Effects of a Short Work/Shorter Rest Intermittent Exercise on Muscle
Metabolic Status, VO\textsubscript{2}, Hemoglobin Saturation and Performance

by

Glen Robert Belfry

A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
Graduate Department of Exercise Science
University of Toronto

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Effects of a Short Work/Shorter Rest Intermittent Exercise on Muscle Metabolic Status, VO₂, Hemoglobin Saturation*and Performance, PhD, 2010, Glen Robert Belfry, Exercise Science, University of Toronto.

Abstract

Many sports require short duration work periods and short duration rest periods (INT). This dissertation examines the engagement of aerobic and anaerobic energy systems in acute exercise with brief (10 seconds) work and recovery (5 seconds) and the effect of chronic exposure on those systems. The differences between INT and continuous exercise (CONT) of deoxygenated hemoglobin saturation (ΔHHb), the pattern of breath-by-oxygen uptake (VO₂) and muscle metabolic status, including, [Pᵢ], [Pᵢ]/[PCr], and [H⁺] were studied in young healthy adults. The physiological and performance responses to a four week, 12 session cycle ergometer training regime of the CONT and INT protocols were observed. Fluctuations in VO₂, ΔHHb, [PCr], and [H⁺] were observed within the work : recovery duty cycle of INT. Fluctuations of VO₂, ΔHHb, and [PCr] may be a function of the priming effects of previous high intensity exercise, inhibition at the locus of metabolic control, and elevated blood flow over the recovery-work transitions. Fluctuations in oxidative phosphorylation were associated with concurrent fluctuations in [PCr]. [Pi] and [PCr] were greater in INT vs CONT, whereas no differences were observed for [H⁺] between conditions. Reduced [PCr] may be the most appropriate indicator of metabolic stress, not increased [Pi] or [H⁺]. Similar improvements in aerobic power were observed for CONT and INT training, whereas anaerobic performance was enhanced post-INT training. Collectively the findings indicate that a unique INT exercise protocol facilitates increased muscle blood flow versus continuous exercise, the temporal association of muscle metabolic status, ΔHHb, and pulmonary VO₂ with work rate, the apt use of [PCr] as a proxy for metabolic stress, and when this unique INT bout is utilized as a training protocol, adaptations in both aerobic and anaerobic metabolism will occur.
Acknowledgements

The studies in this thesis would not have been possible without the help of a number of individuals. First I would like to thank my supervisor Dr. Scott Thomas, for his guidance, availability, patience, and support, and for providing the flexibility to set my own research path.

I also greatly appreciate the timely support and encouragement from Dr Cathy Amara. I thank Dr. Jack Goodman for his honest and poignant feedback and Dr. Tom Overend for his last minute editorial work. Dr. Terry Thompson and Dr. John Kowalchuk, although not on my committees, supported my data collection in their labs at St. Joseph’s Health Care London MRS unit and the breath-by-breath and NIRS unit in the Arthur and Sonia Labatt Health Science Building at the University of Western Ontario. In particular I would like thank Dr. Don Paterson who has given me access to the labs at the Canadian Centre for Activity and Aging and gave unselfishly of his time to comment on many drafts and questions.

I sincerely thank Dr. Alan Salmoni and Dr. Jim Weese for the opportunity to take an education leave to assist in my doctoral studies.

I would also like to thank PhD candidates Juan Murias and Matt Spencer for including me in their discussions of “Science”.

In conclusion I would like to express my unending love to my wife Shannon whose faith, in not only me but in the good Lord, enabled me to bring this dream to fruition, and to my daughters Grace and Emily, who could bring a smile to my face and gladness to my heart no matter what the Day had delivered.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$^{31}\text{P-MRS}$</td>
<td>31-phosphorous magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>10sW</td>
<td>ten seconds work</td>
</tr>
<tr>
<td>Δ</td>
<td>change</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>CONT</td>
<td>continuous constant load exercise</td>
</tr>
<tr>
<td>Cr</td>
<td>creatine</td>
</tr>
<tr>
<td>CK</td>
<td>creatine kinase</td>
</tr>
<tr>
<td>$[\text{H}^+]$</td>
<td>hydrogen ion concentration</td>
</tr>
<tr>
<td>Hb</td>
<td>haemoglobin</td>
</tr>
<tr>
<td>Hbtot</td>
<td>total haemoglobin myoglobin</td>
</tr>
<tr>
<td>ΔHHb</td>
<td>deoxy-haemoglobin myoglobin</td>
</tr>
<tr>
<td>HIT</td>
<td>high intensity training</td>
</tr>
<tr>
<td>INT 1</td>
<td>intermittent exercise consisting repeated cycles of 10s work, 5s light exercise</td>
</tr>
<tr>
<td>INT 2</td>
<td>intermittent exercise consisting repeated cycles of 10s work, 5s moderate exercise</td>
</tr>
<tr>
<td>L</td>
<td>litres</td>
</tr>
<tr>
<td>l</td>
<td>litres</td>
</tr>
<tr>
<td>Mb</td>
<td>myoglobin</td>
</tr>
<tr>
<td>MDP</td>
<td>methylene diphosphate</td>
</tr>
<tr>
<td>NIRS</td>
<td>near-infrared spectroscopy analysis</td>
</tr>
<tr>
<td>$\text{O}_2$</td>
<td>oxygen</td>
</tr>
<tr>
<td>P</td>
<td>probability</td>
</tr>
<tr>
<td>$[\text{PCr}]$</td>
<td>phosphocreatine concentration</td>
</tr>
<tr>
<td>$[\text{P}_i]$</td>
<td>inorganic phosphate concentration</td>
</tr>
<tr>
<td>$[\text{P}_i]/[\text{PCr}]$</td>
<td>inorganic phosphate phosphocreatine ratio</td>
</tr>
<tr>
<td>R</td>
<td>recovery</td>
</tr>
<tr>
<td>RM</td>
<td>repeated measures</td>
</tr>
<tr>
<td>S</td>
<td>seconds</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
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</tbody>
</table>
SD standard deviation
SID strong ion difference
T1 longitudinal relaxation
VeT ventilatory threshold
VO$_2$ oxygen uptake
VO$_{2\text{max}}$ maximal oxygen uptake
VO$_{2p}$ pulmonary oxygen uptake
vs versus
W/R work recovery cycles during interval exercise
W watts
$\approx$ approximately
$<$ less than
$>$ greater than
Chapter 1: Introduction, Review of Literature and Overview

Introduction

Numerous sports demand short duration work periods interspersed with short duration recovery periods during training and performance. Observations incorporating time motion analysis of track pursuit cycling\textsuperscript{42}, rugby\textsuperscript{26} and ballet\textsuperscript{55} have been performed. A variety of durations of short work and short recovery periods, of various intensities during training and competition, have been observed. Furthermore, competitive swimming pool facilities of different sizes (25 m vs 50 m) will change the duration of work and recovery periods during competition and training. The time gliding off the wall after each turn reduces the distance swum while also providing a short period of recovery. Swimming in a 25 m pool gives four opportunities for these short rest periods every 100m, while in a 50 m pool there are only two. The cumulative effect of these periods of relative inactivity while gliding after a push off the wall, reduces the total volume of work performed and increases the duration for physiologic recovery.

Moreover, swim training sessions regularly use short duration recovery intervals (5 s) for variety and to enable greater swimming speeds to be maintained. Little is known about the acute physiologic response to short periods of high intensity work (10 s) followed by shorter periods of recovery (5 s) versus continuous high intensity exercise. The following thesis will study the acute physiological responses of a 10 s work : 5 s recovery intermittent exercise bout, followed by the quantification of the physiological and performance adaptations to a high intensity training program, using a comparable short work: shorter rest intermittent and continuous training protocol.
Review of Literature

Acute Physiological Response to Short Work and Rest Interval Exercise Bouts

The initial work done on the acute response to repeated short duration high intensity work with short rest was performed by Astrand et al.³ (1960). This group studied the effect of these rest intervals on respiration and circulation, pursuant to a similar supra threshold work and observed and compared the effects of an intermittent work : recovery, 30 s work : 30 s recovery protocol and a 3 min work : 3 min recovery over a period of 60 min. The VO₂ max of their single subject was 4.6 L/min. They observed work : recovery VO₂’s for the 30 s : 30 s protocol that were 37% (2.9L/minO₂) lower during the work, and 50% higher (2.3L/minO₂) during the recovery than the 3 min : 3 min protocol. This shorter work and recovery protocol elicited a lower peak VO₂, a reflection of reduced metabolic stress. The difference in VO₂ between the work and recovery periods of the 30 s : 30 s protocol was 21%, whereas the VO₂ during Astrand et al.’s 3 min work : 3 min recovery protocol did reach maximum (4.6L/min O₂) during each work period but then dropped ≈78% to 1.0L/min O₂ during the 3 min rest period. This demonstrated a more constant VO₂ during the intermittent exercise.

Blood lactates, at the conclusion of a work period, of 12mmol/L (3 min : 3 min) and 2mmol/L (30 s : 30 s), 20 min after the work session had begun, were also observed providing a further indication of the increased metabolic stress of the longer work periods of the 3 min work : 3 min recovery protocol relative to the 30 s work : 30 s recovery protocol. Astrand et al. hypothesized that with the combination of a shorter work than rest period (10 s work : 20 s rest) of intermittent exercise, aerobic conditions would prevail as actual O₂ transport and stored intramuscular O₂ during the recovery period would be sufficient to maintain the required O₂ uptake. If this was not the case there would have been an accumulation of lactate, and fatigue would occur before the completion of the 30 min exercise bout. The lactate concentrations corresponding to the 10 s : 20 s intermittent protocol at 400w of work (close to the work rate at
VO$_{2\text{max}}$) were low (2mmol/l) and the VO$_2$ during the work periods was 2.8 L/min or 0.47L per 10 s. To manage the 400w load, an O$_2$ supply of 5.6L/min is required, or 0.91L per 10 s period. Consequently a deficit of .43 L O$_2$ per 10 s work period occurred but was repaid during the recovery period. The limited subject population observed make their conclusions speculative.

Investigators then turned to the 10 s work : 5 s recovery protocol for a 30 min exercise session but with only two subjects$^{12}$. The workload performed (411W) was similar to the peak work rate achieved on an incremental VO$_{2\text{max}}$ test. This work rate elicited fatigue during continuous exercise after $\approx$ 180s. During the 10 s : 5 s protocol, the subjects were at or near their VO$_{2\text{max}}$ during the work periods and then experienced a decrease of $\approx$18% in VO$_2$ during the rest period. They also studied work : recovery durations of 5 s : 5 s, 10 s : 10 s, and 15 s and 15 s. Again, with only two subjects, it is difficult to make firm conclusions from this study.

Increasing the duration of work will decrease maximal power output$^{40}$, and increasing the recovery period will reduce average VO$_2$$^{44}$. Subsequently the aforementioned 10 s work : 5 s recovery (INT 1) intermittent exercise will enable a high and constant VO$_2$ to be maintained while performing heavy intensity power outputs during the 10 s work period$^{16, 45}$, and as such, was selected for this current research.

An additional intermittent exercise protocol utilizing the same heavy intensity 10 s work rate, but using a moderate intensity work rate during the 5 s recovery period (INT 2), which elevates average VO$_2$, was also performed. This INT 2 protocol results in a VO$_2$ profile that most resembles continuous constant load exercise, while still incorporating the large fluctuations in work rate between the work and recovery periods.

The necessary increase of the subject population to facilitate statistical power was also implemented.

In the current study cycle ergometry was utilized to collect gas exchange data from breath-by-breath analysis and muscle deoxygenation was collected from the INT 1, INT 2, and
CONT exercise bouts. Due to technical constraints between the $^{31}$P-MRS unit and the plantar flexion ergometer interface, only the INT 1 and continuous protocols were performed in this unit.

**Bioenergetics of Substrate and Oxidative Phosphorylation During Work and Recovery**

The fluctuations of short high intensity work (10 s) and shorter recovery (5 s) intermittent exercise (INT) present bioenergetic challenges to the exercising muscles which include repeated phosphorylation of ADP to perform work and for repayment of the O$_2$ deficit during recovery. If a moderate intensity work rate is superimposed on the 5 s recovery period, phosphorylation will also contribute to the ATP demand for the moderate intensity work rate during recovery. The ATP required during the work and recovery of INT exercise originates from the creatine kinase reaction, and glycolytic and oxidative phosphorylation. The integrated nature of metabolism is demonstrated during this intermittent exercise.

There has been limited study of the acute and chronic metabolic responses to these particular INT exercise bouts vs CONT exercise. However, there has been a body of work that has gained substantial knowledge into the acute metabolic responses related to work and recovery and the adaptive responses to various intermittent and continuous exercise training protocols.

The bioenergetics of the cell during exercise and recovery are summarized below.

ATP breakdown (Equ. 1) leads to energy release for actin-myosin interactions to elicit muscle shortening, and for the energy required to phosphorylate ADP and Cr during recovery.

*Equation 1.*

\[
(ATP + H_2O \rightarrow ADP + Pi + H^+)^{17}
\]

There is no appreciable change during the first seconds of high intensity exercise in ATP despite high energy use, as there is a virtually simultaneous reduction in PCr which maintains ATP levels via the creatine kinase (CK) reaction$^{32}$ (Equ.2).
Equation 2.

$$\text{PCr} + \text{ADP} + H^+ \xleftrightarrow{\text{CK}} \text{Cr} + \text{ATP}$$

Control of oxidative metabolism has been linked to this creatine kinase reaction. Creatine kinase knockout mice, under electrically induced contractions, elicited much faster changes in muscle $\text{PO}_2$ and accelerated $\text{VO}_2$ at the onset of exercise vs controls, indicative of the tight coupling between oxidative metabolism and the creatine kinase reaction (Fig.1.1).

Oxidative phosphorylation which tries to maintain this $[\text{PCr}]$ is represented by the following equation (Equ. 3.):

Equation 3.

$$\text{NADH} + 0.5 \text{O}_2 + H^+ + 3\text{ADP} + 3\text{Pi} \rightarrow \text{NAD}^+ + \text{H}_2\text{O} + 3\text{ATP}^{17,34}$$

At the onset of work and/or during work that requires a greater quantity of ATP than can be phosphorylated oxidatively, $[\text{ATP}]$ can be added through substrate phosphorylation either
through the afore mentioned creatine kinase reaction and/or anaerobic glycolytic phosphorylation in the cytosol (Equ. 4):

\[
\text{Equation 4.}
\]

\[
\text{glucose + 2ATP + 4ADP + 2Pi +2NAD}^+ \rightarrow 2\text{pyruvate + 2ADP + 4ATP + 2NADH + 2H}^+ + 2\text{H}_2\text{O}
\]

It is relevant to note that anaerobic glycolytic phosphorylation has been suggested to contribute immediately post exercise for recovery of ATP as well\textsuperscript{13, 24, 48}.

Increases in [Pi], [H\textsuperscript{+}] and decreased [PCr] from ATP hydrolysis have been associated with heavy intensity constant load continuous exercise leading to increased metabolic stress and fatigue\textsuperscript{14, 18, 19, 23, 46, 49}. There is ambiguity in the literature as to which of these variables has the strongest association with increased metabolic stress\textsuperscript{15}. The comparison of this INT exercise to a CONT exercise performed at a similar work rate as the 10 s work period of INT may differentiate these metabolites as the differences in average power output may require different handling of [Pi], [H\textsuperscript{+}] and [PCr].

Observations utilizing \textsuperscript{31}P magnetic resonance spectroscopy (\textsuperscript{31}P-MRS) enables the researcher to quantify ATP breakdown by observing H\textsuperscript{+}, indicative of anaerobic glycolytic phosphorylation and the creatine kinase reaction, Pi from all ATP breakdown, and oxidative phosphorylation via PCr during these CONT and INT exercise protocols.

\textit{\textsuperscript{31}P-NMR Spectroscopy}

\textsuperscript{31}P-NMR Spectroscopy was utilized to observe changes in high energy phosphates and acid-base balance. The following presents the theoretical base behind this mode of data collection.

Nuclei with an odd atomic number have "nuclear spin" (in a similar fashion to the spin of electrons), this includes \textsuperscript{31}P. The overall spin of the charged nucleus generates a magnetic
dipole along the spin axis, and the intrinsic magnitude of this dipole is a fundamental nuclear property called the nuclear magnetic moment. In the absence of a magnetic field, these are randomly oriented but when a magnetic field is applied they line up parallel to the applied field, either spin aligned or spin opposed. In quantum mechanical terms, the nuclear magnetic moment of a nucleus can align with an externally applied magnetic field of strength either reinforcing or opposing this field of strength. The energetically preferred orientation has the magnetic moment aligned parallel with the applied field, whereas the higher energy anti-parallel orientation is of lower magnitude. The more highly populated state is the lower energy spin state.

A schematic representation of these arrangements are shown below (Fig 1.2)

![Figure 1.2.](image)

In NMR, electromagnetic radiation (EM) is used to "flip" the alignment of nuclear spins from the low energy spin aligned state to the higher energy spin opposed state. The energy required for this transition depends on the strength of the applied magnetic but is small and corresponds to the radio frequency range of the EM spectrum. The energy required for the spin-flip depends on the magnetic field strength at the nucleus. With no applied field, there is no energy difference between the spin states, but as the field increases so does the separation of energies of the spin states and therefore so does the frequency required to cause the spin-flip. The rotational axis of the targeted spinning nucleus cannot be orientated exactly parallel (or anti-parallel) with the direction of the applied field but must precess about this field at an angle, called resonance (Fig 1.3.).
Signal intensity is determined by the number of nuclei involved as the radio frequency signal received is proportional to the number of nuclei responding to this signal. The recorded changes in EM are computed by Fourier transformation and signal intensity is plotted against frequency or chemical shift to give the $^{31}$P-NMR spectra (Figure 1.4).

The phosphates Pi and PCr exist in a highly dissociated state including HPO$_4$ and H$_2$PO$_4$. A change in pH of the cellular environment will change the quantities of each and the
change in the frequency of these signals will cause a shift in position between the Pi and PCr peaks of the NMR spectra. This allows calculations of pH or H⁺ from this chemical shift.

The NMR behaviour of ³¹P has been utilized to determine the quantities of phosphorous containing compounds ATP, PCr and Pi, while the chemical shift between Pi and PCr with changes in cellular pH is used to calculate [H⁺] within the muscle during rest and exercise⁷.

**Near Infra Red Spectroscopy**

Near-Infrared Spectroscopy (NIRS) is utilized to observe the changes in deoxyhemoglobin saturation (ΔHHb). The ΔHHb signal is a function of the balance between O₂ delivery and O₂ utilization from the muscle being investigated. The following describes the theoretical basis of this technology.

The electromagnetic spectrum refers to the diverse collection of radiant energy, from cosmic rays to X-rays to visible light to microwaves, each of which can be considered as a wave or particle traveling at the speed of light. These waves differ from each other in length and frequency. Infrared refers to that part of the electromagnetic spectrum between the visible and microwave regions. During infrared spectroscopy, an organic molecule, such as oxyhemoglobin, is exposed to infrared radiation. This infrared radiation is absorbed by the organic molecule and converted into the energy of molecular vibration. When the radiant energy matches the energy of a specific molecular vibration, absorption occurs reducing the light returning to the spectrophotometer. This form of spectrophotometry provides continuous, noninvasive monitoring of the relative concentration changes in oxy- (O₂Hb), deoxy- (ΔHHb), and total Hb (Hbtot). The NIRS signals from skeletal muscle include signal from oxy- and deoxy-myoglobin in addition to those of oxy- and deoxy-haemoglobin because, until recently, the NIR spectra of the oxy- and deoxy- forms of the two molecules have been indistinguishable. In even red skeletal muscle, however, the majority of the NIRS O₂ store signal is still derived from oxy- and deoxy-
haemoglobin. More recently Amara et al. have differentiated between the Hb and Mb signal and have observed not only slightly faster kinetics of Hb versus Mb but a lower relative peak concentration of Mb at rest, and as such the combined ΔHHb signal from the NIRS spectra would give a consistent but lower relative concentration during the exercise perturbations of the INT exercise utilized in this thesis. The differentiation between the ΔHHb and Mb signal in the present study has not been elucidated and as such, the total ΔHHb signal has been interpreted to include both the Mb and ΔHHb signal.

Beer's law states that the absorption of light or in this case the intensity of molecular resonance is related to the properties of the material through which the near-infrared light is traveling and the path length between the optodes emitting the incident light and receiving the transmitted light.

The specific NIRS equipment utilized in this thesis has one fiber-optic bundle carrying the NIR light produced by the laser diodes to the tissue of interest, whereas a second fiber-optic bundle returned the transmitted light from the tissue to a photon detector (photomultiplier tube) in the spectrometer. The intensity of incident and transmitted light was recorded continuously at 2 Hz and, along with the relevant specific extinction coefficients (absorption at a given wavelength) and optical path length, used for online estimation and display of the concentration changes from the resting baseline of ΔHHb. The raw attenuation signals (in optical density units) are transferred to a computer and stored for further analysis.

*Breath by breath Gas-exchange Measurements*

Determination of VO₂, VCO₂ and ventilation rates were determined by the following: the inspired and expired flow rates were measured using a low-dead-space bidirectional turbine, which (calibrated before each test by using a syringe of known volume). Inspired and expired gases were sampled continuously at the mouth and analyzed for concentrations of O₂, CO₂, and
N\textsubscript{2} by mass spectrometry after calibration with precision-analyzed gas mixtures. Changes in gas concentration were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas passing through the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data was collected every 20ms and transferred to a computer, which aligned concentrations with volume data to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al.\textsuperscript{4}.

These algorithms were developed to estimate breath-by-breath alveolar gas exchange by accounting for changes in both functional residual capacity and alveolar gas concentrations during ventilation. These corrections are computed to give accurate total lung gas exchange.

\textit{VO\textsubscript{2} and Deoxyhemoglobin Saturation and its Relationship with [PCr]}

The profile in the change in [PCr] over the recovery to heavy intensity work transition has been shown to be closely linked to changes in pulmonary VO\textsubscript{2} as both exhibit similar monoexponential responses over the rest-work transition (Fig.1.1)\textsuperscript{20, 37, 44}.

Near Infrared Spectroscopy Imaging (NIRS) of the changes of muscle deoxygenation (ΔHHb) have been utilized as an estimate of the balance between O\textsubscript{2} delivery and O\textsubscript{2} utilization at the muscle\textsuperscript{16}. Response profiles similar to PCr over rest-work transitions have been observed with muscle VO\textsubscript{2} and ΔHHb, although the ΔHHb response has been shown to be faster than the VO\textsubscript{2} response as the inherent delay of the cardiodynamic phase between the actual muscle VO\textsubscript{2} and pulmonary VO\textsubscript{2} has been eliminated with this signal.

Since similar changes in [PCr], ΔHHb, and VO\textsubscript{2} are demonstrated over the recovery-work transitions, inferences can be made regarding the association of ΔHHb and [PCr] changes during oxidative phosphorylation during INT exercise.
High Intensity Training

The highest sustainable power output over a 30 min duration (High Intensity Training-HIT) will be employed in both the 10 s work : 5 s recovery and continuous protocols. The superiority of HIT to lower intensity training as a training method for increases in maximal oxygen uptake, ventilatory threshold and buffering is common in the literature. The higher VO₂ and heart rate observed, and moderate recruitment of type 11a and 11b fiber type during this predominantly aerobic training, compared to continuous exercise at lower intensity, may lead to superior physiological and performance adaptation.

The classic early work by Dudley et al. demonstrated that the peak influence of exercise intensity on the adaptive increase in oxidative capacity in all fiber types, in working muscle, occurs above 85% of max VO₂. Although well trained humans have been reported to reach a limit to VO₂max enhancement, several studies have demonstrated that the VO₂max of well trained runners can be increased (4.5-13%) when various HIT protocols known to elicit VO₂max, are included in their training. These findings suggest that training at or near VO₂max is an effective and/or necessary training intervention for the well-trained endurance athlete to enhance VO₂max. In recreationally active individuals, similar and more dramatic results (14-44%) of increased VO₂max have been observed with HIT.

Traditionally, activation of the signaling pathways specific to endurance exercise have been stimulated by endurance bouts of some 60 min in duration. The specific transcriptional coactivators identified are AMP activated kinase (AMP-K) and P38 mitogen activated protein kinase (p38-MAPK) which up regulate peroxisome proliferator receptor gamma coactivator 1α (PGC-1α). Conversely traditional resistance training incorporating short intense stimuli has been observed to preferentially induce signaling cascades via protein kinase B (PKB) for muscle. Recently Gibala et al. has induced the signaling cascades associated with endurance training, (p38-MAPK and AMP-K) with short sprint interval training. Specifically multiple repeats of 30 s
maximal efforts with 4min recovery between bouts were employed. The acute exercise bout response revealed an increase in mRNA PGC-1α within three hours post exercise but increases in actual PGC-1α were not observed until after several exercise sessions. Notably these transcriptional signaling cascades initially identified with the signaling pathways were now observed with sprint training. The up regulation of these transcriptional changes are not unexpected as the maximal 30 s stimulus would require near maximal activation from oxidative phosphorylation would be a potent stimulator for mitochondrial biogenesis. Both of the HIT exercise protocols studied here may also induce similar signaling cascades affecting PGC-1α due to the near maximal and lengthy (30 min) activation from oxidative phosphorylation during training.

This objective of the proposed repeated 10 s work : 5 s moderate recovery intermittent exercise protocol was to perform the highest sustainable power output over the exercise duration thereby fulfilling the aforementioned requirement for maximal physiological and performance adaptation.

*Duration of an Exercise Bout*

The exercise bout duration that leads to the greatest adaptation (cytochrome C activity and mitochondrial content) in a predominantly oxidative muscle, from continuous exercise (CONT) training has been reported to be achieved with a training duration of 30 min\textsuperscript{21}. These researchers found that there is no evidence that an increase in VO\textsubscript{2max} or cytochrome C activity occurs when training durations were increased from 60 min to 90 min. It would seem that a duration limit exists for increased respiratory enzyme activity adaptation to high intensity aerobic training.
The 30 min exercise duration will be utilized in this proposed study as it has been shown to be the duration which has the greatest rate of increased aerobic enzyme activity from training\textsuperscript{21}.

**Progression of Training Intensities within a Training Program**

The most comprehensive study on the rate of performance and VO\textsubscript{2max} adaptation to HIT tested VO\textsubscript{2max} during every training session for 14 training sessions\textsuperscript{8}. Sixty-eight per cent of the performance adaptation to a specific work rate in this study occurred by 5.8 training sessions. Initial changes were observed after only two training sessions\textsuperscript{8}. Other studies have monitored heart rate (HR) during every training session and then increased work rates to maintain the prescribed HR as fitness increased\textsuperscript{30}. Still others have tested VO\textsubscript{2max} and increased work rates weekly\textsuperscript{31}, bi-weekly\textsuperscript{31, 54} and every 5wks\textsuperscript{43}. In this current research, the work rate will be increased 1.5\% per training session\textsuperscript{8} as per previous work.

**Interval Training vs Continuous Training**

The proposed 10 s work : 5 s moderate recovery exercise protocol is a combination of traditional continuous constant load exercise and interval exercise. A wide variety of HIT short work interval duration training programs between 3s and 30s, with total work performed as short as 2.3 min, have been studied\textsuperscript{25, 33, 43, 44, 52, 54}. In these experiments the volume of work completed between the interval and continuous training protocols have been equilibrated. None have utilized the highest sustainable power output over a 30 min intermittent protocol utilizing very short work periods and shorter moderate recovery periods (10 s : 5 s), and compared this to a similar duration and intensity of continuous exercise.

Various durations of continuous exercise training between 15 min and 2h have been observed over periods ranging from 4 to 10 weeks\textsuperscript{25, 30, 31, 35, 39, 43, 52, 54}. Increases in VO\textsubscript{2max} and ventilatory threshold have generally been observed but the differences between continuous and
interval training of similar average work rate and VO₂ have not exhibited differences in all cases. It is possible that the constant metabolic stress of continuous exercise offsets the effects of the higher intensity but intermittent metabolic stress in interval training. If this is true, a training protocol that elicits the higher physiological responses and power output of interval training while maintaining the constant VO₂ characterized by constant load exercise will lead to greater adaptations than either interval or continuous training. The proposed training protocol fulfills this condition as the average VO₂ during the proposed INT 2 exercise protocol is similar to that of a maximal continuous constant load exercise (>85% VO₂max) but with a greater substrate phosphorylation contribution. Consequently, physiological and performance adaptations to both oxidative and substrate phosphorylation may be observed.

A summary of the intermittent and continuous protocols is presented in Table 1.1. Results of both continuous and intermittent training programs have been homogenous in nature. Increases in both VO₂max and ventilatory threshold consequent to both intermittent and continuous protocols have been observed. Overend et al. (1986) did observe greater changes in V̇E:T with continuous training vs intermittent training. Of previously studied training protocols, the protocol used by Tabata et al. (20 s work : 10 s intermittent recovery) is the most similar to the INT protocol utilized. The protocol employed a 20 s high intensity (170% VO₂max) work : 20 s complete rest protocol (2 min 40 s work during each block of training) repeated 8 times for a total work duration of 21 min. Unfortunately, there was no mention of the duration of rest between exercise bouts. One may speculate, due to the much greater anaerobic energy system demand of each set, that recovery would have been at least 5 min in duration. This would bring the total exercise session to 72 min in duration which is significantly longer (58%) than the intermittent protocol utilized here.

Tabata et al. compared the results of this intermittent protocol with 60 min of continuous exercise at 70% VO₂max which is a lower intensity and longer duration than the present study.
These protocols were performed 5 times per week for 6 weeks, which equates to 60% more sessions than this research. Increases in VO$_{2\text{max}}$ were similar in both training groups (≈10%). Other important differences between the protocol used by Tabata et al. and the current protocol are the 18 additional training sessions in the Tabata study, and that their interval group maintained continuous high heart rate and VO$_2$ for only 4 min during each interval. The increase in anaerobic work capacity observed in their high intensity training group suggests that the proposed INT 2 protocol may elicit significant increases in anaerobic capacity but greater increase in VO$_{2\text{max}}$.

<table>
<thead>
<tr>
<th>INT</th>
<th>Work-Rest</th>
<th>Intensity</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overend$^{43}$ 1992</td>
<td>30-30 s</td>
<td>110% VO$_{2\text{max}}$</td>
<td>inc VeT and VO$_{2\text{max}}$</td>
</tr>
<tr>
<td>Burke$^{53}$ 1994</td>
<td>30-30 s</td>
<td>90% VO$_{2\text{max}}$</td>
<td>inc VeT and VO$_{2\text{max}}$</td>
</tr>
<tr>
<td>Tabata$^{54}$ 1996</td>
<td>20-10 s</td>
<td>170% VO$_{2\text{max}}$</td>
<td>inc AWC and VO$_{2\text{max}}$</td>
</tr>
<tr>
<td>Present 2010</td>
<td>10-5 s</td>
<td>90%-30% VO$_{2\text{max}}$</td>
<td></td>
</tr>
<tr>
<td>Overend$^{43}$ 1986</td>
<td>40 min</td>
<td>80% VO$_{2\text{max}}$</td>
<td>&gt; inc VeT and VO$_{2\text{max}}$</td>
</tr>
<tr>
<td>Burgomaster$^{28}$ 2008</td>
<td>40-60 min</td>
<td>65% VO$_{2\text{max}}$</td>
<td>inc VO$_{2\text{max}}$</td>
</tr>
<tr>
<td>Gibala$^{38}$ 2006</td>
<td>120 min</td>
<td>65% VO$_{2\text{max}}$</td>
<td>inc VO$_{2\text{max}}$</td>
</tr>
<tr>
<td>Mckay$^{41}$ 2009</td>
<td>120 min</td>
<td>65% VO$_{2\text{max}}$</td>
<td>inc LaT</td>
</tr>
<tr>
<td>Murias$^{5,21}$ 2010</td>
<td>45 min</td>
<td>70% VO$_{2\text{max}}$</td>
<td>inc VO$_{2\text{max}}$ and LaT</td>
</tr>
</tbody>
</table>

This proposed high intensity interval training protocol optimizes each of the critical determinants responsible for enhanced physiological responses and performance. These include the benefits derived from interval and continuous training, high intensity training, the utilization of the optimal duration for increases in aerobic enzymatic activity to high intensity training (30 min), and regular increases in work rate over the training period to keep pace with the physiological and performance adaptations.

In summary, the enhancement of VeT and VO$_2$ may be elicited from different stimuli involving high intensity intermittent and continuous exercise. By combining these two training
stimuli in one high intensity intermittent protocol eliciting a high and continuous VO₂ may yield improved adaptations.

Training Principles

The proposed intermittent training protocol has been designed to optimize the following key training methodologies that elicit maximal physiologic adaptation and subsequent performance.

The overload principle (improved performance and physiologic adaptation is the result of systematic and progressive training of sufficient frequency, intensity and duration) is an accepted practice within exercise training. Systematic training may include interval training, where work periods are interspersed by recovery periods. This permits greater volumes of a higher work rate to be performed than is possible when exercising at continuous constant loads. Furthermore, if a constant load exercise is partitioned into blocks (≤1 min) followed by a recovery period of similar duration, the average power output will decrease. Conversely, when the duration of the work periods are reduced, without changing the recovery duration, the power output must be increased to elicit the same average work, and metabolic and cardiorespiratory stress. Although work periods with longer relative rest periods allow for higher work rates to be performed, the continuous cardiorespiratory and metabolic involvement that is associated with constant load exercise, and subsequent related adaptation may vanish. The combination of the short 10 s work and 5 s moderate recovery periods permit power outputs that would normally elicit fatigue in several minutes will be possible for 30 min.

It is suggested that training at the highest sustainable work rate over the 30 min duration for the 10 s high work rate : 5 s moderate recovery work rate will elicit the physiological responses of continuous constant load exercise (constant heart rate and VO₂).
Overview

This thesis studies the breath-by-breath VO₂, muscle deoxyhemoglobin saturation, muscle metabolic status, and acid-base balance response to a HIT intermittent exercise protocol (10 s work : 5 s recovery) and HIT continuous protocol to assist in elucidating the different phosphorylation involved in these differing exercise protocols. Subsequently, comparisons of the performance and physiological adaptations from a four week training intervention of this high intensity 10 s work : 5 s moderate recovery exercise training versus a high intensity continuous training protocol are made.

The following outlines the three experiments that have been performed for this thesis in manuscript format.

Chapter 2 will compare the acute response of VO₂ and the change in deoxyhemoglobin of a 10 s heavy intensity work : 5 s light recovery (INT 1), a 10 s heavy intensity work : 5 s moderate recovery (INT 2), and continuous cycle ergometer exercise (CONT) bouts performed at the same power output as the 10 s work period of the intermittent exercise protocols.

The following hypotheses were tested: (1) Oscillations in VO₂ and ΔHHb will be associated with the change in work rate of INT 1. (2) The higher power output during the recovery periods of INT 2 will enhance O₂ delivery, potentially due to the muscle pump effect, and a relative decrease in ΔHHb will be observed relative to INT 1. (3) The higher average power output of INT 2 versus INT 1 will elicit a higher VO₂ during the 10 s heavy intensity work period of INT 2, despite similar energy demands during the 10 s work, and increase the contribution of aerobic metabolism during the 10 s work periods of INT 2, despite s greater average power output.

Chapter 3 will observe the acute responses of a 10 s work: 5 s rest plantar flexion exercise bout and a continuous constant load plantar flexion exercise bout performed at the same
work rate as the 10 s work of the intermittent exercise protocol on $[\text{H}^+]$, $[\text{Pi}]$, and $[\text{PCr}]$ via $^{31}\text{P}$-MRS.

The following hypotheses were tested: (1) Repeated cycles of 10 s work : 5 s rest intermittent exercise will elicit fluctuations in PCr via a reversal of the creatine kinase reaction within these 15 s cycles. (2) Increased $[\text{H}^+]$ will be observed during INT exercise, similar to continuous exercise, despite a lower average ATP demand. Increased $[\text{Pi}]$ but not $[\text{H}^+]$ will be associated with increased metabolic demand of CONT exercise.

Chapter 4 will observe the effects of performing this high intensity INT 2 exercise protocol, 3 times per week for 4 weeks on VO$_2$ max, ventilatory threshold, VCO$_2$ and the energy system contribution to a 60 s Wingate and a 3 min endurance test. Comparisons of these changes to a high intensity continuous protocol will be carried out. The power outputs will be configured to elicit the highest sustainable work rate over a 30 min period.

It is hypothesized that performing this high intensity 30 min INT protocol (3 times per week, 4 weeks) and high intensity continuous training sessions of similar duration, intensity and frequency will result in: (1) similar improvements in VO$_{2\text{max}}$ and associated peak power output by both training groups despite different energy system contributions, and (2) a greater aerobic contribution and greater power output measured during a 60 s Wingate and 3 min endurance performance in the INT group as a function of this high intensity nature of this INT training protocol.
References


Chapter 2: The Effects of a Short-Work : Shorter-Recovery Intermittent Exercise on Breath-By-Breath VO$_2$ and Muscle Deoxyhemoglobin Saturation

Introduction

The intermittent exercise protocol of the present study utilizes repeated 10 s heavy intensity work periods, interspersed with either light (INT 1) or moderate (INT 2) intensity 5 s recovery periods to elucidate and compare the responses of pulmonary oxygen uptake (VO$_{2p}$), and deoxyhemoglobin saturation (ΔHHb) to a CONT exercise performed at the same intensity as the 10 s work period of the INT exercise protocols.

The repeated work : recovery periods of INT exercise are, in essence, a series of repeated square wave exercise bouts with short rest periods. The first objective was to determine whether changes in cellular respiration would track the rapid changes in work rate during these INT exercise protocols. The previously observed exponential changes in muscle VO$_2$ that have been observed at the onset and cessation of square wave exercise, suggest that rapid changes in mitochondrial respiration over this time scale are possible$^{41}$.

The effects on blood flow of muscle contractile activity may be positive or negative. The positive effect of the muscle pump has been suggested to be intensity dependent$^{37}$. Conversely blood flow may be occluded or limited during rhythmical muscle contractions$^{33}$. It has been suggested that the initial decline in exercise muscle blood flow over the first few seconds of loadless cycling recovery, comparable to the 5 s recovery utilized in the present study, is due to the removal of the muscle pump$^{22}$. It is suggested that the various muscle activity performed during the recovery period of INT 1 versus INT 2 versus CONT exercise, will elicit differential responses in ΔHHb saturation reflecting the balances in blood flow, O$_2$ delivery and oxygen utilization.

It is not clear how much muscle activity is required to affect blood flow through muscle pump activity. Moreover, it is possible that the presence of an increased power output and
muscle activity during the recovery period of INT 2 vs INT 1 will enhance the muscle pump effect and increase O₂ delivery. This would reduce O₂ extraction, as expressed by a lower ΔHHb. Thus, the moderate work rate during the recovery period of the INT 2 protocol may elicit enhanced O₂ delivery with a coincident decrease in ΔHHb.

It has been suggested that elevated O₂ delivery during short recovery intervals, similar to INT 1 & 2 exercise, is used to replenish intramuscular O₂ stores that alleviate a portion of the O₂ deficit incurred during suprathreshold heavy intensity exercise¹⁰-¹³, ²⁰, ⁴⁴.

A higher average power output will exist during INT 2 as a function of the increased workload during the recovery period, notwithstanding the fact that the power output during the 10 s work periods are similar between INT 1 and INT 2. Within the constraints of the inherent on-off VO₂ kinetics during this INT exercise, the higher workload during recovery of INT 2 will elicit a higher baseline VO₂ at the onset of the work period and consequently a higher VO₂ during the work period and higher average VO₂ will. It is suggested here that despite the higher average power output of INT 2, the increased VO₂ during the 10 s work period will facilitate a reduction in substrate level phosphorylation contribution.

In the present study the pattern of pulmonary gas exchange and the change in muscle deoxyoxygenated hemoglobin (ΔHHb) saturation were assessed through measures of breath-by-breath VO₂ and NIRS analyses. Observations and comparisons will be made of their response to INT 1 and INT 2 exercise bouts, and a continuous constant load exercise (CONT) performed at the identical work rate as the 10 s work period of the INT exercise bouts.

The following hypotheses were tested: (1) Oscillations in VO₂ and ΔHHb will be associated with the changes in work rate of INT 1 and only in ΔHHb of INT 2. (2) The higher power output during the recovery periods of INT 2 will enhance O₂ delivery, potentially due to the muscle pump effect and a relative decrease in ΔHHb will be observed relative to INT 1. (3) The higher average power output of INT 2 versus INT 1 will elicit a higher VO₂ during the 10 s
heavy intensity work period of INT 2 and increase the contribution of aerobic metabolism to the 10 s work periods of INT 2.

Methods

Subjects. Seven adult males, 24±4 years of age, participated in this study (see Table 2.1) which was approved by The University Review Board for Research Involving Human Subjects. All subjects were healthy and moderately active.

Testing protocol. Subjects were asked to refrain from eating and/or ingesting caffeine three hours prior to their testing sessions. The subjects performed a series of cycle ergometer tests on four separate Days. Testing Day 1: One incremental ramp test to fatigue, 25 W / min was performed. This test was used to determine VO\(_{2\text{max}}\). Testing Day 2: Subjects performed a 3 min no load warm-up followed immediately by 120 cycles (30 min) of this 10 s high intensity : 5 s 20 W cycling (INT 1) protocol. This was followed immediately by a 10 min warm down period at 50W. Testing Day 3: A minimum of 48 hrs after Testing Day 2 the subjects returned to the lab and performed a 3 min no load warm-up followed immediately by 120 cycles (30min) of this 10 s heavy intensity : 5 s moderate intensity (INT 2) protocol. This was followed immediately by a 10 min warm down period at 50W. The work rate during the 10 s high work period for both INT 1 and INT 2 was set at \(V_{ET} + 50\%\) of the difference between the VO\(_2\) at the \(V_{ET}\) and VO\(_{2\text{max}}\) (\(\Delta50\%\)) of each subject. The work rate during the 5 s moderate period of INT 2 was set at 50% of the difference between the subject’s no load cycling VO\(_2\) and ventilatory threshold VO\(_2\) (\(\Delta50\%VET\)). This moderate work rate was performed to increase the VO\(_2\) during the recovery period and reduce the fluctuations in VO\(_2\) during this INT 2 exercise. Due to the submaximal nature of the INT 1 and INT 2 tests, randomization of the order of these tests was not required. Testing Day 4: Subjects returned to the lab a minimum of 48 hrs after Testing Day 3. Subjects performed a 3 min 20 W warm-up followed immediately by a continuous constant load exercise
at an identical power output to the 10 s high of their INT protocols. Subjects were asked to
maintain this power output until volitional fatigue or were stopped at 10 min, whichever was
longer. This was followed immediately by a 10 min warm down period at 50W. Inspired and
expired gases, breath-by-breath, were measured using a mass spectrometer and volume turbine,
while simultaneous measures of deoxygenated hemoglobin (ΔHHb) were collected using NIRS
throughout each test.

Measurements. Gas-exchange measurements have been previously described in detail\(^2\). Briefly,
inspired and expired flow rates were measured with a low dead-space (90 ml) bidirectional
turbine (AlphaTechnologies VMM 110), which was calibrated before each test with a syringe of
known volume. Inspired and expired gases were sampled continuously at the mouth and
analyzed for concentrations of O\(_2\), CO\(_2\), and N\(_2\) by mass spectrometry (Perkin Elmer 1100) after
calibration with precision-analyzed gas mixtures. Changes in gas concentration were aligned
with gas volumes by measuring the time delay for a square-wave bolus of gas passing the turbine
to the resulting changes in fractional gas concentrations, as measured by the mass spectrometer.
Data collected every 20 ms were transferred to a computer, which aligned concentrations with
volume data to build a profile of each breath. Breath-by-breath alveolar gas exchange was
calculated using algorithms of Beaver et al.\(^6\). Heart rate (HR) was continuously monitored by
electrocardiogram.

NIRS measurements have been described in detail previously\(^17\). Briefly, local muscle
oxygenation profiles of the quadriceps vastus lateralis muscle were made with NIRS
(Hamamatsu NIRO 300, Hamamatsu Photonics). Optodes were placed on the belly of the
muscle midway between the lateral epicondyle and greater trochanter of the femur. The
interoptode spacing was 5 cm. The optodes were housed in an optically dense plastic holder,
thus ensuring that the position of the optodes, relative to each other, was fixed and invariant.
The optode assembly was secured on the skin surface with tape and then covered with an
optically dense, black vinyl sheet, thus minimizing the intrusion of extraneous light and loss of NIR-transmitted light from the field of interrogation. The thigh, with attached optodes and covering, was wrapped with an elastic bandage to minimize movement of the optodes while still permitting freedom of movement for cycling. This preparation essentially prevented any optode movement relative to the skin surface.

The intensity of incident and transmitted light was recorded continuously at 2 Hz and, along with the relevant specific extinction coefficients and optical path length, used for online estimation and display of the concentration changes from the zero-set during the resting baseline of O$_2$Hb, ΔHHb and Hbtot. The raw attenuation signals (in optical density units) were transferred to a computer and stored for further analysis. At present, NIRS instrumentation is unable to accurately determine the relative contribution of Mb to the total NIRS signal because the Mb absorption spectrum overlaps that of Hb$^{14}$. However, Mb levels are small relative to those of Hb, and several studies$^8, 9, 38, 43$ have suggested that intracellular Mb contributes $<10\%$ to the total NIRS signal. Thus the preponderance of evidence in the literature would suggest that NIRS primarily monitors changes in vascular Hb oxygenation and deoxygenation. In this study, we used an interoptode spacing of 5 cm. Given the uncertainty of the optical path length in the vastus lateralis at rest and during exercise, NIRS data are presented as delta (ΔHHb) arbitrary units (a.u.). NIRS-derived signal was zero set before the onset of exercise while subjects were quietly seated on the cycle ergometer. The raw attenuation signals (in optical density units) were transferred to a computer for later analysis. Changes in light intensities were recorded continuously at 2 Hz.

The HHb signal is regarded as a reliable estimator of changes in intramuscular oxygenation status and O$_2$ utilization in the field of interrogation$^{14, 21}$. 
Analysis

The VO$_2$ and NIRS profiles of the different exercise protocols were analyzed from the onset to the end of exercise. Breath-by-breath gas-exchange data were filtered for aberrant data points, interpolated to 1 s intervals, and then averaged into 5 s time bins to yield a single response for each subject. The arrival of the initial deoxygenated blood contributing to the measure of VO$_2$ was detected by a combination of the first drop in P$_{ET}$O$_2$ and RER. The previous VO$_2$ data (ie. the Phase 1 or cardiodynamic phase) were deleted. This time point, end of Phase 1, was utilized to time align the ΔHHb signal with the pulmonary VO$_2$ (VO$_2$p) for purposes of observing the subsequent changes in the ΔHHb with the change in muscle VO$_2$. The recent point/counterpoint discussion in the Journal of Applied Physiology on the time-delayed phase at the onset of exercise initiated by Whipp$^{45}$ is most helpful, as the consensus from these discussions enables the formulation of appropriate time alignment strategies of the individual VO$_2$ data which may have been problematic in previous work where observations of fluctuations during 10 s work : 20 s recovery were unable to be discerned$^{44}$.

The three data points (5 s-10 s-15 s) of each subsequent 15 s cycle of INT exercise from 80 – 480 s were overlaid to observe the effect of changes in VO$_2$ and ΔHHb with the changes in work rate during each 15 s cycle. It was assumed that the lowest mean 5 s VO$_2$ within each 15 s cycle within each subject was a consequence of the lowest power output of each of the INT protocols and the two subsequent mean 5 s VO$_2$ data were considered to be linked to the following two data points of the 10 s work period. The three mean VO$_2$ data points of all the subjects, within each 15 s cycle of each of the intermittent exercise groups were then compared statistically (RM ANOVA-P<0.05).

The NIRS-derived ΔHHb were time aligned and ensemble averaged to 5 s time bins to yield a single response for each subject. Since time alignment is straightforward due to the
prompt ΔHHb response to changes in work rate, these data were matched in real time with changes in power output from 80 to 480s.

The fundamental responses of the VO₂p on transient to CONT were modeled as a monoexponential beginning after the cardiodynamic phase to the end of phase 2. Phase 3 was identified by increased VO₂ relative to the end of Phase 2. The juncture of Phase 2 and 3 was used to estimate the actual O₂ cost of performing the 270 W of the INT exercises. The aerobic/anaerobic contribution to the 10 s INT was determined by the following method: Initially the estimated O₂ cost per watt for this supra threshold work rate (270W) during CONT was determined by the VO₂ observed at the end of Phase 2 at the onset of the CONT exercise. The VO₂ after this point increased, independent of a change in power output, as a function of the VO₂ slow component that begins at this juncture and decreases efficiency. The O₂ cost per watt at this juncture was then multiplied by the 10 s work rate of the INT exercise to estimate the O₂ demand of this work rate.

Statistical analysis  Analysis of the results between each exercise condition (CONT, INT 1, INT 2) on changes in VO₂p, and ΔHHb were calculated by two-way repeated-measures RM ANOVA. Within group comparisons were calculated using a RM ANOVA. Significant differences were further tested by Student-Holman-Sidak, or Tukey post hoc analysis.

Results

Summary of the anthropometric characteristics and aerobic parameters assessed during ramp incremental testing are presented in Table 2.1.

The mean power outputs of the three exercise conditions were different (p<0.05) ( CONT -270W, INT 1-187W and INT 2-210W). The mean VO₂ for the time period 80 s to 480 s of the three exercise conditions were utilized to describe the steady state response after the initial onset of exercise. VO₂ was different between CONT (3.62 l/min ± 0.61), INT 2 (3.08 l/min ± 0.49) and
INT 1 (2.89 l/min ± 0.36) (P<0.05) (Fig.1.). Consecutive 15 s work and rest duty cycles were overlayed between 80-480s to give a mean VO₂ response for each 10 s of the work and the 5 s recovery periods of both INT exercise bouts. Systematic oscillations of VO₂ during INT 1 were observed over the repeated 10 s work : 5 s recovery periods (P<0.05). Similar oscillations within INT 2 (Fig.2.2) were not observed.

The observed VO₂ of CONT at the end of Phase 2 at time = 133 s was 3.52 lO₂/min and was utilized as the specific VO₂ demand for the 10 s work of the INT protocols and the actual O₂ cost associated with the work rate during CONT. The difference between the predicted VO₂ from CONT and the actual VO₂ recorded during INT 1(2.84 lO₂/min) and INT 2 (3.09 lO₂/min) at this same inflection point was 420mls/min for INT 2, and 680mls/min for INT 1 (p<0.05) (Fig.2.1). The 5 s recovery period reduced average VO₂, as well as the VO₂ of the 10 s work period. The increase of average power output between INT 1 (187 W) and INT 2 (210 W) was 11%, and from INT 2 (210 W) and CONT (270 W) was 22%.

The average ΔHHb response of the different exercise protocols is presented in Fig. 2.3. The average ΔHHb over each 5 s of the 15 s work : recovery cycles was analyzed from 80 s to 480 s (Fig.2.4. A & B). The amplitude of the ΔHHb was greater during CONT than INT 1 and INT 2 (P<0.05) while there was no difference between INT 1 and INT 2 (P<0.05) (Fig.2.3.). The amplitude of the ΔHHb response early in INT 2 exercise (20 s-60 s) was notably smaller indicative of greater blood flow or matching distribution (Fig.2.3). ΔHHb during INT 2 was different from CONT (P<0.05) while INT 1 was not statistically different from either INT 2 or CONT exercise over this time period (Fig. 2.3).

Oscillations in ΔHHb during both intermittent protocols were observed (Fig.2.4 A & B). During INT 1, ΔHHb was the lowest at 5 s of recovery and then progressively increased through 5 s and 10 s of work. Within INT 2- ΔHHb, differences were observed between the 5 s of recovery and the 10 s of work (P<0.05). The ΔHHb/VO₂ exhibited differences (P<0.05)
between INT 2 and CONT early in exercise, with differences observed between INT 1 and INT 2, and CONT later in exercise. (Fig.2.5).
Table 2.1 *Subject Characteristics*: Maximal Oxygen Uptake (VO$_{2\text{max}}$), Maximal Carbon Dioxide Production (VCO$_2$), Power Output at VO$_{2\text{max}}$ (PO), Ventilatory Threshold (V$_{ET}$), Power Output at Ventilatory Threshold (PO at V$_{ET}$), Weight in Kilograms (Kg), Height in Centimeters (Hgt Cms), and Age in Years (Age)

<table>
<thead>
<tr>
<th>VO$_2$ l/min</th>
<th>VCO$_2$ l/min</th>
<th>PO W</th>
<th>V$_{ET}$ l/min</th>
<th>PO at V$_{ET}$ W</th>
<th>Mass Kg</th>
<th>Height cms</th>
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<tbody>
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<td>4.40</td>
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</table>

*SD* | 0.68 | 0.80 | 55 | 0.63 | 58 | 9.8 | 5.67 | 2.19 |
Figure 2.1. VO₂ during (○) INT 1 (10 s work : 5 s recovery (20W cycling), (●) INT 2 (10s work /5 s recovery moderate cycling), and (•) CONT. (Difference comparing INT 1 and INT 2 with CONT after 70 s of exercise: P<0.05)
Figure 2.2  Average VO$_2$ (SE) from INT 1 (A) and INT 2 (B) Averaged from multiple 15 s work/recovery cycles at 5 s time intervals from 180-480 s. * Indicates significant difference from 5 s of recovery.
Figure 2.3. ΔHb during (•) INT 1 (10 s work : 5 s recovery 20 W cycling, (○) INT 2 (10 s work : 5 s recovery 89 W moderate cycling, and (●) CONT . INT 1 & 2 different from CONT after 30s (p< 0.05)

INT1 different from INT 2 (p<0.05).
Figure 2.4. Average Oscillations in ΔHHb (SD) INT 1 (A) (open bars) and INT 2 (B) (grey bars) at 5 s Recovery, 5 s Work and 10 s Work. * Different from 5 s Work and 10 s Work, α different from 5 s Recovery and 5 s Work, β different from 5 s Recovery and 10 s Work (p<0.05)
Figure 2.5. (o) INT 1 (10 s work : 5 s recovery 20 W cycling) (●) INT 2 (10 s work : 5 s recovery moderate cycling (89 W), and (●) CONT, depicts ΔHHb/VO2 over the first 2 min of exercise. The ΔHHb/VO2 was different between INT 2 and CONT exercise over this period, differences between INT 1 and 2 and CONT (p<0.05).
Discussion

It has been customary to compare physiological variables between continuous and intermittent exercise performed at similar average power outputs\(^{20}\). Differentially, the 10 s high intensity work periods of the intermittent exercises (INT 1 and INT 2), and of the constant load continuous protocol (CONT) in this present study were performed at similar heavy intensity work rates. This facilitated comparison of the physiological effects of the repeated 5 s recovery periods of differing power outputs (INT 1= 20 W; INT 2= 89 W) after each similar 10 s period of work (270 W), to continuous constant load exercise performed at the same work rate (270W).

The present study is the first to examine and compare the effects of the two unique INT exercise models versus a CONT protocol on the breath-by-breath VO\(_2p\) and ΔHHb response.

The major findings were:

1. Oscillations in VO\(_2p\) and ΔHHb associated with the change in work rate were observed during INT 1 (Fig. 2.2 & 2.4). It is suggested that the amplitude of these oscillations are a balance between opposing mechanisms. Enhanced O\(_2\) delivery and the effects of previous priming exercise which would increase the amplitude of the VO\(_2\) over the exercise : recovery transitions, whereas constraints within the locus of metabolic control may be responsible for the imprecise matching of the fluctuations in mitochondrial respiration with the fluctuations in work rate during INT exercise. (2) The average ΔHHb was similar between INT 1 and INT 2 and different from CONT after 120 s of exercise (Figs. 2.3 & 2.5). It is suggested that the similar ΔHHb for a higher VO\(_2\) in INT 2 versus INT 1 represents a relative increase in O\(_2\) delivery and decreased O\(_2\) extraction during INT 2. It was also observed in direct contravention to earlier reports\(^{20}\) that lower relative blood flow occurs during CONT vs INT exercise (Fig 2.5). (3) The higher VO\(_2\) during the 10 s work period of INT 2 vs INT 1, suggests a greater oxidative phosphorylation contribution for the identical power output. This would increase the oxidative phosphorylation contribution to the 10 s heavy intensity work period of INT 2 versus INT 1.
Change in VO₂ and ΔHHb during INT 1

It has been suggested that the rate of change of VO₂ over a rest : work transition is, in part, dependent upon blood flow and that capillary blood flow and muscle VO₂ are tightly coupled. Grassi et al. observed speeded VO₂ kinetics at the onset of high intensity exercise in the presence of elevated blood flow at exercise onset in dogs. The involvement of Type 2 fibres would be necessary for this high intensity work and they suggested that improved perfusion of these fibres elicited these speeded VO₂ kinetics. Others have observed a similar result in humans during transitions from moderate to severe supine exercise. This speeded rate of change in VO₂ was also attributed to increased blood flow to Type 2 fibers and it has been suggested that these fibres are particularly sensitive to restrictions in O₂ delivery. The present INT exercise protocol constructs an elevated blood flow model by utilizing a recovery duration that ensures high muscle blood flow at the onset of each 10 s work period. It is suggested that a similar speeding in the rate of change in VO₂ over the recovery : work transitions as has been observed elsewhere under similar blood flow condition would ensue.

Furthermore, the priming effect of previous exercise has been suggested to speed the rate of change in VO₂ at the onset of square wave exercise. This has been attributed to the increased amplitude of the VO₂ Phase 2 component, and a reduction in amplitude of the VO₂ slow component. Each previous work period of this INT exercise may, in essence, be priming exercise.

Others have suggested that over the transition from moderate to severe exercise, fluctuations in PDH, the locus for metabolic control for oxidative phosphorylation, is limited. Conversely the elevated activity of this enzyme during heavy intensity INT exercise has been correlated with speeded VO₂ kinetics. The blunted response of PDH that would maintain an elevated mitochondrial respiration rate, and inhibit the capacity of oxidative phosphorylation to match the fluctuations in energy demand of this INT exercise, are tempered by elevated O₂
delivery and/or improved marching of blood flow distribution and priming which would speed changes in mitochondrial respiration.

**Similar ΔHHb Despite Differences in Average Power Output.**

The ΔHHb response has been utilized as a measure of the balance between O₂ utilization and O₂ delivery\(^{15-17}\). Turner et al.\(^{44}\) observed increases in ΔHHb over a 10 s work period with modest changes during the initial 5 s of the work period and then a substantial increase over the later 5 s of the work period. Decreases were also observed during their 20 s recovery period. The present study demonstrated similar results over a shorter 5 s moderate or light recovery period.

In this study, average ΔHHb was highest during CONT (P<0.05), contrary to earlier reports\(^{20}\) while validating our hypothesis, ΔHHb was similar during INT 1 and INT 2 (P<0.05) despite differing VO₂s and power outputs. It is suggested that this similar ΔHHb of both INT protocols despite different VO₂ reflects greater blood flow and O₂ delivery, during INT 2 vs INT 1.

Ferreira et al.\(^{22}\) studied the effects of a transition from loaded to unloaded cycling on muscle blood flow, similar to the work rate transitions of INT 1 in the present study, and suggested that reductions in blood flow occur consequent to the removal of the muscle pump. It is proposed here that this is the mechanism expressed during INT 1. The elevated ΔHHb compensates directly for this lowered muscle blood flow during the 5 s recovery vs INT 2. The increased contractile activity related to the increased power output during the 5 s recovery period of INT 2 (89w), would increase the muscle pump effect, increasing O₂ delivery and reducing ΔHHb.

Others have suggested that not only does the muscle pump increase muscle blood flow as work rate increases, but that increases in muscle blood flow occur through vasodilation, independent of the muscle pump\(^{1, 19, 32, 30, 36}\). There is some controversy as to the time course of
humoral stimulated vasodilation to exercise (4 s\textsuperscript{,25} to > 20 s\textsuperscript{,25}) and there was no literature found to suggest that even with previously observed fast vasodilatory responses that these fluctuations would be possible during the present work/recovery transitions.

Finally, early work by Essen et al.\textsuperscript{20} concluded since arterial-venous oxygen differences (a-\textit{v}O\textsubscript{2} diff) was greater during intermittent exercise that relative blood flow was higher during CONT exercise. The current \(\Delta\text{HHb/VO}_2\) data suggest otherwise. The observed increases in \(\Delta\text{HHb}\) infer that the a-\textit{v}O\textsubscript{2} diff during CONT exercise was much greater than during these unique INT protocols. This demonstrates a relative reduction in \(O_2\) delivery during CONT vs INT exercise. This is in agreement with Lutjmeyer et al.\textsuperscript{37}, utilizing knee extension exercise with similar muscle contraction rates (40 contractions min\textsuperscript{-1}) and range of motion to this present study and concluded that the muscle contractions necessary for greater workloads during this rhythmical knee extension exercise elicited greater impedance to blood flow to the working muscle and higher \(\Delta\text{HHb}\) versus moderate exercise.

\textit{Greater Substrate Phosphorylation (Anaerobic) Contribution During the Heavy Intensity Work Period of INT 1 vs the Work Period of INT 2.}

During the 10 s work period of the INT exercise a \(\Delta50\% \text{ VO}_2\text{max}\) power output (270 W) was performed, and as such, included energy contributions derived from anaerobic or substrate level phosphorylations\textsuperscript{46}. The aerobic/anaerobic contribution to the 10 s INT was determined by the following method. Initially the estimated \(O_2\) cost per watt for this supra threshold work rate (270 W) during CONT was determined by the \(\text{VO}_2\) observed at the end of Phase 2 (3.52 l/min). The \(\text{VO}_2\) after this point would begin to increase, independent of a change in power output, as a function of the \(\text{VO}_2\) slow component\textsuperscript{42}. An \(O_2\) cost per watt of 13.0 ml/min / W was observed at this point. This \(\text{VO}_2\) was then used as the predicted \(\text{VO}_2\) for the 270 W for the 10 s work period of INT exercise (Equ 1.)
Equation 1

\[ *13\text{mlsO}_2/\text{min/w} \times 270\text{w} = 3.51 \text{L/min or 585mls/10s} \]

*calculated from the CONT VO2 at the end of Phase 2*

The highest actual 5 s average VO2 within a 15 s cycle of INT 1 was matched with the 10 s of the work period (2.84 L/min or 477mls/10s). The VO2 equivalent (Eq. 1) during the 10 s work was estimated to require 585 mls. An O2 deficit of 108 mls / 10 s would be accrued. This O2 deficit of 18.4% (108 mls / 10 s ÷ 587 mls / 10 s * 100 = 18.4%) requires a similar increase in substrate phosphorylation to meet the energy demand. The aerobic contribution at 10 s of the work period of INT 1 is 81.6%.

The highest 10 s VO2 (3.09 L / min or 515 mls / 10s) within a 15 s cycle of INT 2 was matched with the 10 s work period. The VO2 equivalent (Eq. 1) during the 10 s work was 585 mls therefore an O2 deficit of 70 mls / 10 s (585 mls / 10 s – 515 mls / 10 s) = 70 mls /10 s) was accrued. This O2 deficit of 11.9% (70 mls / 10 s ÷ 585mls / 10 s * 100 = 11.9%) must be accompanied by a smaller increase in substrate phosphorylation to meet the energy demand during the 10 s work period than INT 1. The aerobic contribution at 10 s of the work period in INT 2 was 88.1%. Because the higher average power output of INT 2 versus INT 1, a greater aerobic energy contribution (6.8%) was utilized during the 10 s work period of INT 2.

The difference in work rates between INT 1 and INT 2 increased the average intensity from 77% to 83% of VO2 max (6% change) while the average work rate increased by 11%. It is suggested that the remainder of the energy required is derived from substrate level phosphorylations.

Summary

In summary, this study demonstrates that short repeated changes in metabolic rate, consequent to changes in power output, elicit $\Delta$HHb and VO2 oscillations over the recovery:
work transitions during high intensity INT exercise. The rate of change may be determined by the balance between the speeding effects of increased $O_2$ delivery and priming exercise on the $VO_2$ kinetics, and the inherent limitations of changes in PDH activity during this INT exercise. It is also suggested that the greater $\Delta Hb$ during CONT is a function of a reduction in relative blood flow versus INT, and that the similar $\Delta Hb$ during INT 2 and INT 1 is a function of a relative increase in oxygen delivery during INT 2. Finally, the inclusion of a moderate work rate during the 5 s recovery period of INT 2 while increasing the average power output, increased the aerobic contribution to the work period.

Practically, these intermittent exercise protocols increase Type 11 fibre recruitment while reducing the fluctuations usually elicited by intermittent exercise.
References


Chapter 3: Muscle metabolic status, acid-base balance and metabolic stress during 10 s work : 5 s rest intermittent and continuous exercise.

Introduction

Early research was limited to comparisons of oxygen uptake and plasma lactates between high intensity continuous exercise (CONT) and high intensity short work: shorter rest exercise (INT). Fluctuations of oxygen uptake over the work : recovery transitions and reduced plasma lactate, reflecting reduced metabolic stress during INT exercise were observed on a limited subject population (n = 2)\(^10\). Recently, this early work has been supplemented with observations of oxygen uptake (VO\(_2\)) and deoxyhemoglobin saturation (\(\Delta\text{HHb}\)) comparing similar exercise bouts of INT and CONT exercise\(^4\). It was suggested that the amplitude of the observed fluctuations of these variables, over the work : recovery transitions of INT exercise, was a balance between the speeding effects of increased O\(_2\) delivery\(^25\) and priming exercise\(^28\), and the inhibitory effects on the locus of metabolic control for oxidative phosphorylation\(^26\).

The present study revisits this specific intermittent exercise protocol using \(^{31}\text{P}-\text{Magnetic Resonance Spectroscopy (}^{31}\text{P-MRS})\) to assess and compare the bioenergetic responses of similar INT exercise versus CONT exercise. Utilizing \(^{31}\text{P-MRS}, near instantaneous observations of intramuscular [P\(_i\)], [PCr], and [H\(^+\)] are possible\(^33,39,40\).

INT exercise is a series of on-off, exercise-recovery, perturbations. Consequently, the changes in [PCr] may respond in like fashion to the fluctuations in work rate. Observations of [PCr] recovery kinetics to moderate exercise have indicated an early fast component\(^17,33,51,52\) (16% recovery at 6 s, with a half time of \(\approx 21\) s\(^30\) in duration) while more recently, Newcomer et al.\(^43\), utilizing a 4.1-Tesla magnet enabling 0.5 s temporal resolution for \(^{31}\text{P-MRS}, observed similar recovery kinetics of [PCr] following heavy intensity exercise. If Newcomer et al. and others have correctly assessed these recovery profiles, the current INT exercise protocol utilizing 5 s recovery periods, should be sufficient time for partial recovery of [PCr].
PCr breakdown has been closely associated with changes in oxidative phosphorylation at the onset of square wave moderate exercise and more recently, during the on and off transients to heavy intensity exercise. PCr breakdown at the onset of heavy intensity square wave exercise has been shown to be faster than the rate of PCr formation during recovery, and as such, PCr resynthesis rather than PCr breakdown may modulate the amplitude of the [PCr] fluctuations between the repeated work : rest periods of this INT protocol.

The action of the PCr shuttle mechanism suggests that previously observed fluctuations in VO2 and muscle deoxygenation (ΔHb) will be associated with similar temporal fluctuations of [PCr] during a similar INT protocol.

Increased [Pi], [H+] and decreased [PCr] have been observed during the increased metabolic stress of heavy intensity constant load exercise leading to fatigue. There is ambiguity in the literature as to which of these variables has the strongest association with increased metabolic stress. The work rate fluctuations of INT exercise may induce a dissociation of the increased [Pi] and [H+] response to heavy intensity exercise. Elevated [H+] during the recovery period of INT may occur due to a reversal of the creatine kinase reaction without the concomitant increase in [Pi] as an increase in the phosphate potential (ATP/ADP + Pi) is facilitated. Furthermore, previously observed increases in [H+] during the initial seconds of recovery originating from glycolytic contributions to ATP formation may result in elevated [H+] during the recovery periods of INT exercise. Likewise the heavy intensity CONT exercise performed here may elicit higher [Pi] and similar [H+] stemming from the necessary substrate level phosphorylation contribution due to the higher average power output versus the INT exercise.

In this study the [PCr], [Pi], and [H+] were recorded during a single bout of heavy intensity constant load exercise (CONT) and compared to an intermittent exercise bout (INT) utilizing the same workload during the 10 s work period, and a 5 s recovery period.
The following hypotheses were tested: (1) Repeated cycles of 10 s work : 5 s rest intermittent exercise will elicit fluctuations in [PCr] via the reversal of the creatine kinase reaction and [PCr] reflected changes in mitochondrial respiration within these 15 s cycles and; (2) increased [H\(^+\)] will be observed during INT exercise, similar to continuous exercise, despite a lower average ATP demand. Increased [P\(_i\)] but not [H\(^+\)] will be associated with increased metabolic demand of CONT exercise.

Methods

Subjects. Recreationally active male subjects (\(n = 8\), 27 yr ± SD 8.7) participated in this study. Before the experiment, all procedures and potential risks were explained to each subject and an informed consent was signed. The study was approved by The Ethics Review Board for Health Sciences Research Involving Human Subjects.

Experimental Procedures. Subjects were studied on three different Days, with a minimum 48 h between tests, completing one experimental protocol each Day. A plantar flexion ergometer\(^{45}\) compatible with a 3-Tesla MRS unit was used to implement the exercise protocols and allow observation of changes in [P\(_i\)], [H\(^+\)], and [PCr] of the lateral gastrocnemius muscle during exercise.

Testing Day 1: A 3 min rest period was followed by an incremental (i.e. ‘ramped’) plantar flexion exercise test to fatigue with contractions at the rate of 80 plantar flexions per min. This test was used to determine the power output associated with the onset of intracellular [H\(^+\)] accumulation and the status at fatigue of [H\(^+\)], [PCr], and [P\(_i\)] / [PCr]. Acidification corresponds to a sudden increase in [H\(^+\)] during the incremental ramp test\(^{39}\). Testing Day 2: Subjects performed a plantar flexion continuous exercise test (CONT) for a maximal duration of 10 min with a range of motion (ROM) over 35\(^\circ\). They were asked to stop exercising if the ROM
dropped below $35^\circ$ or when INT or CONT exercise had been performed for 10 min. Infrared beams triggered a light source visible to the subjects to ensure appropriate ROM. The exercise power output in CONT was 50% of the difference between the onset of intracellular [H$^+$] accumulation and fatigue. *Testing Day 3:* Subjects performed the 10 s work : 5 s rest protocol performing plantar flexion. Exercise involved alternation of 10 s work periods with 5 s rest periods. This 15 s cycle was repeated 40 times (i.e. for 10 min). Work rate during the 10 s work was identical to the work rate performed during continuous constant load testing (i.e. Day 2). Subjects reported to the lab at least 2 h after eating with no ingestion of caffeine containing foods or drinks over that time period. Subjects lay supine with the dominant leg positioned in the plantar flexion ergometer securely and then inserted into the bore of the magnet.

$^{31}P$-MRS.

Intracellular muscle metabolism was studied using $^{31}P$-MRS with the ankle exercise ergometer positioned within the bore of the magnet. Data were obtained using a 64-cm-bore, 3.0-T superconducting magnet interfaced with a SMIS/IMRIS console (Surrey Medical Imaging Systems, Guilford, UK; Innovative Magnetic Resonance Imaging Systems, Winnipeg, Canada). A 4-cm square $^{31}P$ surface coil was securely fastened over the belly of the lateral gastrocnemius of the dominant leg used for exercise. An external reference standard containing methylene diphosphate (MDP) was affixed to the opposite side of the surface coil. All spectra were acquired with 3-ms, 90$^\circ$-adiabatic radio-frequency pulse, a 5.0-kHz receiver bandwidth, and 2,048 complex data points. Before the experimental protocol commenced, two baseline spectra were acquired. The first baseline spectrum was an average of six acquisitions having a repetition time (TR) of 30 s. The second baseline spectrum was the average of the final 24 acquisitions of a 30-acquisition spectrum with TR = 5 s. Calculation of the longitudinal relaxation (T1) correction
factors to account for steady-state precession was therefore obtained from the differences in amplitude of the \( ^{31}\text{P} \) metabolites in the first baseline spectrum (no T1 effects) from the second baseline spectrum (T1 saturated) (see Raymer et al.\textsuperscript{45} for a detailed description of this protocol). All subsequent spectra obtained during the experimental protocol were collected continuously, with each spectrum collected every 5 s.

Data Analyses

Quantification of the \( ^{31}\text{P} \)-MRS metabolite data was performed in the time (acquisition) domain by fitting each \( ^{31}\text{P} \) free induction decay to a sum of damped sinusoids, which could be varied in terms of amplitude, phase, delay time, damping constant, and frequency. This method utilized prior knowledge and a nonlinear least squares algorithm to iteratively reduce the difference in error between the actual data and the experimental model\textsuperscript{46}. The concentrations of the phosphate compounds, \([\text{PCr}]\) and \([\text{P}_i]\), were determined from the amplitude of the exponential model function at time equal zero.

Phosphate peaks were corrected for partial saturation\textsuperscript{53} and absolute concentration values were calculated by assuming a resting ATP concentration of 8.2 mM, resting \([\text{PCr}] + [\text{P}_i] = 42.5\) mM\textsuperscript{29,35} and that the creatine kinase reaction was at equilibrium. The equilibrium constant was adjusted for \([\text{H}^+]\) and for free \([\text{Mg}^{2+}]\) of 0.6 mM\textsuperscript{24}. The \(\text{pH}_i\) was determined from the chemical shift of \([\text{P}_i]\) relative to \([\text{PCr}]\)\textsuperscript{53}. \([\text{H}^+]\) was calculated from \(\text{pH}_i\).

Statistical Analysis

A two-way repeated measures (RM) ANOVA was performed on the mean of the \([\text{PCr}], [\text{P}_i] / [\text{PCr}], \text{pH}, \) and \([\text{H}^+]\) data points at 5 s - 10 s - 15 s from 80 s to 480 s, as well as of each min of the INT and CONT. Post-hoc analyses on the two way RM was performed using the Holm-Sidak or Tukey method as determined by the Sigma-Stat software. Within group comparisons of
[PCr], [P_i] / [PCr], pH, and [H+] for the continuous and INT were analyzed using an ANOVA across the time of the exercise period and the Tukey test was used post-hoc for one way RM ANOVA. Significance was set at P < 0.05 for all statistical analyses.

Results

Results from the incremental test to fatigue to determine acidification threshold are presented in Table 3.1.

Due the severity of the work load, five of the seven subjects in CONT and three subjects in the INT were unable to complete the full 10 min duration of the exercise bout. Consequently observations and statistical comparisons of seven subjects were analyzed for the first 5 min of CONT and 8 min of INT exercise.

[PCr] Response to INT and CONT

The 5 s averaged fraction of resting [PCr] data for CONT and INT is presented against time (Fig. 3.1a) and against accumulated work (Fig. 3.1b). The values for accumulated work during CONT were derived by adding one unit of work for each subsequent time period. The accumulated work for INT exercise was calculated by multiplying each relative work unit of CONT by 0.66. Over each 15s cycle the INT exercise group does one third less work. The change in [PCr] was similar between the CONT and INT groups over the first 2 min of exercise (Fig. 3.2). By the fourth min of exercise the [PCr] during CONT exercise was continuing to decline (P < 0.05) compared to INT exercise. Further statistical analysis revealed that within INT exercise [PCr] was unchanged from 2 min to 5 min of exercise (P = 0.162), while within CONT exercise, [PCr] continued to decline from 2 min to 5 min of exercise (P < 0.05) (Fig. 3.2). When the fraction of [PCr] was plotted against accumulated work there were no differences between CONT and INT exercise (Fig. 3.1b).
Overlaying the 15 s work/rest cycles, from 80 s - 480 s, revealed fluctuations in [PCr] between 4 s of the recovery period and 9 s of the work period (9.6% - 20.80 - 18.57mM; P < 0.05) (Fig. 3.3).

\([\text{Pi}] / \text{[PCr]} \) Response to CONT vs INT

The ratio of \([\text{Pi}] / \text{[PCr]} \) is plotted against time (Fig. 3.4a) and accumulated work (Fig. 3.4b). A trend towards an increase in the CONT fraction of \([\text{Pi}] / \text{[PCr]} \) vs INT was observed when plotted against time. These trends disappeared when these data were plotted against accumulated work.

\([\text{Pi}] \) Response to CONT vs INT

Increased fraction of resting \([\text{Pi}] \) during INT vs CONT occurred 90 s after the onset of exercise (Fig. 3.5a). The fraction of resting \([\text{Pi}] \) when plotted against accumulated work, is greater than the fraction of resting \([\text{Pi}] \) during CONT exercise (Fig. 3.5b).

\([\text{H}^+] \) Response to CONT vs INT

The 5 s averaged data for fraction of resting \([\text{H}^+] \) is plotted against time (Fig. 3.6a) and accumulated work (Fig. 3.6b). Similarities in \([\text{H}^+] \) between CONT and INT after 2 min of exercise were observed whether these data were plotted against time or accumulated work. When the mean data at 4 s rest, 4 s work and 9 s work were averaged over each min (Fig. 3.7) the only differences observed (P < 0.05) were from 1 to 2 min of exercise. Fig. 3.8 reflects the oscillations of \([\text{H}^+] \) over the 15 s cycle of INT, at 4 s rest, 4 s exercise and 9 s of exercise from 2 min to 6 min of exercise (P < 0.05).
Figure 3.1a. Mean [PCr] fraction of resting values for continuous (●) and interval (○) exercise at 5 s intervals from rest to 5 min of exercise for CONT and INT. Each point represents an average across the 8 study participants.
Figure 3.1b. Mean [PCr] fraction of resting values for continuous (●) and interval (○) exercise at 5 s intervals plotted against relative accumulated work. There was no difference between groups when performing similar work rates.
Figure 3.2. Mean [PCr] fraction of resting values (mean and SD for all subjects) for continuous (●) and interval (○) exercise at one min intervals from rest to 5 min of exercise. Heavy line denotes onset of exercise. * denotes significance between INT and CONT, ** denotes differences within CONT from 2 min to 5 min (P < 0.05).
Figure 3.3. Mean [PCr] and SE during interval exercise at 4 s rest, 4 s work, and 9 s work, averaged from 90 s to 180 of exercise. Denotes significance between α 4 s work, β 9 s work, * 4 s rest.
Figure 3.4a. Mean ratio of \([P_i] / [PCr]\) plotted against time of continuous (●) and interval (○) exercise. There were no statistical differences between CONT and INT exercise.
Figure 3.4b. Mean ratio of $[P_i] / [PCr]$ plotted against accumulated work of continuous (●) and interval (○). There were no statistical differences between CONT and INT exercise.
Figure 3.5a. Mean fraction of [P_i] plotted against time for continuous (●) and interval (○). Differences after 90 s of exercise (P < 0.05).
Figure 3.5b. Mean fraction of [P_i] plotted against accumulated work for continuous (●) and interval (○). Differences between CONT and INT exercise (P < 0.05).
Figure 3.6a. Mean fraction of $[H^+]$ plotted against time for continuous (●) and interval (○). Differences between CONT and INT exercise from 60 s - 120 s ($p < 0.05$).
Figure 3.6b. Mean fraction of $[\text{H}^+]$ plotted against accumulated work for continuous (●) and interval (○ $P<0.05$).
Figure 3.7. $[H^+]$ (Mean and SE for all subjects at 4 s – 9 s – 14 s over each min) for continuous (●) and interval (o) exercise. * Significant difference between continuous and interval exercise during second min of exercise.
Figure 3.8. Mean [H+] and SE during interval exercise at 4 s rest, 4 s work, and 9 s work, averaged from 2min to 6min of exercise. *Denotes significance from 4 s rest.
Table 3.1. Results from Incremental Test to Determine Acidification Threshold (AT)

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<td>0.77</td>
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</table>
Discussion

This study is the first to assess the intracellular \([\text{H}^+]\) and high energy phosphate metabolism of a high intensity 10 s work : 5 s rest intermittent exercise protocol (INT). This INT exercise was contrasted with a CONT exercise performed at an identical work rate as the 10 s work period of INT. The major findings were: (1) fluctuations in [PCr] (10.7%) within the 15 s work : rest cycle of INT; this change may reflect an amalgamation of the echoed fluctuations in ΔHb and VO₂ fluctuations⁴ (8.6%) observed previously during similar INT exercise, and the reversal of the creatine kinase reaction. (2) Despite the lower average power output of INT vs CONT exercise, elevated \([\text{P}_\text{i}]\) and similar increases in \([\text{H}^+]\) were observed. Thus, an exercise protocol with lower average power output and lower metabolic stress has elicited responses usually associated with higher power outputs.

The Oscillations in [PCr] During the Oscillations of INT

The rate of decline of [PCr] at the onset of a step-increase to constant work rate exercise is essentially a mirror image of VO₂ ³⁸, ⁴⁰, ⁵⁰, ⁵⁵. This has been attributed to the instant synthesis of Cr via oxidative phosphorylation in the mitochondria and the immediate transport of PCr across the mitochondrial membrane into the cytosol⁵⁴ (Fig.3.9).

Elevated muscle and capillary blood flow during the initial seconds of recovery⁴, ¹⁸ have been observed to accelerate muscle VO₂ at the onset of high intensity exercise²⁵, ⁴⁴, ⁵¹. These effects have also been modeled through priming exercise with similar speeding effects on VO₂ ², ²⁷, ³¹, ³², ⁴⁴ and associated [PCr]⁴⁰, ⁵⁰. This elevated blood flow would exist over the work : recovery transitions of INT exercise and subsequently this speeding may be reflected in the amplitude of the fluctuations of [PCr] during INT (Fig. 3.3 & 3.4) via the mitochondrial [PCr] shuttle⁵⁴ (Fig. 3.9⁴²).
Comparable fluctuations in VO$_2$p and ΔHHb have been observed during a similar INT exercise protocol$^4$. This suggests that, in part, observed fluctuations in [PCr] pursuant to the changes in work rate are associated with fluctuations of mitochondrial respiration via the [PCr] shuttle.

PDH, as the regulatory enzyme for substrate to the Kreb’s cycle, dictates mitochondrial respiration rate$^{48}$. The instantaneous fluctuations in work rate during INT elicited a non instantaneous [PCr] response. The preserved PDH activity over the rest : work transitions observed elsewhere$^{26}$, may limit the rate of change in [PCr] originating from oxidative phosphorylation over the rest : work transitions of INT exercise. Furthermore, the inhibition of PDH, as a consequence of the greater ATP availability during INT, may also fetter this enzyme’s ability to respond more closely to the changes in work rate.

The decrease in $[H^+]$ during the work period of INT would suggest a concurrent reversal of the creatine kinase reaction. This would increase the amplitude of the fluctuations in PCr

Figure 3.9. ATP is generated by oxidative phosphorylation inside the mitochondria. This ATP is exported by the adenine nucleotide transporter (ANT) to the intermembrane space (IMS), where it is phosphorylated to Cr by mitochondrial CK (MtCK) to give PCr and ADP. The mitochondrial isozyme of creatine kinase (MICK) within the mitochondrial membrane transports the PCr into the cytosol$^{42}$.
during exercise and recovery, and contribute to the energy requirement of the suprathreshold work rate of INT exercise.

In summary, if blood flow and O$_2$ delivery are maintained across the 10 s work : 5 s rest transitions of INT exercise, it is suggested that a portion of the change in [PCr] over the work recovery transitions are associated with previously observed changes in VO$_2$ and ΔHHb during INT$^4$, and as such, are associated with oxidative phosphorylation via the [PCr] shuttle$^5$.

*The Effects of the Lower Average Power Output of INT vs CONT on [P$i$], [PCr] and [H$^+$]*

Similar mean [PCr] responses (Fig. 3.1) were observed during CONT and INT groups over the first 2 min of exercise. Subsequently the [PCr] during INT reached a plateau from two to five min of exercise ($p < 0.162$), whereas [PCr] during CONT continued to decline ($P < 0.05$) (see Figs. 3.1 & 3.2.). These differences are the expected [PCr] responses to moderate and heavy intensity exercise and reflect the greater average metabolic stress of CONT exercise$^{22}$.

Increased [H$^+$] has also been correlated with increased metabolic stress$^{19, 20}$ and is indicative of the changes in strong ion difference within the cell during heavy intensity exercise$^{37, 39}$. It is suggested that the similar [H$^+$] between INT versus CONT occurs, not only as a function of the anaerobic glycolytic contribution$^{12}$ to the work periods of INT exercise, but also as a consequence of the ATP breakdown via oxidative and glycolytic phosphorylation during recovery$^{13}$. Conversely, the elevated [H$^+$] during CONT would be a function of the increased anaerobic glycolytic phosphorylation$^{11, 41}$ and the decrease in [H$^+$] associated with the increased [PCr] breakdown. The similar [H$^+$] of INT and CONT may result from differing origins within metabolism.

Both increased [P$i$] and [H$^+$] have been associated with increased power outputs above the acidification threshold$^{7, 14, 15, 20, 47}$. Differentially, the present results demonstrate similar [H$^+$]
and in particular, higher \([P_i]\) during INT exercise compared to CONT exercise despite lower metabolic stress (Figs. 3.5a & 3.5b).

The increased \([P_i]\) during INT may be a function of differences in membrane transport rate of \(P_i\). It has been suggested that the transport of \([P_i]\) from the cytosol to the mitochondria as substrate for oxidative phosphorylation may be limited by a mitochondrial membrane carrier protein\(^{19}\). It is conceivable that accumulation of cytosolic \([P_i]\) from substrate phosphorylation during the work and rest periods of INT would occur because of the saturation of this mitochondrial membrane transport mechanism. The rate of this carrier mediated transport system is diametrically related to \([H^+]\)\(^{8,34}\). The increased \([H^+]\) during the 5 s rest period (Fig. 3.8), compared to the work period of INT which inhibits membrane transport of \([P_i]\), as well as reducing the transportable form \((\text{HPO}_4^{2-})\) into the mitochondria\(^{23}\), may lead to the higher \([P_i]\) during INT exercise. Furthermore, it has been demonstrated elsewhere that previous exercise increases PDH activity and increases oxidative phosphorylation thereby decreasing the accumulation of \([P_i]\)\(^{48}\). The higher oxidative phosphorylation rates of CONT versus INT exercise\(^4\), may also account for the decreased \([P_i]\).

Experiments to determine the temperature effects of \([P_i]\) (15\(^\circ\)C - 30\(^\circ\)C) on force generation suggest decreased deleterious effects with increased temperature\(^{15}\) and that the correlation between \([P_i]\) and muscular fatigue is lower than has been previously hypothesized. This proposed mechanism may be at work during both INT and CONT as much higher cellular temperatures would be present due to the heavy intensity work rate.

Others\(^7\) have observed increased \([P_i]\) in patients with myophosphorylase deficiency and observed a linear correlation between \([P_i]\) and fatigue during isometric contractions\(^{36}\). Our protocol seems to uncouple this correlation despite the evidence linking \([P_i]\) accumulation with reduced work output such as the inhibition of cross bridge attachment\(^{20}\), or Type 1 fibers increased sensitivity to high \([P_i]\)\(^{15}\) which would reduce metabolic efficiency. When the \([P_i]\) data
are plotted against accumulated work the differences in \([P_i]\) between protocols are even more pronounced. This would indicate that some or all of the above mechanisms stated may elicit increased \([P_i]\) during INT compared to CONT.

It is suggested that the similar \([P_i] / [PCr]\) between INT and CONT in the face of the increased \([P_i]\) during INT is a function of the elevated \([PCr]\) observed during INT.

Our results show that during a single bout of CONT and INT, \([H^+]\) reaches similar concentrations while increases of \([P_i]\) were greater during INT exercise. It would seem that, within the constructs of our two exercise protocols, \([PCr]\) is a more appropriate measure of metabolic stress than either \([P_i]\) or \([H^+]\).

**Summary**

These data suggest that the amplitude of the fluctuations in \([PCr]\) during INT may be a sum of the reversal of the creatine kinase reaction and the oxidative phosphorylation response to changes in work rate. This INT exercise model has elicited greater increases in \([P_i]\) and similar increases in \([H^+]\) despite a lower average power output, uncoupling these metabolites from greater power outputs and metabolic stress. Finally, under the current exercise conditions \([PCr]\) seems to be the most appropriate measure of metabolic stress.
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Chapter 4: Effects Of A High Intensity Short Work: Shorter Recovery Intermittent and Endurance Training on VO2, VCO2 and Performance

**Introduction**

Comparisons of the effects of CONT and INT training stimuli are complicated by the various characterizations of the stimuli and training responses. A review of the current literature points to specific work : recovery cycle durations and intensities that should elicit maximal physiological and performance adaptations. Long duration intervals (> 100 s) performed at very high intensity require maximal contributions from the aerobic system and an ≈ 40% contribution from the anaerobic system \(^{30}\). Shorter duration intervals may require greater anaerobic and reduced aerobic systems contributions depending on the duration of the recovery period \(^{30}\). As duration of recovery increases, greater power output and anaerobic contribution during the work period are possible and vice versa \(^{30}\).

High intensity interval and continuous training elicits greater adaptations compared to lower intensities of exercise training as documented by changes in VO2\(_{\text{max}}\) and power output \(^{5, 7, 17, 23}\). Several studies have proposed that the time spent at, or near VO2\(_{\text{max}}\) is important to achieving the greatest adaptations of aerobic power and performances during events that require extended duration at VO2\(_{\text{max}}\) \(^{8, 27, 41, 43}\). These performance and aerobic power adaptations from high intensity exercise have been attributed to the recruitment of Type 11 fibres and associated metabolism during training \(^{14}\) and are part of the template utilized when designing the current CONT and INT exercise protocols.

The optimal duration of exercise for changes in muscle aerobic enzyme concentration and activities, and mitochondrial content have been presented in the classic work of Dudley \(^{14}\). He demonstrated in rodents that a 30 min CONT exercise duration elicited superior adaptations
versus durations of 60 or 90 min. All groups utilized the highest sustainable work rate for each these durations.

Comparisons of the chronic effects of intermittent training protocols with continuous exercise at matched average power outputs have been made\textsuperscript{20, 29, 42}. The actual power outputs during the continuous protocols of two of these studies were below the \( V_{ET} \) with the exception of the study by Overend et al.\textsuperscript{29}. This group increased the intensity of the continuous training group to 80\% of the subjects’ \( VO_2_{max} \) over the training period. The power outputs of the 30 s work : 30 s recovery intermittent protocols were 110\% : 30\% of \( VO_2_{max} \). The most notable result from this study was a greater increase in \( V_{ET} \) of the CONT group versus the INT group. This suggests the superiority of high intensity CONT training over this specific INT training for increasing \( V_{ET} \). The objective of the current INT protocol was to preserve the \( VO_2 \) profile and consequent \( V_{ET} \) adaptation of CONT exercise during an INT protocol.

The majority of previous intermittent training studies have examined work durations of 30 seconds or more, with work to rest ratios of approximately 1 : 1\textsuperscript{15, 19, 23, 27}, whereas studies of short work : short rest stimuli may provide unique information about the adaptive process\textsuperscript{1, 2, 11}.

To this end, a variation of an INT protocol from early research by Christensen et al.\textsuperscript{11}, and pilot work from this lab has demonstrated that if the highest sustainable supra-threshold 10 s work period is coupled with a 5 s moderate recovery period, a high and near constant \( VO_2 \) over a 30 min exercise duration is possible. The near maximal resultant \( VO_2 \) over the time course of such an INT exercise bout may elicit similar adaptations as have been observed elsewhere to similar intensities of CONT exercise that elicit near maximal \( VO_2 \) for extended periods\textsuperscript{6, 22}. This INT protocol will be compared with a 30 min CONT protocol at a similar intensity.

Previous studies of exercise training have seldom employed a full spectrum of performance measures. As a consequence, nuances within the performance and physiological adaptations may have been overlooked. In the present study maximal power output over short
and intermediate durations (10 s - 60 s), longer durations (3 min), and peak oxygen uptake and associated power output were measured. The measurement of VO$_2$ during all these maximal performances enabled quantification of the contribution of the aerobic system to relatively short duration performance$^{30}$. Pilot work has shown that the final 20 s of the 60 s Wingate and the final 90 s of the 3 min endurance test elicit power outputs, VO$_2$, and aerobic energy system contributions that are comparable to the 10 s work period of the INT protocol.

It is hypothesized that performing 12 of these high intensity 30 min INT and CONT protocols of similar duration, will result in: (1) increased VO$_2$ max and associated peak power output on an incremental aerobic power test; (2) similar improvements in peak power output by both training groups over the course of the training sessions; and (3) greater aerobic and anaerobic power, and greater power output during a 60 s Wingate and 3 min endurance performance in the INT group versus the CONT trained group.

**Methods**

*Subjects:* Fourteen male subjects, weight 83.1 kg SD 6.1, height 180 cm; SD 2.1, age 24yrs; SD 2 volunteered to participate in this study. The subjects were randomly assigned to one of two groups: the maximal interval training group (INT) and a maximal continuous constant load group (CONT). The Review Board for research involving Human Subjects approved of this study and the subjects provided informed consent prior to participating.

*Measures*

A cycle ergometer (Lode) ramp (25 W/min) test was used to determine VO$_{2\max}$. This test was initiated following 2 min of loadless cycling (20 W)$^{9,44}$. Tests were terminated when the subject was no longer able to maintain a pedal cadence of 60 rev*min$^{-1}$, despite verbal encouragement. During each test inspired and expired flow rates were measured using a bi-
directional turbine (Alpha Technologies, VMM 110), which was calibrated daily using a syringe of known volume (3.01 l). Respired oxygen, carbon dioxide and nitrogen (O₂, CO₂, N₂) were sampled continuously (1 ml s⁻¹) at the mouth and analyzed by a mass spectrometer (Perkin-Elmer, MGA-1100). The mass spectrometer was calibrated daily with precision-analyzed gas mixtures. Analog signals were sampled and digitized every 10 ms. Changes in gas concentration signals were aligned with the inspired and expired volumes by measuring the time delays for a square wave bolus of gas passing the turbine to the resulting change in O₂, CO₂ and N₂. Breath-by-breath computation of gas exchange variables was performed using the algorithms of Beaver et al.³ Data were then filtered for aberrant data points, interpolated to 1 s intervals, and then averaged into 5 s time bins to yield a single response for each subject. VO₂max was averaged over the last 20 s of the incremental test and peak work rate (watts) was recorded. Criteria for confirmation of reaching VO₂max included two of the following indicators, RER > 1.15, heart rate > 95% of age predicted max, and a plateau of VO₂ (<125 ml increase/min) over the last 30 s of the incremental tests. If two of these criteria were not attained the test was repeated after a 24 hour period.

Wingate tests

Anaerobic ATP-PCr and glycolytic peak power was calculated over the initial 20 s and maintenance of anaerobic glycolytic power was demarcated from 21-40 s²⁴,³⁷,⁴⁰. VO₂ (breath-by-breath collection) was collected from 0 to 60 s. VO₂ within these 20 s durations was converted to a per cent aerobic contribution by calculating the required VO₂ from the power output (VO₂ (ml/min) = work rate (kpm/min) x 2ml/kpm + sitting VO₂ of 300ml/min)¹² divided by the actual VO₂ recorded. This calculation assumes similar efficiencies over a broad range of power outputs³⁶.
Progression of Exercise Session Intensity

The work rate was increased three watts per exercise training session. An increment that has been suggested to be manageable from previous work\(^4\). Heart rate and volitional fatigue were used as markers of intensity and if heart rate decreased below 80% of the subject’s maximum heart rate during an exercise session the work rate was increased a further 1.5% for the following training session. Rating of Perceived Exertion (RPE), using the Borg scale 0-10, was also recorded during each training session at 5min intervals during both CONT and INT protocols for additional confirmation that the intensity at the conclusion of each session was maximal\(^15\).

Testing Schedule

Testing Day 1: One incremental ramp test to fatigue was performed on the LODE cycle ergometer to determine VO\(_{2\text{max}}\), V\(_{\text{ET}}\), maximal heart rate and power output. Verbal encouragement was given to facilitate maximal efforts. The work rate increment for this test was 25 W / min\(^{13,29}\). This test was also used to determine the initial work rates for the maximal INT and CONT training bouts. Ventilatory threshold was determined by an increased Ve/VO\(_2\) and the change to a non linear increase in VCO\(_2\) during the incremental test.

Tests for Days 2 and 3 were done in random fashion. On both Days pedal revolutions were recorded over 5 s intervals with an electronic cadence device attached to the bottom bracket of the cycle ergometer.

Testing Day 2: Subjects performed a 5 min warm-up on the LODE cycle ergometer at 105% V\(_{\text{ET}}\) followed by a 10 min rest period. A 3 min endurance test with breath-by-breath VO\(_2\) collection was then performed. This duration of maximal exercise has been observed to require >75% contribution from aerobic metabolism\(^30\) and from observation of pilot work results in power outputs similar to the 10 s work rate of the INT exercise. The work rate was set at a
power output equal to 50% of the difference between \( V_{ET} \) and \( VO_{2max} (\Delta 50\%) \) at 80 rpm. The subjects were instructed to begin the test at 110 rpm and to maintain the highest cadence possible until test termination at 3 minutes. Verbal encouragement was given to facilitate maximal efforts. Pedal revolutions were recorded over 10 s intervals with an electronic cadence device attached to the bottom bracket of the cycle ergometer. Day 2 testing took place a minimum of 48h after Day 1.

**Testing Day 3:** A five minute warm-up at 50 W was followed by a 60 s Wingate Test was performed at .075kp/kg body mass\(^{34}\). Subjects began no-load maximal pedaling rate for 5 s before the workload was added. \( VO_2 \) was collected throughout the Wingate test. Verbal encouragement was given throughout the test. This duration of Wingate was selected to include a high aerobic energy system contribution during the last 20 s of this 60 s Wingate. During this time period \( VO_2 \) is near maximal with the power output comparable to the 10 s work period of INT exercise.

**Post-training testing:** Post-training measures of \( VO_{2max} \), 60 s Wingate and 3 min endurance performance were made at least 48 hours following the final training session using the same procedures as for the pre-training measures.

**Study Training Protocols INT and CONT**

Group 1 - INT: Training consisted of 12 exercise sessions on alternate Days. The heavy intensity work period was 10s and the moderate intensity recovery period was 5 s. The initial work rate during the work period of the interval exercise was 50% of the difference between the \( VO_2 \) at the ventilatory threshold (\( V_{ET} \)) and \( VO_{2max} (\Delta 50\%) \). Work during the 5s moderate recovery period was 50% of the difference between \( V_{ET} \) and baseline resting \( VO_2 (\Delta 50\% V_{ET}) \). During pilot work it was possible to sustain these work / recovery power outputs for 30 min. The
work rate during the 10 s work phases was increased three watts every training period as has been previously described⁴. All training was performed on electronically braked LODE cycle ergometers.

Group 2 - CONT: Training consisted of 12 exercise sessions performed on alternate Days. Continuous training was performed at the highest work rate possible for 30 min. Initial power outputs were set at the equivalent of 120% of \( V_{ET} \). This intensity was based on the aerobic contribution for a 30 min maximal performance³⁰. Work rates were increased three watts per training session until the end of the training period. All training was performed on electronically braked LODE cycle ergometers.

A rating of perceived exertion chart (Table 4.1) was used to monitor exercise intensities.

Statistical Analysis

Baseline and post-training data were analyzed for statistical differences using repeated measures ANOVA. Significance was set at \( p<0.05 \). Tukey post-hoc tests were used if a significant main effect or interaction was found. All statistical analyses were carried out using Sigma Stat 3.1 and Sigma Plot 9.0.

Results

\( VO_2 \) and Power Output from the Incremental \( VO_2_{max} \) Tests and the Training Sessions

All subjects completed all training and testing sessions.

Both training groups achieved significant increases (\( p<0.05 \)) in their \( VO_2_{max} \) (CONT 8.3%; INT 8.1%), peak aerobic power output (CONT 5.7%; INT 7.6%) and increases in \( V_{ET} \) (CONT 9.1%; INT 12.8%) and associated power output (CONT 10.6%; INT 15.5% (\( p<0.08 \))) (Table 4.1) on the post-training incremental ramp test to fatigue, an indication that the different stresses specific to each protocol on aerobic metabolism elicited similar adaptations.
The objective of achieving maximal performances during the training sessions was confirmed as the average percent of VO$_{2\text{max}}$ 6 min into a training session was 85% of VO$_{2\text{max}}$ for CONT and 82% VO$_{2\text{max}}$ for INT (Fig. 4.1) and was 90% VO$_{2\text{max}}$ for both groups by 30 min. The average power output was lower in INT than CONT (208 W versus 218 W, p<0.05) whereas the power outputs during the 10 s work interval for INT and the constant load for CONT were different (77% of peak work rate vs 66% of peak work rate) respectively (p<0.05) of peak power output reached on the post VO$_{2\text{max}}$ test. The power output of the 10 s work period resulted in the significant anaerobic contribution to INT training. The average RPE during the training sessions was 8.5/10$^{32}$.

Power output increased over the course of the 12 training sessions (p<0.05) for both training groups, while CONT showed a trend towards greater improvement over the course of the training period (CONT 15.6% & INT 8.9%), (Fig. 4.2). Linear regression also showed a trend towards greater increases in CONT over the course of the training period [CONT = 197.314 + (3.118 * session number) ($R^2 = .919$). INT = 253.327 + (2.641 * session number) ($R^2 = .915$)].

60 s Wingate

Average power output was increased in both groups pre-training to post-training (CONT: 551 W to 570 W; INT: 432 W to 454 W (p<0.05). For INT, PO increased from 41 s - 60 s pre-training to post-training (p<0.05) with no changes observed for CONT (Fig. 4.3a & 4.3b). Mean VO$_2$ during INT and CONT was also increased pre to post-training (p<0.05). Total VCO$_2$ was higher in CONT, while VCO$_2$ from 20 s - 60 s was increased only for INT (Fig. 4.4a & 4.4b). The percent aerobic contribution was elevated post-training from 21 s – 40 s of INT (p<0.05) (Fig. 4.5a & 4.5b).
3 Min Endurance Test

Power output increased over the last minute (Fig. 4.6a & 4.6b) in both training groups (CONT: pre - post means: 273 W – 311 W; INT: pre - post means; 278 W – 327 W, p<0.05). Mean VO₂ was elevated significantly in both groups post-training (Fig. 4.7a & 4.7b), while VCO₂ was increased only during CONT (pre 3.89 L/min- post 4.44 L/min) (Figures 4.8a & 4.8b).
Table 4.1. VO$_{2\text{max}}$, peak power output on VO$_{2\text{max}}$ test (PO$_{\text{max}}$), ventilatory threshold (V$_{E\text{T}}$), power output at V$_{E\text{T}}$ (PO at V$_{E\text{T}}$), respiratory exchange ratio (RER) on the Incremental VO$_{2\text{max}}$ test pre and post-training for both CONT and INT trained groups. * p>0.05 vs pre-training values. No differences between training groups.

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<th>CONTINUOUS TRAINING GROUP</th>
<th>INTERVAL TRAINING GROUP</th>
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**Mean** | 3.75 | 4.09* | 1.26 | 1.25 | 332 | 353* | 2.41 | 2.64* | 151 | 169* |

**SD** | 0.62 | 0.52 | 0.04 | 0.07 | 44.78 | 43.84 | 0.52 | 0.44 | 40.04 | 41.91 |
Figure 4.1. Mean VO$_2$ during CONT (•) training session and INT (o) training session.
Figure 4.2. Power output over the course of the 12 training sessions of CONT and INT exercise during a CONT and INT training session. Mean values for all subjects.
Figure 4.3a. Power output on 60 s Wingate tests for CONT training group pre and post training. Mean power output higher post training (p<0.05).

Figure 4.3b. Power output on 60 s Wingate tests for INT training group pre and post training. Mean power output higher post training (p<0.05) and * denotes difference pre and post training (p<0.05).
Figure 4.3a. Mean INT VO₂ during WG. α Total mean VO₂ increased pre post values different at P<0.05 (2 way RM ANOVA)

Figure 4.3b. Mean CONT VO₂ during WG. α Total mean VO₂ increased pre post values different at P<0.05 (2 way RM ANOVA)
Figure 4.4a. Mean VCO₂ during WG CONT. α Total mean VCO₂ increased pre post values different at P<0.05 2 way RM ANOVA

Figure 4.4b. Mean VCO₂ during WG INT. α Total mean VCO₂ increased pre post values different at P<0.05 2 way RM ANOVA
Figure 4.6a. Percent aerobic contribution during Wingate test for CONT. Values are group mean ± SD.

Figure 4.6b. Percent aerobic contribution during Wingate test for INT. Values are group mean ± SD. * pre and post differences (p<0.05)
Figure 4.7a. Power output (watts) during 3 min constant load endurance test for CONT, pre (o) and post (•) training. Values are group mean * pre and post differences (p<0.05)

Figure 4.7b. Power output (watts) during 3 min constant load endurance test for INT, pre (o) and post (•) training. Values are group mean * pre and post differences (p<0.05)
Figure 4.8a. VO₂ during 3-min constant load endurance test for CON, pre (○) and post (●) training.

Figure 4.8b. VO₂ during 3-min constant load endurance test for INT, pre (○) and post (●) training. *differences from 20s-60s of exercise (p<0.05) compared to what - CONT
Figure 4.9a. VCO\(_2\) during 3 min constant load endurance test for CONT. Total VCO\(_2\) (0-60s) pre and post values different (p<0.05).

Figure 4.9b. VCO\(_2\) during 3 min constant load endurance test for INT. Pre and post . *pre post differences from 10 s to 60 s (p<0.05)
Discussion

The major findings following 12 training sessions of INT and CONT exercise training were: (1) both groups increased peak aerobic power, peak power output, $V_{ET}$, and power output at $V_{ET}$ on the incremental VO$_2$ max test. There were no significant differences between groups but there was a trend towards increased power output at $V_{ET}$ ($p < 0.08$) within the INT post-training. This suggests that the significant anaerobic contribution to the INT training sessions did not inhibit changes in aerobic metabolism and subsequently aerobic power, and that this INT training may elicit greater improvements in the power output at $V_{ET}$ versus CONT training (Table 4.1): (2) power output increased significantly during both the 10 s work period of INT and the constant load work rate of CONT over the training period (Fig.4.2). This work demonstrates that a similar magnitude in aerobic adaptations incorporating different training work rates requiring differing contributions from aerobic and anaerobic metabolism: (3) the INT trained group increased their power output on the 60 s Wingate from 41 s - 60 s, and their VO$_2$ and per cent aerobic contribution from 21 s – 40 s. This increased aerobic contribution may have led to the increased power output in the latter stages of the 60 s Wingate due to a reduced accumulation of metabolites from anaerobic glycolysis from 21 s – 40 s post-training; and (4) the increased VO$_2$ and similar VCO$_2$ during the last min of the three min performance test post-INT training may reflect a higher power output at $V_{ET}$.

High Intensity training

Early work by Dudley et al.$^{14}$ demonstrated that the greatest influence of exercise intensity on the adaptive increase in oxidative capacity in all fiber types occurred when work rates above 85% of VO$_{2max}$ were utilized.$^{15}$ The configuration of the work rates in the current study fulfilled these intensity requirements. The CONT exercise protocol would recruit predominantly Type 1 fibres while the INT protocol power output would recruit a much greater
percentage of Type 1 fibres, as the intensity is well above the work rate where these fibres would be recruited and as such would facilitate anaerobic adaptation. Both training groups were at ≈83% of their VO\textsubscript{2max} 6 min into the training session and were approaching their VO\textsubscript{2max} by 30 min. This occurred within the INT group by utilizing a significantly lower average power output (208 W versus 218 W) and repeated large fluctuations in work rate. Since this intermittent protocol elicited a similar VO\textsubscript{2} response profile as the CONT exercise, despite utilizing different power output configurations, and both exercise programs achieved similar adaptations in VO\textsubscript{2max} and associated peak power output, it is concluded that this high intensity intermittent exercise program is as effective at improving aerobic power as a high intensity CONT training protocol of similar duration. This is in agreement with several studies that have demonstrated that the VO\textsubscript{2max} of well-trained individuals can be increased (4.5%-13%) when various HIT protocols known to elicit oxygen uptakes in the region of VO\textsubscript{2max} are included during training. The reduced adaptation of VE\textsubscript{T} post high intensity INT training that has been observed elsewhere seems to be prevented by maintaining a similar VO\textsubscript{2} profile between the INT and CONT training protocols. Our findings confirm that training at or near VO\textsubscript{2max} is an effective stimulus for enhancement of VO\textsubscript{2max} and VE\textsubscript{T} within the different work rate configurations of the CONT and INT training protocols.

Progression of Training Intensities within Training

Adjustments in the work rates based on VO\textsubscript{2max} testing over the course of a training program has been performed, weekly\textsuperscript{23,42} bi-weekly\textsuperscript{23} and every 5 wks\textsuperscript{29}. Previous HIT aerobic training performed on alternate Days for 14 sessions, included VO\textsubscript{2max} measurements during each training session. They suggested that the adaption in VO\textsubscript{2max} over their training period would
accommodate work rate increases of 1.2% each training session. This progression of increased
time was utilized during this training study.

The progression of these increases was temporarily suspended during the 6th training
session in CONT and 7th training session in INT as the subjects were unable to complete the
prescribed work rate. During those sessions power output was reduced to enable the subjects to
complete the training session. Planned increases in work rates were again achieved from the
eighth until the 11th session, where again, presumably, physiological adaptation was not fast
enough and fatigue began to occur once again.

This overtraining is a function of the imbalance between the training stimulus and the
allotted recovery time. The imbalance between these variables led to the fatigue observed at
the mid-point of the training program. This overtraining may be attributed to the effects of the
decrease in pH from chronic high intensity exercise. It has also been postulated that the
peripheral component of overtraining includes reduced activities in enzymes responsible for

glucose entry into metabolism (hexokinase), as well as the regulatory enzyme within the Kreb’s
cycle, citrate synthase. The high intensity aerobic nature of both these protocols requiring high
carbohydrate oxidation, would involve both of these enzymes. Planned recovery Days within the
training period may have facilitated the adaptation process in this present study. Previous
protocols utilizing this model of work rate progressions performed two eight min and one five
min HIT work intervals with an average of 10 min rest between intervals. The reduced volume
of work and longer rest intervals may have elicited lower metabolic stress compared to the 30
min intervals employed during this study. These results suggest that previous work
demonstrating that a 3 W sessional increase in power output during HIT aerobic exercise may
have been to high for the specific training protocols utilized in this present study.
60 s Wingate Tests

The INT trained group demonstrated increased aerobic energy contribution from 21 s - 40 s and increased power output from 41 s - 60 s post-training (Figs. 4.3a & 4.4b). The faster VO₂ response observed during the 60 s Wingate performance post-INT training coupled with the increased aerobic power and aerobic energy system contribution from 21 s - 40 s (Fig. 4.4a & 4.4b) would seem to be a precursor to the increased power output at 41 s – 60 s (Fig. 4.3a & 4.3b). It is suggested that the reduction of the anaerobic energy system contribution and presumably subsequent reduced accumulation of lactate from 21 s – 40 s and subsequent increased availability from anaerobic glycolysis may have facilitated the improved performance during the final 20 s of the post-training Wingate. The deleterious effects of decreased pH associated with this anaerobic contribution would have been diminished. The post-training increases in power output during the last 20 s (309 W) may also have been a function of metabolic adaptations specific to the comparable power output performed (284 W) during the 10 s work periods of the INT training sessions at the end of the training period.

The increased VCO₂ post-training (Fig. 4.5b) for the INT group, relative to the changes in VO₂, suggests greater ventilatory buffering during the latter stages of this 60 s Wingate. The repeated recovery periods of INT may have increased carbonic anhydrase activity, increasing non-metabolic production of CO₂ and enabled faster acid-base balance. This may have been reflected in the increased production of VCO₂. The increased power output over the last 20 s of this 60 s WG would facilitate improved performances in this duration and as such should become a key component in the training programs of those athletes involved in similar duration maximal performances.
**3 Min Endurance Test**

Increased power output in both the CONT and INT were observed. Each protocol may have elicited these improved performances by different mechanisms. CONT training may have enhanced aerobic metabolism specific to Type 1 fibres and an increase in ventilatory buffering as is evidenced by the increased VCO$_2$ (Fig. 9a). INT training did not elicit an increased VCO$_2$ response (Fig. 4.9b), despite the increases in power output and VO$_2$ that were observed. The observed reduction in VCO$_2$ relative to VO$_2$ post-INT training has been ascribed to lower lactate efflux from the muscle and related bicarbonate buffering. This may have resulted as a function of the observed trend to increase power output at V$_{E}$T post-INT training$^{47}$. It is also possible that the lower VCO$_2$ was associated with a greater carbohydrate oxidation$^{21}$ versus fat oxidation$^{46}$ due to enhanced recruitment and oxidative adaptation of Type 11a fibres. These fibres would preferentially utilize carbohydrates as substrate$^{21}$ and increase relative VCO$_2$$^{46}$. It is suggested that the 10 s - Δ50% VO$_2$ max work rate of INT would recruit these specific fibres and accelerate associated metabolic adaptation.

**Summary**

Twelve sessions of maximal short work: shorter rest intermittent training elicited similar training load adaptations and increased peak aerobic power and power output as an identical number of maximal continuous constant load training sessions.

The INT protocol was superior in maintaining power output during the last third of the 60 s WG test. CONT and INT training elicited comparable increases in power output on a 3 min endurance.

Our data also suggest that this INT training program elicited greater aerobic power during the middle of the 60 s WG which facilitated increased anaerobic power and improved buffering during the latter stages of the 60 s Wingate vs CONT training, as well as improved buffering
post-INT training during the 3 min endurance performance. In conclusion the specific physiological and performance adaptations elicited from both aerobic and anaerobic training may be realized from this single INT training regime and are particularly applicable to 60 s and 3 min maximal performances, and those sports demanding repeated and rapid changes in power output above and below $V_{\text{ET}}$. 
References


Chapter 5: Summary, Limitations, and Future Studies

Summary

The overall objectives of this series of studies were to: (1) increase our understanding of the change in VO2, ΔHHb, [PCr], [P_i], and [H+] , consequent to repeated cycles of a 10 s work : 5 s recovery intermittent exercise vs continuous exercise, and (2) to observe the physiological and performance changes after performing these differing protocols three times a week for four weeks.

To elucidate the oxidative phosphorylation response of these exercise perturbations, study number one incorporated two intermittent exercises and a continuous exercise performed on cycle ergometry. The supra-threshold power output during the 10 s work period of INT ensured a much greater anaerobic contribution (12%) vs the CONT exercise (2.5%) based not only on our calculations but previously quantified energy contributions for different durations of maximal exercise21.

Concurrent measures of VO2p and ΔHHb were recorded. Our first hypothesis was validated as oscillations in ΔHHb were observed pursuant to the changes in work rate in both intermittent protocols, whereas oscillations in VO2 were only observed during the intermittent exercise that utilized the light intensity exercise (as opposed to moderate intensity) during the 5 s recovery period. These results were consistent with previous studies demonstrating fluctuations in VO2 during short work : short recovery exercise1,2,6,10, while in disagreement with others7,24. It would seem that these fluctuations are not only dependent on the difference in the energy demand of the work and recovery periods, the arterial-venous O2 differences, but as a combined effects of elevated O2 delivery13, the presence of previous priming exercise15, and the locus of metabolic control15,22 over the rest-work transitions11.
Our second hypothesis was also validated as the elevated power output of the recovery period during INT 2 (moderate intensity) elicited reduced O₂ extraction. The mechanism responsible for this result may have been the increased O₂ delivery consequent to the moderate contractile activity leading to enhanced muscle pump effects during the recovery periods of INT 2. It was also observed, contrary to earlier reports, that the ΔHHb was lower implying that relative blood flow was greater during INT vs CONT, presumably as a consequence of the contractions associated with heavy intensity exercise increasing impedance to blood flow during CONT cycling ergometer exercise. Whereas the INT allowed a short recovery period of a potential hyperemia.

The second study of this thesis examined the response of [PCr], [H⁺], and [Pi] to repeated cycles of 10 s work : 5 s rest to determine whether the fluctuations observed in VO₂ and ΔHHb in study one would be observed in [PCr], thereby, theoretically, linking these [PCr] fluctuations with oxidative phosphorylation.

Results demonstrated changes in [PCr] similar to previously observed oscillations in VO₂ and ΔHHb, over the work-recovery transitions, suggesting, the association of these oscillations in [PCr] with oxidative phosphorylation. It is also suggested that the oscillations of [PCr] and [H⁺] observed with the oscillations in work rate during the INT exercise demonstrate a virtually immediate response to the changes in work rate within not only oxidative phosphorylation, but the creatine kinase reaction and glycolytic substrate level phosphorylation as well.

The second stated hypothesis was partially validated as, indeed, [H⁺] was elevated to similar concentrations in both protocols, but contrary to initial expectations [Pi] was higher during the INT exercise compared with the CONT exercise. This suggests that the increased average power output and metabolic stress of CONT exercise, which has previously been linked to higher [H⁺] and [Pi], has been inhibited.
The similar $[H^+]$ of CONT and INT exercise and the increased $[Pi]$ of INT associated with the lower power output and metabolic stress of INT, usually associated with greater metabolic stress, would indicate that $PCr]$ is a more appropriate measure of metabolic stress under these exercise perturbations.

The final study examined the effects of a four week, three sessions per week, training regimen to compare the effects of maximal 10 s work : 5 s moderate intermittent exercise bouts with a maximal continuous exercise of similar frequency and duration. Hypotheses encompassed increased power output within the 12 training sessions, enhanced VO$_2$ max and associated peak power, and enhanced aerobic and anaerobic contributions via the INT training stimulus.

Increases in work rate over the course of the four week training period were similar between training groups while, distinctively, the work rate employed during the 10 s work period ($\Delta50\%$ VO$_2$ max), was $\approx20\%$ greater than the work rate during the continuous exercise. This demonstrates a similar adaptive response between two protocols despite requiring differing aerobic and anaerobic contributions and fibre recruitment patterns.

The similar VO$_2$ response during training in both protocols may have been responsible for the similar adaptations in aerobic power, despite differing energy system contributions during CONT and INT training.

Increased power output during 60 s Wingate performance post-INT training, was hypothesized. The increased power output from 41 - 60 s may have been a function of: (1) increased aerobic contribution from 21 - 40 s and the consequent delayed lactic acid accumulations and; (2) the increased VCO$_2$ observed during the latter stages, which may have been related to increased ventilatory buffering post-training, stemming from the repeated suprathreshold work : recovery transitions.
The three min performance test demonstrated similar increases in power output between INT and CONT training. It is suggested that the higher VO₂ during the initial 50 s of INT may have occurred as a function of the stimulus of the fluctuating power output and subsequent oscillations of oxidative phosphorylation stimuli impersonating, repeatedly, the onset of exercise.

While the decreased VCO₂ after training has been ascribed to higher VO₂ and subsequent lower lactate efflux from the muscle and related bicarbonate buffering²⁶,²⁵ and / or to the change from carbohydrates to fat utilization due to training⁸, it is also possible that the lower VCO₂ is associated with a greater carbohydrate¹⁴ vs fat oxidation²⁵ due to enhanced recruitment and adaptation of Type 11a fibres. These fibres preferentially utilize carbohydrates as substrate¹⁴. An increased reliance on oxidation of carbohydrates by the Type 11 fibres would decrease VCO₂ at any given work rate post-INT training²⁵. It is suggested that the 10 s Δ50% VO₂max work rate of INT would facilitate these specific fibre type and metabolic adaptations.

This unique intermittent training protocol utilizing a short duration but high power output 10 s work period and a 5 s moderate intensity recovery period was effective in improving aerobic as well as anaerobic indices of performance. Both the INT and CONT protocols maintain an average work intensity that averages over 90% of VO₂max. Work intensities that demand near-maximal rates of VO₂ have been recommended for the maximal adaptation to training sessions⁵,¹⁸.

Athletes performing sports that require repeated short work periods above the V₅₇T followed by short moderate recovery intervals such as pursuit cycling, rugby, short track speed skating, and basketball would experience significant benefits from the specific physiological and performance adaptations subsequent to a training program utilizing this 10 s work : 5 s recovery intermittent protocol, performed at the highest sustainable power output over a 30 min duration.

Furthermore, coaches should be cognizant of the fact that short reductions in exercise intensity during training sessions will have a significant reduction on the intensity of the work
that is being performed. These changes in exercise intensity would also be affected by training in different sized facilities. Examples would be training for hockey on an NHL size rink versus an Olympic size ice surface, short track speed skaters performing on a long track ice surface and vice versa, and swimmers performing in 25 m and 50 m pools. These situations would elicit a reduced aerobic metabolic stress as the requirements of the larger “playing” surfaces require longer duration work periods and shorter rest periods during competition and training.

Collectively there were a few general conclusions that surfaced from this thesis. First, the results suggest that during short work : short recovery intermittent exercise VO₂, ΔHHb, and [PCr] demonstrate similar temporal responses linking oxidative phosphorylation with [PCr] through the creatine kinase reaction. Secondly, [PCr], not elevated [H⁺] or [Pi] is the most appropriate measure of metabolic stress under these two exercise and conditions; and thirdly, when this maximal INT and maximal CONT exercise are utilized as training interventions, INT training will elicit adaptations in both oxidative and substrate level phosphorylation.

Limitations

Barstow et al.³ utilized a similar model comparing VO₂ data during cycle geometry and [PCr] data during plantar flexion exercise. Similar time constants between the VO₂ during cycle ergometry and [PCr] during plantar flexion exercise, collected via ³¹P-MRS, were observed. This was deemed problematic by McCreary et al.²⁰ as different muscle groups were being observed at different times despite the application of the similar relative work rates. This latter group collected measures of VO₂ and [PCr] on an identical muscle group and did see similar results. That being said, the inferences that are made between plantar flexion and cycle ergometry in the present study must be considered a limitation as the same muscle groups and similar absolute work rates were not utilized and any inferences between the two are speculative.
The use of $^{31}$P-MRS data collection systems observe relatively large areas of the muscle and associated microvasculature. One makes the assumption that the area of interrogation is homogenous in fibre type and bioenergetic responses and high energy phosphate concentrations\textsuperscript{9}. The signal that generates the data may be recording information from thousands of muscle fibres and local differences have been observed within the gastrocnemius muscle\textsuperscript{12}. Similar reservations can be made when collecting the NIRS data, as different fibre types within the area being investigated may have differing capillary densities, red blood cell concentrations, blood flow velocities and hematocrit, which will all affect $\Delta$HHb status\textsuperscript{4,17}.

Another possible limitation to the training paper was the activity patterns of the subjects. In an attempt to recruit subjects with a higher VO$_2$ max, subjects were necessarily participating in regular activity. Subjects involved in the training study were given instructions to maintain their activity levels but it is possible that these coincidental activities may have blunted or enhanced pre-post performances as well as exacerbated fatigue during the training program.

Future Studies

A training study utilizing 30 min of 10 s maximal work : 5 s rest INT exercise would allow for a greater contribution of substrate phosphorylation, while presumably the high VO$_2$ realized during the work, and maintained during the recovery period, would lead to an enhanced substrate phosphorylation response while maintaining the high aerobic adaptive response that was observed during this present study.

Interrupting the current INT protocol with a 3 min rest period after 5 min and repeating this 5 min INT exercise six times would enable increased power outputs during the work period thereby increasing the anaerobic contribution while maintaining near maximal oxygen uptakes. This protocol may facilitate greater improvements in anaerobic performance while maintaining the adaptation specific to aerobic performance.
Finally, the efficacy of short (60 min) high intensity training is well documented. Elite athletes involved in endurance sports (eg. cycling, rowing, and swimming) believe it is necessary to perform long duration exercise daily (4 – 6 hrs per Day) during a significant portion of their seasonal and or career preparation. Little is known of the acute and chronic physiological and performance effects of these extended duration training sessions. A research endeavor of this sort would be a significant contribution to applied physiology.
References


