying magnetic resonance spectroscopy to the study of energy metabolism is that several metabolic pathways exist, each capable of synthesizing ATP for energy production.

The preferred mechanism is to produce energy by oxidizing carbohydrates and fatty acids. However, because these pathways take time to activate, short term energy demands may be met by glycogenolysis or by the breakdown of creatine phosphate. However, it is now known that only oxidative processes are important during the recovery from exercise and the resynthesis of PCr after exercise gives a direct measure of oxidative metabolism [55].

THE METABOLIC RANGE OF SKELETAL MUSCLE

Measurement of oxidative phosphorylation and glycolysis in skeletal muscle at rest bears little relation to the metabolic potential displayed over a wide range of exercise intensities. The limitations imposed on an organism suffering a disease of skeletal muscle, be it extrinsic (muscle blood flow, oxygenation, substrate shortfall) or intrinsic (post-viral syndrome, uncoupling of oxidative phosphorylation, PFK deficiency) are only noticeable when the organism is required to perform a task, i.e., when the metabolic potential of the tissue is exposed. Adaptive strategies in response to disease or environmental stress are accompanied by up or down changes in work capacities of two types: (i) adjustments of the performance range or metabolic scope for activity (defined as the difference between BMR and $\dot{V}O_{2max}$), or (ii) adjustments of the duration (endurance) range or fatigue resistance.

The plasticity of the skeletal muscle metabolic range forms the basis for a research strategy gaining insight from comparisons between normal muscle and disease situations in which the range of skeletal muscle metabolism is adaptively or maladaptively altered. In effect, this research strategy uses

the unique adaptations or adjustments brought on by disease, training, or genetic selection as information-rich biological deviations from the norm; then exercise is used as an immediate experimental parameter to perturb (mildly or maximally) the metabolic system in order to expose and explore its regulatory properties. The two regulatory properties of particular theoretical and clinical significance are those which set the upper end of the performance range and those which set the upper limits to work duration, i.e., which set the point of fatigue or the metabolic duration range.

Performance Range

During intense exercise, skeletal muscle can increase its metabolic rate 100 fold [2]. This increase in metabolic rate is accompanied by increases in cardiac output from 5 $l \cdot min^{-1}$ at rest to as high as 40 $l \cdot min^{-1}$ at maximal exercise; and increases in pulmonary ventilatory rates of from 5 $l \cdot min^{-1}$ at rest to 250 $l \cdot min^{-1}$ at fatigue. Although maximal oxygen consumption measured in the whole organism seldom exceeds 80 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$, skeletal muscle alone is capable of oxygen consumption rates as high as 350 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ [2].

The factors which control the performance range of skeletal muscle are the same factors which control $\dot{V}O_2$. Briefly, two current theories are used to describe the control of $\dot{V}O_2$. One views O_2 as a rate-limiting substrate in the coupling between ATP supply and demand. For example, greater rates of oxidative phosphorylation under conditions of hypoxia may require greater levels of ADP, Pi and NADH with the increases in ADP (and therefore AMP via the adenylate kinase equilibrium) and Pi stimulating glycolysis; and the resulting increase in cytosolic NADH shifting the LDH

equilibrium toward increased lactate production [33]. An alternative hypothesis views $\dot{V}O_2$ as being thermodynamically driven by the phosphorylation potential, i.e., $\dot{V}O_2$ as a linear function of PCr/Cr or ADP x Pi/ADP [12, 13, 15].

Whole body evidence exists to indicate that skeletal muscle is O_2 limited at maximal work rates (impaired performance in conditions which limit O_2 supply to working muscle - induced hypoxia, altitude, and cardiorespiratory disease). Since available methods (including freeze clamped MbO_2 cryomicrospectrometry) lack the spatial resolution necessary to measure the PO_2 in an isolated mitochondrion of a working muscle in an intact organism, no conclusions can be drawn about the condition of individual mitochondria if normoxic and hypoxic populations of mitochondria are measured simultaneously. Potential limiting factors to oxygen supply in the whole organism which may not be considered in isolated preparations include muscle blood flow and fiber recruitment [37], RBC transit time within the pulmonary [20] and muscle [27] capillaries, cardiac output [2] and ventilatory fatigue [6]. Reduced O_2 delivery during exercise in humans is associated with greater levels of lactate at similar workloads [39].

Duration Range

As noted in the following chapters, changes in the endurance range of skeletal muscle may be observed independent of changes in $\dot{V}O_{2max}$. Thus, progressive decrements in muscular performance (as measured by velocity of movement, or inability to maintain an expected submaximal work rate) can occur well before the maximal metabolic capacity of the muscle is

reached. For example, myophosphorylase or phosphofructokinase deficiency leads to early fatigue while hypothyroidy delays the onset of muscular fatigue [21].

Fatigue in finely-tuned healthy systems occurs at the limits of energy supply to energy demand coupling, while in non-healthy systems, the decrements in muscular performance occur at much lower relative work rates despite equivalent substrate and enzyme concentrations at rest. Fatigue results from a number of factors which are synergistic in their actions including substrate depletions (PCr, glycogen, ATP), energetic limitations (due to down regulation by adenylates, redox potential and/or proton levels), end-product accumulations (protons, lactate, inorganic phosphate, ammonia, creatine) and/or ultrastructural muscle damage. Cellular function during augmented flux rates is directed at maintaining homeostasis with regard to the coupling of adenylates to the various ATPases. Any imbalance in this relationship results in an uncoupling of adenylate production and utilization, ultimately resulting in fatigue. Thus, the concept of fatigue as setting the limit of the duration range of skeletal muscle separate from the performance range (controlled by $\dot{V}O_2$) is fundamental to an understanding of the factors which control the adaptations in work capacity which result from disease, training, altitude adaptation, or natural selection.

NMR studies of muscle fatigue [18] show an almost linear relationship between contractile force levels and both the Pi, ADP levels and pH, suggesting that Pi, ADP and/or the hydrogen ion may be directly involved in causing physiologic muscle fatigue. A particularly popular idea is that diprotonated Pi is the main ATPase inhibitor, thus increasing Pi and $[H^+]$

both may correlate with fatigue through $H_2PO_4^-$ inhibition of contraction. However, decreased intracellular pH alone is probably not a major cause of isometric fatigue [41]. The relationship between muscular PCr depletion and fatigue during exercise has been studied following the replacement of PCr with a relatively inert analog (ß-guanidinopropionate phosphate) detectable by NMR. When analog substituted rat muscle is strenuously exercised by electrical stimulation in vivo, the small amount of PCr that is still present is almost immediately hydrolyzed [40, 49] and a precipitous drop in tension development occurs.

Chance and co-workers [10, 34] utilized ^{31}P NMR to investigate the metabolic factors associated with the subjective sensation of fatigue in human subjects during graded, steady state limb exercise protocols. At non-fatiguing muscular work rates that can be comfortably continued for prolonged periods (60 min), a dynamic, metabolic steady state is observed in the exercising muscle. This state is characterized by: (i) an absence of progressive acidosis, (ii) a normal ATP level, and (iii) an elevated mean intracellular Pi/PCr ratio that varies linearly with work rate in this submaximal work range. In vitro studies have shown that the Pi/PCr ratio is directly proportional to the mitochondrial respiration rate at low V/V_{max} [28], presumably because Pi/PCr is proportional to the free ADP level which is considered to be a principal driving stimulus of cellular respiratory activity [32]. As the work rate is increased to a point where Pi/PCr exceeds a critical value [10, 34, 53], a marked transition in the slope of the work rate vs Pi/PCr function occurs with, on average, one sixth the number of work units performed per unit change in Pi/PCr. This transition point, at which mitochondrial respiration begins to approach its

maximum (V_{max}) and subjects begin to complain of local muscle discomfort, and progressive cellular acidosis becomes evident, may represent an intracellular measure of the anaerobic threshold measured by the lactate inflection point.

Anaerobic Threshold

A great number of investigations have been concerned with attempting to explain the anaerobic threshold phenomenon observed during graded exercise to fatigue. The major disagreements concern the interpretation of the nonlinear increase in [lactate] and the work rate at which the ventilation of CO_2 ($\dot{V}CO_2$) becomes nonlinear with respect to the ventilation of O_2 ($\dot{V}O_2$). Despite the lack of a clear understanding of the phenomenon, the anaerobic threshold is a consistently reproducible observation with important clinical application in the prescription of exercise for training and for cardiopulmonary rehabilitation. Current data do not allow a clear delineation as to whether maximum exercise is O_2 limited. Cryomicrospectrophotometric [14] and lactate kinetic [5] measurements would seem to indicate that it is not O_2 limited while whole body measurement of lactate and respiratory gases [4] and steady state exercise studied by NMR [11] would indicate that it is. This question remains unanswered since, as yet, no investigators have simultaneously measured changes in PCr, ATP, and pH in working muscle during conditions of progressive hypoxia at whole body work rates above and below the anaerobic threshold.

EXERCISE AND ENERGY COUPLING

Skeletal muscle metabolism is constrained by a common overriding requirement: rates of energy utilization must be precisely balanced by rates of useable energy production. For practical purposes, this requirement of close energy demand-energy supply coupling means that in any given tissue the rates of ATP utilization must be precisely balanced by the rates of ATP regeneration. If this were not the case, the tissue would quickly deplete its modest endogenous ATP supply. Thus, for any given steady state work rate, ATP catalysis must exactly equal ATP synthesis rates through aerobic and anaerobic processes. Two models for matching ATP supply-demand exist. In tightly coupled systems, flux in both directions could be proportionately increased with no change in substrate concentrations. In loosely coupled systems, flux would be increased at least in part because of change in substrate and product concentrations.

An example of a tightly coupled energy supply-demand system is found in the mammalian heart where work rates can increase three-fold with little or no change in [PCr], [Pi] and [ATP] and no recruitment of glycolytic function. This observation requires that the catalytic behavior of actomyosin ATPase, ATP synthase, and cytochrome oxidase be almost perfectly matched. The mechanism for increasing the catalytic rate in the absence of changes in [ATP], [ADP] or [Pi] is through recruitment (non-allosteric and non-isosteric covalent modification or increased enzyme activity) or allosterically activating the enzyme in step with the increase in work rate. In the case of actomyosin ATPase, Ca^{++} mobilization would represent the main signal for recruitment.

An example of a loosely coupled energy supply-demand system is found in mammalian skeletal muscle. In contrast to the heart, [PCr] and [ATP] concentrations drop at high sustainable work rates while at these same steady states, [ADP] and [Pi] concentrations are elevated. Thus, in the dog gracilis model [15], O_2 uptake rates by the working muscle are not independent of the high energy phosphate metabolites - rather they are linear functions of the phosphorylation potential and the [PCr]/[Cr] ratio. In this situation, actomyosin ATPase becomes increasingly susceptible to product inhibition due to higher concentrations of ADP, Pi and H^+. At increasing work rates, even though more ATPase catalytic capacity is recruited, product concentrations are higher and enzyme function becomes inefficient. Thus, for a given increase in work rate, a proportionately higher ATPase recruitment is required compared with a tightly coupled energy system.

At the level of the mitochondria, the ATP synthases behave as if initially unable to meet myofibrillar demands for ATP. This momentary imbalance between ATP demand and ATP supply leads to altered concentrations of all the high energy phosphates resulting in an elevated phosphate potential or elevated thermodynamic driving force for electron transport chain function. Thus, loosely coupled energy systems utilize an increasing phosphorylation potential as the thermodynamic driving force for oxidative metabolism and ATP synthesis. Since the phosphorylation potential is also a driving force for the reaction:

glucose + ADP + Pi <--------> ATP + lactate

skeletal muscle working at steady state rates primarily by oxidative metabolism may generate lactate under working rates which are never O_2 limited.

In summary, ATP supply and ATP demand can be matched in two ways. Closely coupled systems sustain large changes in the rate of ATP turnover by activation of ATPase and ATP synthase with immeasurable changes in high energy phosphate metabolite concentrations and with minimal glycolytic activation as a result. Loosely coupled systems, on the other hand, can sustain large changes in ATP turnover rate, but in this case, ATP turnover rates (or O_2 consumption rates) vary with the phosphorylation potential or with the [PCr]/[Cr] ratios. The latter are said to be loosely coupled because ATPase flux rates and ATP synthase flux rates must at least momentarily be uncoupled in order to adjust the adenylate and Pi concentrations to new steady state levels. Changes in the phosphorylation potential in loosely coupled systems not only favour accelerated electron transport system function (increased O_2 uptake rates) but also favor increased glycolysis, lactate accumulation, and tissue acidification despite adequate oxygenation.

CONCLUSIONS

^{31}P NMR spectroscopy has proven to be a valuable tool for studying energy metabolism in skeletal muscle and is ideally suited for studies in humans requiring repeated measures over time. Until now, studies in the exercising limbs of human subjects have chiefly been limited to the forearm muscles, setting the stage for the development of new experimental techniques which allow for the study of the larger muscles of

the lower weight bearing limbs. Skeletal muscle is an ideal tissue to study during exercise since it possesses a very wide metabolic range and NMR spectroscopy can provide valuable information regarding the control of metabolism during exercise.

Therefore, the specific objectives of this thesis were:

1. To design and develop experimental methods for stressing lower limb skeletal muscle in vivo simultaneously with ^{31}P NMR data acquisition.

2. To compare the degree of perturbation in the high energy phosphate metabolites, adenylates, and intracellular pH for a given work rate in the exercising skeletal muscles of subject groups separated by distinct differences in their physical training and exposure to chronic hypoxia.

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CHAPTER 2

^{31}P NMR of Electrically Stimulated Rectus Femoris Muscle: An In Vivo Graded Exercise Model

ABSTRACT

This study reports on the development of an in vivo graded exercise model using surface electrical stimulation of human skeletal muscle simultaneously with ^{31}P NMR monitoring. Here, the effects of varying stimulation parameters (duty cycle, stimulation intensity, stimulation frequency) and RF coil geometry were compared using data acquired from the rectus femoris muscle of 10 healthy, fit subjects ($\dot{V}O_{2max}$ = 53 ± 4 ml·kg^{-1}·min^{-1}). With stimulation, the inorganic phosphate to phosphocreatine concentration ratio ([Pi]/[PCr]) and intracellular pH (pH_m) follow curvilinear relationships over the stimulation frequencies from 3-30 Hz with the magnitude of the observed change related closely to stimulation intensity, duty cycle, and RF coil geometry. In this model, oxidative phosphorylation predominates at stimulation frequencies below 12 Hz while anaerobic metabolism predominates above 12 Hz. The results from this study indicate that stimulation parameters and RF coil geometry are important determinants of the reproducibility and interpretability of the data obtained with ^{31}P NMR spectroscopy. These findings are relevant to the use of this model for studying skeletal muscle metabolism through a wide range of exercise intensities.

INTRODUCTION

Electrical stimulation is widely used in clinical settings for restoring function in cases of muscle atrophy resulting from injury [31], denervation [26, 33], or unusual conditions such as zero-gravity [14]. In addition, the fatigue-resistant metabolic adaptations which result from chronic electrical stimulation of skeletal muscle [19, 21] have been studied (in conjunction with the surgical transplantation of latissimus dorsi pedicle grafts [2] or skeletal muscle ventricles [3]) as potential methods of restoring cardiac output in cases of cardiomyopathy. Electrical stimulation has also been used as an experimental method to stress (minimally or maximally) the resting skeletal muscle fiber, allowing comparison of the range of adaptive metabolic responses in normal, trained, and diseased muscle.

The chronic stimulation model has been suggested as an experimental method for studying the plasticity of skeletal muscle [7, 20], while the acute effects of electrical stimulation have been studied as methods for simulating voluntary exercise excluding influences from central innervation [37]. Classically, these types of experiments utilize the needle muscle biopsy technique to document metabolic changes in high energy phosphate metabolites and pH during stimulation periods of up to one hour [6, 23, 25, 39, 40, 41]. The limitations in this experimental approach relate to the frequency with which the muscle can be sampled and the time required for the determination of metabolite levels. By comparison, ^{31}P NMR spectroscopy has been successfully used as a noninvasive serial assay technique to study the temporal relationships between work rate, relative concentrations of key metabolites (Pi, PCr, ATP), and pH_m in exercising

skeletal muscle. For example, Clark et al. [11] used ^{31}P NMR to document a reduced fall in [PCr] in chronically stimulated canine latissimus dorsi muscle during graded electrically paced work rates.

In humans, constraints imposed by the NMR working environment together with the requirement for an exercise apparatus which allows at least a partial range of joint motion, have limited the vast majority of NMR studies of exercising skeletal muscle to the forearm muscles. If a model using electrical stimulation to exercise muscle through a range of intensities were developed, it could potentially overcome some of these difficulties and, with the use of RF surface coils, allow a wide range of muscles to be studied. In humans, Shenton et al. [36] were one of the first groups to combine NMR spectroscopy and electrical stimulation of skeletal muscle, comparing PCr depletion in forearm muscles due to electrically stimulated, voluntary isometric, and isokinetic contractions. More recently Helpern et al. [17] have advocated electrical stimulation as a compliance-independent, well tolerated method for studying skeletal muscle metabolism by NMR spectroscopy. However, the physiology and metabolism of muscle during electrical stimulation differs significantly from that during voluntary dynamic exercise, and the selection of stimulation parameters in vivo is an important determinant of the reproducibility and interpretability of the results.

Since the advantages of continuous noninvasive monitoring of metabolic changes in exercising skeletal muscle are well established, the objective of this study was to design and investigate the use of an in vivo electrical stimulation model which would mimic graded exercise through a range of oxidative and glycolytic work intensities simultaneously with ^{31}P NMR

data acquisition. Optimization of stimulation and data acquisition parameters was deemed essential in this model for several reasons.

Tolerance to surface electrical stimulation varies widely between subjects with the result being that the mass of activated skeletal muscle in 'low-tolerance' subjects may be considerably less than in subjects who tolerate the procedure readily. Thus, the design of the RF antenna must be such that its interrogation volume closely matches the volume of metabolically active tissue in order not to include metabolically inactive tissue in the data acquisition. In addition, since electrical stimulation recruits all types of motor fibers simultaneously, physiological recruitment patterns for graded exercise are not seen and a suitable duty cycle needs to be established to simulate aerobic exercise. Finally, stimulation frequencies need to be selected appropriately to provide a graded exercise protocol.

In this chapter, using stimulation of the rectus femoris muscle in healthy subjects, we studied the effects of duty cycle, stimulation intensity, stimulation frequency and RF coil design on the ^{31}P NMR measurements. Although other investigators have used electrical stimulation in the NMR environment, we are not aware of any reports using this type of systematic development of an electrical stimulation model for use with NMR in humans.

MATERIALS AND METHODS

The procedures used in these experiments were approved by the Committees on Human Experimentation at the Universities of British Columbia and Alberta and informed consent was obtained from the subjects prior to collection of the data.

The rectus femoris muscle was selected for electrical stimulation since it is a superficial, anatomically well defined part of the major muscle mass of the anterior thigh (quadriceps femoris). It contains mixed fiber types [16, 35], and previous needle biopsy studies during stimulation have been reported [6, 9, 22, 23, 24, 25, 37, 39, 40, 41]. Although the rectus femoris muscle comprises only approximately 20% of the knee extensor musculature [1], maximal contractions were assured by selecting subjects who could tolerate stimulation voltages sufficient to develop a knee extensor force of at least 40% of maximal voluntary contraction (MVC) values, as measured by Cybex isokinetic dynamometry. All subjects were familiarized with the electrical stimulation procedures and sufficient exposure to the stimulation was provided to avoid subject apprehension. Considerable inter-subject variability was found in the tolerance to electrical stimulation, and, as a result, 6 potential subjects were excluded from the study due to their inability to tolerate the stimulation. A total of 10 male volunteers were retained as subjects (ages 19-24), all were reasonably fit varsity ice hockey players ($\dot{V}O_{2max}$ = 53 ± 4 ml·kg^{-1}·min^{-1}).

A battery operated, portable, electrical stimulation unit was used (Respond II™, Medtronic Inc.) but was modified by the manufacturer to allow rapid switching between stimulation frequencies in the 3-48 Hz range. The unit is designed as a constant-current source, and so adjusts its output voltage as required to produce an output current proportional to the stimulation intensity selected. For a particular intensity setting, this voltage varied only slightly between subjects since all were fit athletes with little subcutaneous fat. The stimulation unit outputs asymmetric bipolar rectangular pulses with a pulse width of 300 μs. The pulse spacing is

controlled by adjusting the stimulation frequency. Because the average current is quite low, the electrode-tissue interface is assumed to be linear, so that stimulator output current is proportional to output voltage. Fresh batteries were used for each subject and an oscilloscope was used to monitor stimulator output voltage over the duration of the experiment.

Surface electrodes were placed over the rectus femoris muscle in the same position used for the Cybex measurements. One 2 x 6 cm bipolar carbon surface electrode was placed proximally over the rectus femoris motor point while a similar electrode was positioned distally over the muscle and adjusted to produce maximum force output. The skin was cleansed with alcohol, and conducting jelly was applied to the electrodes which were then held in place by tape and kling.

A two-turn, elliptical, 2.5 cm x 5.0 cm RF surface coil positioned over the center of the rectus femoris muscle, was used for excitation (180° tip angle at the center of coil) and acquisition. Depth pulse techniques were used to minimize high flux signals from the surface region in the ^{31}P free induction decay (FID). The coil sampled ≈ 14 cc of tissue in the shape of an ellipsoidal layer 1.5 cm thick directly beneath the coil. The subject was placed supine in the bore of the 1.0 m magnet. At rest, an NMR image was taken to localize placement of the RF surface coil directly over the center of the rectus femoris muscle and to ensure the interrogation volume was within the anatomic borders of the muscle (Figure 2). An alternative circular RF surface coil with a 4.5 cm diameter interrogating a tissue volume of ≈ 30 cc was used to assess the effects of coil geometry in localizing metabolically active skeletal muscle during electrical stimulation. Time-averaged, Fourier transform ^{31}P NMR spectra were obtained from

60 acquisitions (FIDs) using a 1 sec repetition interval both at rest and during electrical stimulation. The spectrometer center frequency was 25.86 MHz and the sampling frequency 2.0 kHz. The raw time domain data were processed using spline baseline correction, two orders of phase correction and a convolution difference procedure with line broadenings of 5 Hz and 15 Hz respectively. The processed spectra were curve fitted using simplex optimization routines to give peak areas (relative concentrations of Pi, PCr and ATP) and chemical shift differences (allowing calculation of intracellular pH). Phosphocreatine (PCr), inorganic phosphate (Pi) and adenosine triphosphate (ATP) resonances were integrated using a computer fitting program and relative concentrations were calculated. Intracellular pH (pH_m) was calculated from the chemical shift difference between PCr and Pi. Representative spectra are shown in Figure 3.

Figure 2

An NMR cross-sectional image of the quadriceps femoris muscle showing the borders of the rectus femoris muscle (arrows) in relation to the RF surface coil (single arrow). A dark shadow is cast on the rectus femoris muscle when the RF coil is pulsed at the same time as the image is taken. VL = vastus lateralis m., VI = vastus intermedius m., VM = vastus medialis m., S = sartorius m.

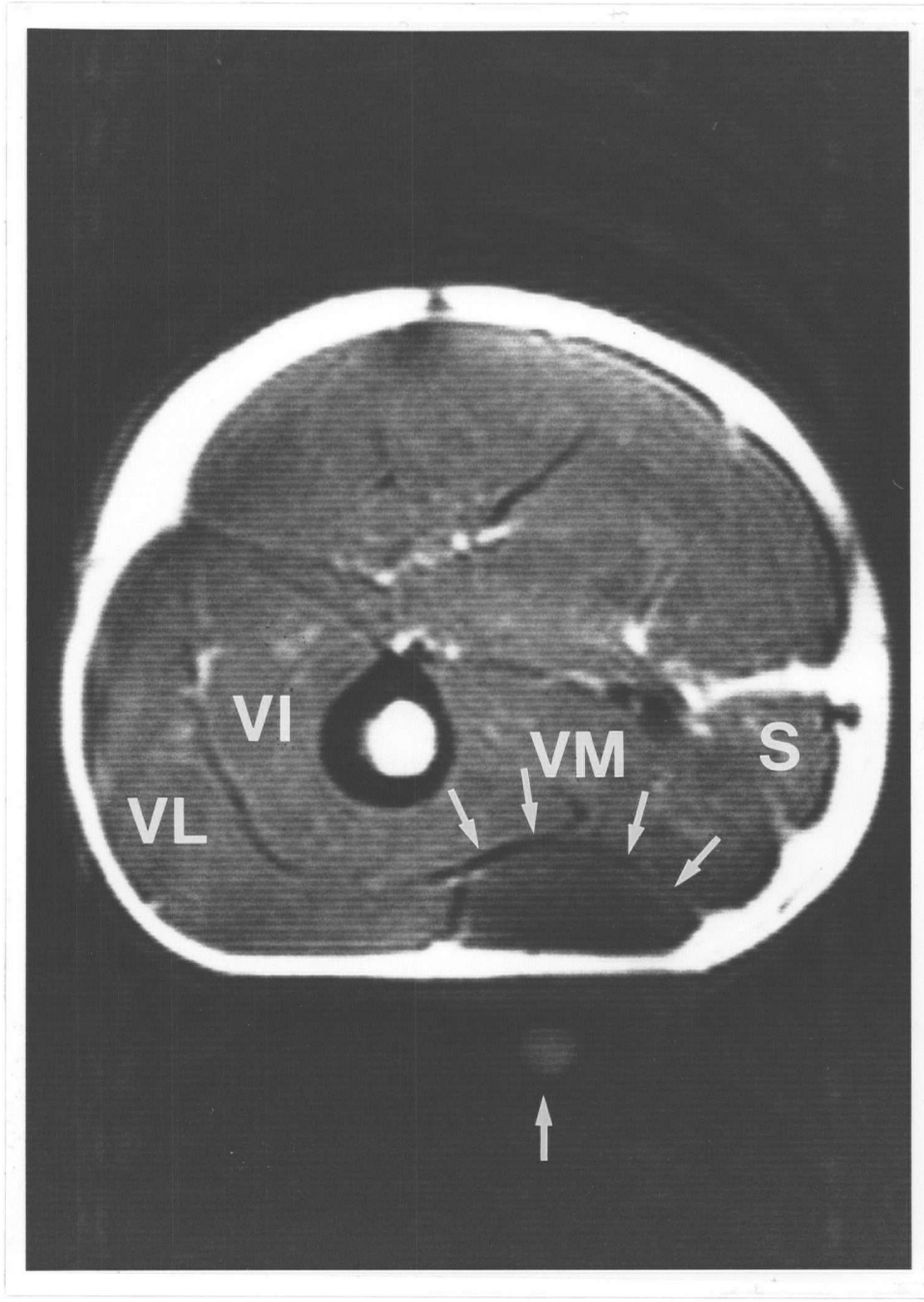
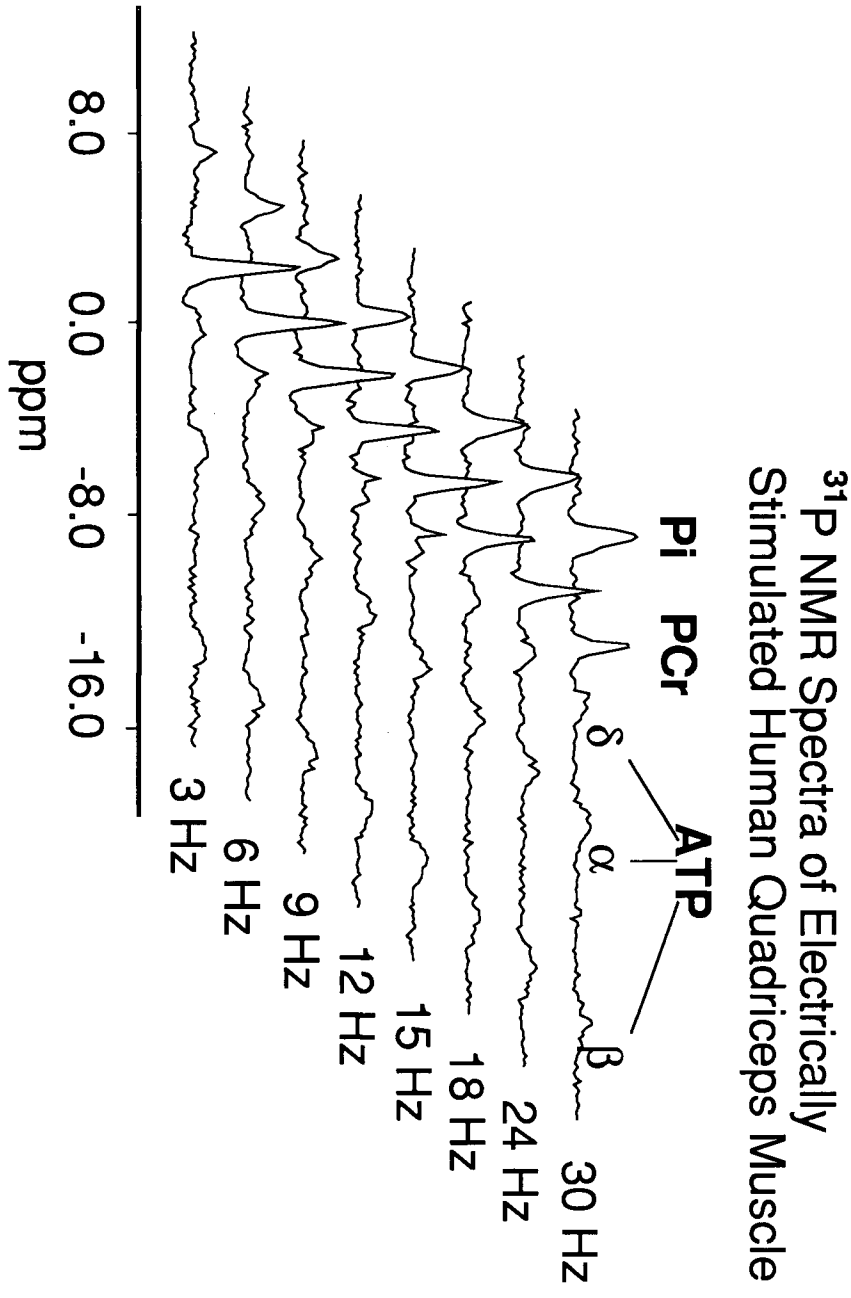

VI
VM
S
VL

Figure 3

A representative series of 60 sec time-averaged ^{31}P spectra acquired from the rectus femoris muscle of a healthy human subject. Stimulation duty cycle was 1 sec on:1 sec off and stimulation duration was 1 min at each frequency.


31P NMR Spectra of Electrically
Stimulated Human Quadriceps Muscle
Pi
PCr
ATP
δ
α
β
30 Hz
24 Hz
18 Hz
15 Hz
12 Hz
9 Hz
6 Hz
3 Hz
8.0
0.0
-8.0
-16.0
ppm

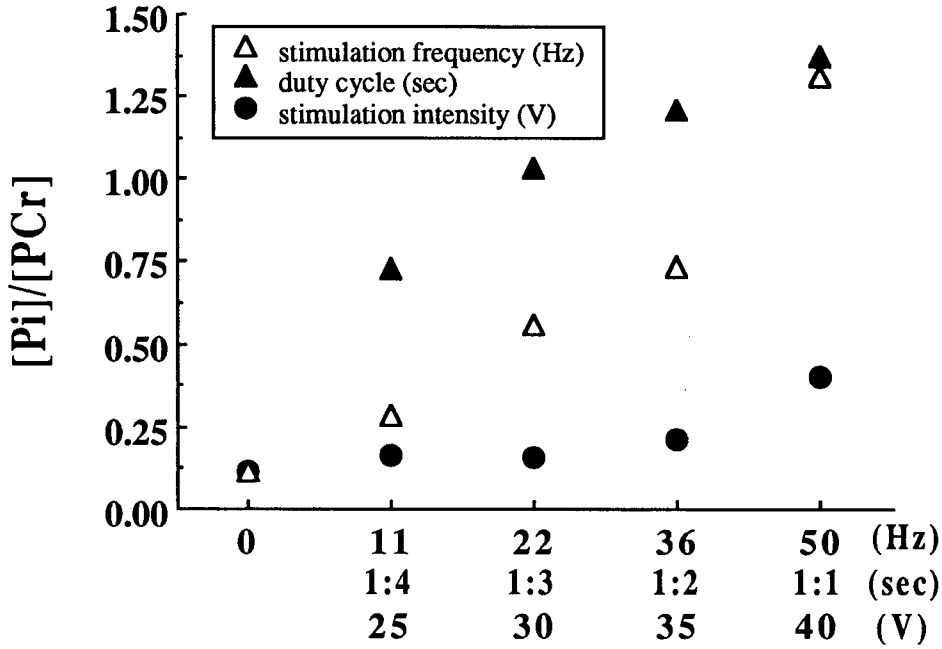
RESULTS

Initial measurements were made of the effects of manipulations on three stimulation parameters which, in theory, could be used to produce step-wise increases in muscular work rate: i) reductions in duty cycle, ii) increases in stimulation frequency, and iii) increases in stimulation intensity (Figure 4). The data from these pilot experiments were used to develop the graded exercise model. When evaluating the duty cycle, gated ^{31}P spectra were found to be insensitive to whether they were acquired during the contraction phase only (30 FIDs), the relaxation phase only (30 FIDs), or both (60 FIDs), over a range of stimulation frequencies between 3-30 Hz, when 1000 ms stimulation trains were applied in a 1:1 duty cycle (30 contractions/min). Gated spectral acquisition when the duty cycle provided longer relaxation phases (e.g. 1:2 or more) showed partial recovery of [Pi], [PCr] and pH_m during the relaxation phase. Continuous stimulation showed an early and rapid fall in pH_m (Figures 5 and 6), a result previously noted in vivo even at low stimulation frequencies [17]. By comparison, 1000 ms stimulus trains in a 1:1 duty cycle produced little net change in pH_m until 12-15 Hz (Figures 7b and 9). Because of these findings, as well as the results of measurements by other investigators (see Discussion section), a duty cycle of 1:1 for 1000 ms stimulation trains was selected.

Using this duty cycle, stimulation frequency was assessed as a parameter for mimicking graded muscular exercise. Increases in stimulation frequency up to approximately 30 Hz produced step-wise changes in [Pi]/[PCr] and pH_m with little further change at frequencies between 30-48 Hz (Figure 6). Using this finding, 30 Hz was selected as the upper limit for

Figure 4

Changes in [Pi]/[PCr] (Figure 4a) and pH_m (Figure 4b) in a single subject resulting from manipulation of the stimulation frequency (Hz), duty cycle, and stimulation intensity. Each data point is the value in the final minute of 4 minutes of stimulation. The muscle was allowed to recover completely prior to the next stimulation. During the manipulation of frequency, the voltage (50 V) and duty cycle (1:1) were held constant. During manipulation of the duty cycle, the voltage (50 V) and frequency (30 Hz) were held constant, while during the manipulation of intensity, frequency (30 Hz) and duty cycle (1:1) were held constant.


[Pi]/[PCr]
1.50
1.25
1.00
0.75
0.50
0.25
0.00
stimulation frequency (Hz)
duty cycle (sec)
stimulation intensity (V)
0
11
1:4
25
22
1:3
30
36
1:2
35
50
1:1
40
(Hz)
(sec)
(V)

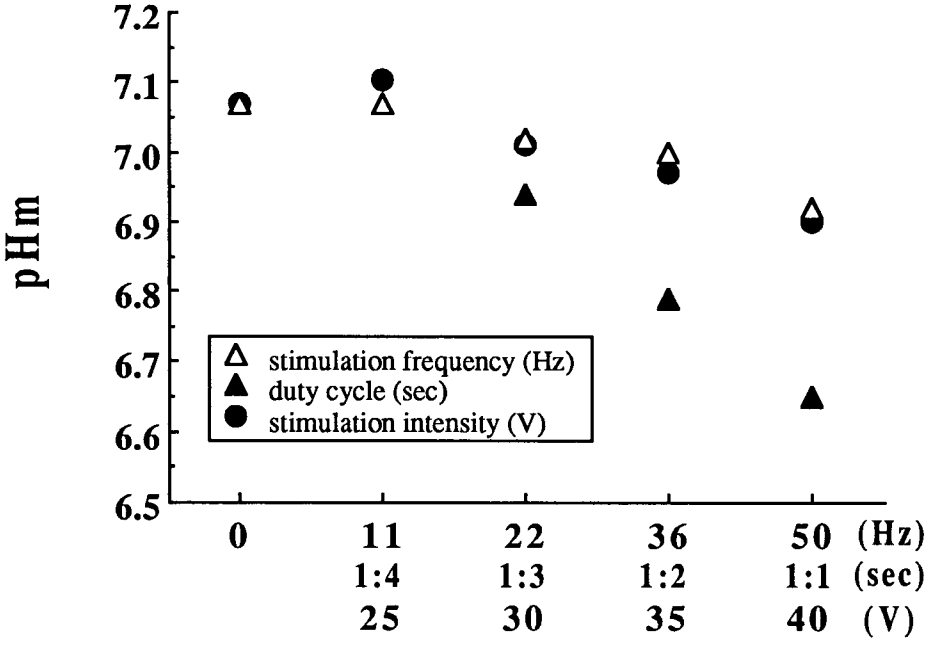

pHm
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
stimulation frequency (Hz)
duty cycle (sec)
stimulation intensity (V)
0
11
1:4
25
22
1:3
30
36
1:2
35
50
1:1
40
(Hz)
(sec)
(V)

Figure 5

Continuous stimulation of the rectus femoris muscle for 15 minutes in 4 subjects. Stimulation frequency was increased every 3 minutes. [Pi]/[PCr] and pH_m values are normalized difference means ± S.E.

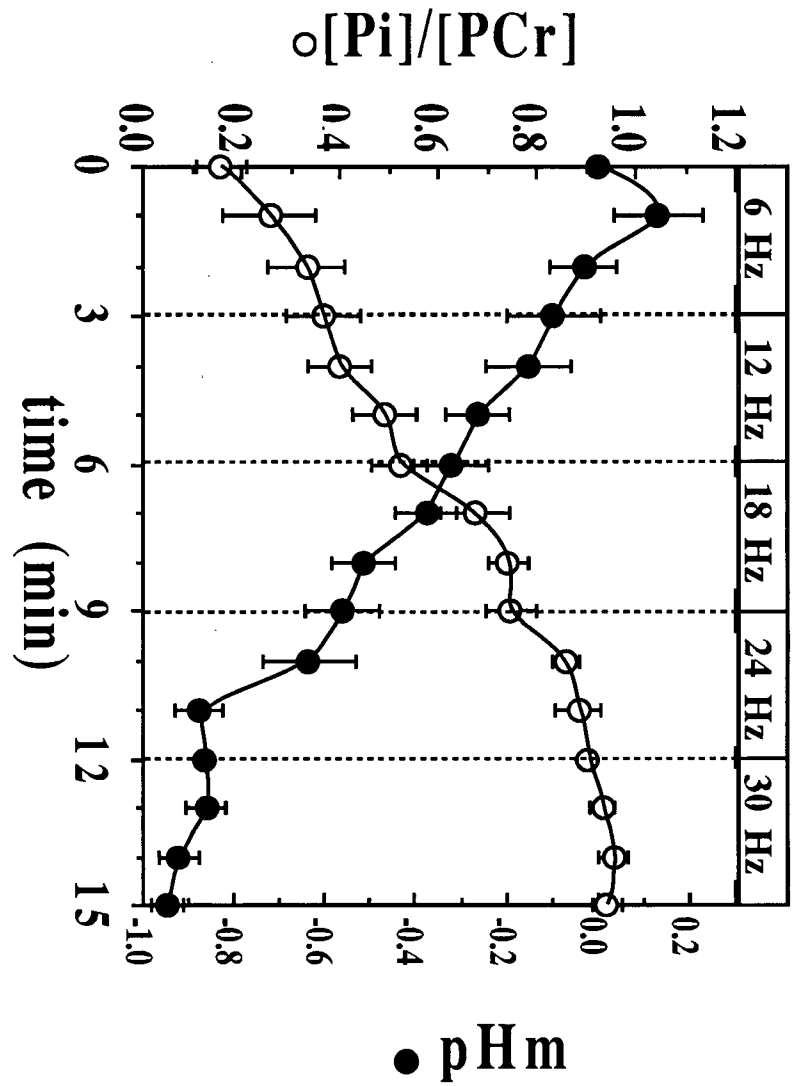
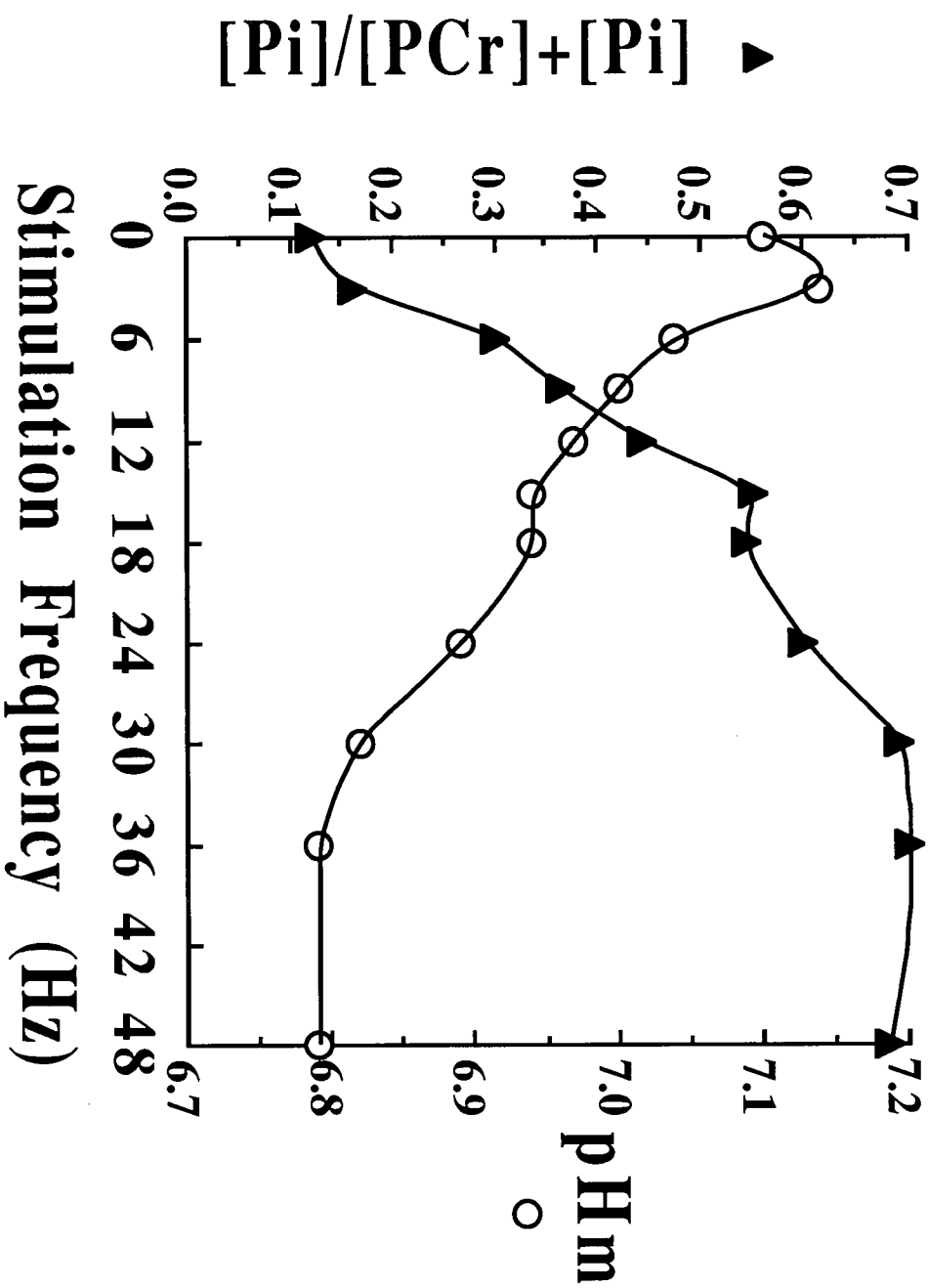

o[Pi]/[PCr]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
3
6
9
12
15
time (min)
6 Hz
12 Hz
18 Hz
24 Hz
30 Hz
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
● pHm

Figure 6

This figure shows the mean [Pi]/[PCr] and pH_m values ± S.E. (n=4) when the rectus femoris muscle was stimulated continuously at frequencies between 4 and 48 Hz. Stimulation was applied for 1 min at each of 10 frequencies.


[Pi]/[PCr]+[Pi] ▲
pHm ○
Stimulation Frequency (Hz)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
6.7
6.8
6.9
7.0
7.1
7.2
0
6
12
18
24
30
36
42
48

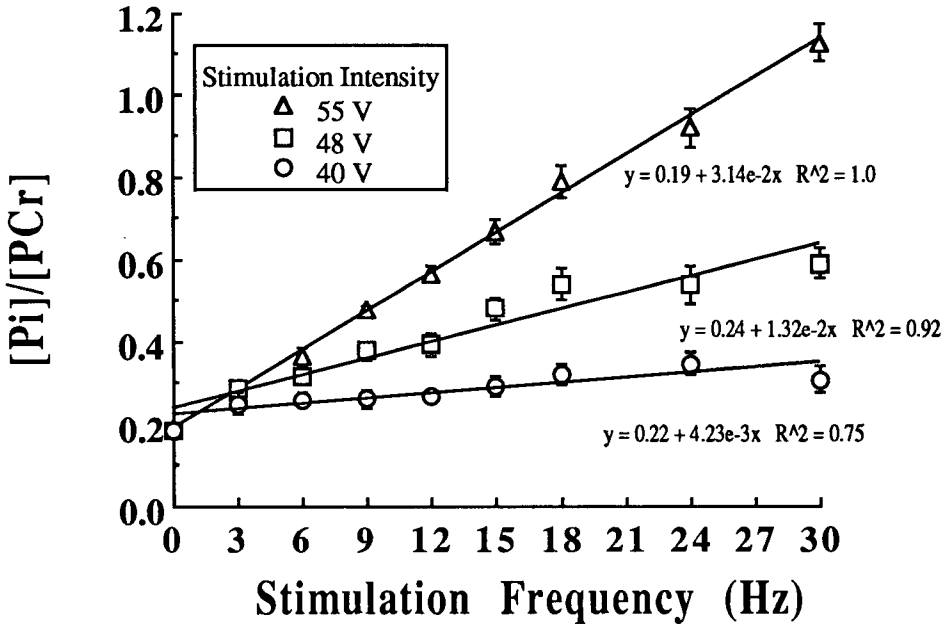
the stimulation frequency in this model.

The effects of varying the stimulation intensity on metabolite concentrations and pH_m values is shown in Figure 7. Using the constant current stimulation device, and assuming no change in tissue impedance during the three tests, [Pi]/[PCr] increased in its sensitivity to stimulation frequency for each increase in stimulation voltage. pH_m remained relatively constant to about 12 Hz at all three stimulation intensities while the values for pH_m at or above 15 Hz showed progressively greater decreases with higher stimulation intensities. Although similar relationships are seen at all three stimulation intensities, the magnitude of the frequency sensitivity of both [Pi]/[PCr] and pH_m correlates well with the voltage applied. The R^2 for a simple curve fit describing the relationship between [Pi]/[PCr] and stimulation frequency increased from 0.75 at 40 V to 1.0 at 55 V (Figure 7a), while R^2 for pH_m over the same stimulation range increased from 0.60 to 0.98 (Figure 7b).

The effects of varying the tissue interrogation volume by changing the dimensions of the RF surface coil are shown in Figure 8. During 8 min of electrical stimulation using a 1:1 duty cycle with 1000 ms stimulation trains at stimulation frequencies between 3-30 Hz, the observed range of values using the smaller interrogation volume RF coil (Figure 8b) is expanded by more than 0.2 pH units and more than 3.0 [Pi]/[PCr] units compared with that of the larger interrogation volume RF coil (Figure 8a). The R^2 value for the equation describing the linear relationship between [Pi]/[PCr] and pH_m improved from 0.87 to 0.98.

Figure 7

Using a 1:1 duty cycle, the rectus femoris muscle was stimulated in 6 subjects at three different intensities. Stimulation was applied for 1 min at each of 8 frequencies with the muscle allowed to recover for 30 min between stimulations. [Pi]/[PCr] (Figure 7a) and pH_m (Figure 7b) values are means ± S.E. The values for 55 V at 0, 3, 6, and 9 Hz are lost in the data points for 40 and 48 V.


[Pi]/[PCr]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Stimulation Intensity
55 V
48 V
40 V
y = 0.19 + 3.14e-2x R^2 = 1.0
y = 0.24 + 1.32e-2x R^2 = 0.92
y = 0.22 + 4.23e-3x R^2 = 0.75
0 3 6 9 12 15 18 21 24 27 30
Stimulation Frequency (Hz)

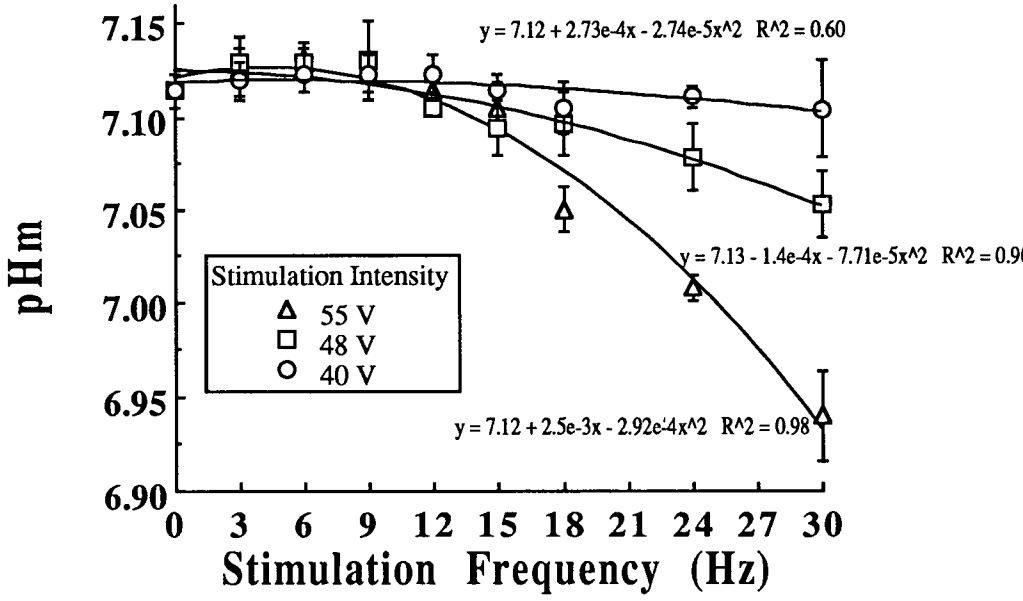
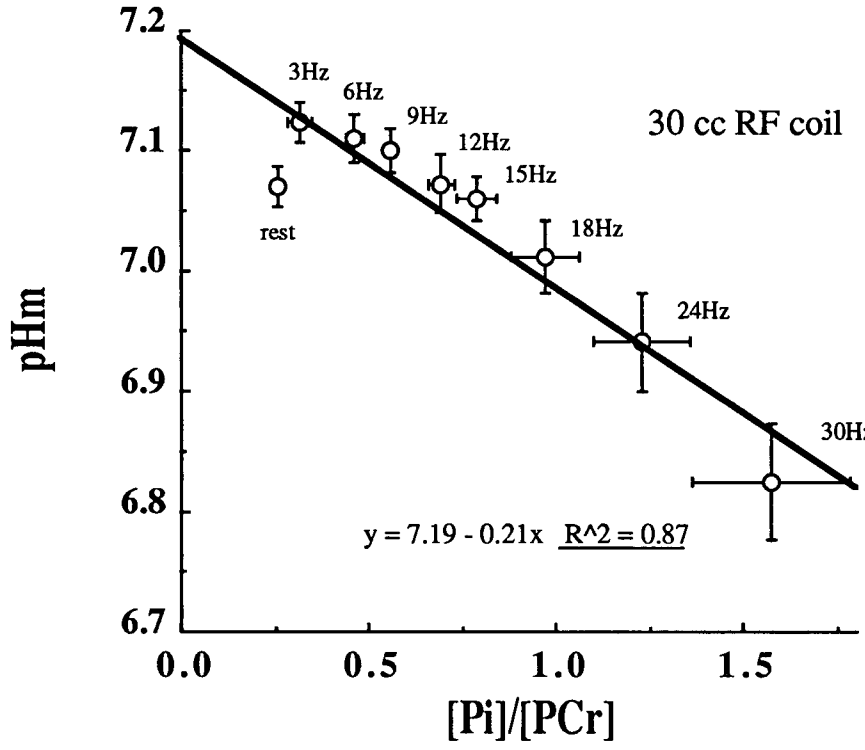

pHm
7.15
7.10
7.05
7.00
6.95
6.90
y = 7.12 + 2.73e-4x - 2.74e-5x^2 R^2 = 0.60
y = 7.13 - 1.4e-4x - 7.71e-5x^2 R^2 = 0.90
y = 7.12 + 2.5e-3x - 2.92e-4x^2 R^2 = 0.98
Stimulation Intensity
55 V
48 V
40 V
0 3 6 9 12 15 18 21 24 27 30
Stimulation Frequency (Hz)

Figure 8

During electrical stimulation, ^{31}P NMR spectra were acquired from the rectus femoris muscle of 8 subjects using two separate RF surface coils: a 4.5 cm (i.d.) coil with a calculated interrogation volume of ≈ 30 cc (Figure 8a) and a 2.5 x 5.0 cm elliptical coil with a calculated interrogation volume of ≈ 14 cc (Figure 8b). Each data point is the mean ± S.E.


7.2
7.1
7.0
6.9
6.8
6.7
pHm
3Hz
6Hz
9Hz
12Hz
15Hz
18Hz
24Hz
30Hz
rest
30 cc RF coil
y = 7.19 - 0.21x R^2 = 0.87
0.0
0.5
1.0
1.5
[Pi]/[PCr]

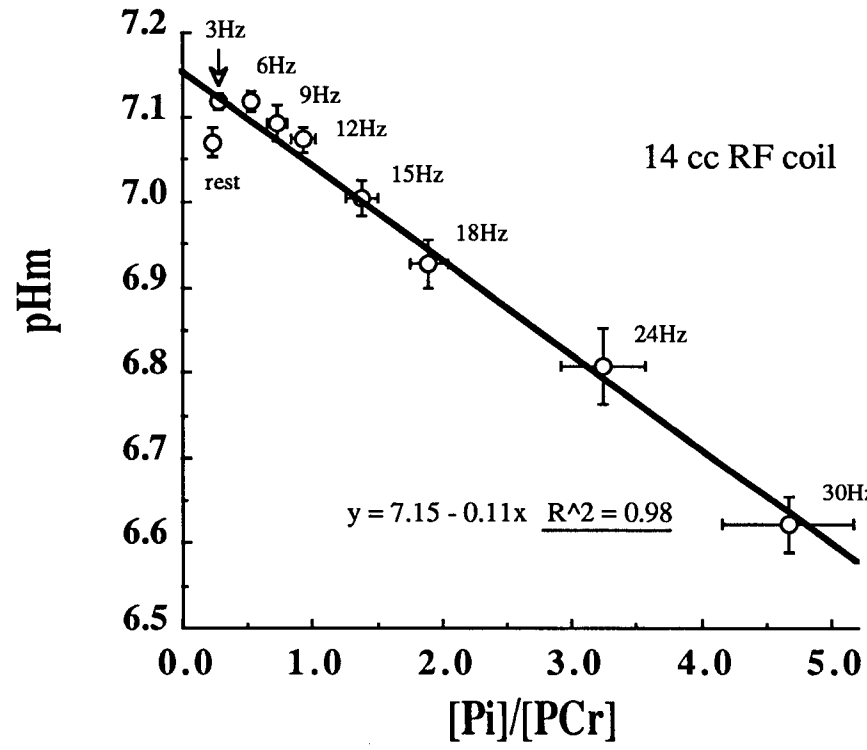
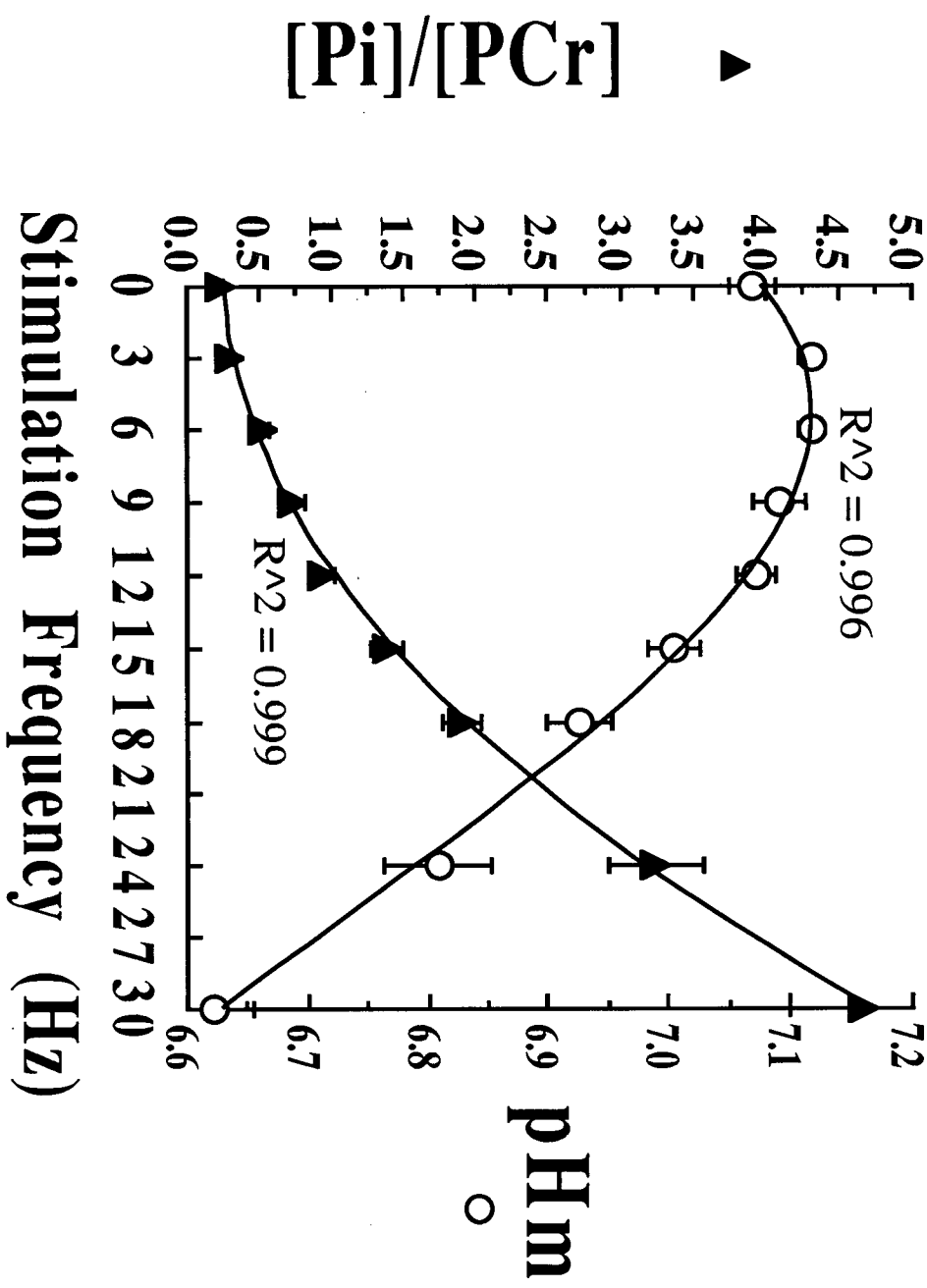

7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
pHm
3Hz
6Hz
9Hz
12Hz
15Hz
18Hz
24Hz
30Hz
rest
14 cc RF coil
y = 7.15 - 0.11x R^2 = 0.98
0.0
1.0
2.0
3.0
4.0
5.0
[Pi]/[PCr]

Figure 9 shows the curvilinear non-steady state relationship between stimulation frequency, [Pi]/[PCr] and pH_m when experimental conditions are optimized; namely, a 1:1 duty cycle for 1000 ms stimulation trains, maximum tolerated stimulation intensity, and a small volume RF surface coil. ß [ATP] decreased an average of 15% from resting values values at the end of 8 min stimulation. The initial skeletal fiber alkalinization reflects PCr hydrolysis (H^+ + PCr + ADP ----> Cr + ATP) with a net drop in pH_m occurring at the 15 Hz stimulation frequency, at which point the [Pi]/[PCr] ratio also begins to change more drastically.

Figure 9

The relationship between stimulation frequency, [Pi]/[PCr], and pH_m when experimental conditions are optimized: duty cycle 1:1, stimulation intensity 60 V, and RF coil interrogation volume 14 cc. Values are mean ± S.E. Equation for pH_m curve is $y = 7.08 + 1.58e^{-2x} - 1.68e^{-3x^2} + 2.17e^{-5x^3}$ and for Pi/PCr is $y = 0.24 + 7.95e^{-3x} - 4.70e^{-3x^2}$.


[Pi]/[PCr] ▲
pHm ○
Stimulation Frequency (Hz)
R^2 = 0.996
R^2 = 0.999
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
6.6
6.7
6.8
6.9
7.0
7.1
7.2
0 3 6 9 12 15 18 21 24 27 30

DISCUSSION

Skeletal muscle energy metabolism during electrical stimulation of the quadriceps femoris muscle in man has previously been studied using the needle biopsy technique [6, 9, 22, 23, 24, 25, 37, 39, 40, 41] whereas ^{31}P NMR investigations of electrical stimulation have been reported on forearm flexors [36] and the tibialis anterior muscle [17]. In the latter two reports, the magnitude of the change in [Pi], [PCr] and pH_m was considerably less than in the present study. Shenton et al. [36] found a 50% drop in [PCr]/[PCr]+[Pi] and an average pH_m of 6.9 following ten 6 sec electrically stimulated isometric contractions of the forearm flexor muscles at 70 Hz. Helpern et al. [17] also reported an approximate 50% drop in [PCr]/[PCr]+[Pi] with average pH_m of 6.9 in measures taken from the tibialis anterior muscle at the end of 5 min of continuous electrical stimulation at 4 Hz. By comparison, in the present rectus femoris study, [PCr]/[PCr]+[Pi] (calculated using the data from Figure 9) decreased by 75% and pH_m dropped to 6.6 after 8 min of intermittent stimulation at frequencies between 3 and 30 Hz. Shenton et al. [36] used shorter stimulation times and higher frequencies than ourselves, while Helpern et al. [17] used longer total stimulation times at lower frequencies. These differences may be responsible for the different changes in metabolite ratios and pH_m values between the studies. As shown in the present study, the selection of stimulation parameters is an important consideration in identifying a reproducible stimulation model for graded exercise.

Duty Cycle

Since stimulation duty cycle comprises two separate components, namely, the contraction duration and the relaxation duration, changes in the ratio of these two components could in theory be used to simulate a graded exercise model (Figure 4). The problem here, is that although modifications in either the contraction or relaxation duration can be made independent of each other, gated acquisition of ^{31}P spectra show greater change in metabolite values using a 1:1 rather than a 1:2 duty cycle with a 1000 ms train - not because the energetics of muscle work are different, but rather because the period of time for recovery is greater in the latter. The interpretation of results from stimulation protocols that manipulate the duty cycle to mimic graded exercise must take into account changes in both work and recovery.

Figures 5 and 6 and data from Helpern et al. [17] show that in the intact human limb, intracellular acidification occurs even at stimulation frequencies as low as 4 Hz. Since the energy cost to the contracting muscle fiber is higher with intermittent contractions [6, 9, 41] (presumably due to increased ATP utilization by actomyosin ATPase during contraction and Ca^{+2}-transport ATPase during relaxation), this result was not expected. In isolated limb preparations, fully aerobic metabolism is seen in the muscle fiber up to stimulation frequencies of approximately 10 Hz [12], but in the present study, the stimulation frequency at which the drop in pH_m is first seen in continuous stimulation is at 4-6 Hz (Figures 5 and 6). Introduction of a duty cycle changes this frequency to 12-15 Hz (Figures 7b and 9). It is thus possible, in the intact limb, that continuous electrical stimulation may

compromise blood flow to the contracting muscle. The mechanism by which this occurs may be related to the sustained reductions in muscle blood flow and oxygen consumption which are found, even at high work rates, to result from simultaneous sympathetic nerve activation during surface electrical stimulation of a mixed nerve [44]. By contrast, constrictor responses to sympathetic nerve stimulation and/or epinephrine are attenuated during voluntary muscle exercise.

Another reason that duty cycle was utilized in the electrical stimulation model of graded exercise concerns the difference in motor fiber recruitment patterns seen in voluntary and electrically stimulated exercise. During voluntary work, recruitment occurs by two primary means, namely, variation of the number of motor units activated (spatial recruitment) and variation of the force generated by a given motor unit (rate coding) by altering the discharge frequency of the innervating alpha-motoneuron (for a review see [4]). Both of these mechanisms can serve to recruit sufficient muscle mass to meet a given steady state work rate at which O_2 consumption, [Pi], [PCr] and [lactate] values are in equilibrium. Since all motoneurons are stimulated simultaneously with supramaximal electrical stimulation [25], the normal physiological mechanism for progressive fiber recruitment is not possible and "steady state" at any given stimulation frequency can only be accomplished by varying the duty cycle.

Finally, a duty cycle of 1:1 with 1000 ms stimulation trains is at least six times longer than is needed to accommodate the prolonged relaxation interval which results from fatiguing contractions [37]. For these reasons, and because [Pi], [PCr] and pH_m values acquired during contraction only

and during relaxation only were no different, a 1:1 duty cycle was selected for the graded exercise model.

Stimulation Frequency

Increases in stimulation frequency are commonly used in isolated animal limb preparations for simulating graded exercise [12, 33, 38]. In the present study of the intact limb, little change is found in pH_m for stimulation frequencies up to approximately 12 Hz but large decreases occurred thereafter (Figures 7b and 9). These data would indicate that aerobic metabolism of the rectus femoris muscle predominates at or below about 12 Hz stimulation frequency and any lactate produced is rapidly oxidized. These findings are in agreement with other data from isolated limb preparations [12, 47] where $\dot{V}O_{2max}$ of the muscle is reached at around 10 Hz.

At stimulation frequencies above 12 Hz, pH_m begins to fall and [Pi]/[PCr] begins to rise more steeply. These findings are attributed to the muscle's inability to sustain ATP synthesis solely through oxidative phosphorylation at the higher stimulation frequencies. Although blood flow compromise cannot be definitively ruled out in this study, a duty cycle of 1:1 allows the muscle fiber to receive normal blood flow 50% of the time and, since the rectus femoris was the only muscle contracting, competitive demands for cardiac output would be negligible.

That stimulation frequencies above 12 Hz lead to a greater rate of glycolytic metabolism is supported by the data of Walker et al. who used an exercising in vivo canine hindlimb model in which the sciatic nerve was stimulated at 4, 8 and 12 Hz [47]. Walker et al. found maximal blood flow

values and O_2 delivery at 12 Hz, although $\dot{V}O_{2max}$ values increased minimally between 8-12 Hz. The largest falls in [PCr], [ATP] and [glycogen] also occurred between 8 and 12 Hz while [lactate] uptake fell from 2.0 $\mu mol \cdot min^{-1} \cdot 100$ g $muscle^{-1}$ at 8 Hz to -0.4 $\mu mol \cdot min^{-1} \cdot 100$ g $muscle^{-1}$ at 12 Hz - ie., net lactate efflux occurred. Thus, the transition to endogenous fuel utilization and net lactate efflux between 8 and 12 Hz stimulation frequencies was not related to blood flow compromise.

The upper limits of the stimulation frequency range for measuring changes in oxidative phosphorylation in the present model is then around 10-12 Hz. This would be in general agreement with Nemeth's results [33] in which denervated soleus muscle of guinea pigs was stimulated at 10 Hz with a 1:1 duty cycle 8-9 hours per day for 4 weeks. This protocol produced near normal restoration of oxidative enzymes including ß-hydroxybutyrate dehydrogenase, cytochrome oxidase, succinate dehydrogenase, and NADH-dependent tetrazolium reductase. In addition, skeletal fiber transformation from fast twitch to slow twitch based on ultrastructural, histochemical and fatigue tests is commonly reported following chronic stimulation at 10 Hz [8, 10].

In studies which have used moderate to high stimulation frequencies, nerve fiber propagation failure could be a simple explanation for the reported findings of metabolic recovery simultaneously with reduced force production during the stimulation. For example, Hultman and Spriet stimulated the quadriceps femoris muscle for 45 min at 20 Hz with a duty cycle of 1:1 using 1600 ms stimulation trains [25], and reported a 30% fall in muscle tension accompanied by large increases in [lactate] and large decreases in [PCr] after 1-2 min. Over the next 45 min of stimulation,

near complete metabolic recovery was found, while tension measurements continued to fall. In practical terms, transmission failure in healthy nerve fibers at 20 Hz stimulation frequency would be extremely uncommon and there are many other potential sites for contractile mechanism fatigue which would give the same pattern of metabolic recovery as reported by Hultman and Spriet [25]. Nevertheless, the amplitude of the compound nerve action potential (CNAP) has been shown to decrease immediately after the onset of continuous stimulation at 20 Hz and drop to 89% of resting values after 30 min [34]. The mechanism is possibly related to functional impairment of the axon membrane Na^+/K^+-activated ATPase resulting in reduced muscle membrane excitability.

For prolonged stimulation periods at or above 20 Hz, the ideal graded exercise model should implement a duty cycle to avoid the possibility of confounding neuromuscular fatigue [15, 30, 32, 46]. The use of a duty cycle at higher stimulation frequencies will help to provide assurance that the sequential measurements of the pH and of the concentrations of Pi, PCr, and ATP are a function of metabolic fatigue.

Stimulation Intensity

In animal studies, electrical stimulation experiments usually utilize isolated limb preparations in which the skeletal muscle is stimulated directly via wire electrodes wrapped around the motor nerve, thereby assuring contraction of all muscle fibers supplied by the stimulated motor nerve. In humans, this approach is not practical and, as in the present study, when using surface electrodes, the current density (determined by subject or patient tolerance) determines the number of fibers which are stimulated. A

rough guide to force production in the limb is provided by independent measurements of the percentage of the MVC that electrical stimulation can produce. However, this does not tell the investigator which portion of the muscle is contracting and at what percentage of maximum capacity.

Figures 7a and 7b show a progressive increase in [Pi]/[PCr] and decrease in pH_m as the stimulation voltage is increased. Two explanations could be used for these data. The electrical current delivered through surface electrodes to the sarcolemma is conducted via motoneurons rather than through transmembranal depolarization [24] and the functional threshold for depolarization of the axon of a motoneuron activated by direct electrical stimulation varies inversely with the axon diameter. At low stimulation intensities, larger diameter fibers in a mixed motor nerve will depolarize before smaller diameter fibers. Thus, the results depicted in Figure 7 could theoretically be explained in terms of the greater recruitment of smaller diameter (slow twitch) motoneurons with higher depolarization thresholds as stimulation intensity is increased.

However, calibration of stimulation intensities at or near the depolarization thresholds reported for fast and slow twitch motoneurons (between 5 and 20 nA [43]) is practically impossible using surface electrode stimulation. For this reason the maximum tolerated stimulation intensities were used in this study. Since axonal depolarization is an all or nothing phenomenon, and since the current required for depolarization in almost all motoneurons does not exceed 20 nA [43], the maximal stimulation intensities used in this study ensure that the current will exceed the depolarization threshold of even the smallest motoneurons and all nerve fibers will depolarize. Thus, a more likely explanation for the findings illustrated in Figure 7 is that

increases in stimulation intensity result in increased numbers of metabolically active muscle fibers within the interrogation volume of the RF surface coil, producing correspondingly greater changes in phosphate metabolite concentrations and in pH_m.

In the present study, the assumption is made that all fiber types in the rectus femoris muscle are stimulated equally. Reference has previously been made to the possibility that electrical muscle stimulation selectively recruits fast-twitch muscle fibers [22, 31, 45], in effect reversing the normal orderly recruitment of muscle fibers according to Henneman's size principle [18]. Experiments which have reported EMG evidence of recruitment order reversal in the cat hindpaw [28] and the human hand [13, 42] generally used low intensity cutaneous stimulation with voluntary exercise to modify afferent impulses to the cerebral cortex or spinal cord in order to produce inhibitory effects on low threshold slow twitch motor units and excitatory effects on high threshold fast twitch units. However, supramaximal direct electrical stimulation evokes the normal EMG pattern of fiber recruitment in less than 200 ms [28].

Finally, current density in the nerve fibers is a function of the stimulation voltage and of the anisotropic tissue impedance. Moreover, for practical purposes, its limiting values are determined by subject tolerance [5]. Preliminary subject testing determined that, even though fit male athletes with low subcutaneous fat were used in this study, 6 were unable to tolerate the stimulation intensity required to produce a 40% MVC. Hultman et al. [24] report that intramuscular electrodes (requiring only 10% of the voltage necessary to elicit the same contraction strength using surface

electrodes) do not overcome the problem of noxious stimulation of afferent sensory nerve fibers.

RF Surface Coil

Selecting a superficial major motor nerve such as the peroneal and measuring evoked EMG potentials [17] is of assistance in localizing the volume of muscle receiving stimulation; however, it does not eliminate the problem of subject tolerance or necessarily ensure a good match between stimulated muscle volume and RF coil interrogation volume. An NMR image was therefore taken to locate exactly the interrogation volume of the RF coil (Figure 2) and this established that the optimization of RF coil geometry was critical. The size and shape of the RF surface coil determine the spatial boundaries of the skeletal muscle interrogation volume, and it is imperative that this coil be designed to maximize the interrogation of metabolically active muscle, while at the same time minimize the interrogation of inactive muscle (Figure 8).

CONCLUSIONS

NMR spectroscopy has established its usefulness for noninvasively monitoring the metabolic changes which occur in skeletal muscle during electrical stimulation. For example, in comparison with Houston et al. [22] who note almost no change in [PCr] and minimal increase in [lactate] in vastus lateralis muscle biopsy samples taken after 60 min of quadriceps stimulation continuously (10 Hz) or intermittently (12 sec on: 48 sec off at 50 Hz), the present study found large changes in [Pi]/[PCr] and pH_m in a shorter period of time. Methodological concerns such as the timing of the biopsy in relation to the contraction, ensuring the sample is from

metabolically active tissue, and rapid excision and immersion of the sample in liquid nitrogen, are all circumvented in a noninvasive NMR study such as that reported here.

The present study attempted to determine those parameters which influence the reliability and interpretability of ^{31}P NMR measurements made during surface electrical stimulation of the rectus femoris muscle. The results show that: i) a duty cycle of 1:1 with a 1000 ms stimulation train should be used to ensure that blood flow limitation is not a confounding variable, ii) stimulation intensity should be maximal, iii) the optimal range of stimulation frequencies for studying aerobic metabolism is below 12 Hz, and iv) RF surface coil design should take into consideration the shape and volume of metabolically active muscle in the stimulation current field.

With careful optimization of stimulation parameters and RF surface coil dimensions and the addition of continuous monitoring of muscle force production (Dunlop et al. in preparation), the relationships between [Pi]/[PCr], pH_m and stimulation frequency shown in Figures 8b and 9 could be used reliably for within-subject designs to compare intervention or treatment effects in relation to energy metabolism and fatigue.

Electrical stimulation has been advocated as a standardized method for assessing contractile behavior and metabolic properties of human skeletal muscle while excluding influences from central innervation [37] and from compliance dependent effort and cooperation [17]. Indeed, the purpose of this investigation was to develop the model sufficiently to allow its use in the study of muscle metabolism in differing subject groups. However, for a number of important reasons, this model was not deemed appropriate for

the between-subject design experiments required in the remainder of this thesis. One important set of points is that surface electrical stimulation does not mimic the physiologic fiber recruitment patterns seen with dynamic exercise, it is associated with lower rates of blood flow than voluntary exercise, and simultaneous autonomic stimulation in mixed motor nerves may impair blood flow at high work rates. A second point is that the muscle force response to stimulation is dependent on surface electrode area and location over the muscle [29] and varies considerably between different individual subjects. Moreover, muscle tension per unit stimulation differs widely between subjects and does not necessarily correlate with the EMG signal [29], thus making a calibration of force production resulting from any given stimulation essential (Dunlop et al. in preparation).

However, even if EMG and other techniques are used to measure force production in all of the muscle groups receiving stimulation, the above considerations still bear consideration. The difficulties associated with subject tolerance, reliably duplicating electrode positioning, and reliably ensuring RF antenna placement; and the questions related to introduction of a duty cycle to simulate graded exercise, led this investigator to conclude that a dynamic, volitional exercise protocol may be more suitable for between-subject experimental designs.

^{31}P NMR spectroscopy of dynamically exercising large muscles is reproducible [27], and, with the development of non-ferromagnetic exercise ergometers, reliable methods of ensuring standardized work rates are available. For this reason, the remainder of this thesis is devoted to the development and use of dynamic calf exercise in four subject groups:

sedentary, aerobically trained (marathon and ultramarathon runners), power trained (sprinters), and acclimated and deacclimated Andean highlanders (indigenous Quechua natives). Muscle metabolism in these four subject groups has been extensively studied and for this reason they were chosen to compare the magnitude of perturbation in high energy phosphates and adenylates for a given work rate. In addition, the Andean highlanders were part of a deacclimation study (from 4,000 m to sea level) designed to expose changes in phosphorus metabolites and intracellular pH after a six week sojourn to sea level. As will be shown in later chapters, the NMR technical developments allowed comparison of skeletal muscle metabolism in these four subject groups without the concerns presented by the electrical stimulation model.

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CHAPTER 3

Skeletal Muscle Metabolism and Work Capacity: A ^{31}P NMR Study of Andean Natives and Lowlanders

ABSTRACT

Two metabolic features of altitude adapted humans are the $\dot{V}O_{2max}$ paradox (improved work rates following acclimatization in the absence of increases in $\dot{V}O_{2max}$) and the lactate paradox (progressive reductions in muscle and blood lactate with exercise at increasing altitude). To assess underlying mechanisms, a study was undertaken of Andean Quechua Indians at their home in La Raya, Peru (4,200 m) and at low altitude (below 700 m) immediately upon arrival in Canada. The experimental strategy compared whole body performance tests (aerobic capacity, anaerobic capacity, strength) and single (calf) muscle work capacities in the Andean natives (n=6) to three groups of lowlanders (n=6 in each group) separated by distinct differences in their training status: i) sedentary, ii) power trained, and iii) endurance trained. ^{31}P NMR spectroscopy was used to monitor non-invasively changes in energy metabolism and pH_m in the gastrocnemius muscle of all subjects exercising to fatigue. In addition, calf muscle work rates plus [PCr], [Pi], [ATP], [PCr]/[PCr] + [Cr], [Pi]/[PCr] + [Cr] and pH_m values were compared in conditions of normoxia and hypoxia (FIO_2 = 0.15). The results indicate that, compared with lowlanders: i) the Andeans appear to be a unique phenotypic group with respect to whole body measures of anaerobic and aerobic work capacity, ii)

despite significantly lower anaerobic capacities, the Andeans are capable of calf muscle work rates equal to highly trained power and endurance athletes, and iii) compared with endurance trained athletes with significantly higher $\dot{V}O_{2max}$ values and power trained athletes with similar $\dot{V}O_{2max}$ values, for a given work rate, the Andeans display, respectively, similar and reduced perturbation of all parameters related to the phosphorylation potential and to measurements of [Pi], [PCr], [ATP] and pH_m derivable from NMR. In the light of the fact that the lactate paradox may be explained on the basis of tighter coupling of ATP demand-supply (based on the findings of greater work capacities with less adenylate and phosphate metabolite changes) a similar mechanism may be used to explain: i) the high calf muscle work capacities in the Andeans relative to measures of whole body work capacity, ii) the $\dot{V}O_{2max}$ paradox, and iii) anecdotal reports of exceptional work capacities in indigenous altitude natives.

INTRODUCTION

It is well known that training induced adaptations are capable of expanding the metabolic range and hence work capacity of skeletal muscle [22]. In these cases, measurement of anaerobic and aerobic capacities show significant increases compared with the pre-trained state. What is not as well understood is man's ability to sustain heavy work in the face of significant hypoxia, under conditions which serve to reduce the metabolic scope for exercise. The purpose of this chapter is to compare skeletal muscle energy metabolism in a group of altitude adapted natives with three groups of lowlanders separated by differences in their training status.

There are two metabolic characteristics of high altitude adapted humans which set the stage for the present study: the $\dot{V}O_{2max}$ paradox and the lactate paradox. The former arises from the observation that man's capacity to perform sustained muscular work improves as a result of endurance training at sea level, as well as after a period of acclimatization to moderate altitude. The exercise performance increments in endurance training are associated with increases in $\dot{V}O_{2max}$ capacities [22] while those seen in altitude adaptation [25, 29] occur with little or no change in VO_{2max} capacities [12, 38, 41, 43]. In altitude adaptation, even if the overall metabolic scope for activity is compressed by altitude ($\dot{V}O_{2max}$ drops $\approx$ 11%/1,000 m altitude [14]), acclimatory adjustments provide significant (between 40-60%) improvements in cycling [29] and treadmill [25] time to exhaustion. Improved muscle work capacity with no change in $\dot{V}O_{2max}$ capacity (the $\dot{V}O_{2max}$ paradox) has not been appreciated nor properly explained by earlier studies in this field.

To this point, the efforts to identify sites of adaptive adjustments in Andean natives have relied upon whole organism exercise performance (especially $\dot{V}O_{2max}$) under normoxic and hypoxic conditions [21, 32]. In whole body aerobic exercise tests, the maximum sustainable power output requires a maximum metabolic power input of about 30 μmol ATP/g muscle/min. In contrast, maximum power output during exercise protocols involving smaller muscle masses in man can reach values some 3-4 times higher. For example, metabolic rates of 350 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ have been reported for the human quadriceps muscle [1]. Since the goal in this study was to assess or expose altitude related adaptations at the peripheral (muscle metabolic) level in Andean natives, the experimental strategy focussed on the functional properties of a single muscle (the gastrocnemius) in an aerobic exercise protocol to fatigue.

Generally, it is believed that the peripheral metabolic adaptations (increased capillary and mitochondrial volume densities plus elevated activities of oxidative enzymes) which accompany improvements in work capacity, allow greater overall O_2 fluxes/gm muscle even if individual mitochondria in muscle may operate at reduced O_2 utilization rates [22]. These types of adaptations are found in the skeletal muscle of endurance trained athletes where increases in [ADP] and [Pi] (and concomitant decreases in [PCr] and [ATP]) at any given work rate are less than in the untrained state [22]. If these types of adaptations were to underlie the improved work capacities of altitude adapted individuals, then similar morphometric and enzyme activity changes would be expected in muscles of altitude acclimatized lowlanders or of indigenous highlanders. The problem is that this is not observed.

In contrast, it is known that the cross sectional fibre areas, mitochondrial and capillary volume densities, and oxidative and glycolytic enzyme activities i) of acclimatized lowlanders returning from altitude, ii) of elite mountaineers who have scaled 8,500 m peaks without supplementary oxygen, iii) of lowlanders decompressed to simulated altitude, and iv) of Himalayan Sherpas, either are unchanged from sedentary lowlander values or are actually somewhat reduced [7, 15, 16, 24, 26, 31, 44].

If the 'classical' molecular adaptations of endurance athletes (which increase $\dot{V}O_{2max}$ capacities), are not part of skeletal fiber adaptation to altitude hypoxia, how are the findings of Maher [29] and Horstman [25] as well as anecdotal reports of notably improved work capacities of indigenous highlanders [28, 39] explained? One possibility may lie in adaptations which improve the efficiency of energy flux at the level of working muscle. While there may be several biochemical strategies for up scaling energetic efficiency, the largest advantages arise from maximizing the work achievable/ATP utilized [19]. In muscles designed this way, it is expected that large changes in work rate would be accompanied by smaller than usual changes in the concentrations of the adenylates, [PCr], [Pi] and pH_m [19]; i.e., the muscles would display relatively tight ATP demand-ATP supply coupling. In most skeletal muscles, a change in phosphorylation potential correlates with change in $\dot{V}O_2$ because the former drives the latter [10]; this situation is referred to as a loosely coupled energy demand-energy supply system, simply because the concentrations of ATP and other key metabolites (including PCr, Pi, H^+, and lactate) change as the rate of ATP cycling changes [19]. Possible mechanisms which characterize tightly versus loosely coupled energy

demand-energy supply muscles are discussed elsewhere [2, 19]. For the purposes of this study, suffice to say that muscles maximizing work/ATP utilized (muscles maximizing energetic efficiencies) would behave as if more tightly energy demand-energy supply coupled than usual and may account for the $\dot{V}O_{2max}$ paradox.

If this were the case, reductions in the magnitude of Δ[ADP], Δ[Pi], Δ[PCr], and $\Delta[H^+]$ (i.e., reductions in the degree of change in the phosphorylation potential or all regulatory parameters related to it during exercise at altitude) could also serve to explain the second major metabolic characteristic of altitude adapted individuals, the so-called lactate paradox. First described over 50 years ago [9, 13] and more recently analyzed by several authors [6, 16, 18, 40], the lactate paradox refers to the effects of altitude acclimatization on muscle lactate formation [16] and on blood lactate accumulation [9, 13, 38, 40] during incremental $\dot{V}O_{2max}$ tests: the higher the altitude of acclimatization, the lower the lactate formation rates and the lower the lactate concentrations observed. It is termed a paradox because acute hypoxia in unacclimatized individuals serves to increase muscle and blood lactate levels at any given work load [5, 30] and because at first glance one would expect the Pasteur effect to come into play (glycolysis increasingly activated with progressive hypobaric hypoxia to meet the ATP shortfall due to tissue hypoxemia). Thus, just as the concept of a more tightly coupled energy demand-energy supply regulation would help to explain the $\dot{V}O_{2max}$ paradox above, it could also explain the lactate paradox in muscles of altitude adapted individuals on the basis of less adenylate perturbation and consequently less stimulation of glycolysis.

In order to experimentally test these concepts, 6 high altitude adapted Quechua Indians who had lived all of their lives at moderate altitude (about 4,000 m) were selected as subjects. The research strategy was to compare whole body and single limb calf muscle exercise capacities of the indigenous highlanders with three other groups of lowlanders separated by distinct differences in their physical conditioning: i) completely sedentary, ii) power trained athletes, and iii) endurance trained athletes. Whole-body comparisons of strength, aerobic and anaerobic capacity were performed using standard human performance measurement techniques. In order to obtain measurements which would shed light on the nature of the energetic adaptations which occur in exercising skeletal muscle, ^{31}P NMR spectroscopy was used since this type of data collection enabled repeated measures of key muscle metabolites to be obtained more frequently than could be realistically or ethically obtained by needle biopsy. This technique allowed us to monitor relative concentrations of ATP, Pi, and PCr, plus pH_m during rest, exercise to fatigue, and recovery in the gastrocnemius muscle.

The results of this study show that acclimatized Andean natives are able to sustain single limb work rates equal to those of elite endurance and power trained athletes with equal or reduced perturbation in high energy phosphate metabolites and Pi.

MATERIALS AND METHODS

Subjects

Four separate groups of volunteers were tested; one group of altitude adapted natives (AHL) and three groups (n=6 in each group) of lowlanders distinguished by their level of training: sedentary (SED), power trained (PWR) and endurance trained (AER). The highlanders (n=6) were Quechua Andean natives, lifetime residents of the La Raya region of Peru (3700-4500 m) and workers at the La Raya veterinary research station (4200 m). These subjects were relatively active in their home environment but did not train regularly or intensively. The 18 Caucasian lowlander subjects were lifetime residents below 700 m and none had previously lived at altitudes exceeding their current residence. The sedentary group (n=6) had occupations which involved sitting at a desk and did not actively train outside of their work place. The power trained group (n=6) were athletes in sports which required strength but not aerobic fitness (e.g. sprinting) and who engaged in regular weight training. The endurance trained group (n=6) were marathoners and ultramarathoners, all record holders in 26 mile, 50 mile and 100 mile road races and all capable of marathon race times below 2 hr 30 min.

Performance Measurements

Each subject received an incremental cycle ergometer test to fatigue to measure $\dot{V}O_{2max}$, a modified Wingate test to measure anaerobic power, and a maximal isokinetic torque test of plantar flexion of the foot to measure maximal voluntary contraction (MVC) of the calf muscle. The Andean natives were tested within 48 hours of arrival at sea level. Each of the

lowlander subjects received the same battery of whole body performance measurements. For the measurement of maximal aerobic capacity, after a four minute warm up, subjects cycled at 50 rev/min with resistance set at 1.0 kg. While continuously sampling expired gases through a mouthpiece, the resistance was increased by 1.0 kg every two minutes until the respiratory exchange ratio (RER) $\approx$ 1.0 and then by 0.5 kg each minute until fatigue. Anaerobic power was measured using a modified cycle ergometer Wingate test [35] in which resistance was set at 0.095 kg per kg body weight. Using a photoelectric cell to count pedal revolutions, work output (W) was calculated for each 5 sec period during the 30 sec test. Measurements of maximum calf strength were made at 60°/sec using a Cybex II isokinetic dynamometer fitted with a foot pedal. Care was taken to ensure that positioning of the subject for this test was identical to that for the NMR test described below, i.e., the position of the knee and ankle, the angular velocity and axis of rotation were similar in the two tests.

NMR Exercise Protocol

The 6 Quechua were tested within 48 hrs of leaving altitude while the lowlanders were tested at their convenience. The right calf muscle (dominant limb as determined by psychometric testing [20]) was exercised to fatigue aerobically in a specially fabricated non-ferromagnetic ergometer. The ergometer bed consisted of a sheet of aluminum 2 m in length machined to conform to the shape of the NMR patient bed and secured in place by the weight of the subject, rubberized backing, and 2 straps running from each end of the NMR machine. On one end of the ergometer a foot pedal was mounted which allowed plantar flexion of the ankle from neutral (0°) to +15°. The right foot was strapped into the foot

pedal device so that the axis of rotation of the foot pedal occurred in the same plane as the anatomic axis of plantar flexion of the ankle. One end of a nylon rope was attached to the base of the foot pedal and the other passed through a wall-mounted pulley system to a plastic container into which weight was incrementally added. Eccentric muscle work was eliminated since the weight attached to the base of the foot pedal served to return it immediately to the neutral position during relaxation.

The exercise protocol was designed as a graduated test to fatigue. Duty cycle was fixed at 1:1 by the subjects depressing the foot pedal for one second and relaxing for one second (30 contractions per minute). Cadence was maintained by the use of an audible beep and a visible light triggered by the pulse program of the data acquisition system. Each minute, 2 kg of weight was added while an electronic device (with a photosensitive diode light constructed to assure full depression of the pedal with each contraction) monitored the exercise. Initial work rate was 42.3 J/min (0.71 W) and this was incremented by 23.5 J/min (0.39 W). Absolute work, work rates and integrated (total) work were calculated directly from the mass being lifted.

Since absolute whole body anaerobic and aerobic power measurements are standardly expressed relative to body mass, relative calf work rates were also calculated as a ratio of calf work (J) to body mass (J/kg). In addition, calf work rates at fatigue were expressed relative to the maximum voluntary contraction of the calf muscle (% MVC).

Fatigue was taken as the inability to maintain rhythm or displacement despite verbal encouragement. With the Caucasian lowlanders,

determining the point of fatigue was straightforward. With the Spanish speaking highlanders, an interpreter facilitated communication throughout the test.

Hypoxia

Each subject performed the NMR test twice - once breathing room air and once breathing an hypoxic gas mixture to simulate the hypobaric conditions in the subjects' native environment ($FIO_2 = 14.5 \pm 0.5\%$). The subjects received one hour of rest between the two tests and the order of the tests was systematically varied to avoid a confounding effect from condition order. A gas cylinder of the hypoxic mixture was connected to a mouthpiece which the subject breathed through during the NMR experiments. Expired gases from the mouthpiece were delivered to a CO_2 sensor (P-61B) and CO_2 analyzer (CD-3A) by a flow control pump (R-1, Applied Electrochemistry AMETEK), allowing continuous monitoring of expired CO_2. This measurement allowed the titration of additional CO_2 from a separate gas cylinder so as to minimize hypocapnea and alkalosis induced by breathing the hypoxic mixture. Subjects were allowed to breathe the reduced O_2 mixture for 20 minutes prior to starting the exercise and, after this adjustment period, in most cases the addition of CO_2 to the inspired gas was negligible. An in line O_2 sensor continuously monitored the concentration of O_2 delivered to the subject and % HbO_2 saturation (range 90-92%) was monitored from the index finger using a pulsed oximeter (N-100 Nellcor). These experiments were conducted in Edmonton, Alberta (barometric pressure 704 Torr).

NMR Data Acquisition

^{31}P spectra were acquired at 1.5 T from the gastrocnemius muscle of the right leg of each subject using a Philips Gyroscan NMR machine. The subject was positioned supine with the belly of the right calf resting centrally on a 4 cm diameter surface coil antennae, the knee comfortably positioned in 30° flexion using foam supports, and both the upper and lower legs firmly secured with velcro strapping.

The spectral volume of ≈ 20 ml centered 2 cm into the calf, was localized using a simple depth pulse sequence [3] that included θ/5 and θ/3 high flux signal suppression to remove signals from the surface region. The interrogation volume was estimated roughly be subtracting two hemispherical volumes, one whose radius corresponded ω the axial displacement 45° tip angle contour and the other whose radius corresponded ω that of the 135° tip angle. Typical resting spectra had a signal to noise ratio of 8:1 for ß-ATP and displayed a clear resolution of the *J* coupling within the three ATP peaks. Phase cycling and signal averaging were accommodated within 60 averages of the free induction decays (FIDs) using a repetition interval of 1 sec. The raw time domain data were processed using Fourier transformation, spline baseline correction, two orders of phase correction and a convolution difference procedure with line broadenings of 5 Hz and 15 Hz respectively. The processed spectra were curve fitted using simplex optimization routines to give peak areas (relative concentrations of Pi, PCr and ATP) and chemical shift differences (allowing calculation of intracellular pH). The curve fitting routines employed could use either Lorentzian, Gaussian or a

mixture of the two for the chosen lineshapes. The lineshape could be chosen independently for each peak, and the choice made minimized the subtraction error between the data and the fitted spectrum. The initial guess for each resonance was made by the operator prior to simplex optimization. The relative peak areas were subsequently corrected for relaxation time differences. Data so acquired permitted the dynamic changes in energy metabolism to be monitored with a 1 min time resolution and with excellent reproducibility (Figure 10).

^{31}P spectra were acquired over 1 min intervals during rest, during exercise to fatigue, and for 20 minutes in recovery. Each FID acquisition was made in intervals between muscle contractions, while the limb was stationary.

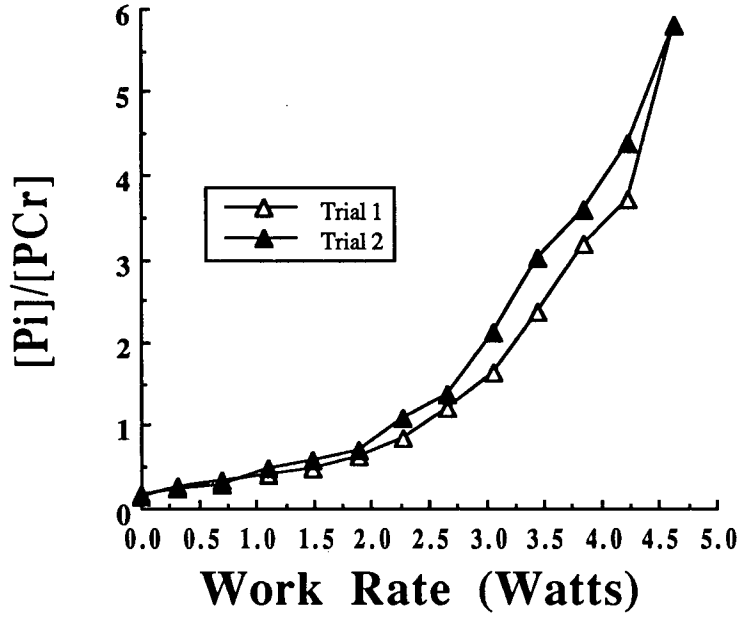
Data Analysis

Comparisons of the four subject groups were made using simple descriptive statistics and analysis of variance (ANOVA). Demographic measures, whole body measures of work capacity, and calf muscle work capacities were compared using a one-way ANOVA design (4 groups). NMR measures were contrasted using a mixed model design - 4 (group) X 7 (time) ANOVA. Alpha was set *a priori* at $p \leq 0.05$ and post hoc Bonferroni testing was used to identify group differences. Post hoc analyses were hypothesis driven with the number of comparisons multiplied by the α per comparison equal to or less than 0.05.

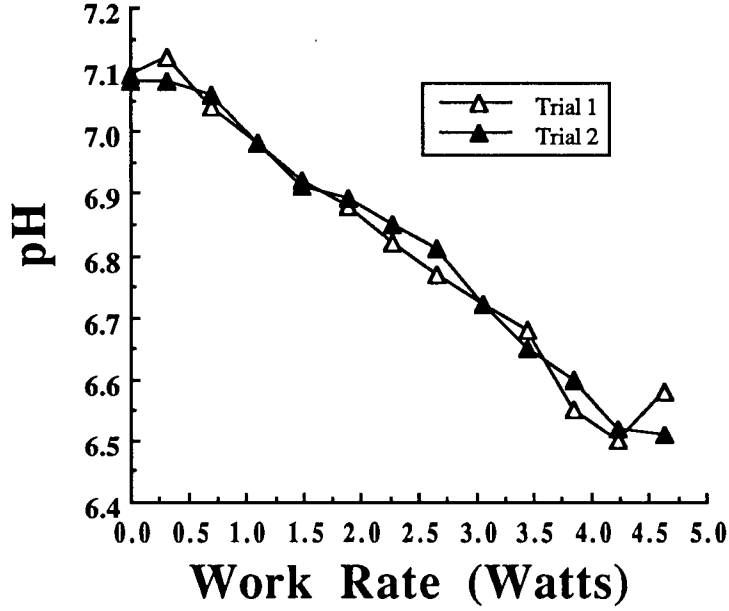
Figure 10

[Pi]/[PCr] (a) and pH_m (b) in the gastrocnemius muscle of a single subject during exercise to fatigue repeated after a one hour rest. Work rate was increased by 0.35 W each minute and total time to fatigue was 12 min.

Reproducibility of [Pi]/[PCr] Measures in Gastrocnemius Muscle During Exercise



Reproducibility of pHm Measurement in Gastrocnemius Muscle During Exercise



RESULTS

Post-hoc results of one-way ANOVA group comparisons of work capacity and results of two-way mixed model ANOVA (group X time) NMR comparisons are presented in Table 3. Significant main effects for time were ignored, main effects for condition (normoxia versus hypoxia) were not significant ($p \geq 0.05$), and there were no significant trends in the interactions to warrant further discussion.

Demographics and Whole-Body Exercise Capacity

Table 1 lists the demographic data and results of whole body work capacity tests on the four subject groups. Single factor univariate ANOVA results an post-hoc Bonferroni results for each of the variables are presented in Table 3. In general, the Andeans and the aerobic group were slightly older, shorter, and had lower body mass compared with the sedentary and power trained groups.

Significant differences were found in $\dot{V}O_{2max}$ between the 4 groups (Tables 1 and 3). The sedentary group had the lowest aerobic capacity, the Andeans and power group were intermediate, and the endurance trained group had the highest aerobic capacity. Although the mammalian "scaling" effect dictates that $\dot{V}O_2$ scales with an exponent less than one (larger individuals display less aerobic capacity per unit muscle mass than smaller individuals), this correction factor was not applied in the present study since all subject groups were from the same species.

Significant between-group differences were also found in the absolute (W) and relative (W/kg) anaerobic capacity tests over the full 30 sec period of

the test (Tables 1 and 3) and in the power output in the first and last 5 sec interval of the test (Figure 11). The Andeans produced the least amount of absolute anaerobic power - about 60% of the sedentary and aerobic values and only 40% of the power trained values. The absolute power output by the aerobic group was the same as the sedentary group. When relative anaerobic power outputs were calculated (W/kg) the Andeans still had the lowest values - 15% lower than the sedentary group (not statistically significant) and 30-35% lower than the aerobic and anaerobic groups respectively (Tables 1 and 3).

The percent drop in anaerobic power output (Δ anaerobic capacity calculated as a percent ratio of work output in the first 5 sec divided by work output in the last 5 sec) is shown in Table 1 and Figure 11. Significant group differences were found with the sedentary and power groups having the largest drop and the aerobic and Quechua groups having the smallest drop.

Isokinetic calf maximum voluntary contraction values are shown in Table 2. Post-hoc testing showed the power group to be significantly stronger than the aerobic group (Table 3).

Calf Muscle Work Capacity in Normoxia and Hypoxia

Table 2 shows calf muscle work capacities in the four subject groups in conditions of normoxia and hypoxia. Single factor univariate ANOVA results for each of the variables and post-hoc test results are presented in Table 3.

Table 1

Demographic measures, maximum heart rate (HR), respiratory exchange ratio at fatigue (RER), maximum aerobic capacity (graded cycle ergometer exercise) and maximum anaerobic power (modified Wingate) in the four subject groups. Δ anaerobic power is calculated as power output in the last 5 sec of the Wingate test divided by power output in the first 5 sec and expressed as a percentage. Values are means ± S.E. The results of the Bonferroni post hoc tests are summarized in Table 3.

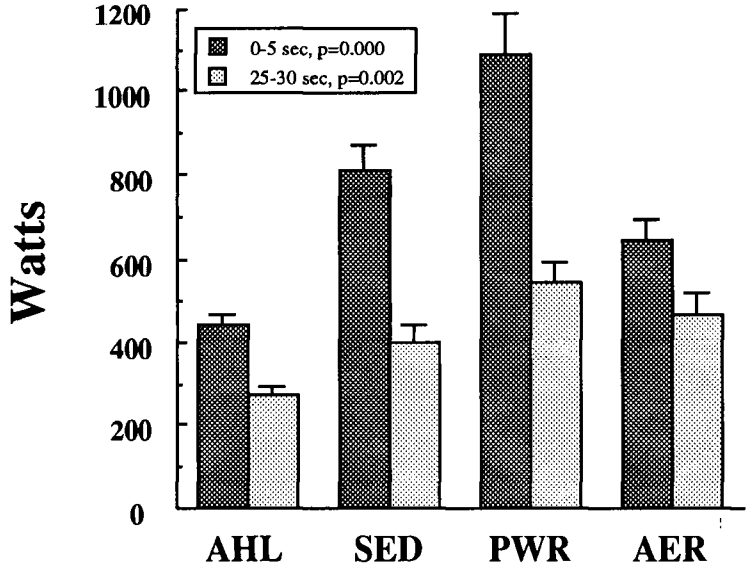
Table 1 - Measurements of Whole Body Work Capacity

	AHL	SED	PWR	AER
age (yr)	34.0±1.1	27.0±1.2	24.3±1.3	29.5±0.4
height (cm)	159.5±2.1	181.6±3.1	181.2±2.8	176.3±2.0
weight (kg)	60.5±1.6	94.3±7.5	89.8±3.6	63.9±2.2
HR_{max}	182.7±3.5	182.3±5.1	188.0±2.5	171.0±4.2
$RER_{fatigue}$	1.25±.04	1.09±.04	1.17±.06	1.11±.03
$\dot{V}O_{2max}$ (l)	3.05±.18	3.32±.34	4.07±.30	4.05±.08
$\dot{V}O_{2max}$ (ml/kg/min)	50.3±2.0	35.4±2.9	45.3±2.9	63.3±1.3
Absolute Anaerobic Capacity (W)	341.2±17.5	598.9±44.7	796.7±70.4	539.3±47.0
Relative Anaerobic Capacity (W/kg)	5.65±.28	6.47±.54	8.79±.48	8.22±.59
Δ Anaerobic Capacity	62.8±1.9	49.2±2.4	50.2±1.6	71.3±4.6

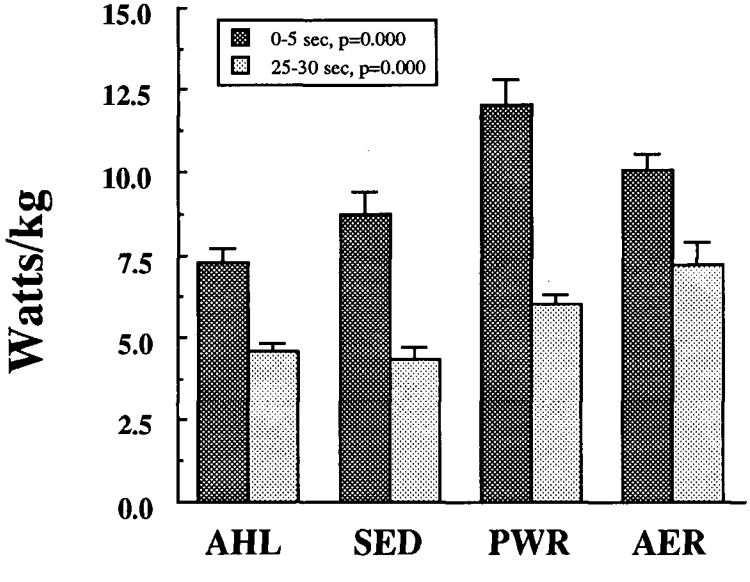
Figure 11

Work output in the first 5 sec of the 30 sec modified Wingate test of anaerobic capacity compared with work output measured in the last 5 sec of the same test. Values are means ± S.E. Figure 11a shows absolute work output in Watts while Figure 11b shows relative work output (Watts/ kg). Comparisons are by group. Relative drop in power output in the last five seconds compared with the first five seconds is presented in Table 2. The results of the Bonferroni post hoc tests are summarized in Table 3.

Absolute Anaerobic Capacity



Relative Anaerobic Capacity



Between-group differences in the absolute work capacities of the calf muscle at fatigue are shown in Table 2. The most consistent finding was a significantly greater amount of calf work at fatigue in the power group compared with the aerobic group (Table 3). Since a positive correlation exists between body mass and absolute muscle strength, these findings were predictable.

Relative (mass-specific) work capacities were calculated as ratios of work output to body weight and maximum voluntary contraction of the calf and the results are reported in Figures 12, 13 and 14. Using these comparisons, the Andean and aerobic groups were similar and generally performed more work at fatigue than the power group (Table 3). In turn, the power group performed more work than the sedentary group. Although normoxia versus hypoxia comparisons were not significant, it is notable that the Andeans performed the same amount of work at fatigue in both conditions while all of the other groups performed less work in the hypoxia condition.

An alternative approach to quantifying calf work rates would have been to use anthropometric techniques to estimate muscle volume or imaging techniques to estimate cross-sectional muscle area. However, it was felt that since both the gastrocnemius and soleus muscles participate in plantar flexion, isolated measurement of the gastrocnemius volume would not prove advantageous. In addition, the Andean natives were observed to have the smallest calf muscles of all four groups tested. In fact, the plexiglass box housing the RF antenna required modification to accommodate the small calves of the Andean group.

Table 2

Maximum voluntary contraction (MVC) of the right calf muscle measured isokinetically at 60°/s plantar flexion, maximum work (J) performed with each gastrocnemius contraction at fatigue, integrated (total) work (J) performed by the gastrocnemius muscle at fatigue, the ratio of work per contraction at fatigue to the MVC of the calf muscle (% MVC), and the integrated work performed by the calf muscle at fatigue as a function of the MVC of that muscle. Values are means ± S.E. The results of the Bonferroni post hoc tests are summarized in Table 3.

Table 2 - Measurements of Calf Muscle Work Capacity

MVC Calf Muscle (J)	86.5±11.1	94.5±15.3	125.8±11.6	71.0±9.2
Work (J) Normoxia	12.7±1.1	9.3±0.5	13.9±1.0	11.2±0.8
Work (J) Hypoxia	12.7±1.1	9.1±0.7	13.0±0.7	10.0±1.1
Integrated Work (J) Normoxia	3365.0±537	1802.0±168	3965.0±510	2633.0±346
Integrated Work (J) Hypoxia	3365.0±537	1783.0±276	3504.0±372	2189.0±483
% MVC Normoxia	15.4±1.8	11.2±1.8	11.3±1.2	17.3±2.9
% MVC Hypoxia	15.4±1.8	10.8±1.6	10.8±1.1	14.8±1.6
Integrated Work/MVC Normoxia	40.6±7.3	21.2±3.4	32.3±4.6	40.6±8.3
Integrated Work/MVC Hypoxia	40.6±7.3	20.1±2.7	28.7±3.6	31.0±5.3

Figure 12

Mass-specific calf work at fatigue in conditions of normoxia and hypoxia. Values are means ± S.E. Univariate F Test results indicate separate overall significance values for group main effect in each of the two conditions. The results of the Bonferroni post hoc tests are summarized in Table 3.

Mass-Specific Calf Work at Fatigue

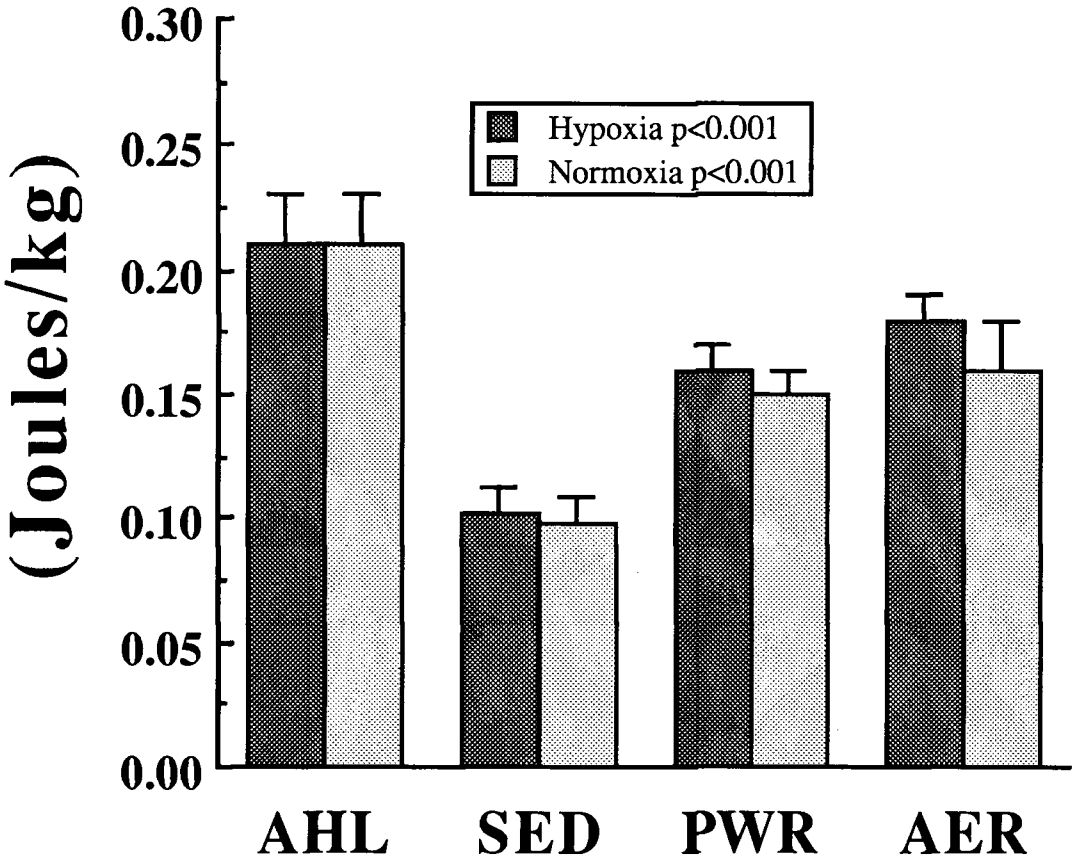


Figure 13

Calf work at fatigue as a function of maximum voluntary contraction of the calf muscle and body weight (kg) in conditions of normoxia and hypoxia. Values are means ± S.E. Univariate F Test results indicate separate overall significance values for group main effect in each of the two conditions. The results of the Bonferroni post hoc tests are summarized in Table 3.

Mass-Specific %MVC at Fatigue

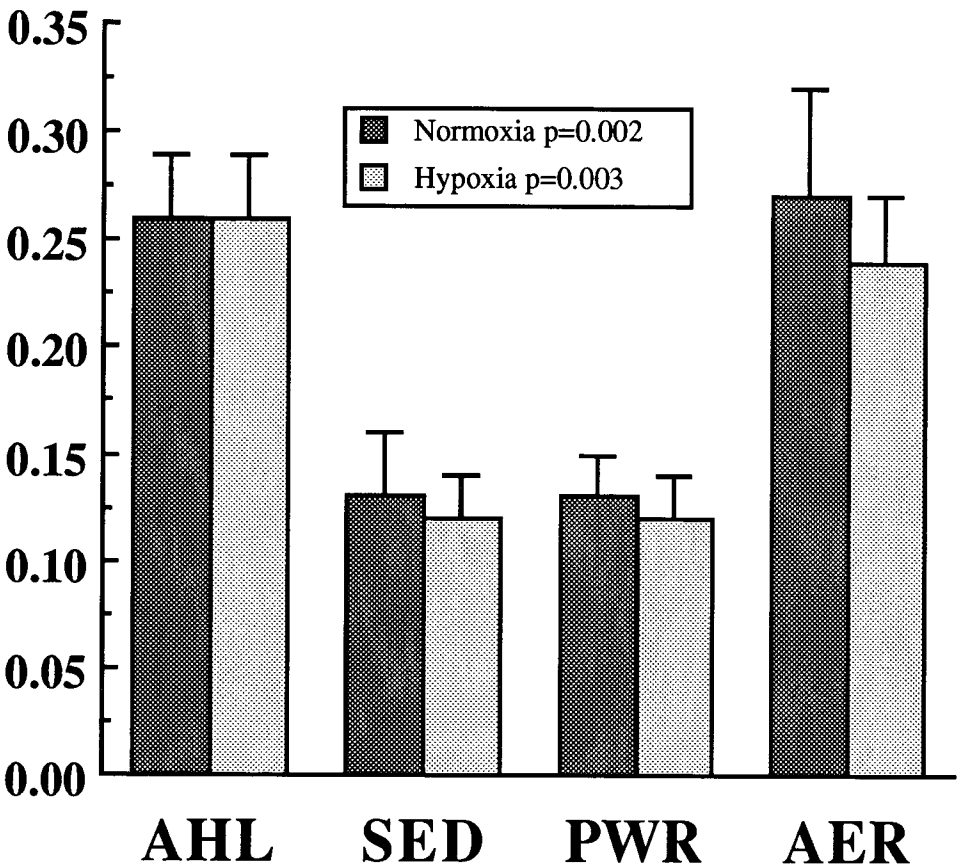
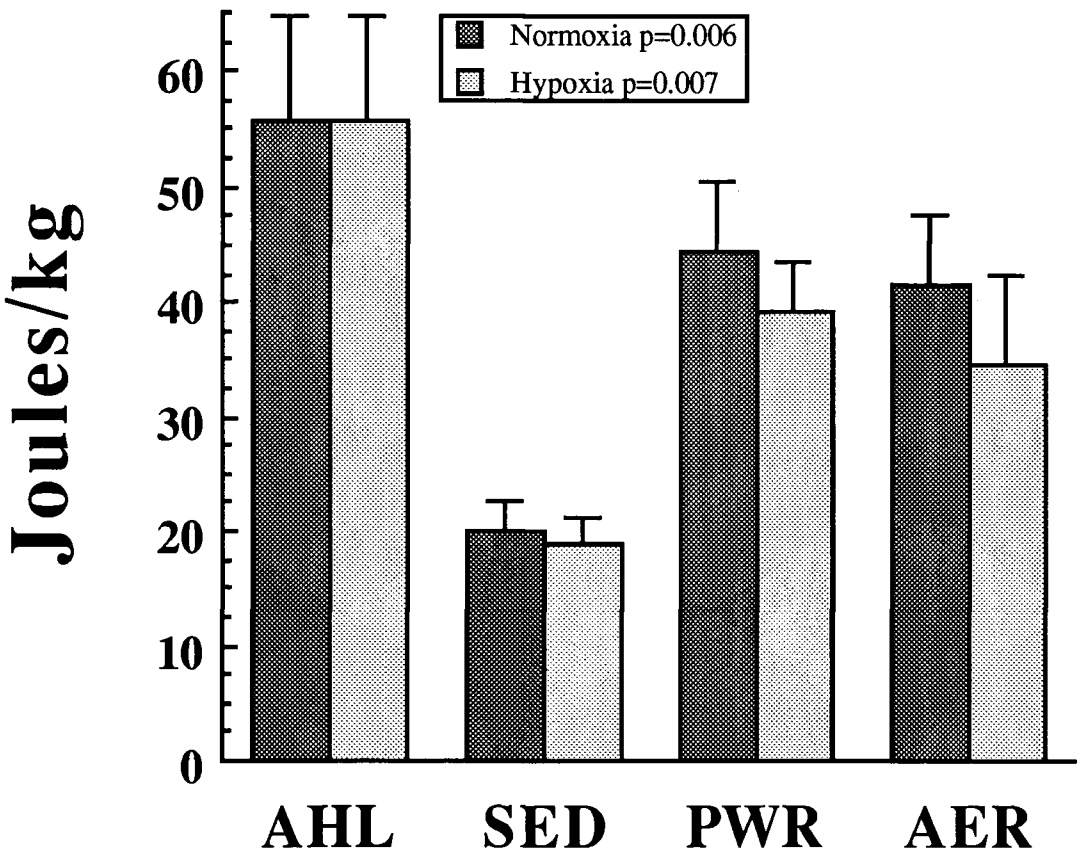


Figure 14

Mass-specific integrated (total) calf work at fatigue in conditions of normoxia and hypoxia. Values are means ± S.E. Univariate F Test results indicate separate overall significance values for group main effect in each of the two conditions. The results of the Bonferroni post hoc tests are summarized in Table 3.

Mass-Specific Integrated Work at Fatigue



^{31}P NMR Spectroscopy

Two factor (group by time) univariate ANOVA results for each of the NMR variables in Figures 15-25 are presented in Table 3 together with the results of Bonferroni post-hoc testing.

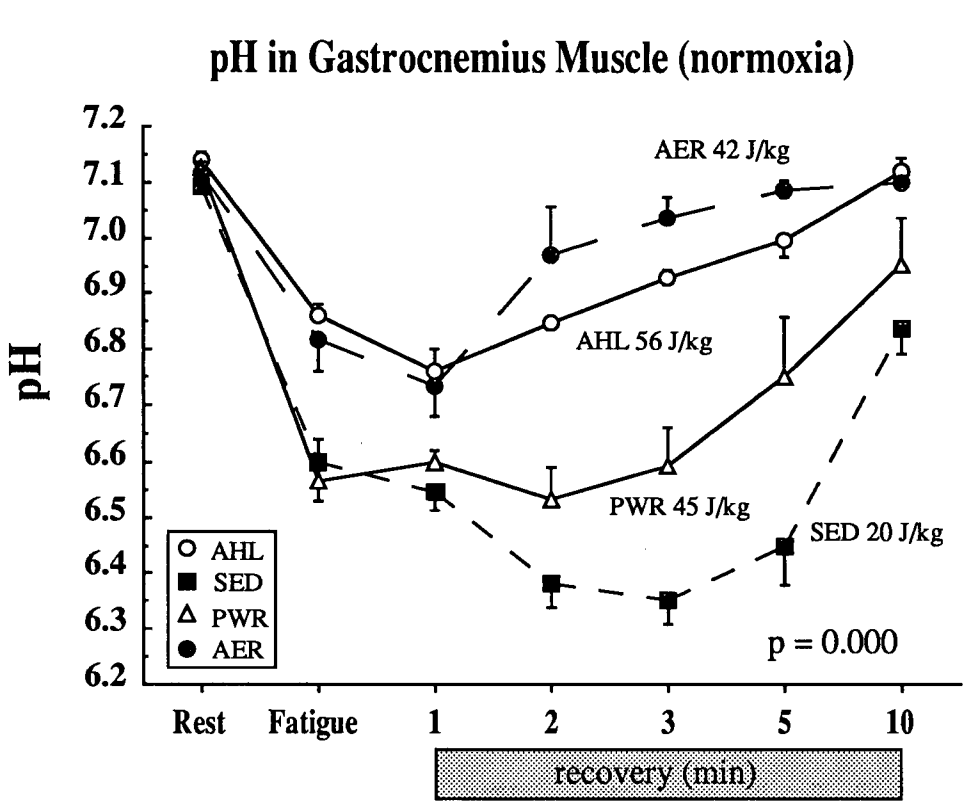
Gastrocnemius muscle pH values at rest, fatigue and at selected points in recovery in the four groups were compared and contrasted in normoxia (Figure 15a) and hypoxia (Figure 15b). Significant overall group differences were found in both conditions. The Andeans and aerobic groups had similar decrements in pH_m at fatigue and similar rates of recovery through the first 10 minutes and these values were significantly higher than for the power and sedentary groups (Table 3). The condition main effect (normoxia vs hypoxia) was not significant across the groups (Figure 16).

[Pi]/[PCr] values at rest, fatigue and through the first 5 minutes of recovery are shown in Figure 17a (normoxia) and 17b (hypoxia). Again, overall significant group main effects were found in both conditions although the condition main effect (normoxia vs hypoxia) was not significant (Figure 18). The power group had the greatest [Pi]/[PCr] at fatigue, followed by the sedentary group, and the Andean and aerobic groups (Table 3).

[ATP] values fell in all groups following aerobic exercise to fatigue (Figures 19 and 20) but significant differences between groups and conditions were not found.

Figure 15

Gastrocnemius muscle pH at rest, fatigue and during recovery from exercise in conditions of normoxia (15a) and hypoxia (15b). Values for mass-specific integrated work at fatigue are included. Values are means ± S.E. Univariate F Test results indicate separate overall significance values for group main effect in each of the two conditions. AHL = Andean, SED = sedentary, PWR = power trained athletes, and AER = endurance trained athletes. The results of the Bonferroni post hoc tests are summarized in Table 3.


pH in Gastrocnemius Muscle (normoxia)
pH
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
AER 42 J/kg
AHL 56 J/kg
PWR 45 J/kg
SED 20 J/kg
AHL
SED
PWR
AER
p = 0.000
Rest
Fatigue
1
2
3
5
10
recovery (min)

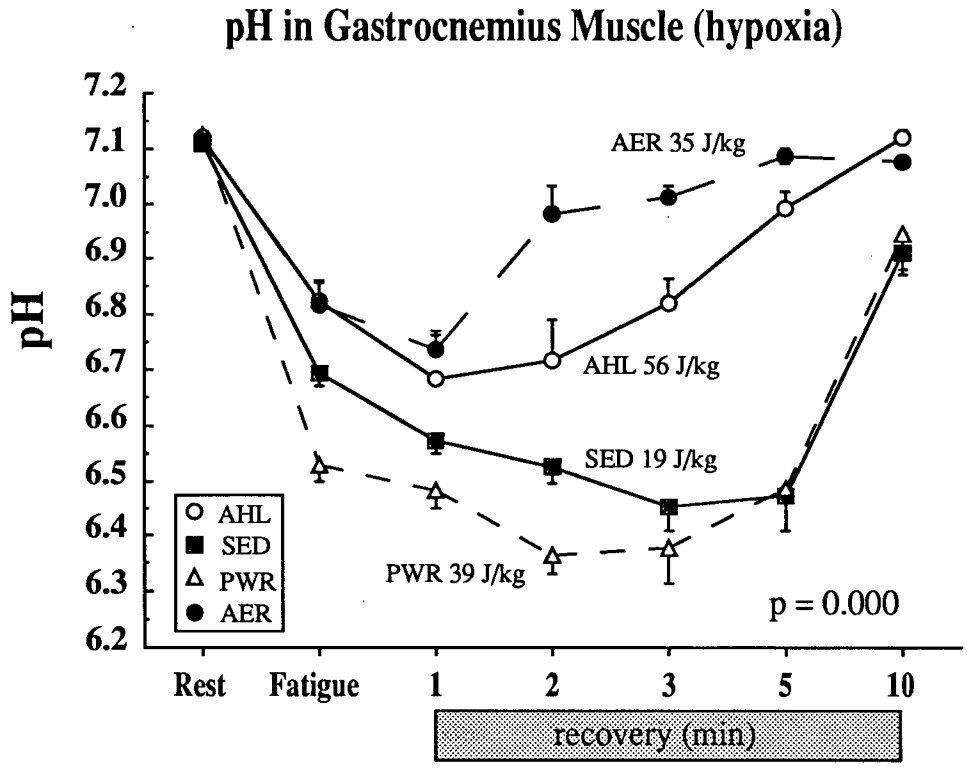

pH in Gastrocnemius Muscle (hypoxia)
pH
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
AER 35 J/kg
AHL 56 J/kg
SED 19 J/kg
PWR 39 J/kg
AHL
SED
PWR
AER
p = 0.000
Rest
Fatigue
1
2
3
5
10
recovery (min)

Figure 16

Gastrocnemius muscle pH during conditions of normoxia (FIO_2 = 0.21) and hypoxia (FIO_2 = 0.15) at rest, fatigue, and during recovery from exercise. Values are means ± S.E. Normoxia versus hypoxia contrasts are not significant ($p > .05$). AHL = Andean, SED = sedentary, PWR = power trained athletes, and AER = endurance trained athletes.

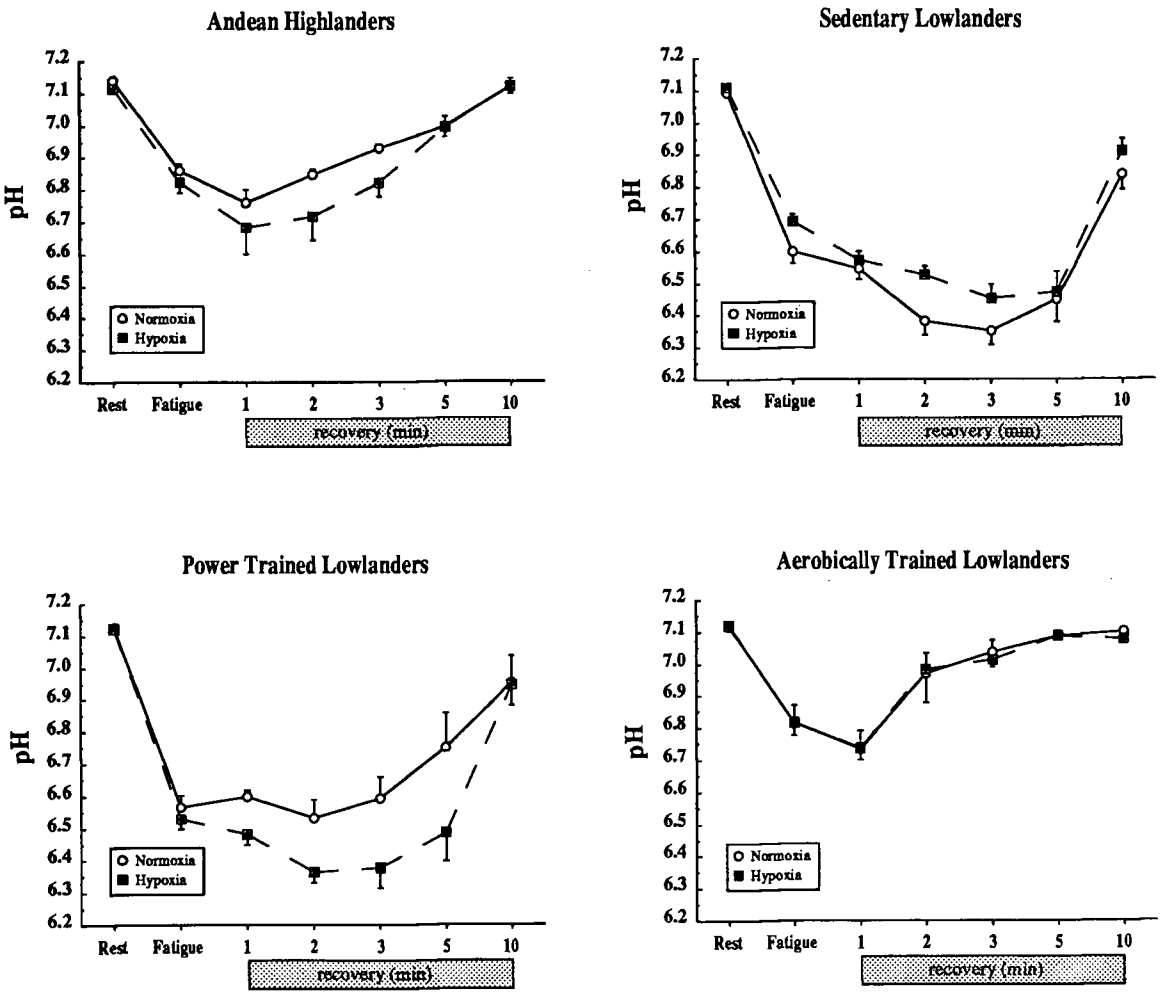
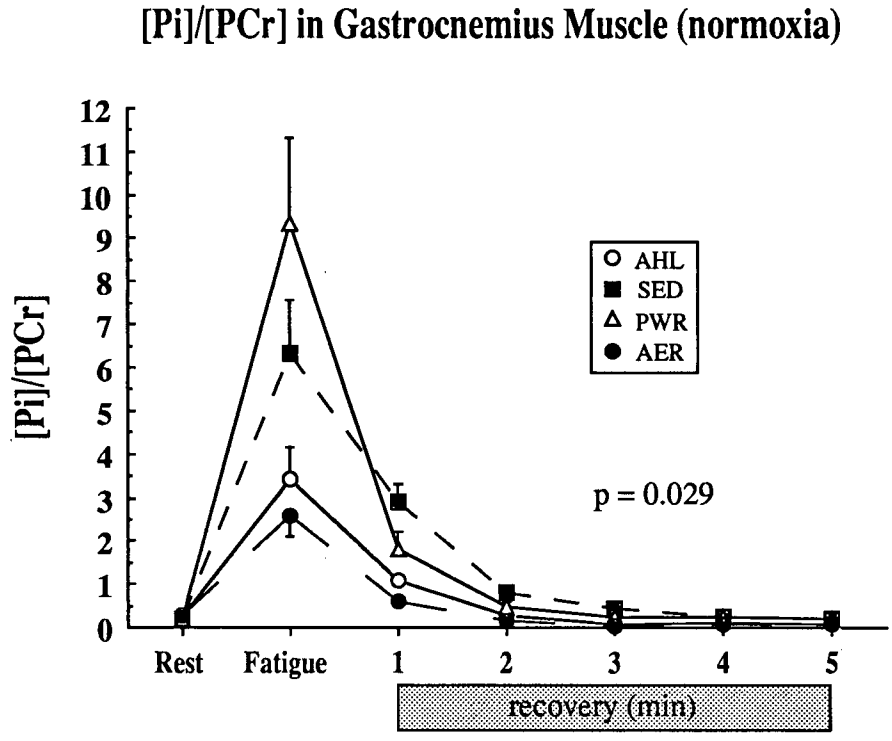

Andean Highlanders
Sedentary Lowlanders
Power Trained Lowlanders
Aerobically Trained Lowlanders
pH
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
O Normoxia
■ Hypoxia
Rest
Fatigue
1
2
3
5
10
recovery (min)

Figure 17

Inorganic phosphate to phosphocreatine concentration ratio ([Pi]/[PCr]) in the gastrocnemius muscle at rest, fatigue and during recovery from exercise in conditions of normoxia (17a) and hypoxia (17b). Values are means ± S.E. Univariate F Test results indicate separate overall significance values for group main effect in each of the two conditions. AHL = Andean, SED = sedentary, PWR = power trained athletes, and AER = endurance trained athletes. The results of the Bonferroni post hoc tests are summarized in Table 3.


[Pi]/[PCr] in Gastrocnemius Muscle (normoxia)
[Pi]/[PCr]
12
11
10
9
8
7
6
5
4
3
2
1
0
AHL
SED
PWR
AER
p = 0.029
Rest
Fatigue
1
2
3
4
5
recovery (min)

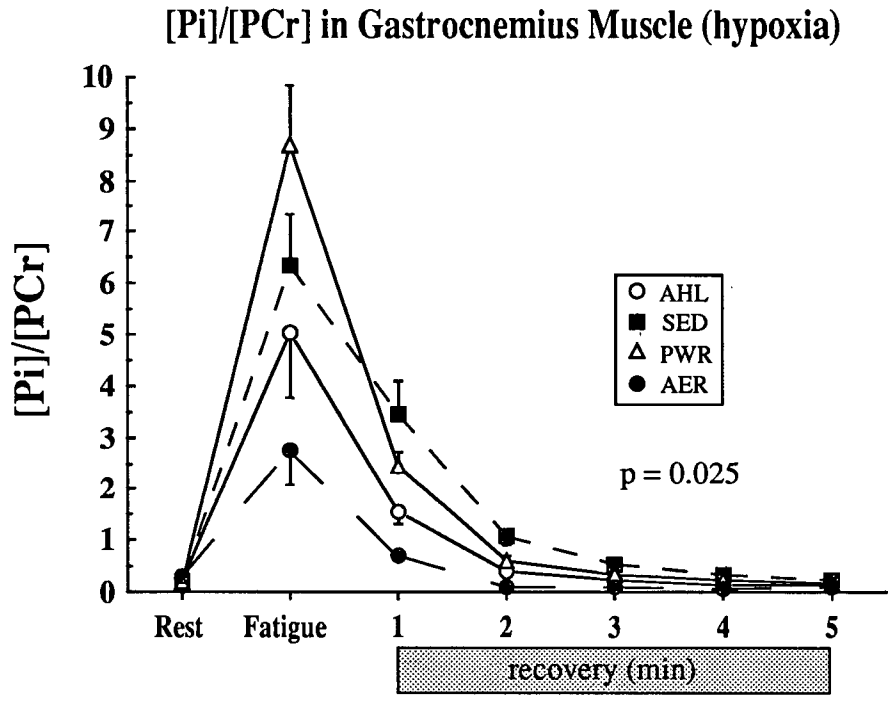

[Pi]/[PCr] in Gastrocnemius Muscle (hypoxia)
[Pi]/[PCr]
10
9
8
7
6
5
4
3
2
1
0
AHL
SED
PWR
AER
p = 0.025
Rest
Fatigue
1
2
3
4
5
recovery (min)

Figure 18

Inorganic phosphate - phosphocreatine concentration ratios ([Pi]/[PCr]) in the gastrocnemius muscle during conditions of normoxia (FIO_2 = 0.21) and hypoxia (FIO_2 = 0.15) at rest, fatigue, and during recovery from exercise. Values are means ± S.E. Normoxia versus hypoxia contrasts are not significant ($p > .05$). AHL = Andean, SED = sedentary, PWR = power trained athletes, and AER = endurance trained athletes.

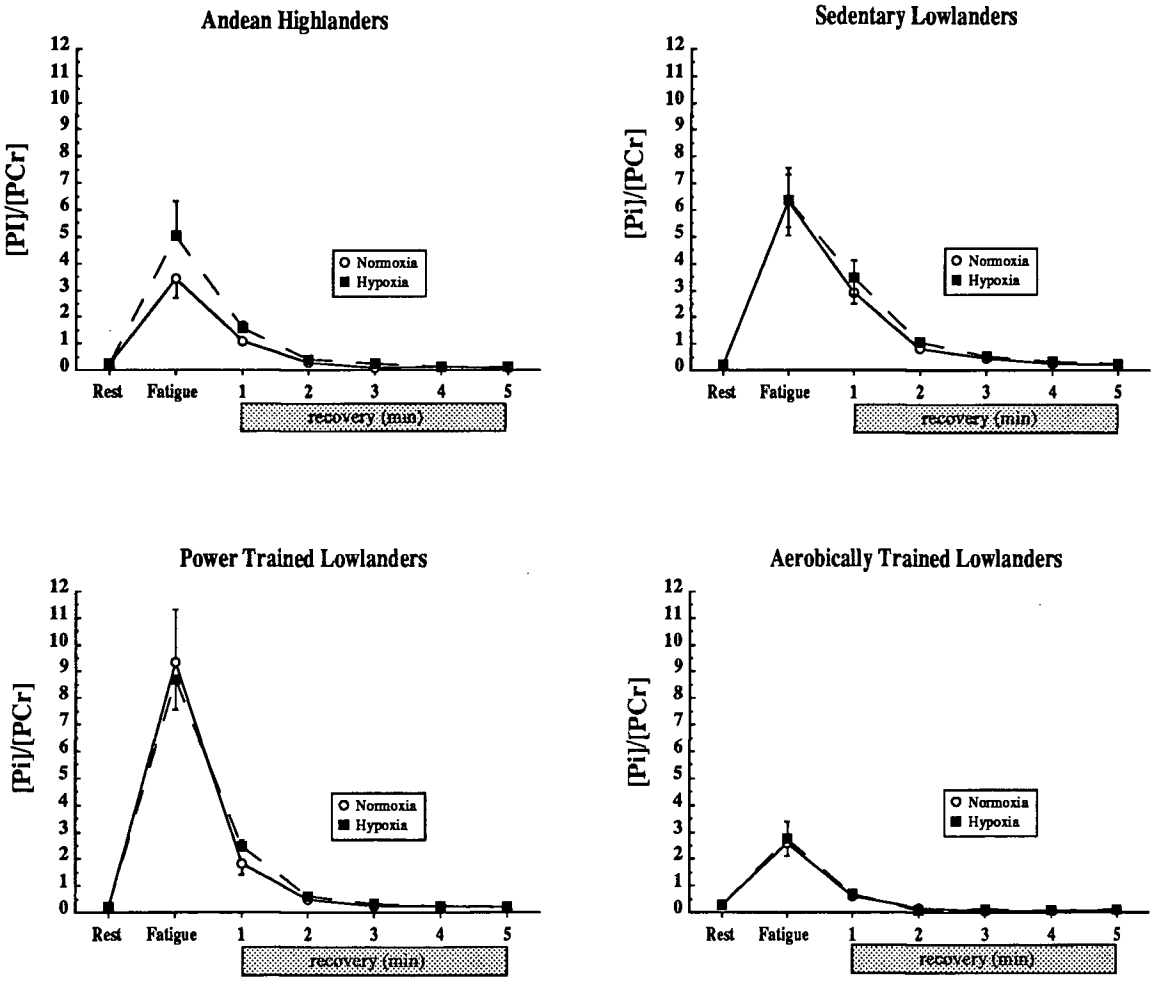
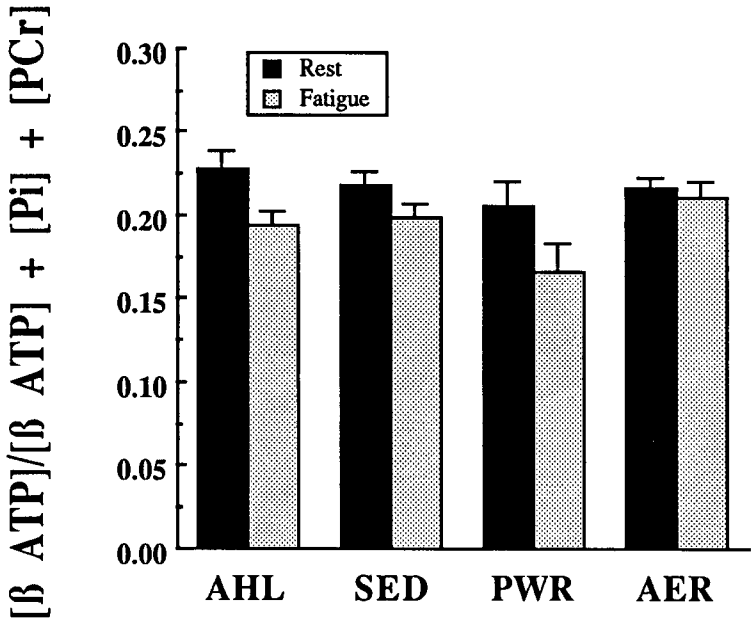

Andean Highlanders
[PI]/[PCr]
Normoxia
Hypoxia
Rest
Fatigue
recovery (min)
Sedentary Lowlanders
[Pi]/[PCr]
Power Trained Lowlanders
Aerobically Trained Lowlanders

Figure 19

[ATP] calculated with reference to total phosphate content in the gastrocnemius muscle at rest and fatigue in conditions of normoxia (19a) and hypoxia (19b). Values are means ± S.E. Group contrasts are not significant ($p > .05$). AHL = Andean, SED = sedentary, PWR = power trained athletes, and AER = endurance trained athletes.

ATP at Rest and Fatigue (normoxia)



ATP at Rest and Fatigue (hypoxia)

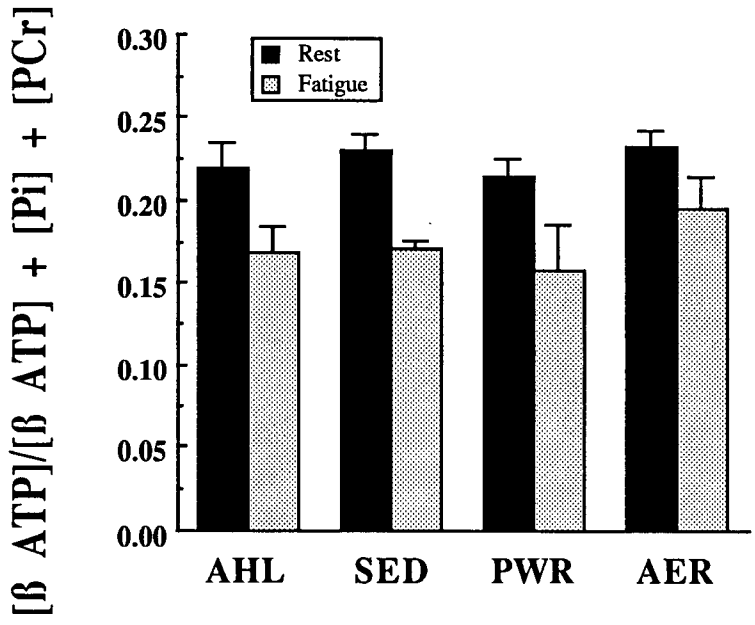
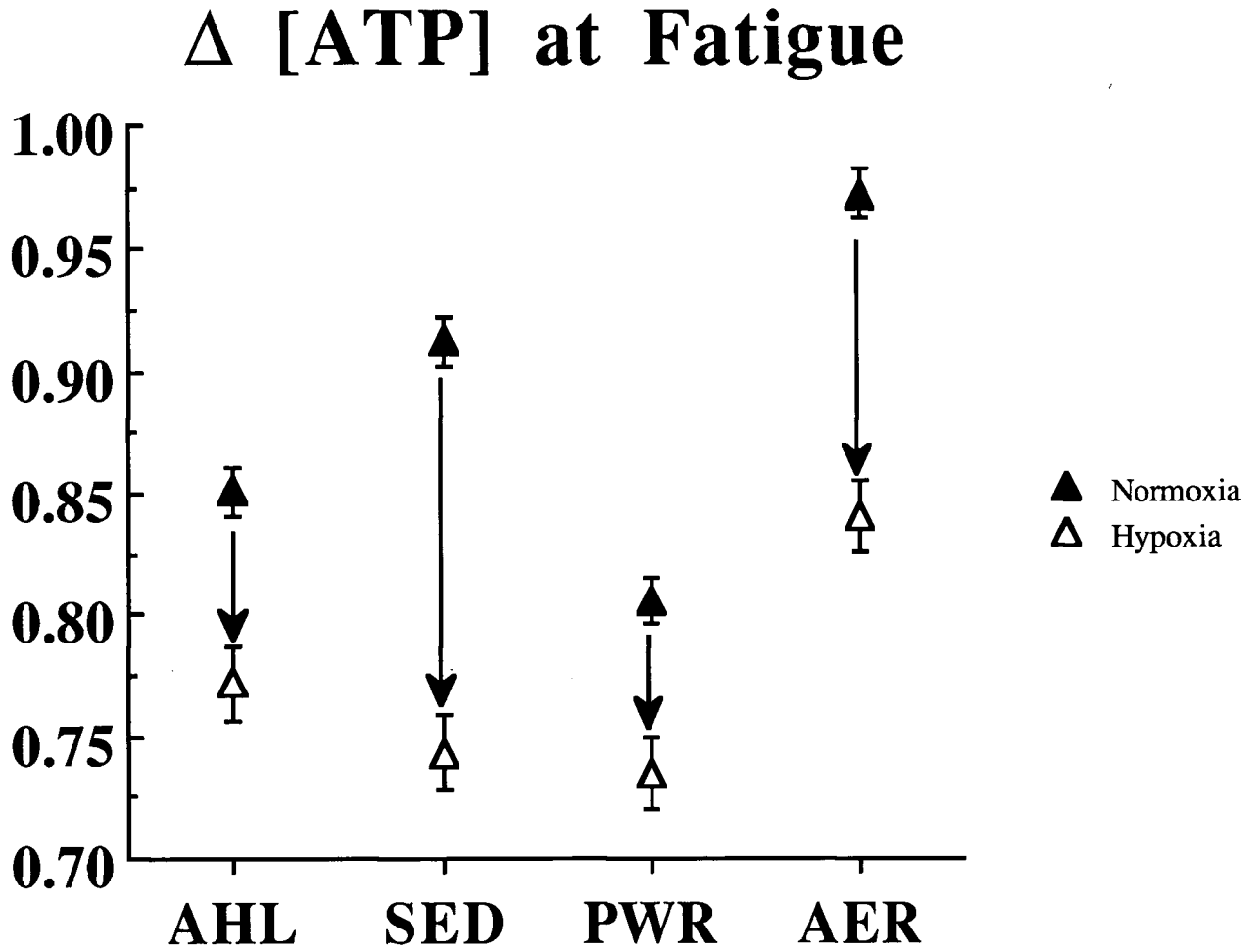


Figure 20

Δ [ATP] in the gastrocnemius muscle at rest and fatigue during conditions of normoxia (FIO_2 = 0.21) and hypoxia (FIO_2 = 0.15). Values are means ± S.E. AHL = Andean, SED = sedentary, PWR = power trained athletes, and AER = endurance trained athletes.


Δ [ATP] at Fatigue
1.00
0.95
0.90
0.85
0.80
0.75
0.70
AHL
SED
PWR
AER
Normoxia
Hypoxia

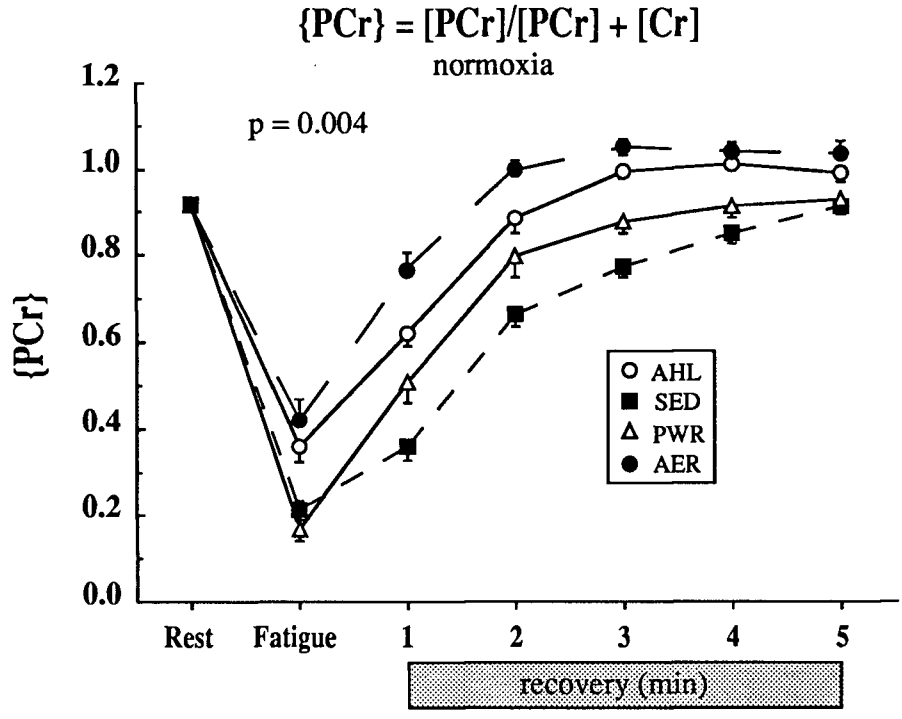
The phosphorylation potential in these experiments was difficult to estimate since its calculation requires knowledge of Mg^{++} and K^{+} concentration changes as well as those of H^{+} and the adenylates per se; however, this problem does not arise for the calculation of the creatine energy charge [PCr]/([PCr] + [Cr]), a value termed {PCr} by Connett and Honig [10, 11] and considered the main determinant of $\dot{V}O_2$ in mammalian skeletal muscles. Since the relative size of the total creatine pool (PCr + Cr) is constant in resting mammalian muscles from various species (assuming 25 μmol/g wet weight of muscle for skeletal muscles in man [11]), it was possible to calculate {PCr} under various conditions (Figure 21). The calculations showed that the [PCr]/[PCr] + [Cr] ratio qualitatively followed the same pattern as observed for the the [Pi]/[PCr] ratios. Comparing rest to fatigue, the greatest change was observed for the sedentary and power trained subjects; the least change was observed for the Andean natives and the endurance trained subjects (Table 3). For the same change in {PCr}, the integrated work/kg achieved by the Andean natives was similar to that achieved by the aerobic athletes (Table 2, Figure 15).

Another regulatory parameter correlating with VO_2 in skeletal muscles is the ratio of [Pi]/([PCr] + [Cr]), termed {Pi} [11]. Since at steady state [PCr] and [Pi] are stoichiometrically related, in principle the regulatory parameter {Pi} should be a kind of mirror image of {PCr}. The expected result is in fact observed, with {Pi} changing the most during exercise to fatigue in sedentary and power trained subjects, and the least in the Andean natives and endurance trained subjects (Figure 22).

As in the {PCr} analysis, it is worth emphasizing that for the same change in [Pi]/[PCr] + [Cr], the calf muscle in Andeans sustained 1.3-1.4 times more work/kg than in endurance trained lowlanders (Table 2, Figure 15).

Figure 21

Calf muscle {PCr} at rest, fatigue and during the first five minutes of recovery from exercise in conditions of normoxia (21a) and hypoxia (21b). Values are means ± S.E. Univariate F Test results indicate separate overall significance values for group main effect in each of the two conditions. Andean (AHL), sedentary (SED), anaerobic (PWR), aerobic (AER). {PCr} = [PCr]/([PCr] + [Cr]). [PCr]/([Pi] + [PCr]) + [ß ATP] = 23 µmol/g at rest; [PCr] + [Cr] = 25 µmol/g (ref 10,11). The results of the Bonferroni post hoc tests are summarized in Table 3.


{PCr} = [PCr]/[PCr] + [Cr]
normoxia
p = 0.004
{PCr}
1.2
1.0
0.8
0.6
0.4
0.2
0.0
AHL
SED
PWR
AER
Rest
Fatigue
1
2
3
4
5
recovery (min)

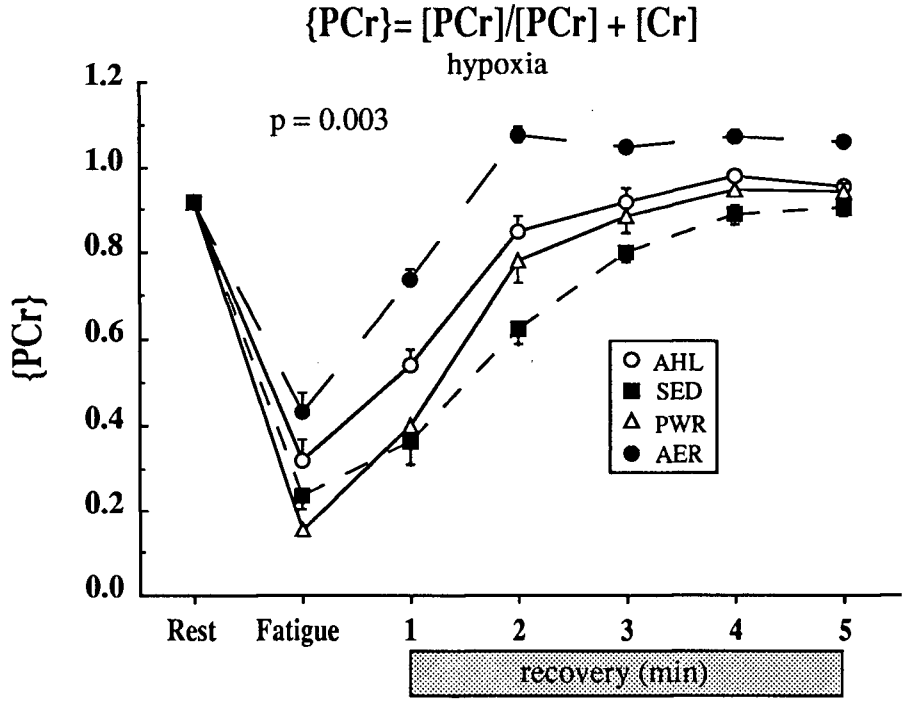
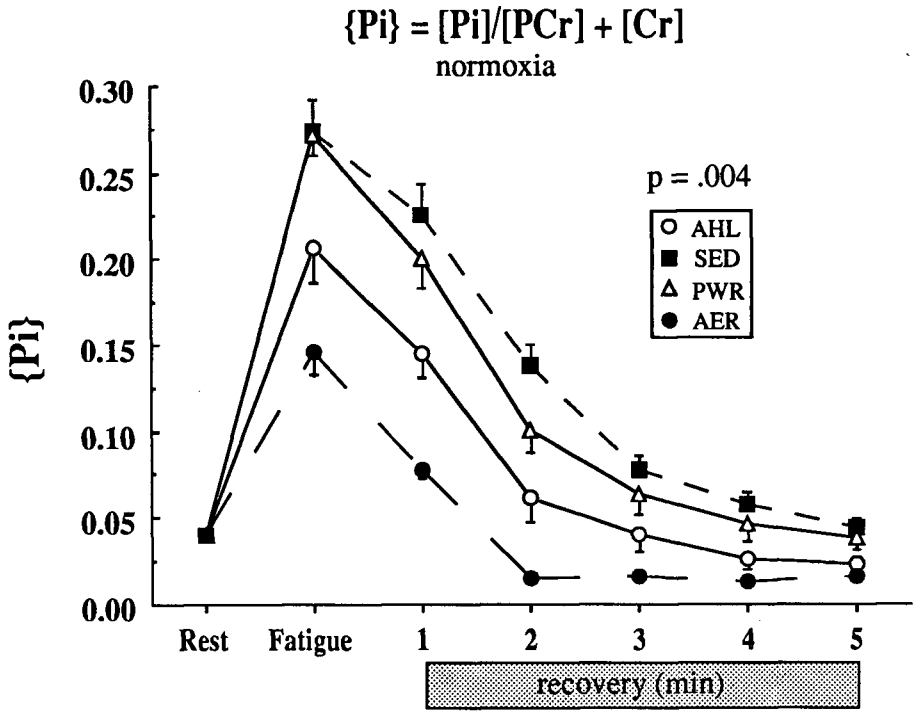

{PCr}= [PCr]/[PCr] + [Cr]
hypoxia
p = 0.003
{PCr}
1.2
1.0
0.8
0.6
0.4
0.2
0.0
AHL
SED
PWR
AER
Rest
Fatigue
1
2
3
4
5
recovery (min)

Figure 22

Calf muscle {Pi} at rest, fatigue and during the first five minutes of recovery from exercise in conditions of normoxia (22a) and hypoxia (22b). Values are means ± S.E. Univariate F Test results indicate separate overall significance values for group main effect in each of the two conditions. Andean (AHL), sedentary (SED), anaerobic (PWR), aerobic (AER). {Pi} = [Pi]/([PCr] + [Cr]). [Pi]/[Pi] + [PCr] + [ß ATP] = 1 µmol/g at rest; {Pi} = 1/25 = 0.04 at rest. The results of the Bonferroni post hoc tests are summarized in Table 3.


{Pi} = [Pi]/[PCr] + [Cr]
normoxia
p = .004
AHL
SED
PWR
AER
{Pi}
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Rest
Fatigue
1
2
3
4
5
recovery (min)

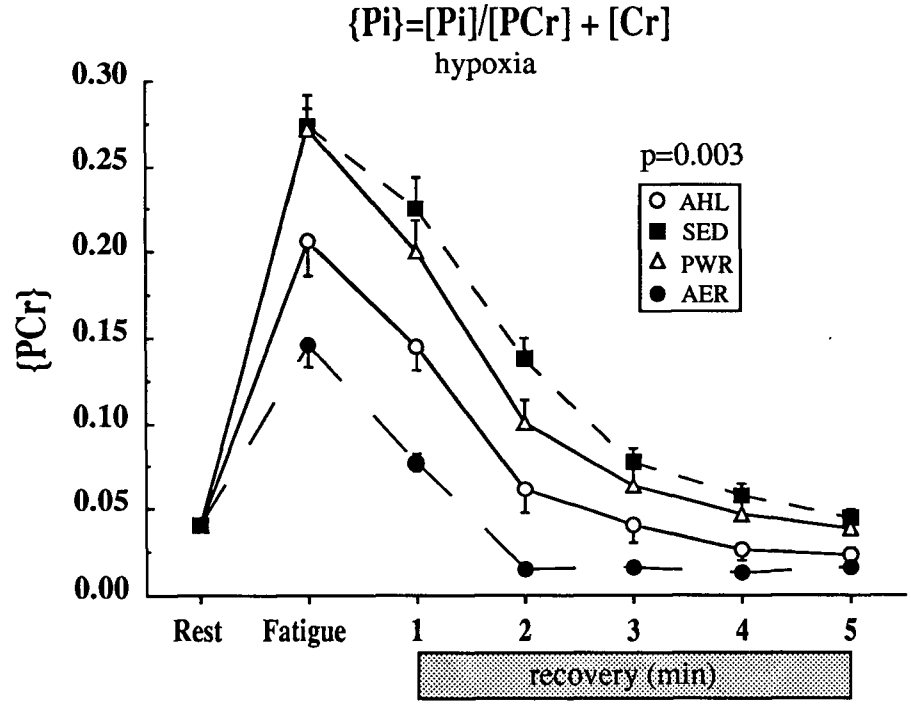

{Pi}=[Pi]/[PCr] + [Cr]
hypoxia
p=0.003
AHL
SED
PWR
AER
{PCr}
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Rest
Fatigue
1
2
3
4
5
recovery (min)

Table 3

Results of all Bonferroni pairwise comparisons if Univariate group statistic results are $p \leq 0.05$. √ indicates significant differences between groups.

Table 3

Dependent Variable	p (Univariate F)	AHL vs AER	AHL vs PWR	AHL vs SED	AER vs PWR	AER vs SED	PWR vs SED
$\dot{V}O_{2max}$ (ml/kg/min)	< .001	√	x	√	√	√	√
Absolute Anaerobic Capacity	< .001	√	√	√	√	x	√
Relative Anaerobic Capacity	= .001	√	√	x	x	√	√
Δ Anaerobic Capacity	< .001	x	√	√	√	√	x
Absolute Anaerobic Capacity 0-5 sec	< .001	√	√	√	√	x	√
Absolute Anaerobic Capacity 25-30 sec	= .002	√	√	x	x	x	x
Relative Anaerobic Capacity 0-5 sec	< .001	√	√	x	√	x	√
Relative Anaerobic Capacity 25-30 sec	< .001	√	√	x	x	√	√
MVC Calf Muscle	= .029	x	x	x	√	x	x
Work (J) Normoxia	= .009	x	x	x	√	x	√
Work (J) Hypoxia	= .016	x	x	x	√	x	√
Integrated Work Normoxia	= .009	x	x	x	√	x	√
Integrated Work Hypoxia	= .023	x	x	x	√	x	√
% MVC Normoxia	= .109						
% MVC Hypoxia	= .077						
Integrated Work/MVC Normoxia	= .121						
Integrated Work/MVC Hypoxia	= .066						

Table 3 continued

Dependent Variable	p (Univariate F)	AHL vs AER	AHL vs PWR	AHL vs SED	AER vs PWR	AER vs SED	PWR vs SED
Mass-Specific Calf Work at Fatigue Normoxia	< .001	x	√	√	x	√	√
Mass-Specific Calf Work at Fatigue Hypoxia	< .001	√	√	√	x	√	x
Mass-Specific %MVC at Fatigue Normoxia	= .002	x	√	√	√	√	x
Mass-Specific %MVC at Fatigue Hypoxia	= .003	x	√	√	√	√	x
Mass-Specific Integrated Work Normoxia	= .006	x	x	√	x	√	√
Mass-Specific Integrated Work Hypoxia	= .007	x	x	√	x	x	x
Gastrocnemius Muscle pH Normoxia	< .001	x	√	√	√	√	x
Gastrocnemius Muscle pH Hypoxia	< .001	x	√	√	√	√	√
Gastrocnemius Muscle [Pi]/[PCr] Normoxia	= .025	x	√	√	√	√	√
Gastrocnemius Muscle [Pi]/[PCr] Hypoxia	= .029	x	x	x	√	√	x
Gastrocnemius Muscle {PCr} Normoxia	= .004	x	√	√	√	√	x
Gastrocnemius Muscle {PCr} Hypoxia	= .003	x	√	√	√	√	x
Gastrocnemius Muscle {Pi} Normoxia	= .004	x	x	√	√	√	x
Gastrocnemius Muscle {Pi} Hypoxia	= .006	x	x	√	√	√	x

DISCUSSION

Andean Natives: Unique Physiological Phenotype

These results from this study indicate that, with respect to whole body measures of work capacity, the Andean natives are not typical of any of the three groups of lowlanders tested. In particular, their absolute and relative anaerobic capacities are very low while their aerobic capacities are intermediate compared with trained and untrained lowlanders. Similar findings have previously been reported [17, 28, 39] and investigators have concluded that altitude adapted indigenous natives do not appear to have enhanced whole body work capacities, at least on the basis of anaerobic and aerobic power outputs. Thus, whole body measures of work capacity in the Andean natives in this study defy categorization in terms of the three groups of lowlanders tested and these findings serve to indicate that adaptations which accrue from power or endurance training are not uniformly or even representatively embodied in the physiological profile of the Andean native.

Absolute calf work results in the groups are only statistically significant for the power group compared with the aerobic and sedentary groups. However, relative calf work results show the Andeans and aerobic group to be similar with values higher than those for the power and sedentary groups. Most importantly, the Andeans' work capacities are associated with equivalent perturbation in pH_m, [PCr] and [Pi] at fatigue compared with endurance trained athletes who possess significantly greater aerobic and anaerobic work capacities. Similarly, the Andean's calf work capacities are associated with significantly less metabolic perturbation

compared with the power trained group with similar $\dot{V}O_{2max}$ values and the sedentary group with similar anaerobic work capacities. In short, in comparative terms, the single muscle work capacity of the Andean natives does not appear to be related to whole body measures of their work capacity while their skeletal metabolic profile most closely resembles that of the aerobically trained group.

Skeletal Muscle Metabolic Capacity with Acclimation

During the process of acclimatization to moderate altitude (4,300 m), at least two studies have demonstrated improved work capacity in the absence of increases in $\dot{V}O_{2max}$. Maher et al. [29] found a 45% increase in cycling ergometer endurance time to fatigue at 75% $\dot{V}O_{2max}$ following 12 days acclimatization to 4,300 m and Horstman et al. [25] reported a 60% increase in time to exhaustion on treadmill running at 85% $\dot{V}O_{2max}$ after 16 days acclimatization to 4,300 m. Anecdotal reports of Himalayan Sherpa and Andean Quechua physical feats have been known for years [28, 39, 42] but it is also well known that whole body measurements of aerobic and anaerobic capacity in indigenous highlanders have failed to show unusual ('superhuman') work capacities [42]. Based on the low anaerobic and intermediate aerobic capacities measured in the Andeans in this study, their relatively high calf work rates were not predicted. Given that at least the quadriceps muscle in man is capable of a metabolic rate on the order of 350 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ when central cardiac limiting factors are accounted for [1] additional adaptive changes within the skeletal muscle (peripheral adaptations) may result from prolonged hypobaric hypoxia and may be exposed in single limb exercise where competitive demands for cardiac output are minimized.

One adaptive strategy to allow the organism to maintain work rates at altitude would see an increase in skeletal fiber capillarity, mitochondrial volume density, and oxidative enzyme capacity. These changes would allow ATP synthesis to occur at lower concentrations of O_2 and would require less increase in [ADP] and [Pi] for any given respiratory chain by using a smaller fraction of the oxidative capacity of the mitochondrial respiratory chain. In effect, these adaptations would allow the muscle to operate at greater O_2 flux rates without excessive demands for high substrate concentrations [10, 11].

However, the available literature has not provided many examples of this strategy in lowlanders acclimatized to altitude or in altitude adapted indigenous highlanders. For example, the total contractile pool of skeletal muscle is reduced in lowlanders returning from long periods at altitudes reaching or surpassing 8,500 m: cross-sectional area of the thigh is reduced by 10% and mean fiber cross sectional area of the vastus lateralis muscle by 20% [24]. Similar findings are found in elite high altitude climbers who have previously scaled peaks above 8,500 m without supplemental oxygen [31] and in Himalayan Sherpas who reside between 3,000 and 5,000 m [7]. In addition, reductions are seen in the capillary to fiber ratio and the volume density of total mitochondria (both interfibrillar and subsarcolemmal) in these three groups. The volume density of total mitochondria is reported to be reduced by 25% in post expedition climbers [24] and was found to be equal in elite climbers compared to sedentaries but considerably lower than endurance trained orienteers [31]. In elite climbers, the capillary to fiber ratio is slightly greater than found in sedentary lowlanders but significantly less than found in endurance runners

and the same is true for the volume density of mitochondria [24, 31]. In Nepalese Sherpas, similar morphometric measures are found [7]. Finally, Green et al. also report similar changes in the acclimation Operation Everest II study [15].

These morphometric findings are reconcilable if the absolute oxidative capacity of the muscle or at least the relative oxidative capacity (oxidative/glycolytic ratio) is increased. Unfortunately, from the studies to date, it would appear that altitude adapted individuals also are unable to appeal to this mechanism. While studies in guinea pigs [33] and rats [34] do not show decrements in oxidative capacities, in lowlanders returning from great altitudes, activities of the oxidative enzymes succinate dehydrogenase (SDH), citrate synthase, malate dehydrogenase, cytochrome oxidase, 3-hydroxyl-CoA dehydrogenase (HADH), and hydroxybutyrate dehydrogenase (HBDH) are reduced 10-30% [26]. These changes (impairment of oxidative capacity) are secondary to loss of mitochondrial structure rather than a qualitative reduction in enzymatic activity since the reduction in enzymatic activity can be accounted for by reductions in mitochondrial volume density. Green et al [15] found similar reductions in oxidative capacity in Operation Everest II. Young showed no effects on oxidative enzyme activities in the vastus lateralis of 5 subjects exposed for 18 days to 4,300 m [44]. Finally, the activities of the glycolytic enzymes phosphofructokinase (PFK) and lactate dehydrogenase (LDH) are reported to be unchanged or reduced following chronic exposure to hypobaric hypoxia [15, 44].

Improved Work Capacity Energy Coupling, and the $\dot{V}O_{2max}$ Paradox

If $\dot{V}O_{2max}$ does not increase after residence at altitude, and if muscle fiber diameter, capillarity, mitochondrial volume, and absolute or relative oxidative capacities are not upscaled (in fact appear to be unchanged or downscaled), we are left to explain the high muscle work rates which occur with altitude and which are found in indigenous highlanders anecdotally and in the present study. The present study demonstrates less perturbation in {PCr}, {Pi}, and [Pi]/[PCr], and perhaps most notably, significantly less drop in pH_m at fatigue compared with subjects having equal aerobic (power group) and greater anaerobic (sedentary group) capacities. Since all of these parameters, especially {PCr} and {Pi}, either reflect the phosphorylation potential or are stoichiometrically related to the phosphorylation potential [10, 11], the data implies that the latter parameter is also less perturbed during skeletal muscle work in Andean natives relative to their aerobic and anaerobic capacities. These findings reflect closer coupling between ATP supply and ATP demand in skeletal muscle during sustained work in Andean natives than in all other groups studied. The mechanisms which are postulated to explain this are reviewed elsewhere [2, 19, 21]. Briefly, in loosely coupled energy demand-supply systems, dog gracilis is an excellent example [10, 11], the phosphorylation potential, {PCr}, {Pi}, [PCr], [Pi], [ADP], [ATP], and pH_m all change in step with work rate increases, thus favoring both increased oxidative phosphorylation rates and increased rates of glycolysis and lactate production.

The NMR data suggest that in Quechua Indians, any given muscle work rate is sustained by lesser change in these key controlling parameters and thus by lesser (kinetic and thermodynamic) activation of mitochondrial metabolism than in lowlanders. That is why improvements in muscle performance observed in high altitude natives are not (indeed, need not) be reflected in $\dot{V}O_{2max}$ capacities [10, 11] and why calculations of conversion of metabolic power input into mechanical power output indicate improved efficiencies in Andean natives [19].

Energy Coupling and the Lactate Paradox

If the above interpretation is valid, the lactate paradox also can be readily explained (on the basis of reduced changes in the phosphorylation potential and in all related regulatory parameters at equivalent workloads [18]) because of reduced activation of glycolysis. Indeed, in the most tightly energy demand-energy supply coupled muscle currently described (heart), no net production of lactate or of H^+ as a function of work rate is observed [2]. In this regulatory characteristic, as in the aerobic ones above, skeletal muscles in Quechua natives behave in a manner intermediate between classical skeletal muscle and cardiac muscle. Thus, the low lactate levels observed at fatigue in $\dot{V}O_{2max}$ tests likely reflects muscle metabolism with somewhat modified (and appropriately intermediate) regulatory properties. This phenomenon is similar to endurance training at sea level where a great deal of time is spent training at work rates near $\dot{V}O_{2max}$. Endurance trained athletes also have lower lactate production in working muscle [23] (together with increased capilarity and oxidative capacity) and in the present study display less change in [PCr], [Pi], and pH_m when compared

with power trained or sedentary controls. It is conceivable that endurance trained athletes also have tighter energy supply-energy demand coupling.

In addition to this theory [18], several other hypotheses have been put forward to explain the lactate paradox. Cerretelli [8] has suggested that reduced buffering capacity during ascent to altitude (principally a reduction in plasma [HCO_3^-] as a consequence of hyperventilation and associated reductions in $PaCO_2$) contributes to greater increases in [H^+] and consequent inhibition of glycolysis, most likely at the level of phosphofructokinase. In the present study, pH_m at fatigue was high compared to lowlanders and certainly not sufficient to inhibit glycolysis. Moreover, the lactate paradox did not deacclimate in the Andean natives examined (no indication of reversal after 6 weeks at sea level [21, 32]). In addition, following 14 days acclimatization to 4,300 m, Young et al. have measured muscle pH 0.15 units higher and venous blood lactate 30% lower following exhaustive exercise [44]. Thus, it would appear that this explanation can be discounted.

From the Operation Everest II study, based on the finding that the integrity of the glycolytic pathway does not appear compromised, Green et al. [16] suggest that the lactate paradox may result from hypoxia-induced decreased activation of the skeletal muscle contractile mechanism either through a disturbance in activation (motoneuron pool) or reduced sensitivity to respond to a calcium stimulus. Although some evidence exists to suggest that CNS inhibition may result from peripherally generated inhibitory reflexes activated by hypoxia [4], this theory is unable to explain the lactate paradox at moderate altitudes where cerebral oxygenation is not seriously impaired; nor is it consistent with the observation that the lactate paradox

does not deacclimate in Andean natives even after 6 weeks at sea level [21]. Thus the interpretation of Green et al. [16] at least tentatively can be dismissed.

Muscle glycogen depletion at altitude has also been suggested as a possible cause of the lactate paradox [8]. However, Green et al. [16] have shown that muscle glycogen content is not reduced at fatigue in subjects who demonstrate the lactate paradox.

Finally, Sutton and Heigenhauser [37] suggest that the alkalemia associated with altitude - being the net effect of respiratory alkalosis (on the basis of alveolar hyperventilation and reduced $PaCO_2$) and metabolic acidosis (on the basis of lowered plasma $[HCO_3^-]$ through renal excretion), may not effect increases in lactate efflux of the same magnitude as changes in strong ion difference (SID) or permeability. However, in studies which have artificially manipulated the extracellular pH through means such as induced respiratory alkalosis, metabolic acidosis and metabolic alkalosis, the controlling factor for lactate efflux is the absolute $[H^+]$ (see [36]). Thus, the alkalemia of altitude would favor lactate efflux at any given cytosol concentration and serve to increase plasma lactate. In any case, this explanation would not hold for the Andean natives whose resting arterial pH and $[HCO_3^-]$ measures were normal [27] but their plasma [lactate] at the end of exhaustive exercise was significantly lower than in trained lowlanders [21].

CONCLUSIONS

Although the Andean natives in the present study display very low anaerobic capacities and intermediate aerobic capacities, they demonstrate

very high calf muscle work capacities compared with power trained and sedentary lowlanders. These findings are not explained on the basis of whole body measures of anaerobic and aerobic capacities but may be explained by ^{31}P NMR measurements. Since it is well established that {PCr} and {Pi} are linear transformations of the phosphorylation potential in vivo over a broad range of pHm values [10, 11], the data from the present study implies that for any given work rate, the calf muscle phosphorylation potential in Andean natives is less perturbed than in other subject groups with similar $\dot{V}O_{2max}$ values and equally perturbed compared with trained subjects with significantly higher $\dot{V}O_{2max}$ values. Presumably, this metabolic regulatory transition is an adaptive response to chronic hypobaric hypoxia and occurs in addition to the central (cardiopulmonary) and erythropoietic adjustments known to result from altitude acclimatization.

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CHAPTER 4

Deacclimation in Andean Highlanders: A ^{31}P NMR Study of Muscle Metabolism

ABSTRACT

The relationships between single muscle (calf) work capacities at fatigue (WF), high energy phosphate metabolites (phosphocreatine (PCr) and inorganic phosphate (Pi)), adenosine triphosphate (ATP), and intramuscular pH (pHm) were determined in Quechua natives from the Peruvian altiplano (barometric pressure 460 Torr) before (A) and after (DA) six weeks deacclimation to sea level. The measurements were made using ^{31}P NMR spectroscopy with an RF surface coil positioned over the gastrocnemius muscle during rest, graded exercise to fatigue, and recovery, in conditions of normoxia and hypoxia (FIO_2 = 14.5%). At the end of the deacclimation period, the Quechua showed slightly reduced calf work at fatigue (WF_A = 12.7 ± 1.1 J, WF_{DA} = 11.4 ± 1.2 J) and integrated work at fatigue (WF_A = 3365 ± 537 J, WF_{DA} = 2831 ± 328 J). Both at fatigue and throughout recovery, [Pi]/[PCr] and {Pi} ([Pi]/([PCr] + [Cr])) were higher and {PCr} ([PCr]/([PCr] + [Cr])) was lower after deacclimation. pH_m showed marked deacclimation after 6 weeks with values 0.2-0.3 pH units lower at fatigue and throughout the first 10 min of recovery after 6 weeks. Calf work capacities, high energy phosphates, and pH_m were refractory to rapid normoxia-hypoxia ($\%HbO_2$ = 90-92%) transitions during exercise and this insensitivity did not deacclimate. These

modest reductions in single muscle work capacity and increases in the indices of the phosphorylation state ratio (for a given respiratory rate in the working muscle) are likely deacclimation effects. The lower pH_m values during exercise and recovery following deacclimation may result from reduced lactate efflux or reduced buffering capacity in the muscle. It is postulated that six weeks following removal of the hypoxic stimulus of altitude hypobaria, the lower [Pi]/[PCr] and {PCr} and the higher {Pi} during exercise and recovery in the gastrocnemius muscle of Quechua natives reflects a looser coupling between ATP supply and ATP demand.

INTRODUCTION

Acclimation to altitude occurs as a result of simultaneous adaptations in several organ systems and at several levels in the O_2 path from the lung to the mitochondria [15, 47]. In man, a great deal of study has concerned the central sites responsible for improved O_2 delivery to the working muscle in altitude hypobaria including cardiac output, control of breathing and the resetting of the CO_2 ventilatory response, pulmonary diffusion capacity, left shift of the oxyhemaglobin dissociation curve, and erythropoiesis (for a review see Ward, Milledge and West [54]). Despite central acclimatory adjustments which serve to maintain tissue oxygen transport [59], the full metabolic scope for activity (defined as the $\dot{V}O_2$ at maximal exercise minus the $\dot{V}O_2$ at rest [21]) is reduced at altitude [53].

In contrast, reports of significant improvements in work capacity (> 50%) during residence at high altitude [28, 35] and following low intensity forearm training at low altitude [38], in the absence of changes in whole body measures of $\dot{V}O_{2max}$, imply a dissociation between improvements in muscle oxidative capacity and mechanical work capacity. The mechanism for this type of adaptation cannot invoke adjustments in blood flow [45] but could theoretically invoke preferential fuel utilization which would serve to maximize the yield of ATP/mole of O_2 consumed [21, 60]. This type of adaptation would require an up scaling of absolute oxidative capacity but, while this is consistent with observations in altitude adapted animal species [25], observations in man show down scaling of absolute oxidative capacities [6, 17, 18, 27, 30, 39, 61].

An alternative explanation for the improved endurance capacity at altitude as well as for the numerous anecdotal reports of unusually high work capacities in altitude adapted natives [1, 13, 14, 33, 55, 58] is a reduction in the phosphorylation state ratio (hence in the requirement for thermodynamic activation of mitochondrial respiration) at a given respiratory rate in the working muscle [22]. This sort of adaptation is made possible through tighter coupling of ATP supply to ATP demand. Although this type of adaptation has not been reported in human acclimation studies, indices of the phosphorylation potential derivable from NMR measurements of Pi, PCr and pH_m presented in Chapter 3 show exactly this type of adaptation in indigenous Andean natives compared with trained and untrained lowlanders. The question addressed in this chapter is: does this functional energetic adaptation deacclimate following removal of the hypoxic stimulus of hypobaria?

Since central adaptations occur within six weeks of acclimation to altitude, any changes in energy metabolism at the level of the working muscle observed within a six week deacclimation period would depend on whether the adaptations are: i) fixed (ie. genetically determined and only adaptable over a time span of generations), ii) medium-term adaptable (over months or years), or iii) short-term adaptable (analogous to the acclimation response of lowlanders). Although Park et al. [41] have reported evidence to suggest a genetic endowment favouring greater oxidative capacity in the forearm muscles of marathon runners, this study did not rule out cross-training effects known to occur with marathon running.

Realizing the advantages and disadvantages of using the mountain as a laboratory on which to address the question of acclimation-deacclimation adaptations [29, 56], the present problem was approached by reversing the methodology used in other altitude studies. Indigenous Peruvian natives, life time residents of altitudes between 3700 and 4500 m, were brought to laboratories at sea-level for six weeks, enabling observation of short-term changes while benefitting from access to modern laboratory equipment [24, 31, 32, 37, 43]. Six weeks was chosen as the appropriate time period to study deacclimation since ventilatory and hematologic adaptations during acclimation to 4,000 m occur as early as three weeks and are complete within six weeks [54].

A common approach to this type of study involves the use of muscle biopsies, which, although allowing determination of a greater variety of metabolites, was impractical with the present subject group since multiple repeated measures of key metabolites were required at two time intervals and in two gas mixture conditions. ^{31}P NMR of skeletal muscle has established itself as a reliable, serial, noninvasive technique for monitoring key metabolites in energy metabolism - phosphocreatine (PCr), inorganic phosphate (Pi), adenosine triphosphate (ATP) as well as intracellular pH (pH_m). Moreover, this technique has the advantage of assaying a relatively large and therefore more representative muscle mass.

The present study compared calf muscle work rates, and ^{31}P NMR measures of cellular metabolic rate and pH_m during aerobic exercise to fatigue in Quechua Indians before and after deacclimation to sea level. A moderate deacclimation effect on skeletal muscle energy metabolism was

found whereby greater decreases in pH_m and greater increases in [Pi]/[PCr] were measured at fatigue and throughout recovery after six weeks at sea level.

MATERIALS AND METHODS

Subjects

The methods used in this study are similar to those reported in Chapter 3. Six healthy Quechua natives, life time residents of the Peruvian Andes (3700-4500 m) and workers at the La Raya Veterinary Research Station (4200 m, average barometric pressure 460 Torr) were selected as subjects. All were Spanish speaking but were accompanied almost continuously by a translator who was with the subjects during the initial experiments in Peru and subsequently throughout the entire deacclimation period. Their demographics, physical characteristics and aerobic and anaerobic work capacities have already been described in Chapter 3. Briefly, these indigenous highlanders had the following physical characteristics (mean ± S.D.): age = 34.3 ± 1.1 years, weight = 62 ± 2 kg, height = 159.5 ± 2.1 cm, $\dot{V}O_{2max}$ = 50.3 ± 2.0 ml·kg^{-1}·min^{-1} (cycle ergometer), anaerobic capacity = 341.2 ± 17.5 W (Wingate). All subjects maintained similar activity levels during their six week sojourn to sea level compared with their activity levels at altitude.

It was established by neuropsychological testing [23] that these 6 subjects were highly motivated, an important factor in voluntary exercise tests to fatigue. The subjects were familiarized with the testing procedures prior to the initiation of the experiments. The measurement techniques used in this paper were approved by the committees on experimentation in humans

at the two Universities involved and informed consent (in Spanish) was obtained from the subjects prior to the experiments.

Calf Exercise Protocol

Maximum voluntary contraction (MVC) of the right (in all cases dominant [23]) calf muscle was measured using a Cybex II isokinetic dynamometer with the subject placed in the same position as for the calf exercise during the NMR experiments (supine, knee flexed to 30°, angular velocity set at 60°/sec and axis of rotation of the foot pedal set at the level of the medial malleolus).

During NMR data acquisition, the right calf muscle was worked to fatigue (see below) using an ergometer fitted with a foot pedal which allowed plantar flexion of the ankle through a 0° to +15° range of motion. The apparatus consisted of a sheet of aluminum 2 m in length machined to conform to the shape of the NMR patient bed and secured in place by the weight of the subject, rubberized backing, and straps. The right foot was held in place in the foot pedal with straps, and the axis of rotation of the foot pedal was set to the same plane as the anatomic axis of plantar flexion of the ankle.

The calf muscle was exercised to fatigue using a graded aerobic protocol with a fixed duty cycle (1 s depressed : 1 s relaxed, total 30 depressions of the foot pedal per minute). Cadence was ensured by the use of an audible beep and a visible light triggered by the pulse program of the NMR data acquisition system. One end of a nylon rope was attached to the base of the foot pedal and the other passed through a wall-mounted pulley system to a set of weights. The initial work rate was 42.3 J/min (0.71 W) and this was increased by increments of 23.5 J/min (0.39 W) each minute to fatigue. Fatigue was defined as the inability to maintain rhythm or full pedal depression with verbal

encouragement (facilitated through a Spanish speaking interpreter). An infrared optical switch monitored pedal excursion to ensure complete depression with each contraction.

The subjects were tested within 48 hrs of leaving altitude and again after 6 weeks at sea level. Measurements included absolute work, work rate and integrated work and calculations were made of mass-specific work capacity (ratio of calf work to MVC and body mass).

Hypoxia

In addition to repeating the testing procedures at the end of the deacclimation period, each subject performed the calf exercise protocol under two different inspired oxygen conditions, with each test separated by 1 hr rest. Assigning a random order, the subjects received one test breathing room air and one breathing an hypoxic gas mixture simulating the hypobaric conditions in La Raya ($FIO_2 = 14.5 \pm 0.5\%$). In the latter test the subject breathed through a mouthpiece. Expired gases were pumped for continuous monitoring to a CO_2 sensor and a CO_2 analyzer (models R-1, CD-3A, and P-61B respectively - Applied Electrochemistry AMETEK). Subjects breathed the hypoxic O_2 mixture for 20 minutes prior to starting calf exercise and the amount of CO_2 required to prevent hypocapnea ($PCO_2 < 30$ Torr) was negligible. An in line O_2 sensor continuously monitored delivered [O_2] and a pulse oximeter (N-100 Nellcor) allowed measurement of % HbO_2 saturation from the index finger (range 90-92%).

^{31}P NMR Measurements

The subject was positioned supine in a 1.0 m bore Philips Gyroscan NMR machine operating at 1.5 T. With the knee in 30° of flexion, the calf muscle of the right leg was positioned with the belly of the muscle resting centrally over a 4.0 cm diameter surface coil antennae. The upper and lower legs and pelvis were firmly secured with velcro strapping to minimize movement during exercise.

The spectral volume of ≈ 20 ml centered 2 cm into the calf, was localized using a simple depth pulse sequence [3] that included high flux signal suppression to remove signals from the surface region. Typical resting spectra had a signal to noise ratio of 8:1 for ß-ATP and displayed a clear resolution of the *J* coupling within the three ATP peaks [36]. Phase cycling and signal averaging were accommodated within 60 averages of the free induction decays (FIDs) using a repetition interval of 1 sec. The raw time domain data were processed using Fourier transformation, spline baseline correction, two orders of phase correction and a convolution difference procedure with line broadenings of 5 Hz and 15 Hz respectively. The processed spectra were curve fitted using simplex optimization routines to give peak areas (relative concentrations of Pi, PCr and ATP) and chemical shift differences (allowing calculation of intracellular pH). The relative peak areas were subsequently corrected for relaxation time differences. Data so acquired permitted the dynamic changes in energy metabolism to be monitored with a 1 min time resolution and with excellent reproducibility [36]. ^{31}P spectra were acquired over 1 min intervals during rest, during exercise to fatigue, and for 20 minutes in recovery. Each

FID acquisition was made in intervals between muscle contractions, while the limb was stationary.

Data Analysis

Standard statistical tests were used for data analysis. Whole body performance measures and calf work rates were compared using Hotelling's T^2 test while paired *t* tests were used to compare ^{31}P NMR measurements in the acclimated and deacclimated conditions at fatigue and in the first minute of recovery.

Table 4

Maximum heart rate (HR), respiratory exchange ratio (RER) at fatigue (graded cycle ergometer exercise), maximum aerobic capacity ($\dot{V}O_{2max}$) and maximum anaerobic power (modified Wingate) in the Quechua Indians before and after 6 weeks deacclimation. Δ anaerobic power is calculated as power output in the last 5 sec of the Wingate test divided by power output in the first 5 sec and expressed as a percentage. Values are means $\pm$ S.E.

Table 4 - Whole Body Work Capacity Measurements

	Acclimated	Deacclimated
HR_{max}	183±4	186±5
$RER_{fatigue}$	1.25±.04	1.27±.02
$\dot{V}O_{2max}$ (l)	3.05±.18	2.94±.24
$\dot{V}O_{2max}$ (ml/kg/min)	50.3±2.0	47.4±2.1
Absolute Anaerobic Capacity (W)	341.2±17.5	357.3±18.9
Relative Anaerobic Capacity (W/kg)	5.65±.28	5.76±.31
Anaerobic Power 0-5 sec (W)	459±35	487±11
Anaerobic Power 25-30 sec (W)	272±18	318±18
Δ Anaerobic Capacity	62.8±1.9	65.2±2.4

RESULTS

Whole body measurements on this subject group have previously been discussed in Chapter 3 and are summarized in Table 4. No significant differences ($p > 0.10$) were found between the acclimated and deacclimated conditions with respect to measurements of whole body work capacity. In general, aerobic and anaerobic capacities remained remarkably constant throughout the 6 week deacclimation period. The small decrease in $\dot{V}O_{2max}$ could be entirely explained by the slight drop in hematocrit over the same time period [37].

Calf Muscle Work Capacity in Normoxia and Hypoxia

The acclimated Andeans achieved slightly higher absolute and relative work rates at fatigue, higher relative work rates (as a function of MVC), and performed a greater integrated (total) amount of work at fatigue (Table 5). However, none of these differences were statistically significant. Considerable individual variation in the amount of change between acclimation and deacclimation, and small sample size (n=6) were factors which contributed to low statistical power. Despite breathing a hypoxic gas mixture which simulated a barometric pressure of 460 Torr ($FIO_2 = 14.5 \pm 0.5\%$), no differences were found in any of the calculations of calf work at fatigue between the conditions of normoxia and hypoxia ($p > 0.10$).

Table 5

MVC Calf Muscle = maximum voluntary contraction of the right calf muscle measured isokinetically at 60°/s plantar flexion, Work at fatigue = maximum work performed with each calf contraction at fatigue, Integrated Work = total work performed by calf muscle at fatigue, % MVC = work per contraction at fatigue as a function of the MVC of the calf muscle, and Integrated Work/MVC = the total work performed by the calf muscle at fatigue as a function of the MVC of the muscle. Values are means ± S.E.

Table 5 - Calf Muscle Work at Fatigue in Normoxia

	Acclimated	Deacclimated
MVC Calf Muscle (J)	86.5±11.1	88.2±15.3
Work (J)	12.7±1.1	11.4±1.2
Work/Weight (J/kg)	0.21±.02	0.19±.02
Integrated Work (J)	3365.0±537	2831.0±328
Integrated Work/Weight	55.6±9.0	46.7±9.4
% MVC	15.4±1.8	14.2±2.0
% MVC/Weight	0.26±.03	0.23±.03
Integrated Work/MVC	40.6±7.3	34.7±6.7

^{31}P NMR Spectroscopy Measures

[Pi]/[PCr] was used as an index of the relative rate of oxidative metabolism in the skeletal fiber. The creatine energy charge ([PCr]/[PCr] + [Cr] = {PCr}) was calculated since it is an important correlate of $\dot{V}O_2$ in mammalian skeletal muscle [9, 11] and since calculation of the phosphorylation potential would have required invasive sampling to determine [Mg^{++}] and [K^+]. Although a standard was not used for spectral intensity comparisons, the size of the total creatine pool ([PCr] + [Cr]) in resting mammalian muscles from various species is constant, and 25 mMol/g wet weight of muscle for skeletal muscles in man [11] was assumed. In addition, the ratio of [Pi]/[PCr] + [Cr] ({Pi} [11]) was calculated as another regulatory parameter correlating with $\dot{V}O_2$ in skeletal muscle. Since at steady state [PCr] and [Pi] are stoichiometrically related, the regulatory parameter {Pi} should, in principle, be a kind of mirror image of {PCr}.

Gastrocnemius muscle [Pi]/[PCr], {PCr}, and {Pi} values at rest, fatigue and at selected points in recovery in the acclimated and deacclimated Quechua are plotted for the normoxia condition in Figure 23. The deacclimated Andeans displayed significantly ($p < 0.05$) greater [Pi]/[PCr] and {Pi} and significantly ($p < 0.05$) less {PCr} values in the first minute of recovery. These figures show that the [PCr]/([PCr] + [Cr]) ratio qualitatively followed the same pattern as observed for the the [Pi]/[PCr] ratio values. [Pi]/[PCr] and {Pi} were higher at fatigue and throughout recovery after deacclimation while {PCr} was lower. In addition, pH_m

was significantly ($p < 0.10, 0.05$) lower at fatigue and in the first minute of recovery following deacclimation (Figure 23).

The effect of hypoxia on [Pi]/[PCr], {PCr}, {Pi}, and pH_m is shown for the deacclimation condition in Figure 24. The condition main effect (normoxia vs hypoxia) was not significant across the acclimation condition.

[ATP] values (the ratio of ATP to total phosphate content) fell an average of 14% at fatigue in normoxia and 20% at fatigue in hypoxia (Figure 25) but these results were not significant for group (acclimated vs deacclimated) or condition (normoxia vs hypoxia) effects.

Figure 23

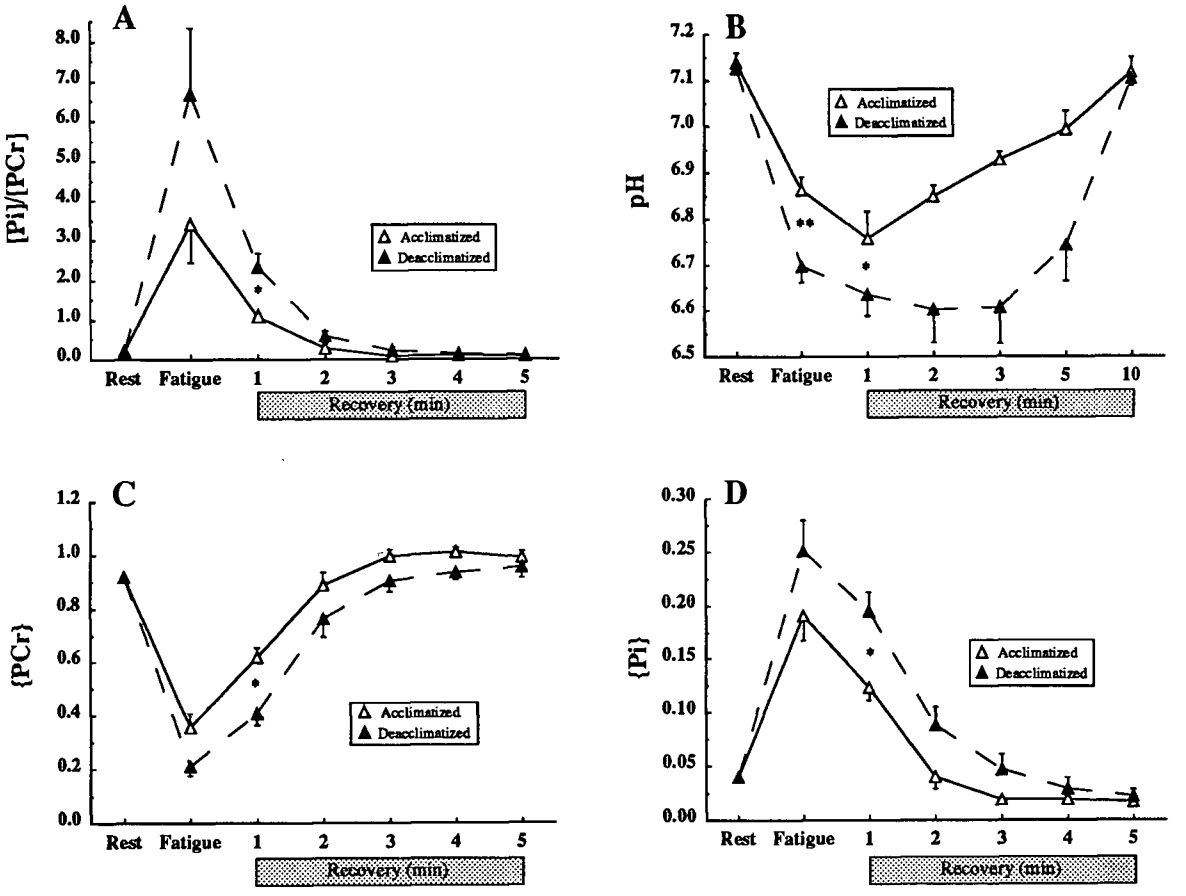
[Pi]/[PCr], pH_m, and the creatine energy charge at rest, fatigue, and through selected points in recovery in the gastrocnemius of Andean natives before and after deacclimation in the normoxia condition. Error bars indicate standard error and in some cases are within the symbols used. * indicates $p < 0.05$, ** indicates $p < 0.10$. Values are means ± S.E.

23a) Inorganic phosphate to phosphocreatine concentration ratio ([Pi/PCr]).

23b) Intracellular pH

23c) Calf muscle {PCr} at rest, fatigue and during the first five minutes of recovery from exercise. {PCr} = [PCr]/[PCr] + [Cr]. [PCr]/[Pi] + [PCr] + [ß ATP] = 23 μmol/g at rest; [PCr] + [Cr] = 25 μmol/g

23d) Calf muscle {Pi}. {Pi} = [Pi]/[PCr] + [Cr]. [Pi]/[Pi] + [PCr] + [ß ATP] = 1 μmol/g at rest; {Pi} = 1/25 = 0.04 at rest.


A
[Pi]/[PCr]
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Acclimatized
Deacclimatized
*
Rest
Fatigue
1
2
3
4
5
Recovery (min)
B
pH
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
Acclimatized
Deacclimatized
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Rest
Fatigue
1
2
3
5
10
Recovery (min)
C
{PCr}
1.2
1.0
0.8
0.6
0.4
0.2
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Acclimatized
Deacclimatized
*
Rest
Fatigue
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2
3
4
5
Recovery (min)
D
{Pi}
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Acclimatized
Deacclimatized
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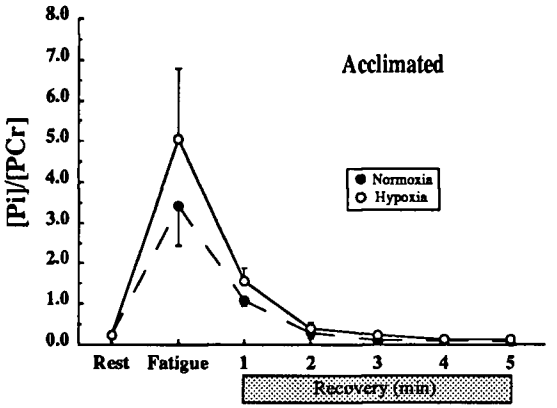
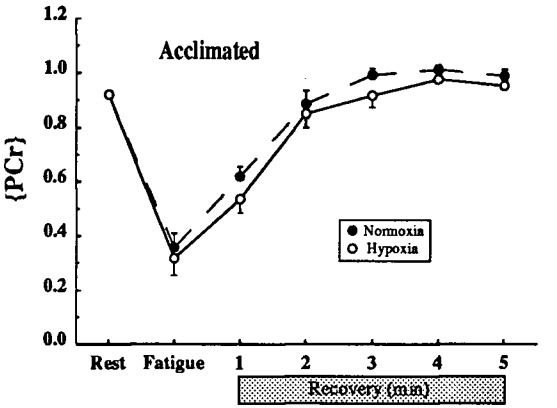
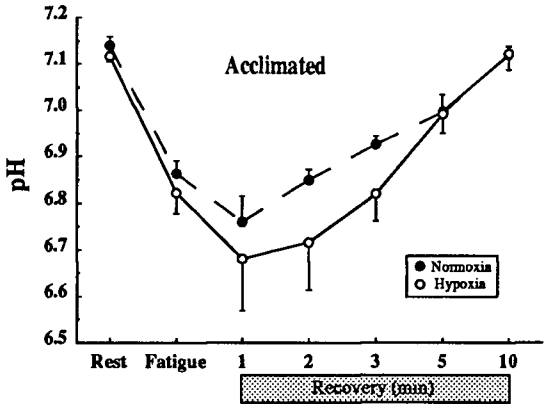
Rest
Fatigue
1
2
3
4
5
Recovery (min)

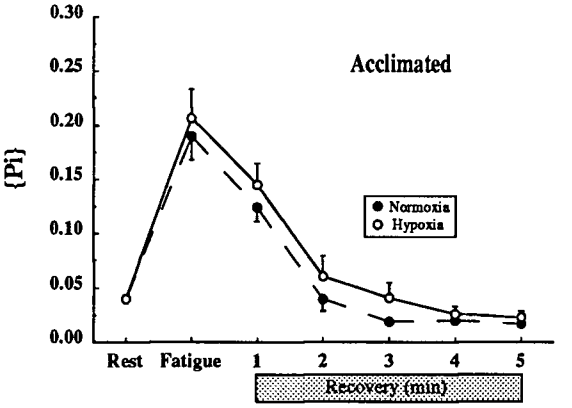
Figure 24

The effect of hypoxia on [Pi]/[PCr], pH_m, {PCr} and {Pi} during gastrocnemius exercise to fatigue and in recovery is shown for the acclimation condition. Values are means ± S.E.


Acclimated
[Pi]/[PCr]
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Normoxia
Hypoxia
Rest
Fatigue
1
2
3
4
5
Recovery (min)


Acclimated
{PCr}
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normoxia
Hypoxia
Rest
Fatigue
1
2
3
4
5
Recovery (min)


Acclimated
pH
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
Normoxia
Hypoxia
Rest
Fatigue
1
2
3
5
10
Recovery (min)


Acclimated
{Pi}
0.30
0.25
0.20
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Normoxia
Hypoxia
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Fatigue
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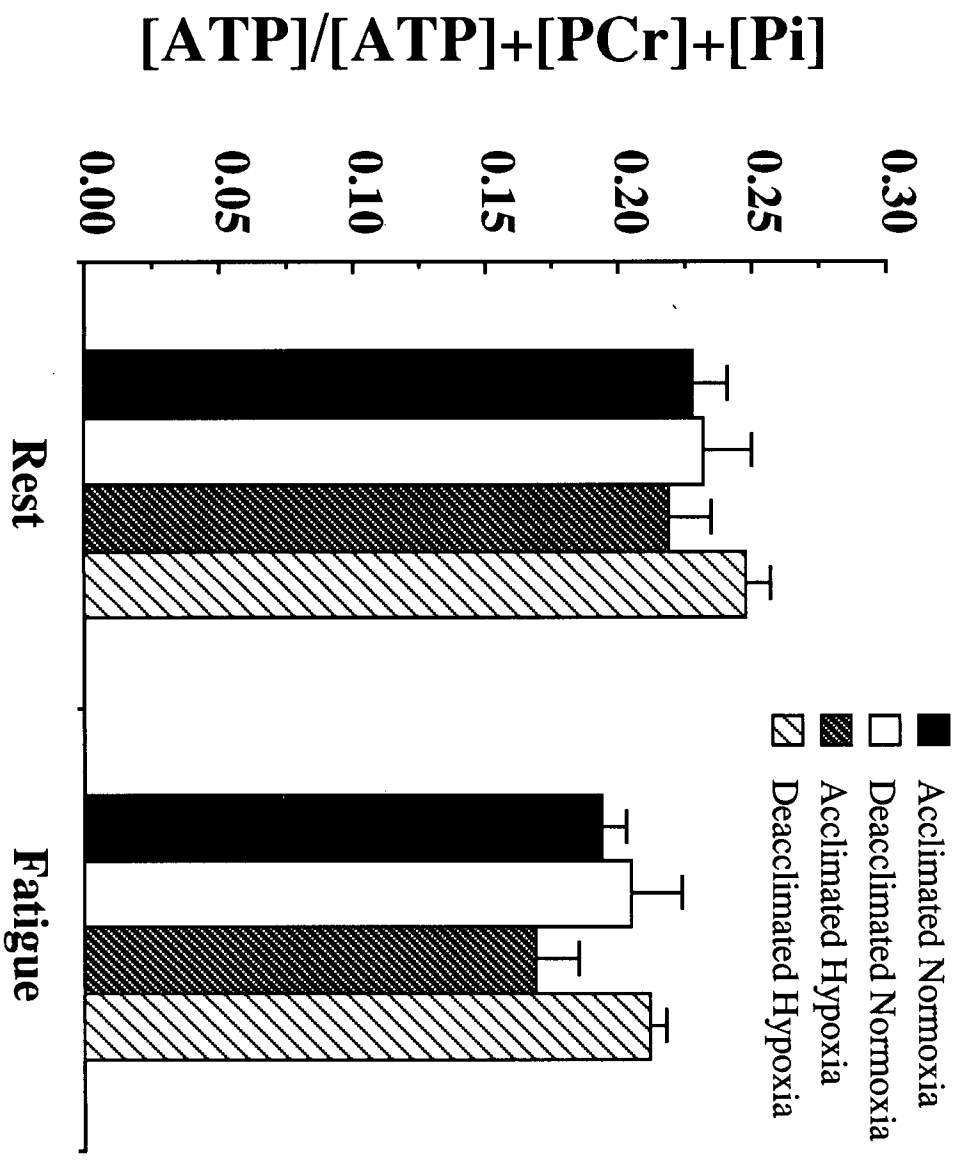
4
5
Recovery (min)

Figure 25

[ATP] at rest and fatigue in the Quechua natives before and after 6 weeks deacclimation and in conditions of normoxia and hypoxia. Values are means ± S.E.


[ATP]/[ATP]+[PCr]+[Pi]
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Rest
Fatigue
Acclimated Normoxia
Deacclimated Normoxia
Acclimated Hypoxia
Deacclimated Hypoxia

DISCUSSION

The present study documents a trend toward reduced calf muscle work capacities in Andean natives after a six week deacclimation period at sea level. These differences were not statistically significant but are consistent in both conditions of normoxia and hypoxia. However, the deacclimated Andeans displayed significantly higher [Pi]/[PCr] and {Pi}, and lower {PCr}, and pH_m in the gastrocnemius muscle at fatigue and throughout recovery following a 6 week deacclimation from 4,200 m to sea level. Since aerobic and anaerobic capacities in this same subject group did not deacclimate (Table 4), the metabolic changes reported here represent adaptations at the level of the working muscle which are independent of whole body measures of oxidative and anaerobic metabolic capacities. Chapter 3 reported lower [Pi]/[PCr] values and higher pH_m values at fatigue in acclimated Andean natives compared with trained and untrained lowlanders, and the present data implies that these functional energetic peripheral muscular adaptations deacclimate.

The drops in single muscle work capacities after six weeks are accompanied by significantly less perturbation in NMR derivable estimates of the energy status in the gastrocnemius muscle in the first minute of recovery (Figure 1). These findings are important with respect to the many anecdotal reports of phenomenal work capacities in altitude adapted natives [1, 13, 14, 33, 55, 58] which have defied explanation on the basis of rather normal whole body aerobic and anaerobic maximum work capacities [5, 7, 58]. Thus, it is not surprising, that in a recent synthesis of the available high altitude literature, Ward, Milledge and West [54] speculate

that the improved exercise performance in altitude adapted natives may largely result from tissue adaptations.

In Chapter 3, the possible mechanisms which could explain improved single muscle work rates in the Quechua were discussed. The most obvious of these would be an up scaling of the oxidative capacity of the skeletal fiber. Unfortunately, animal and human data do not clearly support this hypothesis. While absolute oxidative capacity is increased in the myocardium of the llama, alpaca and taruca [25], guinea pig, rabbit and dog [19] as well as cattle [40] indigenous to high altitude, and anaerobic/aerobic metabolic potentials are decreased [25], anaerobic potentials in the sartorius muscle of rabbits living at 4300 m [2] and in a rat hindlimb model of chronic arterial insufficiency [20] are increased while oxidative capacities are unchanged or decreased.

Just as the enzymatic data from animals exposed to chronic hypoxia are not entirely consistent, the few studies which have been reported on humans also suffer from some uncertainty. In sartorius muscle biopsies of 9 residents from Cerro de Pasco (4400 m) Reynafarje [44] found a 20% increase in the activities of three enzymes in the electron transport chain but no change in glycolytic enzyme activity compared with sea level controls from Lima. Young et al. report no change in oxidative or glycolytic enzyme activities in vastus lateralis biopsy samples following 18 days residence at 4300 m [61] while Saltin et al. [48] report lower activities of the enzymes of terminal substrate oxidation in vastus lateralis samples from sedentary man after six weeks at 3700 m. Green et al. analyzed vastus lateralis samples from the Operation Everest II experiments and found significant drops in the activities of succinic dehydrogenase, citrate

synthase and hexokinase after 5 weeks at simulated altitudes corresponding to pressures as low as 282 Torr [17]. Likewise, Boutellier et al. report a 48% reduction in the activity of succinate dehydrogenase from vastus lateralis samples taken immediately after the expedition to 5200 m on Mt. Lhotse Shar [4]. Finally, Howald et al. [30] studied 7 vastus lateralis biopsies before and after return from 5200 m and above for six weeks and found increases in the activities of glycolytic enzymes and decreases in enzyme activities of the citric acid cycle, fatty acid oxidation, ketone body utilization and respiratory chain.

Thus, if any conclusion regarding skeletal fiber oxidative capacity following altitude adaptation can be drawn, the evidence suggests a down scaling of oxidative capacity. These findings are opposite to the skeletal muscle adaptations found following endurance training at sea level [26] and would not explain the findings of increased calf muscle work capacity in altitude adapted natives [36]. Thus, not knowing the mechanisms responsible for increased work capacity after acclimation to altitude, it is difficult to speculate as to the mechanisms responsible for a deacclimation effect.

Another mechanism to consider in the explanation of increases in endurance capacity without increases in $\dot{V}O_{2max}$, following residence at altitude is increased O_2 flux rates. This type of adaptation would include factors such as a left shift in the oxyhemoglobin dissociation curve (thereby increasing the affinity of Hb for O_2 at the lung) and an increase in facilitated diffusion resulting from increased myoglobin concentration in the myocyte cytosol [44]. Morphometric and ultrastructural changes in the skeletal muscle of altitude acclimatized man tend to support this

mechanism. Reductions in mean cross-sectional fiber diameter without reductions in capillary number (leading to a functionally increased capillary to fiber ratio) have been reported by a number of investigators who have sampled the vastus lateralis muscle after 5-6 weeks at altitude [4, 27] and simulated altitude [17]. More importantly, this reduction in fiber area and increase in capillary fiber ratio has also been reported in elite mountaineers 2-12 months after returning from altitudes at or above 8,500 m [39]. These types of adaptations, even in the absence of absolute up scaling of oxidative potential, could increase the maximum flux capacity for O_2 by virtue of reducing the diffusion distance between the capillary endothelium and the myocyte. However, in contrast to the findings in animal studies [50, 51, 52] muscle oxidative capacity estimated by the volume density of total mitochondria is significantly decreased following a sojourn to altitude [27, 39]. Thus, it is unlikely that morphometric changes can be invoked to explain a deacclimation effect at the level of the working muscle.

Mechanisms other than changes in oxidative enzyme capacity, mitochondrial volume density, and capillary fiber ratios may contribute to the deacclimation of [Pi]/[PCr] seen in this study. There is accumulating evidence pointing to a dissociation between oxidative and anaerobic capacities and work capacity as described by Gleser and Vogel's [16] empirical relationship $\log(t) = A \cdot (\text{load}/\dot{V}O_{2max}) + B$ where t is the endurance time, A is the slope of the log curve, load is the relative exercise intensity, and B is the zero intercept. This relationship predicts that changes in endurance capacity will be reflected by changes in $\dot{V}O_{2max}$, and/or by the rate of glycolytic metabolism, neither of which deacclimated

in the Quechua [24, 43]. $\dot{V}O_{2max}$ does not increase as a result of acclimation [12, 53, 57] while endurance capacity does. Maher et al. report a 45% increase in cycling endurance time at 75% $\dot{V}O_{2max}$ between day 2 and day 12 at 4,300 m [35] while Horstman et al. found a 59% increase in treadmill running time to exhaustion at 85% $\dot{V}O_{2max}$ between day 1 and day 16 at the same altitude [28]. This dissociation between $\dot{V}O_{2max}$ and endurance capacity is also noted by Hoppeler et al. [27] who report a dissociation in the linear relationship between mitochondrial volume density and $\dot{V}O_{2max}$ in lowlanders after a sojourn to altitude. Together, these data imply that the peripheral and central determinants of aerobic work capacity may change independently following exposure to chronic hypoxia. Improvements in endurance capacity without increases in $\dot{V}O_{2max}$ following single muscle training at low altitudes have been reported for dynamic work [38] and chronic electrical stimulation of skeletal muscle [8]. In the former case, following training of the forearm muscles, a lower [Pi]/[PCr] is found at a standardized submaximal workload unaccompanied by increases in blood flow or $\dot{V}O_{2max}$ while in the latter, chronic electrical stimulation of the latissimus dorsi muscle results in a smaller drop in [PCr] for any given stimulation intensity.

Since adaptations in muscular work may occur independent of changes in whole body maximal oxidative capacity, it is postulated that the most likely explanation for the increase in [Pi]/[PCr] at fatigue and throughout recovery following a 6 week period of deacclimation, is a shift to looser coupling between ATP supply and ATP demand (see Chapter 3). As a result, various parameters related to the energy status of skeletal muscle

(Pi/PCr, PCr/(PCr + Cr), Pi/(PCr + Cr), pHm) are more perturbed at fatigue and tend to recover more slowly.

Although exercise blood lactate values in the Quechua did not show any deacclimation after 6 weeks at sea level [24, 43], the present study reports significant increases in $[H^+]$ in the gastrocnemius muscle at fatigue and through the first 10 min of recovery. The possible explanations for this degree of tissue acidification include: i) an increase in glycolytic activation without a corresponding increase in lactate efflux, and ii) a reduction in buffering capacity. Connett et al. [10] report a hyperbolic relationship between lactate efflux rates and tissue [lactate] in the autoperfused pure red muscle (dog gracilis). In this preparation, the steepest rise in lactate efflux occurs between 2 and 12 mM tissue [lactate] with virtually no increase in the efflux rate above $\approx$ 12 mM tissue [lactate], suggesting that the mechanism becomes saturated.

Higher buffer capacities and carnosine levels have been reported in sprinters and rowers compared with marathoners and untrained subjects [42, 46] and the buffering capacity of skeletal muscle has been shown to increase following sprint training [49]. Unfortunately no published data is available to confirm or refute this explanation of the deacclimation changes noted in the Quechua subjects.

CONCLUSIONS

The purpose of this investigation was to determine if certain characteristics observed in the calf muscle of Quechua Indians [36] were constrained or adaptable within a six week time window. This window was chosen because it is known to be sufficient for significant changes in muscle

metabolism to occur in lowlanders as they acclimate to altitude. The characteristics that were evaluated were the values at fatigue for work rate, [Pi]/[PCr], and pH_m, which were respectively higher, lower and higher than corresponding measurements made in trained and untrained lowlanders [36].

The Quechua have higher metabolic efficiencies and lower blood lactate (glycolytic capacities) during fatiguing exercise [24], larger static pulmonary volumes and flow rates [31, 32], and rather low measures of anaerobic capacities [43]. By and large, these adaptations are insensitive to a six week deacclimation period and are tentatively assumed to be genetically or developmentally fixed. In those examples where a measurable change occurred over the six week period, simultaneous adaptation in at least two related variables produced no net change. For example, removal of the hypoxic stimulus of altitude (barometric pressure 460 mm Hg) leads to reduced Hb and slight increases in stroke volume and cardiac output, with the end result being maintained or elevated O_2 delivery [37]. These data imply an alteration in the balance of central mechanisms used to maintain O_2 delivery to the tissues presumably on the basis of the conservation principle [34].

Recognizing that whole body measures represent the "net effect" of many simultaneous adaptations and deadaptations, it is not surprising that improvements in aerobic and anaerobic capacity which result from training at sea level cannot be used as the sole criteria for improvements in work capacity which result from residence at high altitude. Likewise, it is not altogether surprising that a deacclimation effect was found in the cellular measurement of high energy phosphate metabolites when other measures of

whole body work capacity did not deacclimate, since changes in $\dot{V}O_{2max}$ do not always accompany improvements in work capacity.

These new NMR data support the conclusion that significant biochemical and physiological adaptations are at the 'heart' of the frequent claims that Andean natives perform work more capably in their normal environment than do Caucasian lowlanders and moreover, these functional adaptations would appear to deacclimate.

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General Summary and Conclusions

The purposes of this thesis were twofold, namely: i) to develop experimental techniques and hardware which would allow lower limb exercise within the magnetic and spatial constraints of the NMR working environment, and ii) to apply these techniques to the study of skeletal muscle metabolism in subject groups separated by distinct differences in their training status and by their exposure to chronic hypobaric hypoxia. As a general conclusion, both of these goals were realized and the following conclusions arise from this study.

Electrical stimulation has the advantage of minimizing or eliminating limb movement, one of the major causes of poor spectral resolution in NMR studies of exercising muscle. Moreover, although the rectus femoris was the only muscle studied in this thesis using electrical stimulation, the use of RF surface coils and depth pulse localization techniques provide the potential to study a wide range of skeletal muscle at various sites in the body. In addition, the potential confounding variable of volitional effort during exercise to fatigue is removed when electrical stimulation is used and the exercise intensity can be graded and precisely controlled.

The limitations associated with electrical stimulation include subject intolerance and difficulty in precisely duplicating electrode and RF coil placement between subjects. In addition, unless a strain guage, load cell, or EMG is used to monitor force production, accurate calculation of the work performed is very difficult. Even then, uncertainty still exists as to the

exact volume of metabolically active muscle with respect to the RF coil interrogation volume and its specific work rate relative to the total mass of the muscle. Finally, it must be recognized that considerable physiological differences exist between electrically stimulated skeletal muscle and normal voluntary exercise with respect to blood flow and fiber recruitment.

Thus, it was concluded that the electrical simulation model is best suited for within-subject experimental designs and is particularly useful for experiments which utilize an intervention condition within a single subject. For this reason, not to mention the considerable cost of the NMR technique, it was felt that the group comparisons which were planned as part of this thesis were best made by developing a dynamic exercise device to allow standardization of work rates between subject groups.

Dynamic exercise models are necessarily limited by the spatial and magnetic constraints of the NMR working environment. One of the major thrusts in this thesis was to develop hardware to allow the study of lower limb musculature since it is much better adapted for rhythmic work (locomotion at the very least). The calf ergometer used in this thesis was found to be satisfactory for providing a graded exercise protocol to fatigue using a relatively large muscle of the lower weight bearing limb.

This approach is a very useful addition to the vast majority of human NMR experiments reported in the literature. These latter reports almost exclusively use a non-continuous work protocol with a particularly long rest duration (typically nine times the work duration) and at very high work rates. Thus, these measurements largely reflect recovery from intense work rather than the monitoring of continuous graded work. In

addition, most of these experiments were performed using forearm muscles at work rates far greater than those which would be sustainable in a physiological graded protocol. By comparison, the calf ergometer used in these studies provided graded work rates and fatigue occurred at exercise intensities far below those reported with forearm muscles.

The results of the experiments in Chapters 3 and 4 show that, compared with sedentary controls, endurance trained athletes are able to sustain higher calf muscle work rates with significantly less perturbation in pH_m and in the indeces of the phosphorylation potential derivable from NMR measurement of [Pi], [PCr] and [ATP]. Although power trained athletes are able to perform equal work to that of endurance trained athletes, they sustain considerably greater perturbation in the above parameters at any given work rate. The Andean natives are able to sustain calf work rates equivalent to the endurance trained athletes although they possess significantly lower $\dot{V}O_{2max}$ values and anaerobic capacities, and they are able to do this with minimal perturbation of the above parameters (equal to the changes in the endurance trained group).

The trend in the Andean group toward less calf work at fatigue following a six week period of deacclimation to sea level was not significant. However, significant differences were found following deacclimation in the high energy phosphate metabolites and intracellular pH. In particular, [Pi]/[PCr] and pH_m showed greater perturbation for a given work rate after six weeks at sea level.

With respect to the discussion of loose and tight energy coupling systems in Chapter 1, taken together, the above data support the hypothesis of tighter

coupling of the ATP supply-demand in endurance trained individuals and in Quechua natives. Of considerable importance is the fact that exercise training and hypobaric hypoxia are two very different environmental stimuli; the former is accompanied by an elevated scope for physical work (greater O_2 availability to the working muscle) while the latter is associated with a reduced metabolic scope for work (reduced O_2 availability to the working muscle). Thus, the data in this thesis imply a dissociation between whole body measures of aerobic capacity and the ability to perform calf muscle work.

The completion of this study and consideration of future experiments allows reflection as to the appropriateness of the methodologies used and the development of new experimental designs to answer questions which have arisen from the thesis. Since single muscle work does not place significant competitive stress on the central circulatory and pulmonary systems, (little difference was found in work capacities or NMR measures between the conditions of normoxia and hypoxia) future experimental designs will require protocols which elevate the whole body metabolic rate substantially. These experiments will require exercise devices within a whole body NMR machine or an external apparatus with a limb in the machine so that cardiac output and ventilation can reach maximum values. Whole body graded exercise to fatigue, simultaneous with monitoring of a single muscle which is working at a variety of fixed percentages of its maximum capacity, is presently within the realm of experimental possibility.

Continued development will be required to improve the technique of muscle force production measurement relative to the volume of contracting

muscle tissue. In the case of electrical stimulation, EMG electrodes placed in all muscles within the stimulation field, together with tension monitoring, muscle imaging, RF coil design considerations, and depth pulse localization will all be important for reliability. In the case of dynamic muscular exercise, accurate calculation of the muscle mass performing the work (cross-sectional area, volume) will be important for the validity of conclusions comparing subject groups. In addition, steady state experiments which allow the muscle to work at a given percentage of its maximum sustainable metabolic rate will provide important comparative information regarding muscle adaptation in various normal or diseased groups.

POSITIONS HELD

Medical Practice

- 1977-78, Department of Health and Welfare Canada, Inuvik General Hospital, Inuvik, N.W.T.
- 1978-81, Active Staff, Rocky Mountain House General Hospital, Rocky Mountain House, Alta.
- Emergency Physician, 1981-84, Active Staff, Calgary General Hospital, Calgary, Alta.
- Medical Officer (part-time), 1984-90, Ministry of the Attorney General, Province of British Columbia, Vancouver, B.C.
- Sports Medicine Consultant, 1985-present, Division of Sports Medicine, U.B.C.; Medical Staff, Health Sciences Center Hospital, U.B.C.

Research Positions

- Post-doctoral Research Fellow, 1985-88, Faculty of Medicine, U.B.C.
- Research Co-director Department of Family Practice, 1989, U.B.C.

Professional Services

- Medical Services Coordinator, Western Canada Summer Games, Calgary, 1983
- Physician, Canadian National Alpine Ski Team, 1986-present
- Medical Director, Vancouver Sun Run 10k race, 1985-1988
- Team Physician, Canadian Olympic Hockey Team, 1983-88.
- Medical Officer, 1988 Canadian Olympic Team, 1988 Winter Olympics, Calgary
- Team Physician, Vancouver Canucks Hockey Team, 1989-present

PUBLICATIONS

Books

Sport Medicine Manual. International Olympic Committee, Lausanne, 1990.

Refereed Publications

Published:

1. Tomm, K., J. Williams and G.O. Matheson. Understanding the dying process. Can. Fam. Phys., 22:1420, 1976.

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ACADEMIC OR PROFESSIONAL AWARDS AND DISTINCTIONS

- Syntex Pharmaceutical Award for Service in Sports Medicine, 1983
- University of British Columbia, Summer Research Graduate Fellowship, 1985
- Rick Hansen Sports Medicine Research Award, B.C. Medical Association, 1987
- British Columbia Post-Secondary Scholarship, 1987
- Section Editor, Canadian Journal of Sport Sciences, 1986-present
- Fellow, Alberta Heritage Foundation for Medical Research, 1984-88
- Fellow, Medical Research Council of Canada, 1988-present
- Founding Editor, Clinical Journal of Sport Medicine, 1989-present